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Distilled liquid smoke as feed fat protector and it's effect on fatty acids in rumen fluid

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Abstract. This study aims to determine the effect of dietary fat protection using distillated liquid smoke on rumen fluid fatty acids. Crude palm oil (CPO) as a source of feed fat, mixed with skim milk (1:2), then divided into 3 parts: without protected by distillated liquid smoke (P0) and protected by distillated liquid smoke 2,5% (P1) and 5% (P2). For the in vitro test, rumen fluid was used as a microbial donor with elephant grass and soybean meal (60:40) as substrate. A total of 5% CPO was put in a syringe containing 30 ml of rumen fluid, substrate and buffer, then anaerobically fermented at 39 °C for 48 hours. The parameters observed were rumen fluid fatty acids. This study used a completely randomized design, with 3 treatments (P0, P1, P2) each of 3 replications. The results showed that the source of feed fat protection (CPO) used distilled liquid smoke, increasing ($P<0.01$) fatty acid composition of rumen fluid fermented. P1 was better at increasing ($P<0.01$) unsaturated fatty acids in rumen fluids. It can be concluded that liquid smoke can be used as a feed fat protector, because it can reduce hydrogenation and increase unsaturated fatty acids in rumen fluids in vitro fermented.

1. Introduction

Feed with high unsaturated fatty acid (UFA) content is not linear in increasing UFA in ruminant meat (cows, buffaloes, goats and sheep). Even, UFA which is high in feed, will be hydrogenated in the rumen (one part of the stomach in ruminants) into saturated fatty acid (SFA). As a result of this hydrogenation, essential UFA, namely linoleic acid (C18:2) and linolenic acid (C18:3), is converted to SFA stearic (C16:0). This condition causes ruminant meat fat to be more dominated by SFA, so it becomes harder [1] and if it is consumed potentially atheroclerosis in blood vessels, strokes and heart attacks in consumers.

One way that livestock nutritionists do to reduce the hydrogenation of UFA in this rumen is to protect the feed fat UFA source. Recent studies of feed fat protection have been carried out in vitro and in vivo, using several sources of aldehydes as fat protectors, among others formaldehyde (CH_2O) [2–3], *Cinnamomum burmanii* [4] and Kaffir lime (*Citrus hystrix*) leaves [5–6]. Although it can protect fat, the results of this study cannot be applied, because formaldehyde is a prohibited ingredient, while *Cinnamomum burmanii* and *Citrus hystrix* leaves are considered not economical (must be provided in large quantities) and not aesthetically (changing the color and littering of feed).

One source of natural aldehyde that is being studied as a protector of feed fat is distilled liquid smoke. This is because redestilled liquid smoke is a product that is not harmful to consumers and is safe for consumption according to the United States Food and Drug Administration (USFDA) and is a Food Additives Requirements for Taste and Use in Food Products according to SNI 01-7152-2006 [7]. The use of liquid smoke is better in terms of chemistry, microbiology and sensory [8]. Composite compounds of liquid smoke are 11-92% water, 0.2-2.9% phenol, 2.8-9.5% acid, 2.6-4.0% carbonyl and 1-7% tar [9]. One compound is the carbonyl group is formaldehyde [7], which has been used to treat toothache, all kinds of skin ailments by fungi, viruses, bacteria [10].

In this study, CPO is used as feed fat source, because CPO has a relatively good fatty acid composition, especially the relatively high UFA content. According to [11], the percentage of fatty acids in palm oils, among others lauric (C12:0) 0.5%; myristic (C14:0) 0.5-2.0%, palmitic (C16:0) 39.3-47.5%; palmitoleic (C16:1) nd-0.6%, stearic (C18:0) 3.5-6.0%, oleic (C18:1) 36.0-44.0%, linoleic (C18:2) 9.0-12.0% and linolenic (C18:3) nd-0.5%. The fatty acid composition of palm oil is lauric (C12:0) 0.2%; myristic (C14:0) 1.1%, palmitic (C16:0) 44.0%, stearic (C18:0) 4.5%, oleic (C18:1) 39.20%, linoleic (C18:2) 10.1%, linolenic (C18:3) 0.4% and arachidic (C20:0) 0.1%, with total saturated fatty acids (SFAs) 49.9%, monounsaturated fatty acids (MUFAs) 39.2% and polyunsaturated fatty acids (PUFAs) 10.5% [12].

