EFFECT OF CRUDE PALM OIL PROTECTION WITH FORMALDEHYDE ON HYDROGENATION OF RUMEN FLUID UNSATURATED FATTY ACID: ITS EFFECT ON BLOOD AND MEAT FATTY ACID

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

[1]*Faculty of Agriculture, Pattimura University, Jl. Ir. M. Putuhena Ambon 97233*

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

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

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ABSTRACT

This research aimed to determine the effect of crude palm oil protected with formaldehyde on the hydrogenation of unsaturated fatty acids in the rumen and its effect on blood and meat fatty acids. Fifteenth local male lambs aged 9-12 months weighing 14-17 kg, were divided into 3 groups ration treatment. The first group received only the basal ration (R0), the 2nd group received the basal ration and 3% CPO (R1), while the 3rd group received the basal ration and 3% CPO protected with 2% formaldehyde (R2). Basal feed consisted of 60% grass, 30% bran and 10% soybean meal, with the nutrient content of 62.98% TDN, 45.5% DM, 14.48% CP, 4.70% EE and 21.93% CF. Parameters observed were the fatty acid from rumen fluid, blood and meat of sheep. Data were analyzed by complete randomized design direction patterns. Differences between treatments were tested further using Duncan's New Multiple Range Test. The results showed that treatment of R2 can increase unsaturated fatty acids in the rumen, blood and meat (P<0.01). Concluded that the protection of crude palm oil in the ration with formaldehyde can reduce the hydrogenation of unsaturated fatty acids by rumen microbes, which affects the increase in unsaturated fatty acids, both in blood and in the meat.

Keywords: *rumen fluid fatty acid; blood fatty acid; meat fatty acid*

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh proteksi minyak sawit kasar dengan formaldehid terhadap hidrogenasi asam lemak tidak jenuh dalam rumen dan dampaknya terhadap asam lemak darah dan daging. Sebanyak 15 ekor ternak domba lokal jantan umur 9-12 bulan dengan bobot badan sekitar 13-17 kg, dibagi menjadi 3 kelompok perlakuan pakan. Kelompok ternak pertama hanya mendapat ransum basal (R0), kelompok kedua mendapat ransum basal dan 3% CPO tanpa diproteksi formaldehid (R1) serta kelompok ketiga mendapat ransum basal dan 3% CPO yang diproteksi dengan 2% formaldehid (R2). Ransum basal yang digunakan terdiri dari 60% rumput gajah, 30% bekatul dan 10% bungkil kedele, dengan kandungan nutrien 62,98% TDN; 45,5% BK; 14,48% PK; 4,70% LK dan 21,93% SK. Parameter yang diamati adalah asam lemak cairan rumen, darah dan daging domba. Data yang diperoleh dianalisis dengan rancangan acak lengkap pola sederhana. Perbedaan antar perlakuan diuji lanjut menggunakan Duncan's New Multiple Range Test. Hasil penelitian menunjukkan bahwa perlakuan R2 dapat meningkatkan asam lemak tidak jenuh dalam rumen, darah dan daging (P<0.01). Dari penelitian ini dapat disimpulkan bahwa proteksi minyak sawit kasar dengan formaldehid dalam ransum dapat mengurangi hidrogenasi asam lemak tidak jenuh oleh mikrobia rumen, yang berdampak pada peningkatan asam lemak tidak jenuh, baik dalam darah maupun dalam daging.

Kata Kunci: *asam lemak cairan rumen; asam lemak darah; asam lemak daging*

INTRODUCTION

Crude palm oil (CPO) extracted from palm fruit mesocarp [1], has high content in polyunsaturated fatty acids (PUFA). The composition of fatty acids in palm oil are 0.50% lauric acid (C12:0), 0.92% myristic acid (C14:0), 36.84% palmitic acid (C16:0), 4.77% stearic acid (C18:0), 44.51% oleic (C18:1), 11.12% linoleic acid (C18:2) and 0.24% linolenic acid (C18:3) [2]. Palm oil contains saturated fatty acids (SAFA), namely C16:0 44.3%, C18:0 4.6%, C14:0 1.0%, monounsaturated fatty acids (MUFA), namely C18: 1 38. 7%, and poly-

