Original Article

Oil palm frond supplementation can change fatty acid composition of rumen fluid, muscle tissue and blood cholesterol level in crossbred male sheep

Goh Yong Meng[1,2] Mohamed Ali Rajion[1] Saeid Jafari[1,3] Mahdi Ebrahimi[1*]

Abstract

Diet composition is the major factor influencing the fatty acid composition of products derived from ruminants. Thirty 7-month-old, Barbados Black Belly × Malin crossbred male sheep were used for the trial to determine the effects of dietary supplementation of oil palm *(Eleis guineensis)* frond (OPF) on the fatty acid profiles of rumen fluid, muscle tissue and blood lipid parameters. Treatment diets were control diet (CON group, n=10), 25% OPF pellet in diet (% w/w) (HAF group, n=10) and 50% OPF pellet in diet (OPF group, n=10). After 100 days of feeding, eight sheep from each group were slaughtered for sampling of rumen fluid and muscle tissues. The CON group showed fluctuating and increasing total saturated fatty acid (SFA) concentrations in the rumen liquor compared to the OPF group and differed significantly ($P<0.05$) at different times of measurement. The SFA in the *longissimus dorsi*, *psoas major*, *gluteus medius*, *Semimembranosus* and *triceps brachii* muscles of the CON group were also significantly ($P<0.05$) higher than the OPF group. For all muscles, C18:3n-3 fatty acid was significantly higher ($P<0.05$) in the OPF group than in the CON group with minimal impact on the C18:2n-6 and total PUFA n-6. The HDL-Cholesterol values of the OPF group was almost 40% higher than those of the CON group ($P<0.05$). The results demonstrated a feasible way to alter the fatty acid composition of mutton based on feeding practices using indigenous fiber source.

Keywords: blood, fatty acid, muscle tissue, oil palm frond, rumen

[1]*Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[3]*Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, P.O. B.14115-336, Iran*
****Correspondence**: mehdiebrahimii@gmail.com*

Introduction

The use of nutritional strategies to improve the quality of food products from livestock is an approach that emerges at the interface of food science and animal science (Ebrahimi et al., 2015). Nutritional strategies such as dietary manipulation to alter fatty acid composition in meat products derived from ruminants seem to be of interest because of the association of saturated fatty acids with heart-related diseases as the main cause of mortality around the globe (Astrup et al., 2011). In ruminants, modification of fatty acid profiles is made mostly in order to achieve two outcomes; firstly, to control the antimicrobial effects of fatty acids so that additional fat can be fed to ruminants without disrupting rumen digestion and fermentation (Jenkins et al., 2008) and secondly, to regulate microbial biohydrogenation (transformation of unsaturated fatty acid to saturated fatty acid (one of the main causes of coronary diseases)) to alter absorption of selected fatty acids that enhance and improve nutritional values of animal food products (Jenkins et al., 2008). It has been shown that oil palm frond (OPF) supplementation increases the n-3 polyunsaturated fatty acids in plasma and subcutaneous fat in sheep, which has an effect on the health of consumers (Ebrahimi et al., 2013). The by-product of oil palm, which is oil palm frond, is available all year round and provides a sustainable ruminant feed for livestock industry in tropical regions (Ebrahimi et al., 2015).

Moreover, OPF is a rich source of secondary metabolites, such as tannins and phenolic compounds; their effectiveness in reducing rumen biohydrogenation has been approved (Jaffri et al., 2011). Positive effects of OPF supplementation on some characteristics (performance, digestion and microbial populations) in goats and sheep have been reported in previous studies (Hassim et al., 2010; Abubakr et al., 2015); however, until now there is still limited information concerning the effects of the supplementation of OPF on sheep fatty acid profiles of rumen fluid, muscle tissue and blood cholesterol level in literature.

Therefore, the objective of this experiment was to test the effectiveness of dietary manipulation employing feeds with different compositions of oil palm frond pellets on the fatty acid profile of sheep rumen and muscular tissues.

Materials and Methods

Animal management: Thirty individually housed seven-month-old Barbados Black Belly × Malin crossbred male sheep were allotted randomly into three treatment groups.

After a two-week adaptation period, the sheep were each randomly assigned to one of three dietary treatments in a 100-day experiment. The three treatments were control diet (CON group, n=10), 25% OPF pellet supplemented diet (% w/w) (HAF group, n=10) and 50% OPF pellet supplemented diet (OPF group, n=10). The animals were fed twice daily at 3.5% of body weight. Water was provided *ad libitum*. All animal care and sacrifice were in accordance with the country standards; the experimental protocol was reviewed and approved by the University Putra Malaysia Animal Care and Use Committee. Ingredients and chemical composition of the experimental diets are shown in Table 1.

Slaughtering procedure and sampling: At the end of the feeding experiment, four animals from each group were selected randomly for slaughter. The slaughter was performed in accordance with the slaughtering procedure outlined in the Malaysian Standards (2004). Approximately 200 g of the *triceps brachii* (obtained from a point 6 cm to the olecranon), *longissiumus dorsi* and *psoas major* (obtained from the 12th to 13th ribs) *semimembranosus* and *gluteus medius* muscles at their respective sagittal midpoints were obtained.

