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Effect of chemical form, heating, and oxidation products of linoleic acid on rumen bacterial population and activities of biohydrogenating enzymes

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ABSTRACT

Heating polyunsaturated fatty acids (PUFA) produces oxidation products, such as hydroperoxides, aldehydes, and oxypolymers, which could be responsible at least in part for modification of PUFA rumen biohydrogenation (BH). Three in vitro experiments were conducted to investigate the effects of linoleic acid (*cis*-9,*cis*-12-C18:2) oxidation products on BH. In the first experiment, we studied the effects of free linoleic acid (FLA), heated FLA (HFLA, at 150°C for 6 h), triacylglycerols of linoleic acid (TGLA), heated TGLA (HTGLA, at 150°C for 6 h), 13-hydroperoxide (13HPOD), *trans*-2-decenal (T2D), and hexanal (HEX) on BH in vitro after 6 and 24 h of incubation. In the second experiment, aldehydes differing in chain length and degree of unsaturation [pentanal, HEX, heptanal, nonanal, T2D, *trans*-2,*trans*-4-decadienal (T2T4D)] were incubated in vitro for 5 h in rumen fluid. In the third experiment, 9-hydroperoxide (9HPOD), 13HPOD, HEX, or T2T4D were incubated for 1 h in rumen fluid inactivated with chloramphenicol to investigate their effects on enzyme activity. In experiment 1, heat treatment of TGLA generated TGLA oxypolymers, did not affect *cis*-9,*cis*-12-C18:2 disappearance, but did decrease BH intermediates, especially *trans*-11 isomers. Heating FLA decreased *cis*-9,*cis*-12-C18:2 disappearance and *cis*-9,*trans*-11-CLA and *trans*-11-C18:1 production. Treatment with HEX and T2D did not affect *cis*-9,*cis*-12-C18:2 disappearance and barely affected production of BH intermediates. The bacterial community was affected by 13HPOD compared with FLA and HFLA, in parallel with an increase in *trans*-10 isomer production after a 6-h incubation. After 24 h of incubation, 13HPOD decreased *trans*-11 isomer production, but to a lesser extent than HFLA. In experiment 2, some weak but significant effects were observed on BH, unrelated to chain length or degree of unsaturation of aldehydes; the bacterial community was not affected. In experiment 3, 9HPOD inhibited Δ^{9}-isomerization, and both 9HPOD and 13HPOD inhibited Δ^{12}-isomerization. We concluded that oxypolymers did not affect *cis*-9,*cis*-12-C18:2 disappearance. Heating both esterified and free *cis*-9,*cis*-12-C18:2 greatly altered Δ^{12}-isomerization. Aldehydes had few effects. Hydroperoxides are responsible, at least in part, for the effects of fat heating: 13HPOD increased *trans*-10 isomer production (probably by affecting the bacterial community) and decreased *trans*-11 isomer production by inhibiting Δ^{12}-isomerase activity, whereas 9HPOD inhibited both isomerases.

Key words: rumen biohydrogenation, lipid oxidation, linoleic acid, *trans* fatty acids

INTRODUCTION

Trans FA, including various *trans*-octadecenoic acid (*trans*-C18:1) isomers and most conjugated linoleic acid (**CLA**) isomers, which are positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12-C18:2) with conjugated double bonds, have been reported to potentially affect the risk of cancer and cardiovascular diseases in humans (Gebauer et al., 2011). Among CLA isomers, *cis*-9,*trans*-11-CLA has beneficial properties for human health, whereas *trans*-10,*cis*-12-CLA may be harmful (Troegeler-Meynadier and Enjalbert, 2005). Many human foods contain *trans* FA, with dairy products having the highest concentrations. In dairy cows, milk *trans* FA have 2 origins. The first is microbial ruminal biohydrogenation (**BH**) of *cis*-9,*cis*-12-C18:2, which begins with isomerization, mainly producing *cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA (Enjalbert and Troegeler-Meynadier, 2009). Thereafter, initial reduction produces different *trans*-C18:1, in particular *trans*-11-C18:1 (vaccenic acid) and *trans*-10-C18:1. Finally, a subsequent reduction produces stearic acid (C18:0). *Butyrivibrio fibrisolvens* is believed to be responsible for the production of *cis*-9,*trans*-11-CLA and *trans*-11-C18:1 (Enjalbert and Troegeler-Meynadier,

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2009), whereas *Megasphera elsdenii* (Kim et al., 2002) and *Propionibacterium acnes* (Wallace et al., 2007) have been reported to form *trans*-10,*cis*-12-CLA in vitro. A more significant origin of milk CLA is mammary desaturation of *trans*-11-C18:1 into *cis*-9,*trans*-11-CLA (Griinari et al., 2000). *trans*-11-C18:1 is an intermediate of ruminal BH of both *cis*-9,*cis*-12-C18:2 and α-linolenic acid (*cis*-9,*cis*-12,*cis*-15-C18:3). Thus, milk CLA content could be influenced both by factors affecting *cis*-9,*cis*-12-C18:2 and *cis*-9,*cis*-12,*cis*-15-C18:3 BH and by factors affecting desaturation in the mammary gland.

