



Dietary inclusion of oil palm fronds does not change *n*-6 nor *n*-3 content of lamb tissue

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ABSTRACT

Inclusion of oil palm fronds (OPF) in ruminant diets was previously reported to increase the tissue content of unsaturated fatty acids (UFAs) and reduce rumen biohydrogenation, which was in contrast with our former *in vitro* study. Thus, a small scale *in vivo* study was performed to assess the effects of different inclusion levels of OPF on UFA content of intramuscular fat (*longissimus dorsi*, *biceps femoris*) and adipose tissues (*subcutaneous*, *perirenal*). Sixteen lambs were divided into 4 treatment groups, with dietary inclusion levels of OPF of 0, 100, 200 and 300 g/kg DM, respectively. Further, the diet contained a commercial sheep/goat pellet. Diets were supplemented with soybean oil (40 g/kg DM), which was equally mixed with the whole ration. Hence, the diet particularly supplied linoleic acid (LA; 563–569 g/kg total fatty acid (FA) intake). Due to selective refusal of the OPF, actual proportion of OPF in the ingested feed was lower compared with the offered diet, *i.e.* 44, 137 and 170 g/kg DM. The total fat intake also differed among the treatments due to differences in feed intake. After 12 weeks on the experimental diets, the lambs were slaughtered and both intramuscular fat and adipose tissues were collected for FA analysis. No differences were observed in total FA content, proportion of C18:2 *n*-6 and UFA/SFA ratio in OPF inclusion groups for both intramuscular fat and adipose tissues. Hence, OPF does not seem to relatively modify UFA content of adipose or intramuscular fat.

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1. Introduction

The abundance of oil palm fronds (OPF), a by-product of the oil palm industry in Malaysia has resulted in a major interest for their inclusion in ruminant diets. However, OPF show poor metabolizable energy values (4.9–6.5 MJ/kg DM; Hassim et al., 2010), which limits their use in diets of production animals. Nevertheless, reports suggesting that their incorporation (200 g/kg DM) could enhance the unsaturated fatty acid (UFA) proportion in rumen contents (Hassim et al., 2007) and sheep plasma (Rajion et al., 2001), increased the interest in this product in a context of

healthy food development, *e.g.* meat products with greater amounts of UFA. As the low total fat content of OPF (21 g/kg DM; Hassim et al., 2010) excluded its potential as direct supplier of UFA, enhancement of UFA concentration in animal products could be hypothesized to be indirect through modification of the rumen fatty acid (FA) metabolism of an external polyunsaturated fatty acid (PUFA) source, eventually through action of plant secondary metabolites (*e.g.* Lourenço et al., 2008). However, our former *in vitro* study (Hassim et al., 2010) showed that increasing OPF inclusion levels decreased the amount of UFA after 24 h incubation as a result of increased biohydrogenation rate of linoleic and linolenic acids from sunflower and linseed oil, respectively. This confirmed earlier knowledge on the stimulating effect of fibre supply on biohydrogenation rates of linoleic and linolenic acids (Dewhurst et al., 2006). Hence, a small-scale *in vivo* trial was performed to assess this apparent

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contradiction between *in vivo* (Hassim et al., 2007; Rajion et al., 2001) and *in vitro* (Hassim et al., 2010) observations. However, as modified rumen FA metabolism is only beneficial for the consumer if it finally results in increased proportions of UFA in the end products of animal origin, PUFA concentration of intramuscular and adipose fat were emphasized.

Hence, the aim of this study was to assess the effect of different inclusion levels of OPF in combination with an external PUFA source (soybean oil) on FA composition of intramuscular and adipose tissue.

2. Materials and methods

2.1. Animals and diets

The experiment was carried out according to the guidelines of the Research Policy of the Universiti Putra Malaysia on animal ethics. Sixteen four-month old male Barbados Black Belly × Malin crossbred lambs were used in the study carried out at the Experimental Ruminant Unit, Field II, Department of Animal Science, Universiti Putra Malaysia, Malaysia. The average initial weight of the lambs was 11.5 ± 2.8 kg (mean $\pm$ s.d.). The lambs were housed individually in wooden pens with a slatted floor two metres above the ground. The lambs were randomly divided into 4 treatment groups ($n=4$), with dietary OPF inclusion levels of 0, 100, 200 and 300 g/kg DM to each of the treatment groups, respectively. Proximate chemical composition of the OPF (g/kg DM): 33.7 crude protein, 21.0 ether extract, 385 crude fibre, 55.0 ash. Further, the diet contained a commercial sheep/goat pellet (FFM Berhad, Malaysia) consisting of *Leucaena* leaf meal, rice bran, wheat pollard, urea and dicalcium phosphate (ingredients in decreasing order of importance, with proximate chemical composition (g/kg DM): 171 crude protein, 20.3 ether extract, 68.2 crude fibre, 87.1 ash. To each of the diets, an external source of C18:2 *n*-6 (soybean oil) was added at 40 g/kg DM. The soybean oil was equally spread on top of the OPF and the commercial pellet during diet preparation. All dietary compounds mainly provided linoleic acid (C18:2 *n*-6; 466, 395 and 649 g/kg FA for OPF, commercial pellet and soybean oil, respectively), whereas the supply of linolenic acid (C18:3 *n*-3) was 43.9, 32.8 and 0.92 g/kg FA, respectively. The diets were freshly prepared prior to feeding as a total mixed ration. The lambs received the experimental diets in two equal meals (500 g/meal) at 09:00 and 17:00. Water was available *ad libitum*. The lambs were weighed every 2 weeks before the morning feeding. Feed intake was measured daily by weighing the feed orts. Selection against OPF intake was observed, which was visually estimated from the

