MINIMIZE THE HYDROGENATION OF UNSATURATED FATTY ACID IN RUMEN WITH FORMALDEHYDE

Nafly C. Tiven[1,*], Lies Mira Yusiati, Rusman[2], and Umar Santoso[3]

[1]*Program of Post Graduate School, Universitas Gadjah Mada, Jln. Fauna 3, Yogyakarta 55281*

[2]*Faculty of Animal Science and Industries Universitas Gadjah Mada, Jln. Fauna 3, Yogyakarta 55281*

[3]*Faculty of Agriculture Technology Universitas Gadjah Mada, Jln. Sosio Yustisia, Yogyakarta. 55281*

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ABSTRACT

This research aimed to know the ability of formaldehyde to protect unsaturated fatty acid of CPO on the hydrogenation process by rumen microbes. In this experiment, the in vitro fermentation of rumen fluid was carrying out. It was taken from the rumen-trocar of female sheep. The unsaturated fatty acid source was from CPO (Crude Palm Oil) which encapsulated by formaldehyde 37% within 0%, 1%, 2% and 3%. The data was analyzed by Completely Random Design with Duncan's New Multiple Range Test. The difference of means the treatments were tested by Duncan's New Multiple Range Test. Result showed that oleic and linoleic resulting from fermenting CPO protected by formaldehyde was increase if it was compared with the unprotected CPO. It can be concluded that encapsulated CPO with formaldehyde was able to prevent hydrogenating of unsaturated fatty acid, mainly oleic and linoleic.

Keywords: *Hidrogenation, Unsaturated fatty acid, Formaldehyde*

INTRODUCTION

Fats in the rumen through several processes, among others: (a) the lipolysis/hydrolysis, which is a process that causes the release of fatty acid from ester bond [1]. Generally, this process occurs mainly in linoleic fatty acid, which is 60% fat fraction forage and linolenic fatty acids, (b) fermentation, the process that led to glycerol released from hydrolysis process in the rumen, and then it would be fermented into Volatile Fatty Acid (VFA), mainly propionate, (c) hydrogenation/saturation of unsaturated fatty acid to saturated fatty acids (complete or partial) by rumen microbes [1]; (d) other processes, in complete hydrogenation lead to the formation of octadecenoic and octadecadienoic acid isomer [2].

Bihydrogenation of unsaturated fatty acids in the rumen starting from isomerization of double bond configuration on the trans cis-12 to trans 11 which produces two or three trienoic fatty acids. Next step is the hydrogenation reaction, lead to the conversion of unsaturated double bonds into single bonds. Linolenic and linoleic acid decrease on cis-9 double bond into fatty acid trans-11. The last step is the hydrogenation of trans-11 double bond to produce stearic acid (linoleic and linolenic pathway) or trans-15 18:1 (linolenic pathway) [1-3]. There is no species of rumen bacteria has capability on complete hydrogenation of unsaturated fatty acids, so that the rumen bacteria are divided into two groups based on the reaction and the final product. Group A bacteria do hydrogenation of linoleic (C18:2) and α-linoleic to trans-11 C18:1 as a final product, while group B bacteria do hydrogenation of trans-11 C18:1 into stearic (C18:0) as a final product. Biohydrogenation process from unsaturated fatty acids in the rumen can be seen in Fig. 1.

The effect of rumen hydrogenation on feedstuffs unsaturated fatty acids affect no changes on fatty acid composition of the depot, so that lipid entering the duodenum was dominated by saturated fatty acids, primarily stearic (C18:0). Viewed from the aspect of consumer health, the hydrogenation process of unsaturated fatty acid is affects the health of people who consume meat, because rumen hydrogenation making the meat produced it rich with saturated fatty acids, fat of meat relatively harder and constriction of blood vessels [4].