The muscles were sampled from the left side of the carcass after overnight cooling at 4°C. All visible fat was removed from the meat surface, wrapped in aluminium foil, placed in polyvinyl chloride (PVC) plastic bags, gassed with nitrogen and stored at -20°C until fatty acid analysis.

For rumen fluid sampling, two hundred mL of rumen liquor were taken from each animal at 0, 2, 4, 6 and 8 h post-morning feeding on the last day of feeding. The rumen fluid was strained through four layers of cheese cloth to remove feed particles. About 200 mL of the strained rumen fluid was stored at -20°C until further analysis.

Proximate analysis of feed: The standard method of AOAC (1990) was followed to determine the proximate chemical composition of feed samples. Samples of feed were dried in a forced-air oven for 24 h at 105°C to determine dry matter (DM).

Nitrogen was determined by Kjeltec Auto Analyzer and then converted to crude protein (CP = N × 6.25). Ether extract (EE) was determined by extracting the sample with petroleum ether (40-60°C) using Soxtec Auto Analyzer. The samples were ashed in a muffle furnace at 550°C for 4 h to determine the ash content. Crude fiber was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat free samples with 1.25% each of sulfuric acid and sodium hydroxide solutions. Each analysis was performed in triplicate.

Fatty acid profile of experimental diets, rumen liquor and muscle tissues: The fatty acid content in the experimental diet samples, strained rumen contents, and muscle tissue samples was extracted as described by Ebrahimi et al. (2015). In brief, chloroform/methanol 2:1 (v/v) containing butylated hydroxy toluene was used for the extraction of fat. An internal standard, heneicosanoic acid (Sigma Chemical, St. Louis, MO, USA), was added to each sample before transmethylation to determine individual fatty acid concentration within the sample. Transmethylation of the extracted fatty acid to their fatty acid methyl esters (FAME) was carried out using potassium hydroxide in methanol and 14% methanolic boron trifluoride. The FAME was separated by gas chromatography (Agilent 5890A), using a Supelco SP 2560 capillary column of 30 cm × 0.25 mm ID × 0.2 µm film thickness (Supelco, Bellefonte, PA, USA). The fatty acid concentrations were expressed as mg/100 g of

identified fatty acid. Reference standards (mix C16-C20 methyl esters; Sigma-Aldrich, Inc., St. Louis, MO, USA) were used to determine recoveries and correction factors for determination of individual fatty acids.

Table 1 Ingredients and chemical composition of the experimental diets

Ingredients (%)	Experimental diets		
	CON	HAF	OPF
Rice straw	10	7.5	5
Corn grain	65	48.75	32.5
Oil palm frond	0	25	50
Soybean meal	22	16.5	11
Molasses	1.25	0.9375	0.625
Urea	0.75	0.5625	0.375
Salt	0.5	0.375	0.25
Dicalcium phosphate	0.5	0.375	0.25
Chemical composition (g/kg DM)			
Dry Matter	928	931.8	942
Crude Protein	148.1	116.3	90.2
Crude Fiber	99.9	150.4	261.2
Ether Extract	59	42.4	21.3
Ash	62.8	73.5	78.7
Gross Energy (MJ/kg)	18.15	17.8	17.53
Fatty acid (g/100 g of fatty acid)			
Palmitic Acid (16:0)	16.7	18.4	22.9
Palmitoleic Acid (16:1)	1.1	1	2.9
Stearic Acid (18:0)	2.2	2.3	3
Oleic Acid (18:1n-9)	21.9	21.1	20.9
Linoleic Acid (18:2 n-6)	51.5	49.7	37.9
Linolenic Acid (18:3 n-3)	5.6	6.5	9.1
Eicosaenoic Acid (20:1)	1.1	1.1	3.3
Total Saturated Fatty Acids	18.9	20.7	25.9
Total Unsaturated Fatty Acids	81.1	79.3	74.1
Total Monoenes	24	23.2	27.2
Total PUFA n-3	5.6	6.5	9.1
Total PUFA n-6	51.5	49.7	37.9

OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Total saturated fatty acids = sum of C16:0 + C18:0. Total unsaturated fatty acids = sum of C16:1 + C18:1 + C18:2n-6 + C18:3n-3 + C20:1. Total monoens = sum of C16:1 + C18:1 + C20:1. Total PUFA n-3 = sum of C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. Total PUFA n-6 = sum of C18:2n-6 + C20:4n-6. C18 PUFA = sum of C18:3n-3 + C18:2n-6 + C18:1n-9

Determination of blood cholesterol level: Blood was collected from the animals by jugular venipuncture into 10 mL ethylene diamino tetraacetic acid (EDTA) vacutainer tubes (Becton Dickinson, New Jersey, USA). The sheep blood was collected with EDTA coated blood tubes. Blood plasma was separated by centrifuging the blood at 1000×g for 10 min (Heraus Sepatech, GmBH) for blood determinations. Total cholesterol, HDL-Cholesterol and triacylglycerol (TAG) levels were assayed using commercial kits (Pointe Scientific Inc., Michigan, USA) and their values were determined colorimetrically using a Cobas Mira machine (Roche International, Basel, Switzerland).

Statistical analysis: One-way ANOVA analyses were done on the fatty acid data of rumen fluid, muscle tissues and blood cholesterol level to investigate the effects of the treatment diets. Sampling at different times (0, 2, 4, 6 and 8 h) was analyzed using repeated measures. The diets were used as treatment effect, with individual animal as the experimental unit. Treatment means were separated by Duncan multiple range test at $P < 0.05$ (SPSS, 1999).

