

MINIMIZATION OF UNSATURATED FATTY ACID HYDROGENATION IN RUMENT WITH KAFFIR LIME (*Citrus hystrix*) LEAVES

MINIMALISASI HIDROGENASI ASAM LEMAK TIDAK JENUH DALAM RUMEN DENGAN DAUN JERUK PURUT (*Citrus hystrix*)

Nafly Comilo Tiven*

Department of Animal Science, Faculty of Agriculture, Pattimura University, Ambon, 97233

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ABSTRACT

This research aimed to know the ability of *C. hystrix* to protect unsaturated fatty acid on the hydrogenation process by rumen microbes. Cooking oil was tested to determine the fat profile, then mixed with skim milk (1:2). The mixture were added by *C. hystrix* leaves powder with the level of 0%, 1%, 2%, 3% and 4% (dry weight bases) from the weight of the mixture, then mixed evenly to form protected cooking oil. The oil was added into a syringe, containing 300 mg of feed substrates, elephant grass and bran= 60:40, then added with 30 ml of rumen fluid and buffer mixture. The syringe was incubated at 39°C for 48 hours. The data was analyzed by completely random design. The difference of means the treatments were tested by Duncan's new multiple range test. Result showed that oleic and linoleic content resulted from fermentation of cooking oil protected by *C. hystrix* leaves powder was increase compared to unprotected. It can be concluded that cooking oil protected with *C. hystrix* leaves powder reduced hydrogenation of unsaturated fatty acid, mainly oleic and linoleic.

(Key words: Hydrogenation, Kaffir lime (*C. hystrix*) leaves, Unsaturated fatty acid)

INTISARI

Penelitian ini bertujuan untuk mengetahui kemampuan jeruk purut dalam memproteksi asam lemak tidak jenuh terhadap proses hidrogenasi mikrobial rumen. Minyak goreng diuji untuk mengetahui profil lemaknya, kemudian dicampur dengan susu skim (1:2). Kedalam campuran itu ditambahkan bubuk daun jeruk purut dengan level 0%, 1%, 2%, 3% and 4% (berat kering) dari berat campuran tersebut, kemudian dicampur merata sehingga membentuk minyak goreng terproteksi, kemudian dimasukkan dalam syringe yang berisi substrat pakan, yaitu rumput gajah dan bekatul (60:40) sebanyak 300 mg. Ditambahkan 30 ml campuran cairan rumen dan buffer, kemudian syringe diinkubasi pada suhu 39°C selama 48 jam. Data yang diperoleh dianalisis menggunakan rancangan acak lengkap. Perbedaan nilai rerata antar perlakuan diuji lanjut dengan Duncan's new multiple range test. Hasil penelitian menunjukkan bahwa asam lemak oleat dan linoleat hasil fermentasi minyak goreng yang diproteksi dengan bubuk daun jeruk purut mengalami peningkatan dibanding yang tanpa diproteksi. Dari hasil penelitian ini dapat disimpulkan bahwa proteksi minyak goreng dengan bubuk daun jeruk purut dapat mengurangi hidrogenasi asam lemak tidak jenuh, terutama oleat dan linoleat.

(Kata kunci: Asam lemak tidak jenuh, Daun jeruk purut, Hidrogenasi)

Introduction

The process of feed fat digestion in the rumen, occurs in several stages, among others: (i) lipolysis/hydrolysis, (ii) fermentation, and (iii) hydrogenation. Hydrogenation is the process of saturation of unsaturated fatty acids (complete/partial) to

saturated fatty acids by rumen microbes. Biohydrogenation unsaturated fatty acids in the rumen starts from isomerization of the double bond configuration at the *cis*-12 to *trans*-11 produce 2 or 3 *trienoic* fatty acids (Jenkins *et al.*, 2008). The next step is the hydrogenation reaction, resulting conversion of unsaturated double bonds become single