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

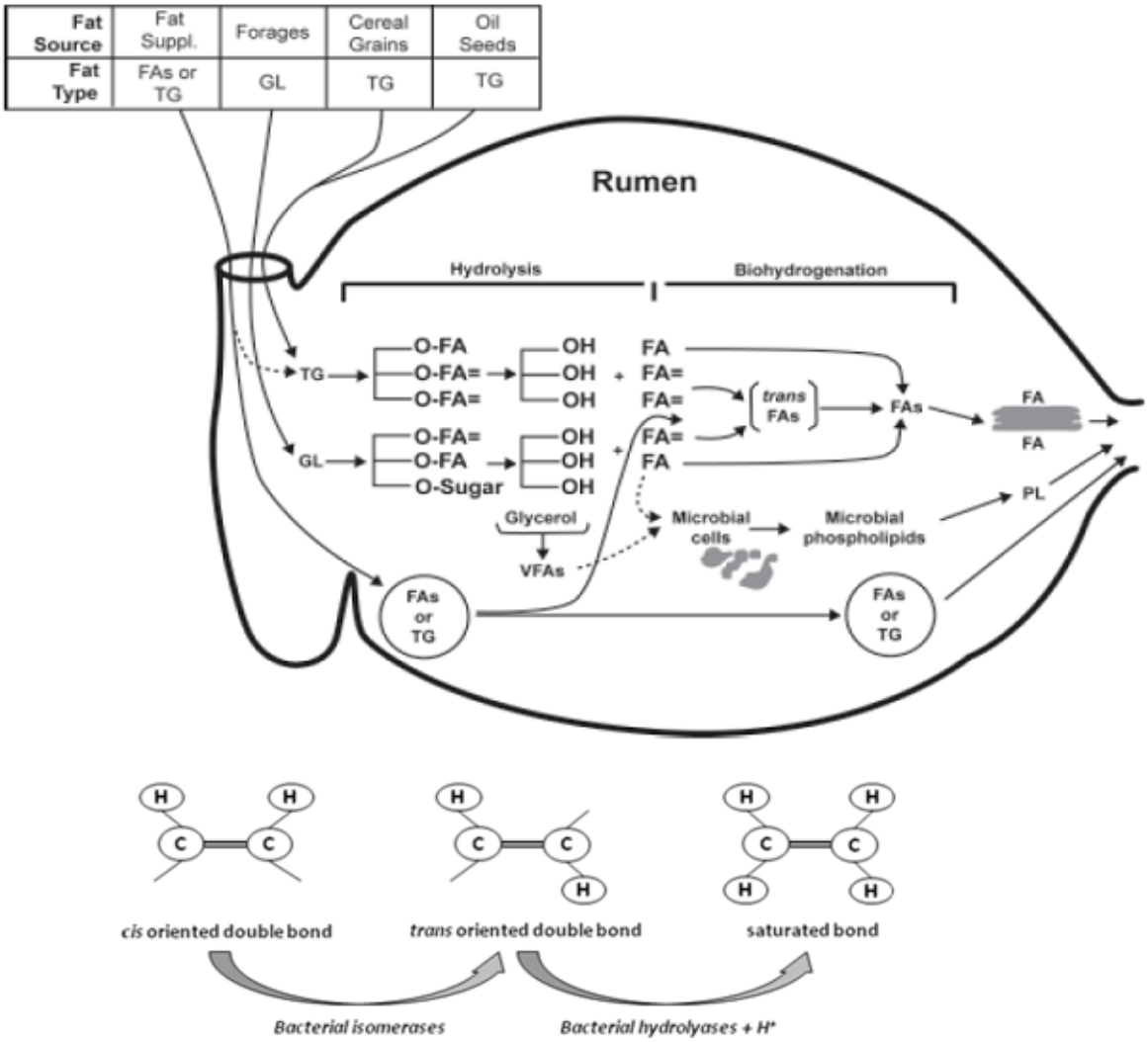

Fig 1. Illustration hydrogenation of unsaturated fatty acids

unsaturated fatty acids (PUFAs), namely C18:2 10.5% and other 0.9% [3]. The high unsaturated fatty acid in CPO can be used as a source of unsaturated fatty acids in the diet. The diet which is supplemented with fats rich in polyunsaturated fatty acids may increase the polyunsaturated fatty acids in meat [4], so that it can decrease cholesterol level and also reducing the risk of coronary heart disease in consumers [5-6].