Many nutritionists try to prevent the hydrogenation of unsaturated fatty acids by rumen microbes using formaldehyde to protect fat content of diet (rumen by pass mechanism). Fat protection by formaldehyde has been developed to save unsaturated fatty acid [5]. This technique seemed to the cheap and easier than other protection, and also has high effect on ruminant product. The formaldehyde in foods is relatively harmless because it would resolve into CO_2 within 1.5 min and will be released through respiration [6]. Giving formaldehyde for 10 and 20 g/kg crude protein did not affect protein digestibility in the small intestine [7].

* Corresponding author. Tel/Fax : +62-81328668298
Email address : nafly_tiven@yahoo.co.id

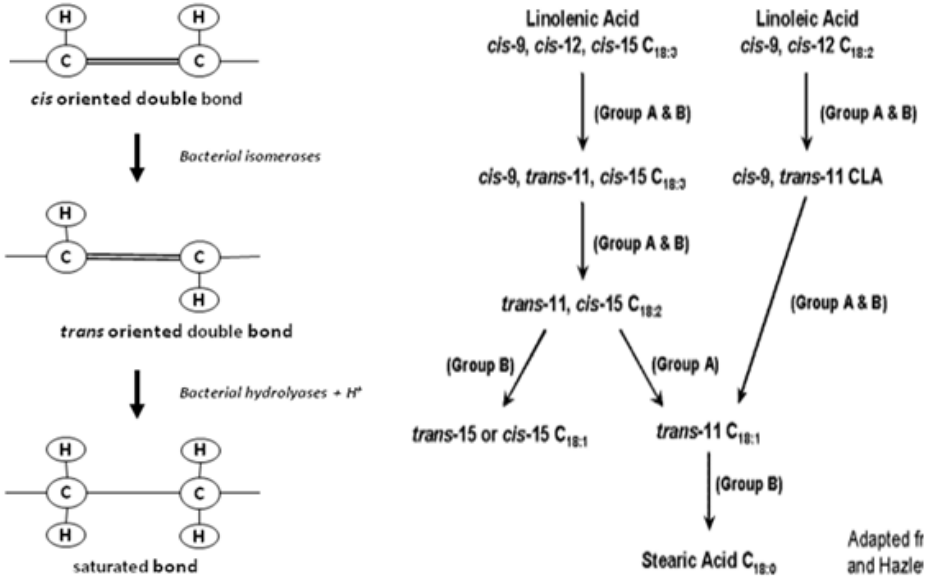

Fig 1. Illustration hydrogenation of unsaturated fatty acids in the rumen [1-3]

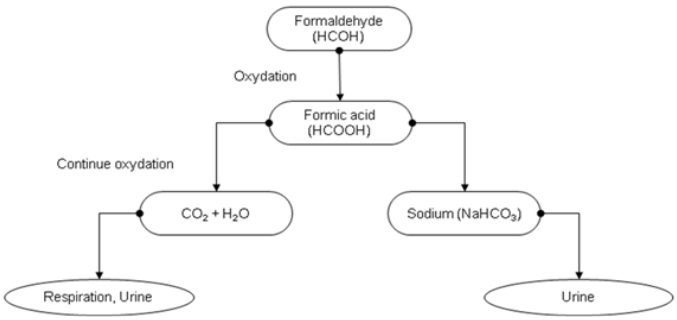

Fig 2. Process detoxification of formaldehyde

According to Wartew [8], when absorbed into the blood, formaldehyde be metabolized into formic acid then would be excreted through the urine as a sodium salt or further oxidized into CO_2 and H_2O (Fig. 2). This detoxification process is effectively at low concentrations of formaldehyde.

This research was very important to obtain the best concentration of formaldehyde to protect unsaturated fatty acid contented on the diet from rumen microbial hydrogenation, which is not followed by negative affect on the fermentation parameters and microbial activity. This research used crude palm oil (CPO) as a source of unsaturated fatty acids, since CPO contain relatively high polyunsaturated fatty acids (PUFA). Fatty acid composition in the CPO is lauric (C12:0) 0.25%; myristic (C14:0) 1.36%, palmitic (C16:0) 42.59%; stearic (C18:0) 0.13%, oleic (C18:1) 43.24%, linoleic (C18:2) 12.15% and linolenic (C18:3) 0.29% [9]. It was expected by formaldehyde protection of diet fat, unsaturated fatty acid composition of ruminant meat could be enriched.