One way to efficiently increase CLA milk concentration is the addition of a fat supplement to the dairy cow diet, particularly a fat source rich in *cis*-9,*cis*-12-C18:2 or *cis*-9,*cis*-12,*cis*-15-C18:3. In dairy cow diets, fat is usually provided by oilseeds, which are often heat treated; for example, by roasting or extrusion. These processes have been shown to have variable effects on BH in different studies, which need to be described and explained. Heating oilseeds often induces a decrease in *cis*-9,*cis*-12-C18:2 and *cis*-9,*cis*-12,*cis*-15-C18:3 BH in vivo (Gonthier et al., 2005), in situ (Troegeler-Meynadier et al., 2006b), and in vitro (Reddy et al., 1994; Kaleem et al., 2013). Furthermore, heat treatment of oilseeds results in increased milk proportions of BH intermediates, particularly *cis*-9,*trans*-11-CLA and *trans*-11-C18:1 (Chouinard et al., 1997a,b; Chilliard et al., 2009).

A previous study in our laboratory (Privé et al., 2010) showed that increasing the heating temperature of sunflower oil led to partial protection of PUFA against BH in vitro, but contrary to previous reports with heated oilseeds, heated oil increased *trans*-10 isomers and decreased *trans*-11 isomers. These effects were linked to the peroxide value of oil and associated with a modification of the bacterial community. More recently, a study with heated oilseeds (Kaleem et al., 2013) showed that aldehydes, in particular hexanal (**HEX**), were linked to a decrease in PUFA disappearance in ruminal cultures.

These changes in BH caused by heated fat could be mediated by potentially active molecules generated during heat treatment: the lipid oxidation products (mostly FA hydroperoxides and aldehydes). Oxidation of *cis*-9,*cis*-12-C18:2 can be divided into 3 steps (Frankel, 2005). During initiation, *cis*-9,*cis*-12-C18:2 is converted to an alkyl radical. During propagation, oxygen binds with this radical forming a peroxyl radical, which takes a hydrogen radical from another *cis*-9,*cis*-12-C18:2, forming a hydroperoxide [13OOH-*cis*-9,*trans*-11-linoleate (**13HPOD**) and 9OOH-*trans*-10,*cis*-12-linoleate (**9HPOD**) for *cis*-9,*cis*-12-C18:2] and spreading the oxidation reaction to other FA. The third step is termination, which may produce hydroxy-acids, aldehydes, ketones, or triacylglycerol (**TAG**) oxypolymers.

A hypothesis for the action of oxidative products on BH is that hydroperoxides could interfere with BH reactions through competitive inhibition of enzymes because of their structural similarity to PUFA and mainly CLA. Moreover, hydroperoxides could be toxic to bacteria because they have been shown to inhibit the activity of rumen anaerobic microorganisms (Brioukhanov and Netrusov, 2004). Aldehydes have also been reported to have toxic effects on rumen bacteria, depending on their chain length (Kubo et al., 1995) and degree of unsaturation (Deng et al., 1993), and some have been shown to promote BH, in particular *trans*-2-decenal (**T2D**; Lee et al., 2007). They also could protect PUFA from BH, in particular HEX (Kaleem et al., 2013). Moreover, TAG oxypolymers could be less sensitive to lipases because of their steric hindrance, resulting in a partial protection of PUFA from BH (as only free FA can be hydrogenated by rumen bacteria).

The objectives of this study were to investigate whether TAG oxypolymers, hydroperoxides, or aldehydes were responsible, at least in part, for the BH alterations observed with heated fat, focusing particularly on the effects of chain length and degree of unsaturation of aldehydes. Moreover, as a first mechanistic approach, we studied the effects of oxidation products on the bacterial community, using an overall quantitative and qualitative approach, and the effects on enzymatic activities using chloramphenicol to prevent synthesis of enzymes during ruminal incubation.

MATERIALS AND METHODS

Three experiments were conducted in vitro: the first explored the effect on BH and rumen bacteria of TAG oxypolymers, obtained by heating TAG, and the effects of heating pure *cis*-9,*cis*-12-C18:2 or adding pure 13HPOD or 2 pure aldehydes (HEX and T2D); the second investigated the effect of aldehyde chain length and degree of unsaturation on rumen BH and bacteria. The third investigated the effects of 13HPOD and 9HPOD and 2 aldehydes (HEX and *trans*-2,*trans*-4-decadienal, **T2T4D**) on BH enzyme activities.

In Vitro Incubations

Experiment 1. As there is no available commercial preparation of TAG oxypolymer, we produced these compounds by heating pure TAG of *cis*-9,*cis*-12-C18:2 (**TGLA**, purity ≥99%, Sigma Co., St Louis, MO) at 150°C for 6 h, resulting in heated TGLA (**HTGLA**). Pure *cis*-9,*cis*-12-C18:2 (free linoleic acid, **FLA**, purity ≥99%, Sigma Co.) was heated separately at 150°C for