Results

Effect of dietary manipulation on fatty acid content of sheep muscle tissues: The fatty acid contents of the various sheep muscle tissues of the three treatment groups are summarized in Table 2. The total saturated fatty acid (SFA) content of the CON group was the highest in both the *longissimus dorsi* and *psoas major* ($P<0.05$) compared to that of the OPF group (Table 2). It can be seen that in all the treatment groups and all muscles, palmitic and stearic acids and to some extent arachidic acids were the major contributors to the total saturated fat content.

Both the HAF and CON animals had significantly higher amounts of total SFA ($P<0.05$) in psoas *major* muscle than those of the OPF animals. For the *semimembranosus* muscle, the HAF animals had much more total saturated fats compared to both the CON and OPF animals. For *triceps brachii*, a forequarter muscle, the CON group had the most total SFA compared to the OPF group ($P<0.05$).

Both the HAF and CON animals had significantly higher amounts of total unsaturated fatty acid ($P<0.05$) in all muscle groups compared to the OPF animals. In general, the OPF animals had significantly more ($P<0.05$) total n-3 polyunsaturated fatty acid (PUFA) in their muscles (except *triceps brachii*) compared to the other animals (CON and HAF). Both the HAF and CON animals contained similar amounts of n-3 PUFA in their muscle tissues in most cases.

The total n-6 PUFA content in the muscle tissues of all treatment groups was not significantly different from each other, except in the *semimembranosus* muscle, where the CON animals seemed to have the highest amount of n-6 PUFA ($P<0.05$).

Effect of dietary manipulation on fatty acid content of rumen fluid: The fatty acid concentrations in the rumen liquor at post-feeding hours on the last day of feeding are shown in Figures 1-4. All treatment groups showed fluctuating total fatty acid concentrations in the rumen liquor and differed significantly ($P<0.05$) from each other at every time point. The linoleic acid concentration declined rapidly by 8 hours post-feeding among the treatments. At 2, 4, 6 and 8 h post-feeding, the CON group had much more ($P<0.05$) stearic acid in their rumen liquor compared with the OPF and HAF groups. The total SFA in the HAF and CON groups rose rapidly after 2 hours post-feeding ($P<0.05$). However, the OPF group had fewer total SFA at 4 hours post-feeding, but this gradually returned to immediate post-feeding levels at 8 hours post-feeding. At this stage, both the CON and HAF groups had about 2 to 2.5 times more total SFA in their rumen fluid compared to the OPF group ($P<0.05$).

Effect of dietary manipulation on blood cholesterol level: The blood plasma total cholesterol levels were not significantly different between the treatment groups (Table 3). The plasma HDL-Cholesterol values (Table 3) were significantly higher ($P<0.05$) in the OPF group than the other treatment groups at the last two months of the feeding. No significant difference was observed among the treatment groups in terms of plasma triglyceride (TAG) levels (Table 3) at different months of feeding.

Discussion

The fatty acid composition of the ruminant tissues is influenced by breed, diet, sex and environment (Turner et al., 2005; Pedrão et al., 2012). High dietary fiber feeding was reported to increase the SFA content of fat depots in sheep compared to concentrate feeding (Smith et al., 2009), consistent with the findings in the muscles of the OPF group in this study. Both HAF and CON animals had high amount of SFA in their muscle tissues (*longissimus dorsi*, *gluteus medius*, and *triceps brachii*), but in the CON animals, this was counter balanced by the vastly increased total UFA, thereby giving them a significantly better tissue fatty acid unsaturation index among all the treatment groups.

Consistent with our study, Ebrahimi et al. (2011) found that 50% of OPF inclusion in the diet of Kacang crossbred male goats decreased concentration of SFA in the *longissimus dorsi* and *biceps femoris* muscles while it increased the concentration of C18:3n-3 PUFA in the same muscles. They also proposed two reasons for their findings. Firstly, the inclusion of 50% of OPF could increase PUFA in the tissues of the animals because of the increase in fiber feeding (Dewhurst et al., 2006). Secondly, OPF contains different types of secondary metabolites (Ebrahimi et al., 2015) which have shown to reduce the biohydrogenation of PUFA, resulting in lower production of SFA (Li and Qingxiang, 2006).

Differences in fatty acid composition according to anatomical locations were linked with diverse roles of these depots or tissues in physiology (Bas and Morand-Fehr, 2000). Totally, the dietary factors evidently influenced the muscle fatty acid profiles in this study, resulting in the CON animals having the highest UFA content and more n-6 PUFA compared to the OPF animals.

The linoleic acid achieved its peak earlier than the other fatty acids due to the fact that it was only available from the diets. Also, it is an important precursor for the conversion into oleic acid (C18:1n-9) and finally SA via the biohydrogenation process (Jenkins, 2008). The high levels of SA towards the end of the trial especially in the CON group compared to the HAF and OPF groups might be due to biohydrogenation process that converted both linoleic and oleic to stearic, microbial synthesis as well as those from the elongation of palmitic acid (by microbial elongase) (Bessa et al., 2000). However, the rate of biohydrogenation was not calculated in the current study. Plants containing secondary metabolites especially tannins have shown to reduce the extent of ruminal biohydrogenation of fatty acids, which results in less production of SA (Jafari et al., 2016). The reduction in rumen SA at different times of measurement in the OPF group compared to the CON group could be due to the presence of tannins and phenolic compounds in OPF according to Ebrahimi et al. (2015). Consistent with our results, Jafari et al. (2016) reported a reduction in rumen SA due to the

supplementation of papaya leaf after 24 h of *in vitro* incubation. They also attributed their results to the presence of secondary metabolites especially tannin in papaya leaf.