There is a difference between ruminants and non-ruminants in the process of digestion and absorption of fat, due to the large differences of fat metabolism in the rumen. Fat digestion in the rumen (1) hydrolysis/lipolysis, the breakdown of fatty acid ester bond, (2) fermentation of glycerol released from hydrolysis of the rumen to VFA (Volatile Fatty Acid), and (3) hydrogenation of saturated fatty acids by rumen microbes. Hydrolysis of ester bonds is the first step is carried out primarily by bacteria *Anaerovibrio lipolytica* (hydrolyzes triglycerides) and *Butyrivibrio fibrisolvens* (hydrolyzes phospholipids and glycolipids), while the hydrogenation of unsaturated fatty acids is the next step, the main substrate is C18:2 and C18:3 with each level of the hydrogenation about 70-95% and 85-100% [7] (Fig. 1). This condition causes the linoleic acid (*cis-9, cis*-12-18: 2) and linolenic acid (*cis-9, cis*-12, *cis*-15-18:3) in the diet, to be found in the meat with low concentrations [8], which is only about 10% remained in the lipid tissues [9] while 90% is hydrogenated to C18:0 [7]. Hydrogenation of unsaturated fatty acids can not be fully carried out only by a single species of bacterium, so bacteria are divided into two groups based on the final product of hydrogenation, namely group A (hydrogenation C18:2 and C18:3 with the final product *trans*-C18:1), and group B (hydrogenation *trans*-C18:1 with the final product C18:0) [7] (Fig. 2).

One way to prevent rumen microbial hydrogenation is to protect the feed material source of unsaturated fatty acids in the diet with formaldehyde

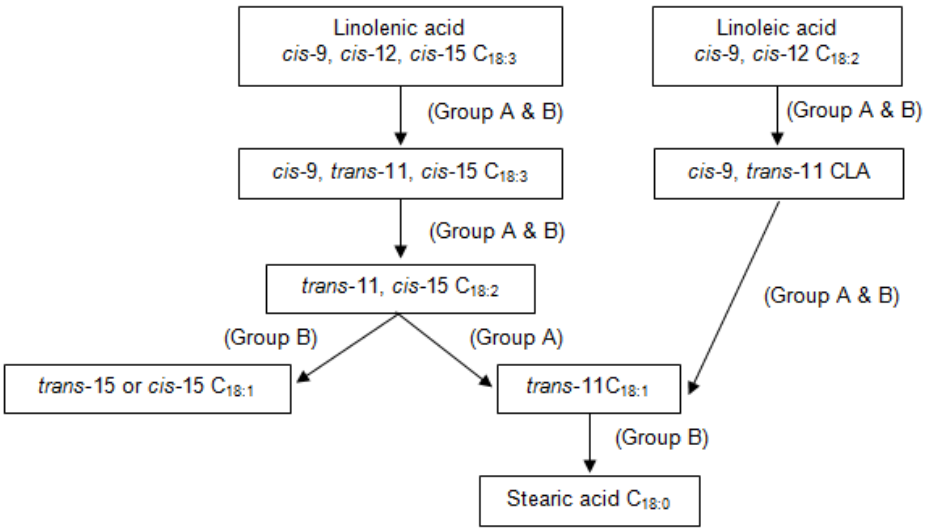

Fig 2. Hydrogenation pathway of linoleic (C18:2) and linolenic (C18:3) by the rumen bacteria [7]

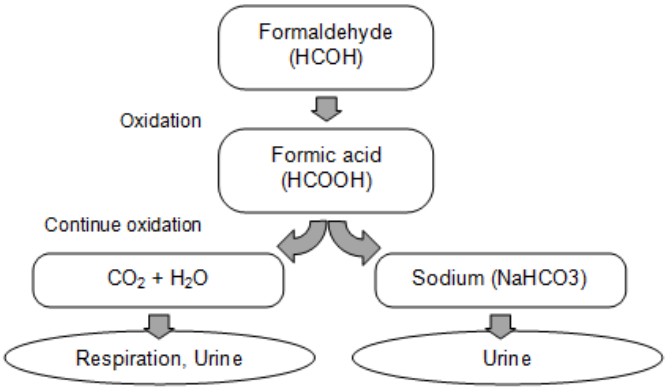

Fig 3. Process detoxification of formaldehyde [13]