* Korespondensi (*corresponding author*):
Telp. +62 81328668298
E-mail: nafly_tiven@yahoo.co.id

bond. Cis-9 double bonds in linolenic (C18:3) and linoleic (C18:2) reduced to trans-11 fatty acids. The final step is the hydrogenation of the double bond of trans-11 to be stearic acid (C18:0) (C18:2 and C18:3 pathway) or trans-15 18:1 (C18:3 pathway). No species of rumen bacteria capable completely hydrogenating unsaturated fatty acids, so the rumen bacteria are divided into two groups based on the reaction and the end product, the group A bacteria and group B bacteria. The group A bacteria capable hydrogenating C18:2 and α -C18:2 to be trans-1 C18:1 as the final product, while the group B bacteria was capable hydrogenating trans-11 C18:1 to be C18:0 (Figure 1). This process causes the concentration of linoleic (*cis*-9, *cis*-12 C18:2) and linolenic (*cis*-9, *cis*-12, *cis*-15 C18:3) is very low in the meat (Jenkins *et al.*, 2008), only 10% remaining and joined the lipids tissue (Wood *et al.*, 2008) whereas 90% hydrogenated to be saturated fatty acids. From the aspect of consumer health, ruminant meats that are high in saturated fatty acids make the meat fat is relatively harder and it may result of the blood vessel disease.

The animal nutrition experts are trying to prevent the hydrogenation of unsaturated fatty acids by rumen microbes with protection of the feed material (mechanism of rumen by-pass). The protection of unsaturated fatty acids source in the diet, has been successfully done with using 3% formaldehyde (CH₂O), can protect unsaturated fatty acids from hydrogenation process (Tiven *et al.*, 2011a), which are not negative to the fermentation parameters and rumen microbial activity *in vitro* (Tiven *et al.*, 2011b) and *in vivo* (Tiven *et al.*, 2013a), even a very significant influence in the blood and meat (Tiven *et al.*, 2013b). The success of this study is relatively not yet widely accepted in the general public, because of the possible dangers posed by formaldehyde.

Kaffir lime (*C. hystrix*) is one of the plants that contain natural aldehydes, namely citronellal. Citronellal (C₁₀H₁₈O) is monoterpena compounds (Figure 2) having aldehyde group, its content in the kaffir lime leaves at 81.49% (Munawaroh and Handayani, 2010). The potential of natural aldehydes will be tested *in vitro* to reduce the hydrogenation of unsaturated fatty acids feed in the rumen. As a source of unsaturated fatty acids, used Tropical (cooking oil twice filtration), with omega-9 fatty acid content of 2325 mg, 6670 mg of omega 3 and omega

29 mg per serving. The success of this research is expected to minimize the hydrogenation and increase unsaturated fatty acids in ruminant meat.

Materials and Methods

Materials

The materials used in this research are Tropical (cooking oil twice filtration) as fat source, Preston skim milk as protein source, rumen fluid as a source of microbial, *C. hystrix* leaves as a source of citronellal, solution for *in vitro* testing consist of (a). Main element (5,7 g Na₂HPO₄ + 6,2 g KH₂PO₄ + 0,6 g MgSO₄.7H₂O dissolved with distilled water in 1 L flask, (b). Trace element CaCl₂.2H₂O 13.2 g + 10.0 g + 1.0 g MnCl₂.4H₂O CoCl₂.6H₂O + 0.8 g Fe³⁺ Cl₃.6H₂O diluted with distilled water to 100 mL, (c) Buffer solution (35 g NaHCO₃ + 4 g (NH₄) HCO₃ 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 Na₂S.7H₂O added to 47.5 mL of distilled water), chloroform : methanol mixture (2:1), Na₂SO₄ anhydrous and saturated NaCl (0.88%).

Equipment used in this study is syringe, gas chromatography (GC) Shimadzu types/kinds of GC-2010 with the column used was RTX 5 30 m, injection and detector temperature 270°C and column temperature 140-250°C. An analytical balance Ohaus PAJ 1003, water bath Memmert WNB 10 and filter paper Whatman 41.

Methods

Fat profile analysis of cooking oil.

Before protected, cooking oil were analyzed to get the fat profile that consist of iodine, saponification, acid number and fatty acid composition (AOAC, 2012). Cooking oil was mixed with Preston skim milk (1:2). The mixture were added by *C. hystrix* leaves powder with the level of 0%, 1%, 2%, 3% and 4% (dry weight bases) from the weight of the mixture, then mixed evenly into protected cooking oil.

The *in vitro* tested. The protected cooking oil was weighed to match each treatments in accordance respectively, included in the syringe as a fermentor. Briefly, 30 mL of rumen fluid and fermented solution mixture were added into the syringe and *in vitro* was performed with a closed system of anaerobic fermentation at a

temperature of 39°C for 48 h (Menke and Steingass, 1998, modified by Ranilla *et al.*, 2001). 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) was removed, while the bottom layer (sediment) were taken and filtered into a test tube to extract the fat then methylated and analyzed the fatty acid composition by gas chromatography (AOAC, 2012).