EXPERIMENTAL SECTION

Materials

The materials used in this research are crude palm oil (CPO), expired skim milk, rumen fluid obtained from female local sheep, which taken by ditrocar, formaldehyde 37% pro analysis, solution for in vitro testing consist of (a). Main element (5.7 g Na_2HPO_4 + 6.2 g KH_2PO_4 + 0.6 g $MgSO_4.7H_2O$ dissolved with distilled water in 1 L flask, (b). Trace element $CaCl_2.2H_2O$ 13.2 g + 10.0 g + 1.0 g $MnCl_2.4H_2O$ $CoCl_2.6H_2O$ + 0.8 g Fe^{3+} $Cl_3.6H_2O$ diluted with distilled water to 100 mL, (c) Buffer solution (35 g $NaHCO_3$ + 4 g $(NH_4).HCO_3$ diluted with distilled water to 1 L; (d). Resazurin solution (100 mg Resazurin diluted with distilled water to 100 mL) and (e). Reduction solution (2 mL NaOH 1 N + 285 mg $Na_2S.7H_2O$ added to 47.5 mL of distilled water), chloroform:methanol mixture (2:1) and saturated NaCl.

Instrumentation

Equipment used in this study included a set of trocar for decision of rumen fluid, fermentor bottles, gas chromatography (GC) Shimadzu types/kinds of GC-2010 the year 2006, an analytical balance, water bath and filter paper.

Procedure

Fat profile analysis of crude palm oil

Before formaldehyde protected, CPO were analyzed to get the fat profile that consist of iodine,

saponification, acid number [10] and fatty acid composition [11].

Capsulated crude palm oil (CCPO)

CPO oil was mixed with skim milk (1:2). The mixture were added by 37% formaldehyde solution with the level of 0%, 1%, 2% and 3% (dry weight bases) to form a kind of capsule formed which called capsulated crude palm oil (CCPO).

The in vitro tested

CCPO weighed that have been prepared to match each treatments in accordance respectively, included in the serum bottles as a fermentor. Into the bottles fermentor inserted 30 mL rumen fluid and fermented solution mixture and tested in vitro with a closed system of anaerobic fermentation at a temperature of 39 °C for 48 h according to the method and Steingass Menke (1998) that has been modified [12].

After the fermentation process is stopped, then added 20 mL mixture of chloroform and methanol (2:1) and set aside some time to form two layers. Top layer (supernatant) removed, while the bottom layer (sediment) were taken and filtered into a test tube to extract the fat [10]. The extract was methylated and then analyzed the fatty acid composition by gas chromatography [11].

Statistical analysis

The data obtained were analyzed by analysis of variance using one way completely randomized design, with 4 formaldehyde treatment (0%, 1%, 2% and 3%) and 3 replications. Differences between treatments were tested further by Duncan's New Multiple Range Test [13].

RESULT AND DISCUSSION

Lipid profile of crude palm oil

The iodine, saponification, acid number and fatty acid composition on crude palm oil used in this research can be seen in Fig. 3 and 4.

Iodine number

Iodine number is expressed as the amount of I_2 (g) are bounded by the 100 g of fat. Amount of bounded I_2 indicates the number of double bonds contained in the fatty acid or oil [14].