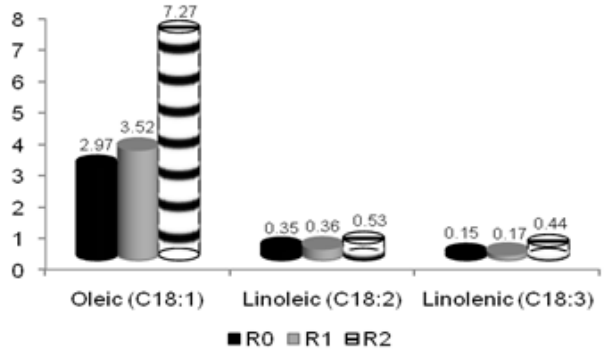

Fig 4. Comparison of increase rumen fluid unsaturated fatty acids of sheep between treatments R2 with R1 and R0

(CH_2O). Feed treatment with formaldehyde can decrease the proportion of C12:0, C14:0, C16:0 and increase C18:1, C18:2 and C18:3 [10], and C20:4, EPA and DHA, but decrease total triglycerides and cholesterol content in sheep [11]. In addition to protecting unsaturated fatty acids, the formaldehyde was chosen because it is cheap, easy to make, and relatively harmless. When absorbed in the blood, formaldehyde will be metabolized to formic acid then excreted through the urine as the sodium salt or further oxidized to CO_2 and H_2O [12] (Fig. 3).

About 3% of CPO mixed with expired milk powder (1:2) and protected with 2% technical formaldehyde of the ingredients are mixed and tested *in vitro*. This treatment can protect C18:1 and C18: 2 from rumen microbial hydrogenation [13], have no negative effect on rumen fermentation parameters and microbial activity [14]. The results were supplemented in the ration and tested *in vivo* to determine its effect on the hydrogenation of unsaturated fatty acids of rumen fluid and its effect on blood and meat fatty acid.

EXPERIMENTAL SECTION

Materials

The materials of this research are CPO, expired SGM milk powder. Rumen fluid was taken from male local sheep using Trocar, 37% technical formaldehyde, chloroform and methanol mixture (2:1), 0.88% NaCl and Na_2SO_4 anhydrous. Basal diet consisted of 60% elephant grass, 30% bran and 10% soybean meal. Nutrient content of basal diet was 62.98% TDN; 45.5% DM; 14.48% CP; 4.70% F and 21.93% CF.

Instrumentation

Instrumentation of this research included are a set of Trocar for sucking rumen fluid, gas chromatography (GC) Shimadzu GC-2010, analytical balance, water bath, filter paper, sterile disposable needles, and test tubes.

Table 1. Rumen fluid fatty acids content (g/100g fat) of sheep with the diet addition of CPO protected

Fatty acids	Treatments		
	R0	R1	R2
Lauric (C12:0)	0.14^{e} ±0.03	0.91^{c} ±0.06	0.70^{d} ±0.16
Myristic (C14:0)	1.46^{e} ±0.05	4.29^{c} ±0.71	3.29^{d} ±0.23
Palmitic (C16:0)	14.12^{d} ±0.28	38.61^{c} ±2.70	34.02^{c} ±3.91
Stearic (C18:0)	28.01^{e} ±0.32	50.39^{c} ±3.07	41.71^{d} ±6.71
Oleic (C18:1)	2.97^{d} ±0.29	3.52^{d} ±0.32	7.27^{c} ±0.43
Linoleic (C18:2)	0.35^{d} ±0.02	0.36^{d} ±0.03	0.53^{c} ±0.07
Linolenic (C18:3)	0.15^{b} ±0.06	0.17^{b} ±0.02	0.44^{a} ±0.15
SFA	43.73^{e} ±0.63	94.20^{c} ±6.28	79.72^{d}±10.17
MUFA	2.97^{d}±0.29	3.52^{d} ±0.32	7.27^{c}±0.43
PUFA	0.50^{d}±0.06	0.53^{d}±0.05	0.97^{c}±0.10
Total	47.20^{d} ±0.73	98.25^{c} ±6.00	87.96^{c} ±9.76

ab : different superscripts in the same row indicate significant (P<0.05).
cde : different superscripts in the same row indicate significant (P<0.01).