Table 2 Fatty acid profiles of the sheep muscle tissues after 14 weeks of experimental feeding trial

Fatty Acids (Mean ± SD mg/100 g)	Tissue	CON (n=8)	HAF (n=8)	OPF (n=8)	P-value
Palmitic Acid (16:0)	*longissimus dorsi*	440.7 ± 76.2^{a}	364.0 ± 50.6^{b}	135.4 ± 34.2^{c}	0.022
	psoas major	402.3 ± 84.5^{a}	356.0 ± 44.2^{b}	234.3 ± 48.7^{b}	0.021
	gluteus medius	192.6 ± 34.5^{b}	271.5 ± 41.5^{a}	129.6 ± 15.0^{c}	0.019
	Semimembranosus	256.3 ± 41.7^{b}	628.1 ± 114.3^{a}	198.3 ± 22.9^{b}	0.022
	triceps brachii	359.2 ± 99.5^{a}	189.0 ± 42.3^{b}	245.6 ± 51.8^{b}	0.036
Stearic Acid (18:0)	*longissimus dorsi*	324.3 ± 41.1^{a}	300.4 ± 52.3^{a}	145.8 ± 28.2^{b}	0.049
	psoas major	329.5 ± 63.4^{ab}	362.6 ± 84^{a}	283.4 ± 55.4^{b}	0.046
	gluteus medius	129.5 ± 13.2^{b}	192.2 ± 33.4^{a}	133.5 ± 24.3^{b}	0.047
	Semimembranosus	214.4 ± 41.2^{b}	497.5 ± 83.9^{a}	215.2 ± 35.4^{b}	0.028
	triceps brachii	290.3 ± 61.2	241.0 ± 48.0	263.5 ± 56.5	0.414
Arachidic Acid (20:0)	*longissimus dorsi*	6.3 ± 0.7	6.1 ± 0.1	6.0 ± 0.5	0.195
	psoas major	9.7 ± 1.2^{a}	7.7 ± 0.8^{b}	4.0 ± 0.2^{c}	0.050
	gluteus medius	ND	9.5 ± 0.6^{a}	6.5 ± 0.7^{b}	0.020
	semimembranosus	6.3 ± 0.6	7.4 ± 1.1	5.8 ± 0.8	0.620
	triceps brachii	ND	ND	5.2 ± 1.0	0.230
Total Saturated Fatty Acids	*longissimus dorsi*	771.3 ± 102.9^{a}	670.5 ± 89.5^{b}	287.2 ± 60.4^{c}	0.025
	psoas major	741.5 ± 142.8^{a}	726.3 ± 109.8^{a}	521.7 ± 99.8^{b}	0.045
	gluteus medius	322.1 ± 41.6^{b}	473.2 ± 58.4^{a}	269.6 ± 37.2^{c}	0.030
	semimembranosus	477.0 ± 72.3^{b}	1133.0 ± 168.8^{a}	419.3 ± 54.3^{b}	0.014
	triceps brachii	649.5 ± 124.9^{a}	430.0 ± 59.6^{b}	514.3 ± 94.0^{b}	0.078
Total Unsaturated Fatty Acids	*longissimus dorsi*	1110.4 ± 79.6^{a}	811.1 ± 128.2^{b}	405.4 ± 56.0^{c}	0.038
	psoas major	958.2 ± 120.2^{a}	809.4 ± 125.1^{b}	606.9 ± 65.2^{c}	0.049
	gluteus medius	562.1 ± 41.9^{a}	569.0 ± 66.7^{b}	418.5 ± 27.9^{b}	0.015
	semimembranosus	832.2 ± 90.6^{a}	1259.6 ± 147.5^{b}	541.9 ± 54.8^{c}	0.023
	triceps brachii	908.1 ± 115.1^{a}	561.6 ± 73.1^{b}	536.9 ± 68.7^{b}	0.031
Total Monoenes	*longissimus dorsi*	930.2 ± 68.5^{a}	635.8 ± 123.8^{b}	229.0 ± 44.9^{c}	0.006
	psoas major	757.5 ± 117.6^{a}	616.0 ± 121.7^{b}	403.0 ± 66.7^{c}	0.050

