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Short communication

# Effect of different inclusion levels of oil palm fronds on *in vitro* rumen fermentation pattern, fatty acid metabolism and apparent biohydrogenation of linoleic and linolenic acid

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ABSTRACT

Effects of different inclusion levels of oil palm fronds (OPF) on rumen fermentation, apparent biohydrogenation of linoleic (C18:2n-6) and linolenic (C18:3n-3) acid and their biohydrogenation intermediates are described after 24 h *in vitro* incubations with buffer (20 ml), rumen fluid (5 ml) and standard dairy concentrate (0.250, 0.225, 0.200 and 0.175 g). Four inclusion levels of OPF (0, 0.025, 0.050 and 0.075 g) were tested in two *in vitro* incubation series: without (Experiment 1) and with (Experiment 2) addition of a mixture of sunflower (10 mg) and linseed oil (10 mg) as an external polyunsaturated fatty acid source. Increasing inclusion levels of OPF changed the rumen fermentation pattern in both *in vitro* incubation series, in terms of a decreased production of short chain fatty acids, a linear increase in acetate and a decrease in propionate proportions. A trend for lower amounts of C18:2n-6 and C18:3n-3 were observed due to a higher apparent biohydrogenation rate of the latter, as the inclusion levels of OPF increased. In order to maximize the valorisation of OPF as a roughage source in Malaysian ruminant production systems, further technological research is needed to improve its digestibility.



## 1. Introduction

A major strategy to enhance the livestock industry in developing countries could be the increased use of indigenous feed resources to reduce import costs. Oil palm fronds (OPF), consisting of leaflets and petioles, is a by-product of the oil palm industry in Malaysia and their abundance has resulted in major interest in their potential use for livestock feed. These OPF cannot be used as a sole source of animal feed mainly because of its poor caloric value, 4.9 to 6.5 MJ (metabolizable energy, ME)/kg dry matter (DM; Dahlan, 2000). Further, there is limited scientific research on the inclusion of OPF in ruminant diets and only some local and technical reports are available (Dahlan, 2000), promoting relatively high OPF inclusion levels of up to 50% and 30% in beef cattle and dairy cows diets, respectively (Ishida and Hassan, 1997). Other reports claimed, appropriate formulation of OPF-based diets could allow live weight gain of beef cattle between 0.6 and 0.8 kg/d and, for local crossbred dairy cows, milk yields of about 22 L/d (Zahari et al., 2003). Furthermore, it was suggested that replacing Napier grass by OPF

*Abbreviations:* ARDC, apparently rumen degradable carbohydrates; FA, fatty acids; LCFA, long chain fatty acids; MOM, microbial organic matter; OPF, oil palm fronds; PUFA, polyunsaturated fatty acids; SCFA, short chain fatty acids.

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on a 20:80, forage:concentrate ratio diet, increased the unsaturated to saturated fatty acid proportion in rumen contents, 8 h post-feeding from 0.22 to 0.37 (Hassim et al., 2007). Although OPF fat is high in unsaturated fatty acids (UFA), the total fat content is low (21 g/kg DM). Therefore, the potential of OPF to serve as polyunsaturated fatty acids (PUFA) supplier is low and the origin of the latter observation requires further investigation. Indeed, alternatively enhancement of PUFA concentration of animal products, *e.g.* milk and meat might be indirect through modification of the rumen fatty acid (FA) metabolism of an external fat source, *e.g.* through action of plant secondary metabolites (Lourenço et al., 2008). Hence, this is a first detailed report evaluating the effect of different inclusion levels of OPF in ruminant diets on rumen fermentation pattern, with respect to short chain fatty acids (SCFA) and rumen methanogenesis, with or without PUFA supplementation, to study the effect of OPF on the biohydrogenation of C18:2n-6 and C18:3n-3 from an external PUFA source.

## 2. Materials and methods

Oil palm fronds were provided by the Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. The fresh OPF was chopped to 1 to 2 cm of length, dried under the sun for 2 to 3 days and made into a pellet of 12 mm diameter in size. The OPF pellet and standard dairy concentrate were ground to 0.5 mm to be included in this incubation. The OPF composition consisted of (g/100 g DM): 3.37 crude protein, 2.10 ether extract, 87.9 non-detergent fibre, 77.4 acid-detergent fibre, 19.9 acid-detergent lignin, 5.50 ash, and 46.6 and 4.39 (g/100 g FA) of C18:2n-6 and C18:3n-3, respectively. The standard dairy concentrate consisted of (g/100 g DM): 20.5 crude protein, 4.75 ether extract, 13.0 crude fibre, 7.40 ash, and 36.7 and 6.30 (g/100 g FA) of C18:2n-6 and C18:3n-3, respectively. Assessment of three OPF inclusion levels was performed by adding OPF (0.025, 0.050 and 0.075 g/incubation) and standard dairy concentrate (0.225, 0.200 and 0.175 g/incubation) for OPF inclusion at 0.10 (OPF 10), 0.20 (OPF 20), and 0.30 (OPF 30), respectively. The control treatment consisted of 0.250 g of standard dairy concentrate without OPF.