Procedure

Livestock

As many as 15 local male sheep's 9-12 months old with body weigh approximately 13-17 kg, maintained in individual cages shaped stage equipped with places to eat and drink. The sheep were divided into 3 groups according to the treatment of feed. The first group received only the basal diet (R0), the 2nd group received the basal diet and 3% CPO (R1), while the 3rd group received the basal diet and 3% CPO protected with 2% formaldehyde (R2). The sheep were maintained for 3 months and were given rations approximately 4.3% from body weight twice a day, at 08.00 a.m. and at 15.00 p.m., while the drinking water supplied by *ad libitum*. During maintenance, the amount of daily consumption of each animal is recorded, and then weighed once a week to determine the weight and adjust the amount of feed given. For data digestibility, a collection made for one week.

Fatty acid tested

At the end of the data collection, 100 mL of rumen fluid was taken using trocar and transferred into an Erlenmeyer flask. The rumen fluid was added with 50 mL of chloroform and methanol (2:1) mixture and allowed to stand. The bottom layer was taken and filtered. The filtrate was added with 10 mL 0.88% NaCl and allowed to stand. The bottom layer was filtered through filter paper containing Na_2SO_4 anhydrous to bind water, and then was blown with N_2. The resultant fat was prepared for the determination of methylated fatty acids and the fatty acid composition by gas chromatography method [15].

Before slaughtering, the blood was taken through the jugular vein using a sterile disposable needle and put in a test tube. Ten g of fresh blood were extracted and methylated in order to determine the fatty acid composition by gas chromatography method [15]. For the determination of meat fatty acids, 10 g of meat from Longissimus dorsi muscle (LD) were used. The fat from meat was extracted and methylated for the determination of the fatty acid composition by gas chromatography method [15]. Fatty acids of rumen fluid, blood and flesh were analyzed for the C12:0, C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3.

Statistical analysis

The data obtained were analyzed with analysis of variance using one way completely randomized design, with 3 ration treatment, namely the basal ration (R0), the basal ration and 3% CPO without formaldehyde treatment (R1) and the basal ration and 3% CPO protected with 2% formaldehyde (R2). Differences between treatments were tested further by Duncan's New Multiple Range Test. Data processing was done with the program SPSS 17.0 for Windows Evaluation Version.

RESULT AND DISCUSSION

Effect of Crude Palm Oil Protected on Rumen Fluid Fatty Acid

Effect of crude palm oil protection on sheep rumen fluid fatty acid can be seen in Table 1. The results showed that the addition of CPO (R1) increased rumen fluid total fatty acid (P<0.01) of 51.05 g/100 g compared to sheep which were given only the basal ration (R0). This was influenced by an increasing of SFA (P<0.01) at 50.47 g/100 g, namely C12:0, C14:0, C16:0 and C18:0. When viewed as a partial, C18:0 is a highest fatty acid. This proves that hydrogenation process on unsaturated fatty acids to saturated fatty acids, namely C18:0, in the rumen occurred. Hydrogenation of unsaturated fatty acids causes a reduction of *cis*-9 double bond into *trans*-11 fatty acids, and then *trans*-11 double bond is hydrogenated further to produce C18:0 [7].

The addition of CPO protected with formaldehyde (R2) increased the total fatty acids (P<0.01) at 40.76 g/100 g compared to sheep which were given only

Table 2. Blood fatty acids content (g/100 g fat) of sheep with the diet addition of CPO protected

Fatty acids	Treatments		
	R0	R1	R3
Lauric (C12:0)	0.15^{a} ±0.02	0.15^{a} ±0.05	0.05^{b} ±0.03
Miristic (C14:0)	1.22^{d} ±0.09	1.43^{c} ±0.10	1.11^{d} ±0.06
Palmitic (C16:0)	18.33^{c} ±0.66	18.70^{c} ±0.68	13.37^{d} ±0.79
Stearic (C18:0)	16.53^{c} ±1.37	16.63^{c} ±1.50	10.21^{d} ±0.78
Oleic (C18:1)	23.49^{d} ±1.25	22.42^{d} ±2.01	27.54^{c} ±0.57
Linoleic (C18:2)	0.90^{b} ±0.11	0.97^{b} ±0.02	1.13^{a} ±0.03
Linolenic (C18:3)	0.11^{e} ±0.05	0.27^{d} ±0.08	1.12^{c} ±0.03
SFA	36.23^{c} ±1.10	36.91^{c} ±2.04	24.74^{d} ±1.51
MUFA	23.49^{d} ±1.25	22.42^{d} ±2.01	27.54^{c} ±0.57
PUFA	1.01^{e} ±0.12	1.24^{d} ±0.08	2.25^{c} ±0.03
Total	60.73^{a} ±2.18	60.57^{a} ±3.08	54.53^{b} ±1.11