	gluteus medius	410.5 ± 37.2[a]	414.5 ± 64.0[a]	249.4 ± 28.8[b]	0.045
	semimembranosus	634.2 ± 82.3[a]	1080.0 ± 139.9[b]	347.4 ± 50.1[c]	0.035
	triceps brachii	723.6 ± 96.1[a]	382.6 ± 72.8[a]	355.3 ± 38.0[b]	0.027
Total PUFA n-3	*longissimus dorsi*	21.0 ± 2.6[b]	20.9 ± 3.8[b]	26.7 ± 2.5[a]	0.038
	psoas major	14.8 ± 2.3[b]	15.8 ± 2.3[b]	18.9 ± 4.1[a]	0.027
	gluteus medius	18.9 ± 1.3[b]	19.7 ± 3.3[b]	24.5 ± 3.5[a]	0.008
	semimembranosus	17.5 ± 2.2[a]	21.2 ± 4.2[b]	31.0 ± 3.7[c]	0.031
	triceps brachii	14.9 ± 3.2	16.0 ± 3.6	15.0 ± 2.6	0.570
Total PUFA n-6	*longissimus dorsi*	159.2 ± 22.9	154.4 ± 18.8	149.7 ± 22.4	0.160
	psoas major	185.9 ± 25.5	177.6 ± 30.4	185.0 ± 14.6	0.240
	gluteus medius	132.7 ± 15.3	130.8 ± 10.3	138.1 ± 14.9	0.250
	semimembranosus	180.5 ± 26.1[a]	158.4 ± 21.5[a]	163.5 ± 19.7[b]	0.047
	triceps brachii	169.6 ± 17.6	163.0 ± 26.6	166.6 ± 19.4	0.471

ND: not detected. OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Total saturated fatty acids = sum of C16:0 + C18:0. Total unsaturated fatty acids = sum of C16:1 + C18:1 + C18:2n-6 + C18:3n-3 + C20:1. Total monoens = sum of C16:1 + C18:1 + C20:1. Total PUFA n-3 = sum of C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. Total PUFA n-6 = sum of C18:2n-6 + C20:4n-6. C18 PUFA = sum of C18:3n-3 + C18:2n-6 + C18:1n-9

Table 3 Blood plasma total cholesterol, HDL-cholesterol and triglyceride level of the sheep after 14 weeks of experimental feeding trial

Blood parameters (mmol/L)	**CON (n=8)**	**HAF (n=8)**	**OPF (n=8)**	**P-value**
Total cholesterol				
1st month	0.90	0.79	0.80	0.051
2nd month	0.97	0.96	0.94	0.090
3rd month	0.92	0.91	0.97	0.200
HDL-Cholesterol				
1st month	0.36	0.39	0.42	0.320
2nd month	0.37[b]	0.40[ab]	0.46[a]	0.047
3rd month	0.37[c]	0.44[b]	0.52[a]	0.028
Triglyceride				
1st month	0.30	0.34	0.31	0.052
2nd month	0.32	0.34	0.28	0.204
3rd month	0.58	0.58	0.45	0.100

OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Different superscript letters [(a,b and c)] in each row denote significant level at P<0.05.

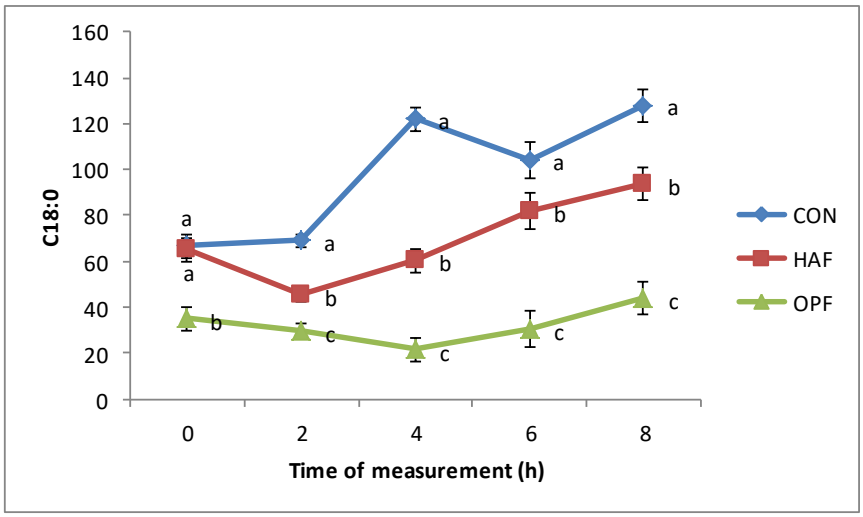

Figure 1 Effect of dietary manipulation on rumen stearic acid (C18:0) concentration. OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Vertical bars are standard error. Values with different superscript letters at the same time differ significantly at P<0.05.

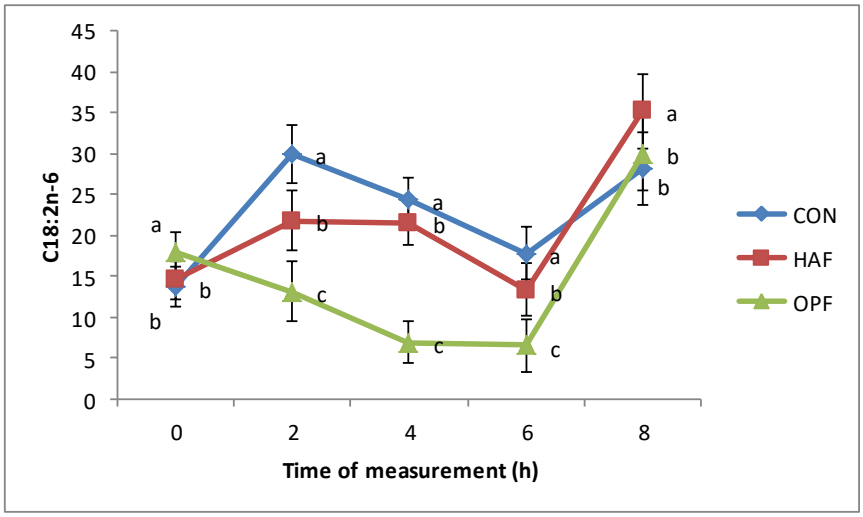

Figure 2 Effect of dietary manipulation on rumen linoleic acid (C18:2n-6) concentration. OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Vertical bars are standard error. Values with different superscript letters at the same time differ significantly at P<0.05.