Statistical analysis. The data obtained were analyzed by analysis of variance using one way completely randomized design, with *C. hystrix* leaves powder level (0%, 1%, 2%, 3% and 4%) and 3 replications. Differences between treatments were tested further by Duncan's new multiple range test (Oramahi, 2008).

Results and Discussion

Lipid profile of cooking oil

The iodine, saponification, acid number and fatty acid composition on cooking oil used in this research can be seen in Table 1.

Iodine number. Iodine number is expressed as the amount of I₂ (g) are bounded by the 100 g of fat. Amount of bounded I₂ indicates the number of double bonds contained in the fatty acid or oil (AOAC, 2012). The results showed that the cooking oil iodine number used in this research was 4.60 g I₂/100 g (Table 1). The cooking oil made from crude palm oil (CPO). As a comparison, iodine number of crude palm oil ranged from 14.5 to 19.0 g I₂/100 g [10], 45.91 g I₂/100 g CPO (Tri-Panji *et al.*, 2005). The lower value of iodine number in

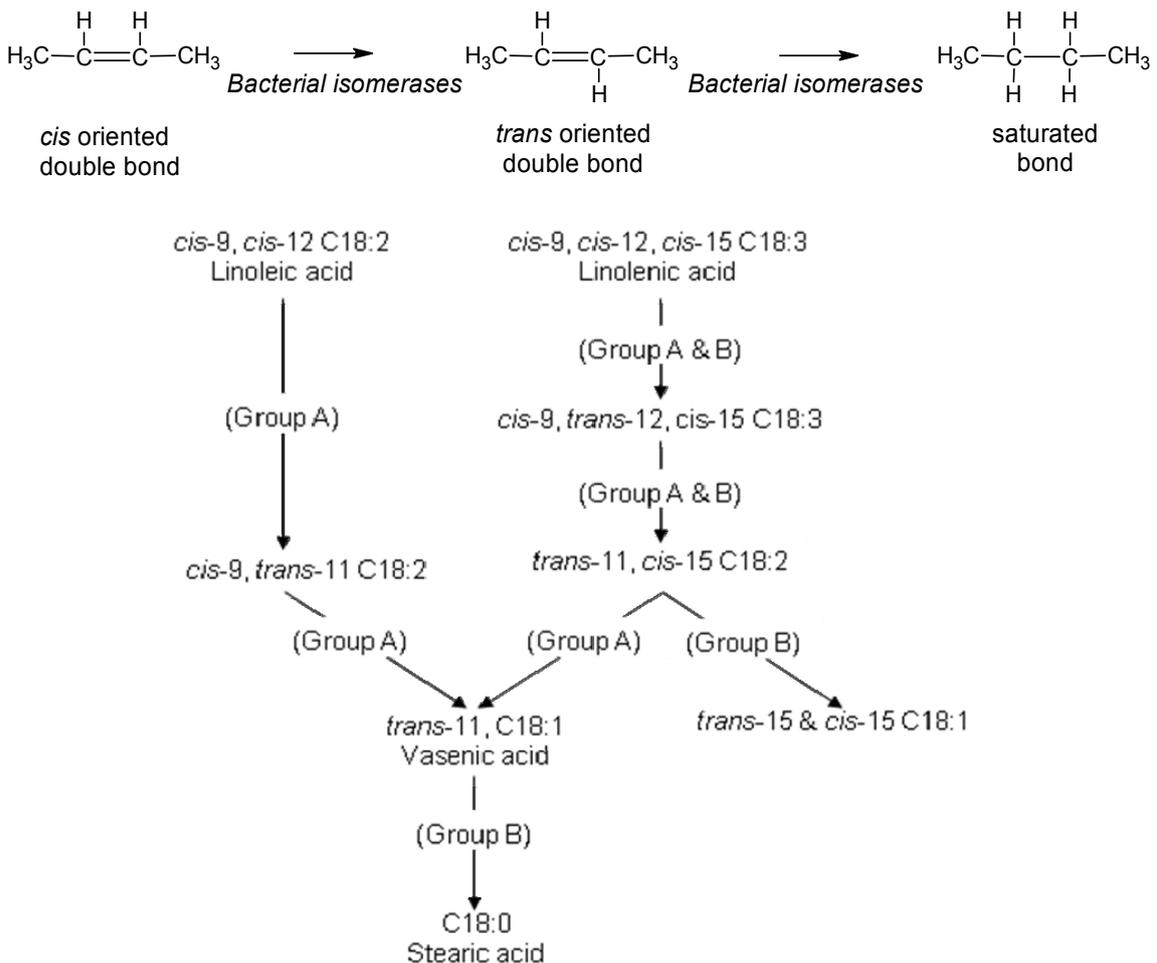


Figure 1. Illustration hydrogenation of unsaturated fatty acids in the rumen (Jenkins *et al.*, 2008).

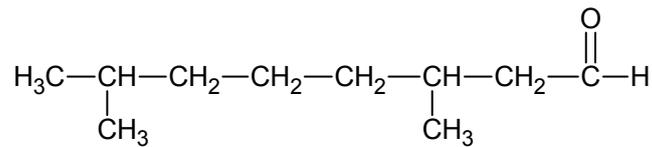


Figure 2. Chemical structure Sitronellal (Ketaren, 2005).

Table 1. The average fat profile of cooking oil that used in the study

Parameters	Unit	Composition
Iodin number	g I ₂ /100g	4.60
Saponification number	mg KOH/g	158.53
Acid number	mg KOH/g	0.90
Fatty acid :		
Lauric (C12:0)	%	0.12
Miristic (C14:0)	%	0.93
Palmitic (C16:0)	%	35.90
Stearic (C18:0)	%	3.57
Oleic (C18:1)	%	58.76
Linoleic (C18:2)	%	0.02
Linolenic (C18:3)	%	0.16
SFA	%	40.52
MUFA	%	58.76
PUFA	%	0.18
Total	%	99.46

this research due to the lower content of unsaturated fatty acids, linoleic acid (C18:2) and linolenic acid (C18:3) in Tropical cooking oil is used.

Saponification number.

Saponification number expressed as the amount of KOH (mg) which needed to saponification 1 g fat (AOAC, 2012). Saponification number indicate the molecular weight of fat or oil roughly. The results showed that cooking oil saponification number used in this research was 158.53 mg KOH/g (Table 1). The cooking oil made from CPO. As a comparison, saponification number of crude palm oil were ranged from 224-249 mg KOH/g (Ketaren, 2005). The low saponification number caused the cooking oil that used in this research had high oleic acid (C18:1) content, which is the fatty acid carbon chain length, but slightly the number of double bond so that small in molecular weight and saponification number.