feed orts, where OPF represented 35% of the orts in the groups offered 100 and 200 g of OPF daily and 50% of the orts of the group offered 300 g OPF daily. Hence, due to selective refusal of the OPF, actual proportion of OPF in the ingested feed was lower compared with the offered diet, i.e. 44, 137 and 170 g/kg DM. Accordingly, dietary treatments are referred to as OPF 44, OPF 137 and OPF 170. The overview of total FA and proportions of individual C18 FA intake by animals from dietary treatments is presented in Table 1.

2.2. Slaughter and sampling procedures

After 12 weeks on the experimental diets, the lambs were slaughtered according to the guidelines of the standard slaughter procedures outlined in the MS1500:2004 (Department of Standards Malaysia, 2004). The lambs were fasted overnight (± 12 h) before the slaughter was performed the next morning at 8.00 am. The carcass was skinned and the head was removed at the atlanto-occipital joint. The fore legs were severed at the carpo-metacarpal joint and the hind legs at the tarso-metatarsal joints.

The pH of warm (0 h) and chilled (24 h) carcass was measured with a rounded-end glass probe attached to a hand held pH meter (Mettler Toledo, USA). The *longissimus dorsi* (LD) muscle was obtained from 12th to 13th ribs and *biceps femoris* (BF) muscle was sampled 6 cm from the trochanter major of the femur. *Subcutaneous* (SC) and *perirenal* (PR) fat as well as muscles were sampled and weighed 24 h after slaughter from chilled carcasses (4 °C). Muscles and fat samples were stored vacuum packed at -20 °C until FA analysis.

2.3. Fatty acid analysis

The FA were extracted from muscle and fat using chloroform/methanol (C/M, 2/1, v/v), as described by Folch et al. (1957) and modified by Rajion et al. (1985). Briefly, 0.5 g of muscle or fat sample was homogenised first in 40 ml of C/M (2/1, v/v) for 1 min by an ultra-turrax mixer (Ultra-Turrax T5, IKA Analysentechnik GmbH, Germany) and FA were extracted overnight with 40 ml of C/M (2/1, v/v) and kept in the dark. The extracts were then filtered (No. 1 Whatman paper, Whatman International Ltd., Maidstone, England) into 250 ml separating funnels. The residue left over on the paper filter was washed twice with 5 ml of C/M (2/1, v/v). Afterwards, 10 ml of normal saline solution (9 g/l NaCl) was added to the separating funnel. The mixture was shaken vigorously for 1 min and was left to stand for 4 h in the separating funnel. Afterwards, the lower phase was collected in a round bottom flask and evaporated by rotary evaporation (Laborota 4000-efficient, Heidolph, Germany) at 70 °C. The dry residue was then re-suspended in 5 ml C/M (2/1, v/v) and transferred to a capped methylation tube.

Table 1

Effect of different levels of OPF on ADG (g/d), feed intake (g/d), OPF and commercial pellet intake (g/kg DM), total fat intake (g/g total feed intake), actual proportions of C18:2 *n*-6, C18:3 *n*-3 and C18:0 intake (g/kg total fat intake), carcass weight (kg), muscle weight (g), and pH of warm (0 h) and chilled carcass (24 h; $n=4$).

	Dietary treatment ^a				SEM	Probability	
	OPF0	OPF44	OPF137	OPF170		Linear	Quadratic
ADG	75.5	81.7	58.2	67.9	12.1	0.407	0.887
Feed intake	886	814	789	739	14.3	<0.001	0.448
OPF intake	–	44.0	137	170	6.07	<0.001	0.003
Commercial pellet intake	886	770	652	570	9.45	<0.001	0.099
Total fat intake	53.4	49.2	47.7	44.7	0.868	<0.001	0.453
C18:0	38.0	38.9	40.9	41.8	0.074	<0.001	0.909
C18:2 <i>n</i> -6	563	565	567	569	0.100	<0.001	0.909
C18:2 <i>n</i> -3	11.7	11.9	12.3	12.6	0.018	0.909	<0.001
Carcass weight	8.22	7.93	7.55	7.92	0.835	0.739	0.698
Muscle weight							
<i>Longissimus dorsi</i>	46.4	42.1	42.8	41.6	7.14	0.675	0.829
<i>Biceps femoris</i>	27.1	31.1	26.2	32.1	3.77	0.567	0.813
Warm carcass pH, 0 h	6.59	6.47	6.27	6.55	0.129	0.611	0.146
Chilled carcass pH, 24 h	5.59	5.63	5.68	5.55	0.089	0.821	0.367

OPF, oil palm frond; ADG, average daily gain.

^a OPF0, OPF44, OPF137, OPF170: dietary inclusion of OPF at 0, 100, 200 or 300 g/kg DM, respectively.

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