2. Material and methods

2.1. Materials

The materials used in this research are CPO, skim milk, rumen fluid from female local sheep, distilled liquid smoke, solution for in vitro testing, chloroform:methanol mixture (2:1) and saturated NaCl. Equipment used in this research are fermentor syringe, gas chromatography (GC) Shimadzu types/kinds of GC-2010 the year 2017, an analytical balance, water bath and filter paper.

2.2. Methods

CPO were analyzed to get the fat profile (iodine value, saponification value, acid value and fatty acid composition [13]. CPO was mixed with skim milk (1:2), then divided into 3 parts: without protected by distilled liquid smoke (P0) and protected by distilled liquid smoke 2,5% (P1) and 5% (P2). A total of 5% CPO was put in a syringe containing 30 ml of rumen fluid, substrate and buffer, then anaerobically fermented at 39 °C for 48 hours according to Steingass Menke (1998) that has been modified [14]. 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. The extract was methylated and then analyzed the fatty acid composition by gas chromatography [13].

The data obtained were analyzed of variance (ANOVA) using a complete randomized design, with 3 treatments (P0, P1, P2) each of 3 replications. Differences between treatments were tested further by Duncan's New Multiple Range Test [15].

3. Results and discussion

3.1. Lipid profile of CPO

3.1.1. Iodine, saponification and acid value. The iodine value of an oil/fat is the number of grams I_2 absorbed by 100g of the oil/fat [13], used to measure of degree of unsaturated in fats/oils [16] and a routine activity in the palm oil industry to control the quality of traded palm oil [17]. Iodine value of CPO used in this research was 36.27 grams I_2/100 grams. Iodine value of CPO according to Malaysian standards is 50.4-53.7 grams I_2/ 100 grams [18]. The lower value of the iodine value in this research due to the lower content of unsaturated fatty acids, oleic (29,98%), linoleic (8.09%) and linolenic (0.17%). The percentage of oleic, linoleic and linolenic acids in CPO is 39.20%, 10.1% and 0.4% [12]. The Iodine value, saponification value and acid value, can be seen in Table 1.

Table 1. The fat profiles CPO used in the study

Lipid profile	Unit	Composition
Iodium value	g I_2/100g	36.27
Saponification value	mg KOH/g	182.84
Acid value	mg KOH/g	6.98
Fatty acid :		
- Kaprilic	%	0.08
- Kapric	%	0.07
- Lauric	%	0.63
- Myristic	%	2.20
- Palmitic	%	54.71
- Palmitoleic	%	0.23
- Stearic	%	2.74
- Oleic	%	29.98
- Linoleic	%	8.09
- Linolenic	%	0.17
- Arakhidic	%	0.15
SFAs	%	60.42
MUFAs	%	30.21
PUFAs	%	8.41
Total	%	99.03

The saponification value is the number (mg) of KOH required to saponify 1 gram of oil/fat, which indicates the molecular weight of oil/fat roughly. Oils/fats contain fatty acids with short carbon chain, have a relatively small molecular weight, so have a large saponification value and vice versa [13]. The saponification value of CPO used in this research was 182.84 mg KOH/g. The saponification value of CPO according to Malaysian standards is 194 to 205 mg KOH/g [18]. The lower saponification number caused high levels of MUFAs and PUFAs.

The acid value is the number (mg) of KOH required to neutralize free fatty acids present in 1.0 g of oil/fat. The acid value of CPO used in this research was 6.98 mg KOH/g. The acid value of CPO according to Malaysian standards is ≤ 10.95 mg NaOH/g [18], so can be said the acid value of CPO in this study are lower relatively.

3.1.2. Fatty acids. The CPO used in this study was high in total saturated fatty acids/SFAs, caused by high in saturated fatty acids (lauric, myristic and palmitate). This CPO is low in total monounsaturated fatty acids/MUFAs and total polyunsaturated fatty acids/PUFAs, caused by low in unsaturated fatty acids (oleic, linoleic and linolenic). The fatty acid composition of palm oil is 0.2% lauric, 1.1% myristic, 44.0% palmitic, 4.5% stearic, 39.2% oleic, 10.1% linoleic, 0.4% linolenic and 0.1% arachidic, with total SFAs, MUFAs and PUFAs, each 49.9%, 39.2% and 10.5% [12].