All *in vitro* incubations were completed in quadruplicate in four runs on separate days. *In vitro* incubations were run in two separate series: one series was not supplemented (Experiment 1), whereas the other series (Experiment 2) was supplemented with a 50:50 mixture of sunflower oil (540 mg/g oil of C18:2n-6 and 10.9 mg/g oil of C18:3n-3) and linseed oil (145 mg/g oil of C18:2n-6 and 546 mg/g oil of C18:3n-3) as an external PUFA source. These oils were added to the empty incubation flasks as an oil-hexane solution (10 mg of each oil) and hexane was evaporated under nitrogen ($N_2$) stream before incubation. Rumen fluid was collected from two rumen fistulated Holstein dairy cows before the morning feeding (08:00 h), fed (on DM basis): 8.83 kg corn silage, 0.2 kg soybean meal, 0.05 kg mineral mix (AVEVE, Belgium) and grass silage *ad libitum*. Incubations were completed as described by Vlaeminck et al. (2008).

After 24 h of incubation, the gas phase was analyzed for methane ($CH_4$) and hydrogen ($H_2$) production with a micro-GC equipped with two gas chromatographic modules and equipped with a thermal conductivity detector (3000 micro-GC, Agilent, USA). Ethane ($C_2H_6$; 1 ml/flask) was used as internal standard. Methane and $H_2$ were separated using module A which contained a molsieve column (10 m × 0.32 mm × 0.12 μm) with a PLOT U as pre-column (3 m × 0.32 mm × 0.30 μm). Ethane was identified on module B containing a PLOT U column (8 m × 0.32 m × 0.30 μm). The operating conditions for the micro-GC were 100 ms for sample injection, 10 s for sampling and inlet temperature at 100 °C. Additional settings for module A were 6 s for backflushing, column temperature at 110 °C and pressure of 40 psi whereas column temperature and pressure for module B were set at 130 °C and 28 psi, respectively. Argon was used as a carrier gas for both columns. After, the pH was measured and remained between 6.26 and 6.46 for all incubations. Samples for long chain fatty acid (LCFA) and SCFA analysis were prepared according to Van Ranst et al. (2010). All FA extractions and methylations were performed as described by Boeckaert et al. (2007) with tridecanoic acid (C13:0) as internal standard, and using a CP-Sil88 column for fatty acid methyl esters (FAME; 100 m × 250 μm × 0.2 μm, Chrompack, Middelburg, The Netherlands). Fatty acid peaks were identified based on their retention times and compared to external standards as described by Lourenço et al. (2008). On average, the percentage of unknown chromatogram peaks was 2.6%. Total fatty acids are expressed gravimetrically (mg/incubation) and include both known and unknown chromatogram peaks.

Net production of SCFA was calculated by subtracting the amounts in rumen fluid before the incubation from amounts after 24 h of incubation. Apparently rumen degradable carbohydrates (ARDC) were calculated according to Demeyer (1991), and apparent biohydrogenation, *i.e.* the disappearance of C18:2n-6 and C18:3n-3, according to Vlaeminck et al. (2008). It was assumed that ME of such a fibre rich source predominantly originated from rumen fermentative processes, *i.e.* energy associated with SCFA and microbial organic matter (MOM) production, with the latter derived from the former assuming a 1:3 ratio (Fievez et al., 2001; Demeyer, 1991). Metabolizable energy content of OPF ($E_{OPF}$, MJ/kg) was then calculated from data of Experiment 1 according to $E_{OPF} = (E_{incub} - aE_{conc})/(1 - a)$, with $E_{incub}$, the ME (MJ) calculated from the net SCFA and MOM production in the incubation expressed per kg of substrate; a the proportion of concentrate relative to the total amount of substrates incubated and $E_{conc}$ the ME of concentrate (MJ/kg), derived from the OPF 0 incubations.

Statistical analyses were performed using SPSS 15.0 (SPSS software for Windows, release 15.0, SPSS, Inc., Chicago, USA), using the general linear model (univariate) according to $Y_{ij} = \mu + A_i + B_j + \varepsilon_{ij}$, where $Y_{ij}$ is the response; $A_i$ the effect of different inclusion levels of OPF; $B_j$ the effect of incubation runs; $\varepsilon_{ij}$ the residual error. Inclusion level and incubation runs were used as a fixed and random factor, respectively and linear and quadratic effects were assessed.