Acid number. Acid number expressed as the amount (mg) of KOH needed to neutralize free fatty acids contained in 1 g of fat (AOAC, 2012). Acid number show the amount of free fatty acids contained in fat or oil. The results showed that the acid number of cooking oil used in this research was 0.90

mg KOH/g. Acid number of crude palm oil were 0.38 mg KOH/g (Tri-Panji *et al.*, 2005). According to Ketaren (2005), one of the factors which determine the quality of palm oil is water and free fatty acid content. 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%). According to Tri-Panji (2005), CPO acid number amount 9.0 mg KOH/g, so the acid number of Tropical cooking oil in this study are lower.

Fatty acids. The results showed that the fatty acids composition of cooking oil used in this research was lauric 0.12%, myristic 0.93%, palmitic 35.90%, stearic 3.57%, oleic 58.76%, linoleic 0.02% and linolenic 0.16%, with total amount of 99.46%. The cooking oil made from CPO. According to Akbar *et al.* (2009), 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%. It can be said that the percentage oleic acid of the cooking oil higher, while linoleic and linolenic lower.

Citronellal content in *C. hystrix* and fatty acid composition of protected cooking oil

Citronellal content in *C. hystrix* leaves powder are used as a source of aldehyde

amounted to 98.89%. *C. hystrix* leaves powder needed for protection of cooking oil was calculated according to citronellal content. Means fatty acid composition of cooking oil protected, as shown in Table 2.

The percentage of fatty acid content of cooking oil protected by citronellal on *C. hystrix* leaves by 0%, 1%, 2%, 3% and 4% were 99.98%, 99.76%, 99.67%, 99.76% and 99.61%, respectively. When compared to the total fatty acid of cooking oil by 99.46% (Table 1), the cooking oil protected is increased, respectively by 0:52%, 0:30%, 0:21%, 0:30%, 0:15%. Fatty acids that increased were palmitic, oleic and linoleic.