3.2. Fatty acid composition of CPO protected by distilled liquid smoke, before and after fermentation

3.2.1. Before fermentation. The results showed that the total fatty acids decreased in P1 compared by P0, because they decreased in PUFA (linoleic and linoleic). This decrease may be caused by CPO mixed with liquid smoke containing water at 11-92% [9]. Fatty acid composition of CPO which protected by distilled liquid smoke before fermentation, can be seen in Table 2.

Table 2. Fatty acid composition (%) of CPO is protected by distilled liquid smoke before fermentation

Fatty acid	% Liquid smoke		
	P0	P1	P2
Lauric (C12:0)	0.87	0.63	0.62
Myristic (C14:0)	2.99	2.17	2.19
Palmitic (C16:0)	53.15	55	57.04
Palmitoleic (C16:1)	0.33	0.23	0.23
Stearic (C18:0)	2.8	2.77	2.65
Oleic (C18:1)	27.92	29.46	28.76
Linoleic (C18:2)	9.97	7.33	7.02
Linolenic (C18:3)	0.22	0.17	0.16
SAFAs	59.81	60.57	62.5
MUFAs	28.25	29.69	28.99
PUFAs	10.19	7.5	7.18
Total	98.25	97.76	98.67

3.2.2. After fermentation. Fatty acid composition of CPO is protected by distilled liquid smoke after fermentation, can be seen in Table 3.

Table 3. Fatty acid composition (%) of CPO is protected by liquid smoke after fermentation

Fatty acid	% Liquid smoke		
	P0	P1	P2
Lauric (C12:0)	0.00^{c}	1.80^{b}	3.77^{a}
Myristic (C14:0)	9.13^{a}	3.39^{b}	5.95^{c}
Palmitic (C16:0)	59.97^{a}	56.77^{b}	55.60^{b}
Palmitoleic (C16:1)	0.00^{c}	1.39^{b}	2.58^{a}
Stearic (C18:0)	6.56^{b}	7.18^{b}	9.69^{a}
Oleic (C18:1)	13.75^{b}	19.70^{a}	12.18^{c}
Linoleic (C18:2)	6.10^{a}	6.46^{a}	4.44^{b}
Linolenic (C18:3) ^{sn}	3.10	2.88	2.76
SAFAs ^{sn}	75.66	69.14	58.34
MUFAs	13.75^{b}	21.08^{a}	14.76^{b}
PUFAs	9.19^{d}	9.34^{d}	7.20^{e}
Total ^{sn}	98.61	99.56	96.97

ns : non significant
a,b,c : different superscript in the same row indicate significant (P<0.01).
d,e : different superscript in the same row indicate significant (P<0.05).

Fatty acid composition of CPO is protected by distilled liquid smoke after fermentation, shows significant effect (P<0.01) on fatty acids (except for linolenic and SFAs), MUFAs and PUFAs. When compared between treatments, the SAFAs at P1 is higher, because it has a increase in myristic and palmitic. MUFAs on P1 is higher, because it has an increase in oleic. When compared between feed fat sources protected with liquid smoke, before and after fermentation, there is an increase in total fatty acid on P0 and P1, because it has an icrease in SAFAs (myristic, palmitic and stearic) after fermentation. There was a decrease in MUFAs after fermentation, because there was a decrease in oleic. There was an increase in PUFAs on P1 after fermentation, because there was an increase in linoleic acid. These results indicate that CPO as a feed fat source, which protected with distilled liquid

smoke, can reduce hydrogenation of unsaturated fatty acids by rumen microbes. CPO protected with formaldehyde can reduce oleic and linoleic due to hydrogenation by rumen microbial [2–19].

4. Conclusion

It can be concluded that distilled liquid smoke can be used as a feed fat protector, because it can reduce hydrogenation and increase unsaturated fatty acids in rumen fluids in vitro fermented.

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