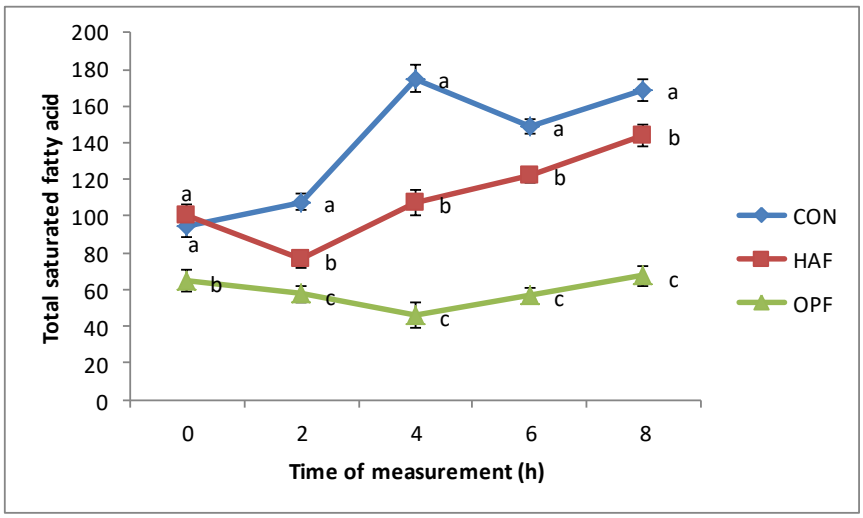

Figure 3 Effect of dietary manipulation on rumen total saturated fatty acid concentration. OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Vertical bars are standard error. Values with different superscript letters at the same time differ significantly at P<0.05.

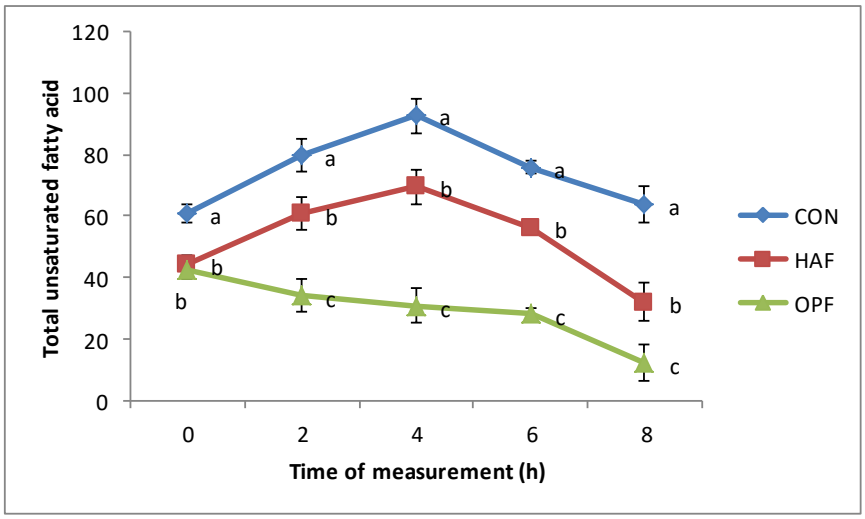

Figure 4 Effect of dietary manipulation on rumen total unsaturated fatty acid concentration. OPF diet = 50% w/w Oil Palm Frond in diet. HAF diet = 25% w/w Oil Palm Frond in diet. CON diet = control. Vertical bars are standard error. Values with different superscript letters at the same time differ significantly at P<0.05.

The lower amounts of SFA observed in this study in the OPF group compared to the CON group in both muscle tissues and rumen fluid could be due to the fiber characteristics of OPF (Goh et al., 2003) as well as secondary metabolite in OPF (Ebrahimi et al., 2015). A similar trend and decrease in SFA and increase in PUFA were also reported in steers fed grass with lowered amounts of concentrate in the diet (French et al., 2000) and in goats fed argan tree leaves compared to grain feed (Bas et al., 2005).

Effect of dietary manipulation on blood plasma cholesterol level: Carbohydrates were found to increase plasma triacylglycerol concentration and to reduce the mean particle size of LDL-Cholesterol (Mensink et al., 2003). Therefore, by assuming that carbohydrates have similar effects in ruminants as in rodent models, it could be explained that the inclusion of starch-rich concentrate diet for the CON group and slightly for the HAF group resulted in almost two-fold increase in the plasma triacylglycerol levels at the end of the trial. The total cholesterol for the animals in this trial was neither significantly affected by the dietary manipulation nor the time frame of the treatment itself. This may be due to the fact that the alteration in the rumen biohydrogenation pattern was not drastic enough to bring about fatty acid composition changes that could induce changes in the total cholesterol metabolism in the animals. In the HAF and CON groups, the high amounts of dietary lipid and starch exerted their effects on the triacylglycerol, while n-3 PUFA and possibly other undetermined factors acted in the OPF group to elevate the plasma HDL-Cholesterol levels. Our animals' HDL-Cholesterol values were similar to the recently published values by Ponnampalam et al. (2001b) as a result of the dietary fatty acid manipulation in sheep.