Fatty acids content of rumen fluid fermentation

The effect of different levels of *C. hystrix* leaves powder and cooking oil on fatty

acid content of rumen fluid fermented, can be seen in Table 3. The results showed that cooking oil protected with *C. hystrix* leaves powder has a significant ($P<0.01$) effect on the fatty acid content of the rumen fluid fermentation. When compared to the total fatty acid protected cooking oils (Table 2), total fatty acid content tend to decrease in line increased level of *C. hystrix* leaves powder, by 0.72%, 1.76%, 1:18, 1:17% and 3.70% respectively. This is caused by decrease ($P<0.01$) content of saturated fatty acid (SFA) are lauric, myristic, palmitic and stearic. The increase levels of *C. hystrix* leaves powder, increase content of monounsaturated fatty acid (MUFA) ($P<0.01$) and polyunsaturated fatty acid (PUFA) ($P<0.01$). The increase of unsaturated fatty acids showed that citronellal which is a natural aldehydes in the *C. hystrix* leaves

Table 2. The average fatty acids composition of the cooking oil protected by *C. hystrix* before fermentation (g/100 g)

Fatty acid	% <i>C. hystrix</i>				
	0%	1%	2%	3%	4%
Lauric (C12:0)	0.20	0.04	0.28	0.28	0.28
Miristic (C14:0)	1.07	1.25	1.34	1.28	1.42
Palmitic (C16:0)	37.24	37.33	37.07	37.24	36.68
Stearic (C18:0)	0.22	0.39	0.41	0.42	0.44
Oleic (C18:1)	60.89	59.93	59.51	59.44	59.15
Linoleic (C18:2)	0.31	0.12	0.98	1.10	1.49
Linolenic (C18:3)	0.06	0.72	0.10	0.00	0.16
SFA	38.72	39.00	39.08	39.21	38.81
MUFA	60.89	59.93	59.51	59.44	59.15
PUFA	0.37	0.84	1.08	1.10	1.65
Total	99.98	99.76	99.67	99.76	99.61

Table 3. The average rumen fluid fatty acid content of cooking oil protected by *C. hystrix* after fermented (g/100 g)

Fatty acid	% <i>C. hystrix</i>				
	0	1	2	3	4
Lauric (C12:0)	14.01 ^b	14.94 ^a	12.64 ^c	10.92 ^d	9.64 ^e
Miristic (C14:0)	7.71 ^a	7.68 ^a	7.13 ^{ab}	6.91 ^b	5.33 ^c
Palmitic (C16:0)	56.03 ^a	53.28 ^b	52.13 ^c	51.13 ^d	36.78 ^e
Stearic (C18:0)	1.68 ^a	1.59 ^a	1.12 ^b	0.56 ^c	0.55 ^c
Oleic (C18:1)	17.02 ^e	18.07 ^d	23.37 ^b	20.87 ^c	39.89 ^a
Linoleic (C18:2)	1.42 ^b	1.66 ^b	1.83 ^b	1.94 ^{cb}	3.45 ^a
Linolenic (C18:3)	0.06 ^c	0.11 ^b	0.27 ^a	0.27 ^a	0.27 ^a
SAFA	79.43 ^a	77.49 ^b	73.02 ^c	69.52 ^d	52.30 ^e
MUFA	17.02 ^e	18.07 ^d	23.37 ^b	20.87 ^c	39.89 ^a
PUFA	2.82 ^b	2.44 ^b	2.10 ^b	2.21 ^b	3.72 ^a
Total	99.26 ^a	98.00 ^b	98.49 ^b	98.59 ^{ab}	95.91 ^c

^{a,b,c,d,e} different superscript at the same row indicate significant differences ($P<0.01$).

powder was able to protect unsaturated fatty acids in cooking oil thus avoid rumen microbial hydrogenation process. This is in line with research by de Veth *et al.* (2005), that feed treatment with formaldehyde can decrease the proportion of fatty acid C12:0, C14:0, C16:0 and increase C18:1, C18:2 and C18:3 in milk fat.

Conclusion

It could be concluded that protection of the cooking oil with *C. hystrix* leaves powder can decrease the hydrogenation of unsaturated fatty acids, especially oleic and linoleic.

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