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Medium-chain fatty acids from coconut or krabok oil inhibit *in vitro* rumen methanogenesis and conversion of non-conjugated dienoic biohydrogenation intermediates

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ABSTRACT

Myristic (C14:0) and lauric (C12:0) acid have been suggested to synergistically influence rumen methanogenesis. This experiment compared the effect of krabok and coconut oil on rumen fermentation, in an attempt to assess this synergism using two natural oil sources which contain similar amounts of C12:0 but with krabok oil containing greater proportions of C14:0 than coconut oil. As a simultaneous action on both rumen methanogenesis and biohydrogenation has been reported for another medium chain fatty acid (C10:0), rumen biohydrogenation also was monitored during the current *in vitro* study. Five treatments were used: one control (CON), without supplementation of coconut or krabok oil, two coconut oil and two krabok oil supplemented incubations. Coconut and krabok oil were supplemented in two doses, providing either 80 (C80 and K80) or 120 mg (C120 and K120) of C12:0 + C14:0 per 100 ml of incubation fluid. A standard concentrate typically fed to ruminant livestock in Thailand (200 mg), buffer (20 ml) and rumen fluid (5 ml) were added to each incubation flask, with or without an external PUFA source (20 mg of a mixture of sunflower and linseed oil). All flasks were incubated at 39 °C for 24 h. Both krabok and coconut oil reduced methane production ($P<0.05$) and increased propionate production ($P<0.05$) at the expense of acetate ($P<0.05$) and butyrate production ($P<0.05$). Krabok and coconut oil induced similar changes and effects were stronger in combination with linseed and sunflower oil, whereas the latter, in the amounts supplemented here, did not change methane production nor induced shifts in the production of any of the VFA. A trend for lower amounts of C18:2 n-6 and C18:3 n-3 after 24 h incubation was observed indicating a higher rate of lipolysis and isomerization of C18:2 n-6 and C18:3 n-3, as the inclusion levels of krabok oil increased. Overall, the effect of krabok and coconut oil on rumen biohydrogenation was limited.

Abbreviations: DHA, docosahexanoic acid; FA, fatty acids; FAME, fatty acid methyl esters; LCFA, long chain fatty acid(s); MCFA, medium chain fatty acid(s); PUFA, polyunsaturated fatty acid(s); RRF, relative response factor; VFA, volatile fatty acid(s).

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Table 1
Fatty acid composition (g/100 g FAME) of coconut oil, krabok oil, tallow, sunflower oil, linseed oil and concentrate ($n = 4$).

Fatty acids	Coconut oil	Krabok oil	Tallow	Sunflower oil	Linseed oil	Concentrate
C8:0	1.86 ± 1.40	0.04 ± 0.04	0.01	0.00	0.00	0.00
C10:0	4.38 ± 2.31	2.26 ± 0.67	0.05	0.00	0.00	0.28
C12:0	44.1 ± 1.52	46.1 ± 3.66	0.12	0.00	0.00	0.12
C14:0	18.5 ± 1.79	43.3 ± 2.55	3.97	0.11	0.05	0.38
C16:0	9.16 ± 2.01	3.95 ± 0.66	26.8	3.08	5.19	15.7
C18:0	4.54 ± 1.77	0.36 ± 0.06	25.7	3.79	3.47	6.42
C18:1c9	8.64 ± 3.26	2.53 ± 0.78	23.5	32.7	13.9	25.9
C18:2 n-6	1.96 ± 0.53	0.36 ± 0.10	0.53	54.5	16.4	42.2
C18:3 n-3	0.04 ± 0.06	0.00 ± 0.00	0.39	1.13	60.6	0.00

1. Introduction

Lauric (C12:0) and myristic (C14:0) acid show potential *in vitro* to reduce rumen methanogenesis and significantly reduce the number of methanogens (Dohme et al., 2001), although the mitigation potential of C14:0 was suggested to be limited when not combined with C12:0 (Soliva et al., 2003). Further, Dohme et al. (1999) reported coconut oil, which is particularly rich in C12:0 (470 g/kg) and C14:0 (180 g/kg) to reduce methane by half when 756 mg/d was fed in a Rusitec fermentation study. *In vivo* methane (CH_4) emissions in sheep have been reduced by 26% up to 73% through the incorporation of coconut oil (70 g/d) (Machmüller and Kreuzer, 1998; Machmüller et al., 2000). Coconut oil (250 g/d) reduced (CH_4) output of beef heifers by 39% (Jordan et al., 2006).