ab : different superscripts in the same row indicate significant (P<0.05).
cde : different superscripts in the same row indicate significant (P<0.01).

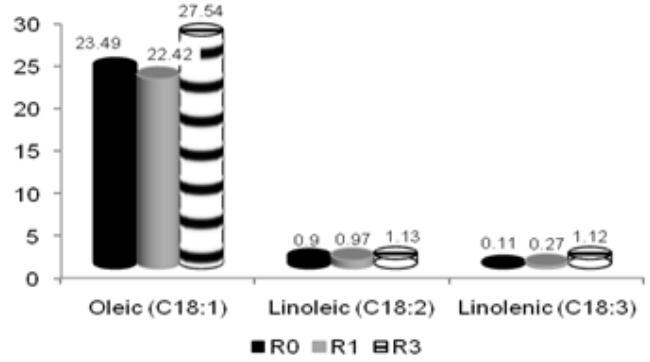

Fig 5. Comparison of increase blood unsaturated fatty acids of sheep between treatments R2 with R1 and R0

the basal ration (R0). It is affected by the increase of SFA, MUFA and PUFA (P<0.01) at 35.99 g/100 g, 4.30 g/100 g and 0.47 g/100 g, respectively. In comparison with R1 treatment, R2 treatment tends to decrease the total fatty acids but not significant. Treatment of R2 decreased SFA (P<0.01) at 14.48 g/100 g, but increased the MUFA and PUFA (P<0.01), at 3.75 g/100 g and 0.44 g/100 g, respectively. Unsaturated fatty acids increased in rumen fluid in R2 treatments as compared with R1 and R0, as shown in Fig. 4. The increasing of PUFA and MUFA of R2 treatment suggests that protection of CPO with formaldehyde may prevent the hydrogenation of unsaturated fatty acids by rumen microbes, suggesting that feed ingredients that is protected with formaldehyde will be resistant to rumen microbial degradation.

Effect of Protected Crude Palm Oil on Blood Fatty Acid

Effect of protected crude palm oil on blood fatty acid of sheep can be seen in Table 2. The results showed that the addition of CPO (R1) causes insignificant increase of the total fatty acids, SFA and MUFA, but increased the PUFA (P<0.01), when compared with sheep that were given only the basal ration (R0). The increasing of PUFA caused by C18:3 (P<0.01), which escaped from the rumen microbial hydrogenation. There are about 10% linoleic acid (*cis*-9, *cis*-12-18:2) and linolenic acid (*cis*-9, *cis*-12, *cis*-15-18:3) remains in the feed and escapes from the rumen microbial hydrogenation [9].

When compared to sheep which were given only the basal ration (R0) and the addition of CPO (R1), total fatty acids decreased (P<0.05) by 6.20 g/100 g and 6.04 g/100 g, respectively when sheep were given ration with the addition of protected CPO with formaldehyde (R2). That was caused the decrease in SFA (P<0.01), at 11.49 g/100 g and 12.17 g/100 g, respectively, which is caused by a decrease in C12:0, C14:0, C16:0 and C18:0 (P<0.01). The treatment of R2 increased MUFA (P<0.01), by 4.05 g/100 g and 5.12 g/100 g, respectively and increase PUFA (P<0.01), by 1.24 g/100 g and 1.01 g/100 g, respectively. Comparison of the increase of unsaturated fatty acids in blood between R2 treatments with R1 and with R0 can be seen in Fig. 5. The increase in MUFA and PUFA caused by an increase of C18:1, C18:2 and C18:3 (P<0.01) on the R2 treatment as compared to R0 and R1 treatment.