The results showed that the CPO iodine number used in this research was 4.02 g I_2/100 g (Fig. 2). Iodine number of crude palm oil ranged from 14.5 to 19.0 g I_2/100 g [15], 45.91 g I_2/100 g CPO [16]. The lower value of iodine number in the present research due to the lower content of unsaturated fatty acids, linoleic (C18:2)

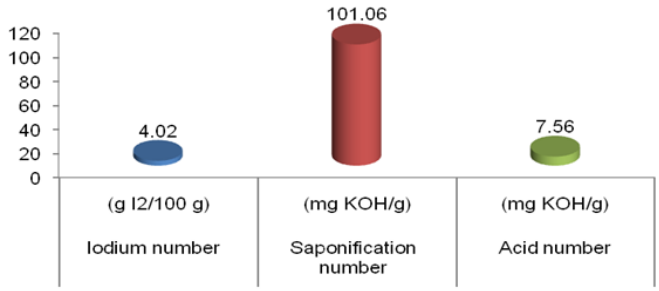

Fig 3. Test results of the iodine value, saponification value and acid value of CPO used in this research

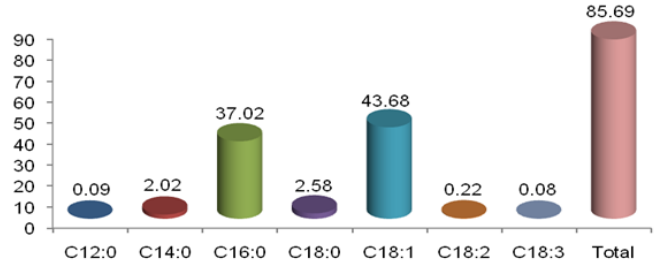

Figure 4. Test results of the fatty acid composition of CPO

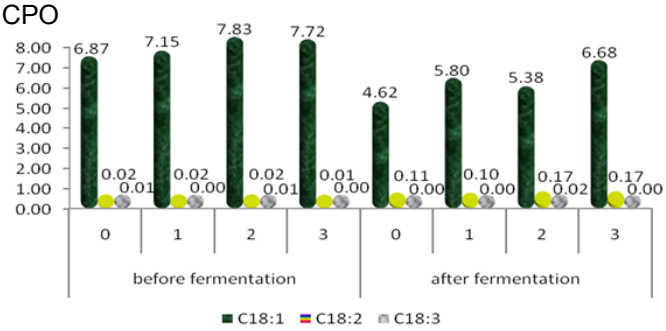

Fig 5. Illustration is a rise in unsaturated fatty acids before and after fermentation

and linolenic (C18:3) acid in CPO is used, ie 0.21% and 0.08% (Fig. 3). The percentage of linoleic and linolenic acids in CPO, were 10.1% and 0.4% respectively [17].

Saponification number

Saponification number expressed as the amount of KOH (mg) which needed to saponification 1 g fat. Saponification number indicate the molecular weight of fat or oil roughly [14].

The results showed that CPO saponification number used in this research was 101.06 mg KOH/g (Fig. 2). Saponification numbers of crude palm oil were ranged from 224 to 249 mg KOH/g [15], 191.66 mg KOH/g [16]. The low saponification number indicated the CPO that used in this research had large molecule weight. This was caused the CPO had high oleic acid (C18:1) content (43.68%) (Fig. 3).

Table 1. Composition of the CPO capsulated with skim and formaldehyde before fermentation (g/100 g CCPO)

Fatty acids	% formaldehyde in CCPO			
	0	1	2	3
Lauric (C12:0)	0.46	0.44	0.52	0.38
Myristic (C14:0)	0.68	0.63	0.78	0.61
Palmitate (C16:0)	5.32	5.42	6.06	5.32
Stearic (C18:0)	0.57	0.55	0.63	0.57
Oleic (C18:1)	6.87	7.15	7.83	7.72
Linoleic (C18:2)	0.02	0.02	0.02	0.01
Linolenic (C18:3)	0.01	0.00	0.01	0.00
Total	13.91	14.20	15.83	14.61

Table 2. Means fatty acid composition of rumen fluid on fermentation of CPO, which is protected with formaldehyde (g/50 mL of liquid fermentation)