Krabok oil is another source of C12:0 and C14:0, with a similar C12:0 (444 g/kg) but much greater C14:0 (437 g/kg) content than coconut oil (Wongsuthavas et al., 2007). This oil which is derived from krabok seeds or barking deer's mango (*Irvingia malayana* Oliv.ex A. w. Benn) are widely available in South-East Asian forests. As these seeds contain large amounts of fat, they are of interest to be used as a fat source in animal diets by local farmers. Moreover, due to their specific fatty acid profile and given the synergistic effect of C14:0 when combined with C12:0 (Soliva et al., 2003), we hypothesized that krabok oil, would reduce rumen methanogenesis to a larger extent than coconut oil. Moreover, simultaneous action on both methanogenesis and rumen biohydrogenation of poly-unsaturated fatty acids (PUFA) has been reported through *in vitro* supplementation of another medium chain fatty acid (MCFA), i.c. capric acid (C10:0) (Goel et al., 2009). The effect of C12:0 and C14:0 on rumen biohydrogenation is unknown.

Therefore, the aim of this *in vitro* study was to evaluate the replacement of tallow by either krabok or coconut oil at two inclusion levels. Rumen fermentation parameters, with respect to methane and volatile fatty acid (VFA) production were assessed after 24 h incubations in combination or not with sunflower and linseed oil. In the PUFA supplemented incubation, rumen biohydrogenation from these external PUFA sources was also monitored.

2. Materials and methods

2.1. Treatments

The treatments in this study were: (1) control (CON) without supplementation of krabok or coconut oil; (2) 20 mg/incubation flask of C12:0 + C14:0 from coconut oil (C80); (3) 20 mg/incubation flask of C12:0 + C14:0 from krabok oil (K80); (4) 30 mg/incubation flask of C12:0 + C14:0 from coconut oil (C120) and (5) 30 mg/incubation flask of C12:0 + C14:0 from krabok oil (K120). Given the amount of incubation fluid in the current experimental set-up (25 ml), this corresponded to the addition of 80 and 120 mg C12:0 + C14:0 per 100 ml of incubation fluid. This concentration reference was used throughout the paper to better allow comparison with other *in vitro* studies. Coconut and krabok oil were derived from four different batches and their fatty acid composition is given in Table 1. These treatments were incubated with and without an external PUFA source: a mixture of sunflower oil (10 mg) and linseed oil (10 mg). Both oils were added as an oil–hexane solution, with hexane being evaporated under N_2 flow before incubation. Fat added through coconut or krabok oil supplementation was compensated by tallow, which was added to the incubation flasks as an ethanol solution. Tallow is a largely saturated fatty acid source (Table 1) which is considered a rumen inert fat, just as lard, which had been used for this purpose earlier (Fievez et al., 2007). An overview of fat and oil added to the incubation flasks is given in Table 2.

2.2. Substrate

The substrate used was a standard concentrate typically fed to ruminant livestock in Thailand (g/kg product) (cassava chips, 421; rice straw, 211; dry tomato pomace, 158; molasses, 73.7; rice bran, 52.6; soybean meal, 31.6; urea, 21.0; salt, 10.5; di-calcium phosphate, 7.4; oysters meal, 5.3; mineral premix, 5.3; sulfur, 3.2), of which 200 mg was added as basal substrate in all the treatments. The proximate chemical analysis of the substrate was (g/kg DM, except for DM: g/kg FM): dry matter (DM) 925, organic matter (OM) 896, crude protein (N × 6.25; CP) 124, neutral-detergent fibre (NDF) 437, acid-detergent fibre (ADF) 272, crude ash 110.