Effect of Protected Crude Palm Oil on Meat Fatty Acid

Effect of protected crude palm oil on meat fatty acid of sheep can be seen in Table 3. The results showed that the addition of CPO (R1) in the diet was not significant to the meat total fatty acids compared to the basal ration (R0). The addition of CPO and protected with formaldehyde (R2) decreased meat total fatty acids (P<0.05), of 2.99 g/100 g and 4.51 g/100 g, respectively compared to sheep which were fed only the basal ration (R0) and the addition of the CPO (R1), which caused the decrease of SFA (P<0.01) due to the decrease in C16:0 and C18:0 (P<0.01). Treatment of R2

Table 3. Meat fatty acids content (g/100 g fat) of sheep with the diet addition of CPO protected

Fatty acids	Treatments		
	R0	R1	R2
Lauric (C12:0)	0.09^{e} ±0.01	0.16^{c} ±0.00	0.12^{d} ±0.02
Miristic (C14:0)	2.88^{ab} ±0.29	3.2^{a} ±0.51	2.27^{b} ±0.14
Palmitic (C16:0)	16.54^{b} ±0.39	19.81^{a} ±1.80	14.25^{b} ±1.94
Stearic (C18:0)	15.36^{e} ±0.48	14.75^{c} ±0.77	9.66^{d} ±0.04
Oleic (C18:1)	17.72^{b} ±1.48	16.15^{b} ±2.15	23.16^{a} ±2.32
Linoleic (C18:2)	0.23^{d} ±0.02	0.26^{d} ±0.02	0.32^{c} ±0.03
Linolenic (C18:3)	0.09^{d} ±0.02	0.10^{d} ±0.01	0.14^{c} ±0.01
SFA	34.87^{c} ±0.45	37.92^{c} ±2.55	26.30^{d} ±2.05
MUFA	17.72^{d} ±1.48	16.15^{d} ±2.15	23.16^{c} ±2.32
PUFA	0.32^{d} ±0.03	0.36^{d} ±0.02	0.46^{c} ±0.04
Total	52.91^{a} ±1.49	54.43^{a} ±0.58	49.92^{b} ±1.63

ab : different superscripts in the same row indicate significant (P<0.05).
cde : different superscripts in the same row indicate significant (P<0.01).

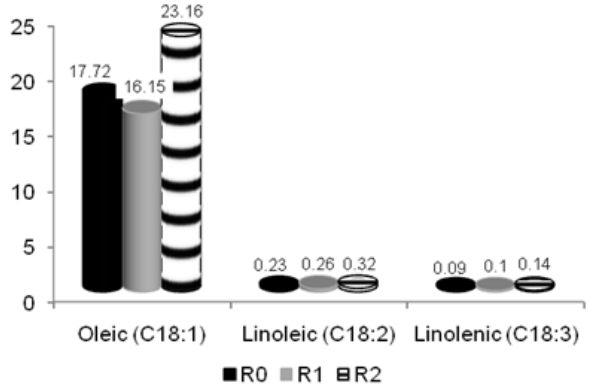

Fig 6. Comparison of increase meat unsaturated fatty acids of sheep between treatments R2 with R1 and R0

also increased MUFA and PUFA (P<0.01), namely C18:1, C18:2 and C18:3. Comparison of the increase of meat unsaturated fatty acids between R2 treatments with R1 and R0 can be seen in Fig. 6. Supplementation of soybean oil and tuna oil (70:30) protected with formaldehyde (PTO) in sheep lactation diet can decrease C16:0 from 302 g/kg (control) to 296 g/kg (PTO), C18:0 out of 12 g/kg (control) to 8.6 g/kg (PTO), but increase C18:2 is almost twice that of 29 g/kg (control) to 56 g/kg (PTO) and C18:3 of 8 g/kg (control) to 13 g/kg (PTO) of milk fat [16]. Protection of tuna oil with formaldehyde decreases C18:0, but increases C18:1 and C18:2 in the sheep meat [13]. Protection of fat using a casein-formaldehyde (Canola Lipid) can decrease C14:0, C16:0 and C18:0, but increase C18:1, C18:2 and C18:3 in adipose tissue of Brangus cattle [17].

CONCLUSION

Protection of crude palm oil with formaldehyde can decrease the hydrogenation of unsaturated fatty acids by rumen microbes, indicating a positive impact for the increase of unsaturated fatty acids in the blood and meat.

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