Fatty acids	% formaldehyde			
	0	1	2	3
Lauric (C12:0) ^{ns}	0.35	0.27	0.30	0.32
Myristic (C14:0) ^{ns}	0.69	0.52	0.53	0.56
Palmitate (C16:0)	5.92^{a}	4.28^{b}	4.02^{b}	3.73^{b}
Stearic (C18:0) ^{ns}	1.35	1.03	1.13	1.08
Oleic (C18:1)	4.62^{c}	5.80^{ab}	5.38^{bc}	6.86^{a}
Linoleic (C18:2)	0.11^{b}	0.10^{b}	0.17^{a}	0.17^{a}
Linolenic (C18:3) ^{ns}	0.00	0.00	0.02	0.00
Total	13.04	12.01	11.55	12.73

ns : non significant
a,b : different superscript in the same row indicate significant differences (P<0.05)

Acid number

Acid number expressed as the amount (mg) of KOH needed to neutralize free fatty acids contained in 1 g of fat. Acid number show the amount of free fatty acids contained in fat or oil [18].

The results showed that the acid number of CPO used in this research was 7.56 mg KOH/g (Fig. 2) with the content of free fatty acids (FFA) as much as 2.5%. Acid number of crude palm oil was 0.38 mg KOH/g [16]. According to Ketaren [15], one of the factors which determine the quality of palm oil, most content of water and free fatty acid. Palm oil is good to have a water content of less than 0.1% and free fatty acid content as low as possible (less than 2%). On that basis, it can be said that palm oil used in this study has a free fatty acid which is high relatively. Nevertheless, when compared with the acid value on the CPO research results from Suharyanto [9] amount 9.0 mg KOH/g, the acid value of CPO in this study are lower relatively.

Fatty acids

Fatty acid composition of palm oil used in this research can be seen in Fig. 4.

The results showed that the fatty acid composition of palm oil used in this research was lauric 0.09%, myristic 2.02%, palmitic 37.02%, stearic 2.58%, oleic 43.68%, linoleic 0.21% and linolenic 0.08%, with total amount of 85.69%. According to Akbar, et al. [17], fatty acid content of palm oil is lauric 0.2%, myristic 1.1%, palmitic 44.0%, stearic 4.5%, oleic 39.2%, linoleic 10.1% and linolenic 0.4%. The percentage of lauric, palmitic, stearic, linoleic and linolenic acid found in this study were relatively lower, but myristic and oleic were higher than results [17].

Capsulated crude palm oil composition (CCPO)

Fatty acid composition of CCPO before fermentation

Fatty acid composition before fermentation CCPO can be seen in Table 1.

The results showed that the CPO was treated with formaldehyde 2% have fatty acid composition 15.83%, then 3% (14.61%), 1% (14.20) and 0% (13.91%), respectively. Total crude palm oil fatty acid was 85.69% (Fig. 4). Since CCPO was a mixture of skim milk and CPO with the ratio of 2:1, it's total fatty acids was a third part of CPO's, and there fore it would be 28.56%. Compared with the total fatty acids of CPO (Fig. 4), then the total fatty acids of CCPO was lower.

The decrease of fatty acid content was ranged 38.20 to 43.95%. It was due to the decreasing of palmitic, stearic, oleic, linoleic and linolenic during CCPO processing.

Fatty acid composition of CCPO after fermentation

CCPO fatty acid composition after fermentation can be seen in Table 2.

The level of formaldehyde have significant effect on average content of oleic and linoleic acid ($P<0.01$) as well as on the average content of the palmitic ($P<0.05$), but have no significant effect on the average content of lauric, myristic, stearic and linolenic acid during fermentation of rumen microbe.

In comparison with total fatty acid content of CCPO (Table 2), total fatty acid of CCPO after rumen microbial fermentation, tended to decrease in linear with levels increase of formaldehyde. Total fatty acid content tended to decrease as the level of formaldehyde increased. It is due to the decreasing of saturated fatty acid content (lauric, myristic, palmitic and stearic), although unsaturated fatty acid (oleic, linoleic and linolenic) significantly increased by the increasing of formaldehyde level. Ashes et al. [19] stated, that the formaldehyde treatment can decrease the proportion of lauric (C12:0), myristic (C14:0) and palmitic (C16:0), but increased oleic (C18:0), linoleic (C18:1) and linolenic (C18:3) in milk fat so it will be very beneficial for human health. Illustration of rise unsaturated fatty acids before and after fermentation can be seen in Fig. 5. The increasing of unsaturated fatty acid along with the increasing of formaldehyde showed that formaldehyde could protect the fatty acids in CPO and avoid them becomes hydrogenation process during rumen fermentation. Gilberth et al. [11] stated, that the fatty acid composition in a feed which protected with formaldehyde was better if compared with unprotected with formaldehyde.