However, the values reported by Ponnampalam et al. (2001b) in their temperate crossbred wethers ([Merino × Border Leceisters] × Polled Dorset) were comparatively low, ranging from 0.17-0.23 mmol/L, compared with the values obtained from our tropical crossbred animals (Barbados Black Belly × Malin), which ranged from 0.21-0.57 mmol/L. On the other hand, the temperate crossbreeds were also shown to have higher plasma total cholesterol compared to our tropical animals (1.33-1.63 mmol/L VS 0.81-1.19 mmol/L, respectively). These differences might be attributed to, apart from dietary regimens, factors like breed, climatic condition and production system differences. The HDL-Cholesterol levels were always high in the OPF group perhaps due to the fact that higher levels of n-3 PUFA fatty acids were found in the plasma of these animals (Rajion et al., 2000).

Oil palm fronds are low-cost easily available feed resources in Malaysia that could be included in diets of sheep up to the presently used 50% level with more beneficial than detrimental effects on the fatty acid profile of rumen fluid, muscle tissues and blood plasma lipid parameters. In summary, our current approach demonstrated a feasible way to alter the fatty acid levels of mutton based on feeding practices using indigenous fiber sources.

Acknowledgements

The authors are thankful to the Faculty of Veterinary Medicine, Universiti Putra Malaysia and Dr. Taradon Luangtongkum from Department of Veterinary Public Health, Chulalongkorn University, Thailand.

References

Bas P and Morand-Fehr P 2000. Effect of nutritional factors on fatty acid composition of lamb fat deposits. Livestock Prod Sci. 64: 61-67.

Bauchart D, Legay-Carmier F, Doreau M and Gaillard B 1990. Lipid metabolism of liquid associated and solid adherent bacteria in rumen contents of dairy cows offered lipid supplemented diets. Br J Nutri 63: 563-578.

Bessa RJ, Santos-Silva BJ, Ribeiro JMR and Portugal AV 2000. Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. Livestock Prod Sci. 63: 201-211.

Cercı IH, Cıftcı M, Bahsı M and Kılınc U 2011. Cholesterol and fatty acid composition of lamb serum and offal as affected by alfalfa and concentrate. Veterinarski arhive. 81: 575-584.

Dewhurst RJ, Shingfield K J, Lee MRF and Scollan N D 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim Feed Sci Technol. 131: 168-206.

De Smet S, Raes K and Demeyer D 2003. Meat fatty acid composition as affected by genetics. Anim Res. (in press).

Dimov K, Kalev R, Tzankova M and Penchev P 2012. Fatty-acid composition of the lipids in m. longisimus dorsi of bovine and buffalo calves and buffalo cows. Bulgarian J Agri Sci. 18: 778-783.

Ebrahimi M, Rajion MA, Goh YM, Shokryzadan P, Sazili AQ and Jahromi MF 2015. Feeding oil palm (*elaeis guineensis*, jacq.) fronds alters rumen protozoal population and ruminal fermentation pattern in goats. Italian J Anim Sci. 14: 3877.

Ebrahimi M, Rajion MA, Goh YM, Sazili AQ, Soleimani AF and Schonewille JT 2013. Oil palm (*Elaeis guineensis* Jacq.) frond feeding 288 of goats in the humid tropics. J Anim Vet Adv. 12: 431-438.

Ebrahimi M, Rajion MA, Goh YM and Sazili AQ 2012. Impact of different inclusion levels of oil palm (Elaeisguineensis Jacq.) fronds on fatty acid profiles of goat muscles. J Anim Physiol Anim Nutri. 96: 962-969.

Guo TJ, Wang JQ, Bu DP, Liu KL, Wang JP, Li D, Luan SY and Huo XK 2010. Evaluation of the microbial population in ruminal fluid using real time PCR in steers treated with virginiamycin. Czech J Anim Sci. 55: 276-285.

Gurr M and Harwood JL 1991. Lipid Biochemistry: An Introduction. 4th Edn. London, Chapman & Hall.

Hassim HA, Lourenc M, Goh YM, Baars JP and Fievez V 2012. Rumen degradation of oil palm fronds is improved through pre-digestion with white rot fungi but not through supplementation with yeast or enzymes. Can J Anim Sci. 92: 79-87.

Heath D 1995. An Introduction to Experimental Design and Statistics for Biology. UCL Press, London.

Jafari S, Meng GY, Rajion MA, Jahromi MF and Ebrahimi M 2016. Manipulation of rumen microbial fermentation by polyphenol rich solvent fractions from papaya leaf to reduce green-house gas methane and biohydrogenation of C18 PUFA. J Agri Food Chem. DOI: 10.1021/acs.jafc.6b00846.

Jaffri JM, Mohamed S, Ahmad IN, Mustapha NM, Manap YA and Rohimi N 2011. Effects of catechin-rich oil palm leaf extract on normal and hypertensive rats' kidney and liver. Food Chem. 128:433-441.

Jenkins TC 1993. Lipid metabolism in the rumen. J Dairy Sci. 76: 3851-3863.

