Polymers of triglycerides generated during heating of fat do not protect linoleic acid from ruminal biohydrogenation

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Introduction: Heating fats often induces a decrease of *cis*-9, *cis*-12 C18:2 and *cis*-9, *cis*-12, *cis*-15 C18:3 biohydrogenation (BH) *in vivo* (Gonthier et al. 2005), *in situ* (Troegeler-Meynadier et al. 2006) and *in vitro* (Privé et al. 2010). This is of interest because it could increase polyunsaturated fatty acids (PUFA) content of ruminant products. Temperature and duration of heating of sunflower oil affect ruminal BH of PUFA, in part due to peroxide value (Privé et al., 2010). Our hypothesis was that polymers of triglycerides (TG), formed during heating of TG but not of free FA, could be responsible for partial protection of PUFA from BH.

Objective: To compare *in vitro* the BH of linoleic acid as free fatty acid (FLA) or TG (TGLA), heated or not.

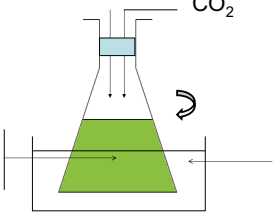
Materials and Methods:

Pure FLA
Pure TGLA
} Heated at 150 °C for 6h

40 ml rumen fluid

CO_2

40 ml bicarbonate buffer
+ 1g hay
+ 0.2 g soybean meal
+ 0.2 g maize grains
+ 52 mg TGLA or heated TGLA **or**
50 mg LA or heated LA



Temperature ---39 °C
Incubation time --- 6 **or** 24 h

Chemical analysis:
Fatty acids ----- Gas chromatography (Park and Goins, 1994)
Polymers of TG ----- HPLC
Hydroperoxides ----- HPLC (Browne et al., 2000)
Aldehydes ---- Gas chromatography

Statistical analysis:
General Linear Model (Systat)
Pairwise comparison (Tukey's test)

Results:

	TGLA	Heated TGLA	FLA	Heated FLA
Added LA (mg)	49	48	48	45
TG polymer (mg)	-	13	-	-
Hydroperoxide (µg)	41	739	ND	1310
Aldehydes (µg)	9	100	7	62

Table 1. Quantities of linoleic acid and oxidative compounds added to flasks

Incub. time		TGLA	Heated TGLA	FLA	Heated FLA	SEM	P fat	P heat	P fat ×heat
6h	mg	41.7^{b}	43.1^{b}	48.8^{a}	44.2^{b}	0.7	<0.01	0.03	<0.01
6h	%	75.1^{b}	77.7^{b}	88.7^{a}	85.4^{a}	1.2	<0.01	0.76	0.03
24 h	mg	49.7^{ab}	49.1^{b}	51.4^{a}	46.7^{c}	0.4	0.48	<0.01	<0.01
24 h	%	90.0^{b}	88.8^{b}	93.7^{a}	89.8^{b}	0.8	<0.01	<0.01	0.09

Table 2. Disappearance of linoleic acid after 6h and 24h incubations with heated or non heated trilinolein or free linoleic acid

Table 1 presents the oxidative status of the different fat sources. As expected, heated TGLA contained 27% of TG polymers contrary to other fat sources. Both heated fats contained high amounts of hydroperoxides, particularly heated LA, and aldehydes. Heated LA contained less non oxidized LA.

Table 2 shows that after 6h, disappearance of LA was not different between TGLA and heated TGLA. The highest quantity disappeared was obtained with non heated FLA. Percentage of disappearance was not affected by heating, but after 6h, TG led to a lower disappearance than free FA. Percentage of disappearance was not different between LA and heated LA, because cultures with heated LA initially contained less LA (Table 1).
After 24h, heating slightly but significantly decreased BH, but only with FLA.

Discussion: Heating TGLA did not significantly decrease BH, which was contrary to our hypothesis and so no effect of TG polymers was demonstrated. On the contrary, heating FLA, resulting in high hydroperoxide formation, slightly reduced BH.

Conclusion: Polymers of TG generated during heating of fat have no effect on ruminal BH, and in particular are not responsible of the protection of PUFA from BH, observed with heated oils and oilseeds. Among other oxidative products, hydroperoxides could be BH modulators.

Browne et al. (2000, Clinical Chemistry 46(6):829-836)
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