CONCLUSION

It could be concluded that protection of the CPO with formaldehyde prevent hydrogenation of unsaturated fatty acids, mainly oleic and linoleic. The implication of CCPO as feed additive in the ruminant diets need to be studied later to know it's effect on fatty acid composition (in rumen fluid, blood and meat), the parameters of rumen fermentation, microbial activity, production performance and meat quality of local sheep.

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REFERENCES

1. Jenkins, T.C., Wallace, R.J., Moate, P.J., and Mosley, E.E., 2008., *J. Anim. Sci*. 86, 397–412.
2. Parakkasi, A., 1999, *Ilmu nutrisi dan makanan ternak ruminant*, UI-Press, Jakarta, 102–142.
3. Bauman, D.E., Perfield, J.W., de Veth, M.J., and Lock, A.L., 2003, *Proc. Cornell Nutr. Conf. Feed Manuf*., 175–189.
4. Gillis, M.H., Duckett, S.K., and Sackmann, J.R., 2004, *J. Anim. Sci.,* 82, 1419–1427.
5. de Veth, M.J., Gulati, S.K., Luchini, N.D., and Bauman, D.E., 2005, *J. Dairy Sci*.,8, 1685–1693.
6. Yuswanto, 2006, *Formalin di makanan tak berbahaya, suatu kontroversial*?, Radar Jogja, 8 January, http://indocina.net/phpBB3/viewtopic.php.
7. Antoniewicz, A.M., van Vuuren, A.M., van der Koelen, C.J., and Kosmala, I., 1992, *Anim. Feed Sci. Tech*., 39, 111–124.
8. Wartew, G.A., 1983, *J. Appl. Toxicol.,* 3, 121–165.
9. Suharyanto, Panji, T., Abdullah, M.I., and Syamsu, K., 2006, *Menara Perkebunan,* 74, 2, 97–108.
10. Pike, O.A., 2003, *"Fat Characterization" in Food Analysis*, 3rd ed., Nielsen, S.S., Springer Science & Business Media Inc., New York, 227–246.
11. Gilbert, C.D., Lunt, D.K., Miller, R.K., and Smith, S.B., 2003, *J. Anim. Sci.,* 81, 2457–2468.
12. Ranilla, M.J., Carro, M.D., Lopez, S., Newbold, C.J., and Wallace, R.J., 2001, *Br. J. Nutr.,* 86, 717–724.
13. Oramahi, H.A., 2008, *Analisis data dengan SPSS & SAS. Studi kasus bidang pertanian, kehutanan dan peternakan*, Ardana Media, Yogyakarta, 113–150.
14. Knothe, G., 2002, *J. Am. Oil Chem. Soc.*, 79*,* 847–854.
15. Ketaren, S., 2005, *Pengantar teknologi minyak dan lemak pangan*, UI-Press, Jakarta, 263–272.
16. Tri-Panji, Suharyanto, Gunawan, and Syamsu, K., 2005, *Menara Perkebunan*., 73, 2, 63–73.
17. Akbar, E., Yaakob, Z., Kamarudin, S. K., Ismail, M., and Salimon, J., 2009, *Eur. J. Sci. Res.*, 29, 3, 396–403.
18. Kardash, E., and Tur'yan, Y.I., 2005, *Croat. Chem. Acta.,* 78, 1, 99–103.
19. Ashes, J.R., Welch, P.V., Gulati, S.K., Scott, T.W., Brown, G.H., and Blakely, S., 1992, *J. Dairy Sci*, 75, 1090–1096.