Jenkins TC, Wallace RJ, Moate PJ and Mosley EE 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J Anim Sci. 86: 397-412.

Li Y and Qingxiang M 2006. Effect of different types of fibre supplemented with sunflower oil on ruminal fermentation and production of conjugated linoleic acids in vitro. Arch Anim Nutri. 60: 402.

Lunn J and Theobald HE 2006. The health effects of dietary unsaturated fatty acids. Br nutri foundation nutria Bulletin. 31: 178-224.

Mensink PR, Zock PL, Kester ADM and Katan MB 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clinical Nutri. 77:1146-1155.

Moibi JA and Christopherson RJ 2001. Effect of environmental temperature and a protected lipid supplement on the fatty acid profile of ovine.

Oldick BS and Firkins JL 2000. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. J Anim Sci. 78: 2412 2420.

Ponnampalam AJ, Sinclair AR, Egan SJ, Blakeley and Leury BJ 2001. Effect of diets containing omega-3 fatty acids on muscle long-chain omega-3 fatty acid content in lambs fed low- and medium-quality roughage diets. J Anim Sci. 79: 698-706.

Rajion MA, Goh YM, Dahlan I and Salam-Abdullah A 2001. Dietary manipulation and increase in plasma unsaturated fatty acids in sheep. Asian-Australasian J. Anim. Sci. 14: 1073-1077.

Rajion MAR, Hassan J, Ripin MS, Shahudin K, Mariappan and Salam-Abdullah A 1996. Increasing polyunsaturated fatty acids in the goat. In: Proceedings of the 8th AAAP Anim Sci Congress, Chiba, Japan, 13-18 October 1996. pp. 156-157.

SPSS 1999. SPSS 10.0 Applications Guide. SPSS Inc., USA.

Turner KE, Wildeus S and Collins JR 2005. Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. Small Ruminant Res. 59: 15-23.

บทคัดย่อ

อาหารเสริมใบปาล์มสามารถเปลี่ยนส่วนประกอบของกรดไขมันในรูเมน กล้ามเนื้อ และระดับโคเลสเตอรอลในแกะพันธุ์ผสมเพศผู้

โก ยัง เมง[1,2] โมฮัมเมด อาลี ราจีอน[1] ซาอิด จาฟารี[1,3] มาดิ อิบราฮิมี่[1]*

องค์ประกอบของอาหารเป็นปัจจัยสำคัญที่มีอิทธิพลต่อองค์ประกอบกรดไขมันของผลิตภัณฑ์ที่ได้จากสัตว์เคี้ยวเอื้อง การศึกษานี้ทำการทดลองในแกะเพศผู้ จำนวน 30 ตัว เพื่อหาผลของการเสริมธาตุอาหารน้ำมันปาล์ม *(Eleis guineensis)* ต่อโปรไฟล์ของกรดไขมันในของเหลวในกระเพาะอาหาร เนื้อเยื่อกล้ามเนื้อและไขมันในเลือด แบ่งแกะออกเป็น 3 กลุ่ม ได้แก่ กลุ่มควบคุมอาหาร (CON, n=10), กลุ่มที่ได้รับอาหารเม็ด OPF 25% ในอาหาร (HAF, n=10) และกลุ่มที่ได้รับอาหารเม็ด OPF 50% ในอาหาร (OPF, n=10) หลังจากเลี้ยงครบ 100 วันแกะแต่ละตัวในแต่ละกลุ่มได้ถูกการุณยฆาตเพื่อเก็บตัวอย่างของของเหลวในกระเพาะอาหารและเนื้อเยื่อของกล้ามเนื้อ ผลการทดลองพบความผันผวนและการเพิ่มขึ้นของความเข้มข้นของกรดไขมันอิ่มตัวในของเหลวในกระเพาะในกลุ่ม CON เมื่อเทียบกับกลุ่ม OPF และพบความแตกต่างกันอย่างมีนัยสำคัญในแต่ละช่วงเวลาของการวัด พบว่า SFA ใน *longissimus dorsi*, *psoas major*, *gluteus medius*, *semimembranosus* และ *triceps brachii* ของกลุ่ม CON มีค่าสูงกว่ากลุ่ม OPF อย่างมีนัยสำคัญทางสถิติ สำหรับกล้ามเนื้อทุกชนิด กรดไขมัน C18: 3n-3 ของกลุ่ม OPF สูงกว่ากลุ่ม CON อย่างมีนัยสำคัญทางสถิติ โดยเฉพาะอย่างยิ่งในกลุ่ม C18: 2n-6 และ PUFA n-6 ระดับของ HDL-Cholesterol ของกลุ่ม OPF สูงกว่ากลุ่ม CON ถึง 40% ผลการทดลองแสดงให้เห็นถึงวิธีที่เป็นไปได้ในการปรับเปลี่ยนองค์ประกอบของกรดไขมันในเนื้อแกะโดยการให้อาหารจากแหล่งเส้นใยพื้นเมือง

คำสำคัญ: เลือด กรดไขมัน เนื้อเยื่อของกล้ามเนื้อ ใบปาล์มน้ำมัน กระเพาะอาหาร

[1]*Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

[2]*Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

[3]*Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, P.O. B.14115-336, Iran*

****ผู้รับผิดชอบบทความ** E-mail: mehdiebrahimii@gmail.com*