J. Dairy Sci. 100:1–15
https://doi.org/10.3168/jds.2017-13117

New insights on the metabolism of ricinoleic acid in ruminants

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ABSTRACT

Dairy goats were fed a total mixed ration with or without the inclusion of castor oil (40 g/kg of DM) to study the metabolism of ricinoleic acid (12-OH,*cis*-9–18:1). Ten goats, at 39.7 ± 4.0 d in milk, were individually penned and allocated at random to the 2 experimental diets. Goats were manually milked twice a day. Milk fatty acids (FA) were analyzed as methyl esters and hydroxyl groups were derivatized in trimethylsilyl ethers. Apart from ricinoleic acid, 6 FA were only detected in the milk of the castor oil group. Ricinoleic acid composed 0.3% of total FA in milk of the castor oil group, whereas the hydroxy-FA (8-OH-14:0, 10-OH-16:0, and 12-OH-18:0) and oxo-FA (8-oxo-14:0, 10-oxo-16:0, and 12-oxo-18:0) reached 7.5% of total FA in milk. We anticipate that these FA were derived from the metabolism of ricinoleic acid, although it was not clear if they were produced in the rumen or in the tissues. To confirm that, we conducted in vitro batch incubations repeated for 3 consecutive weeks with castor oil (40 g/kg of DM) and strained rumen fluid from 2 fistulated sheep. To examine the products formed over time, incubation tubes were stopped at 0, 6, 12, 24, 48, and 72 h. The results of the in vitro experiment showed that ricinoleic acid was metabolized in the rumen at a slow rate and the main products formed were 12-OH-18:0 and 12-oxo-18:0, by hydrogenation of the *cis*-9 double bond, followed by oxidation of the hydroxyl group, respectively. Our results suggest that the 12-OH-18:0 and 12-oxo-18:0 escape rumen and are further metabolized through partial β-oxidation in ruminant tissues. We propose that the 10-OH-16:0 and 8-OH-14:0 found in goat milk of the castor oil group are successive products of the β-oxidation of 12-OH-18:0, and the 10-oxo-16:0 and 8-oxo-14:0 are successive products of the 12-oxo-18:0 in tissues. Overall, our results indicate that ricinoleic acid is extensively metabolized in the rumen and tissues, producing mainly oxo- and hydroxy-FA that are further excreted in milk.
Key words: castor oil, ricinoleic acid, milk, rumen, fatty acid

INTRODUCTION

Castor oil plant (*Ricinus communis* L.) is one of the most important industrial crops in developing countries mostly due to its wide range of industrial use. The castor oil, which is obtained by pressing the castor seeds, has unique characteristics including a high oxidative stability promoting longer shelf-life compared with other vegetable oils. Castor oil contains about 80 to 94% of ricinoleic acid (Binder et al., 1962), a hydroxy-unsaturated fatty acid (12-OH,*cis*-9–18:1), which gives the oil its distinct features. Ricinoleic acid is known to have antimicrobial properties (Novak et al., 1961); thus, it has been studied as a possible modulator of ruminal fermentation to be used as an alternative to ionophore additives to improve growth, feed intake, and efficiency. Indeed, inclusion of castor oil in diet of dairy cows (Gandra et al., 2014; de Jesus et al., 2016), bulls (Cruz et al., 2014; Silva et al., 2014), steers (Gandra et al., 2012), sheep (Maia et al., 2012a), or goat kids (Maia et al., 2012b) did not impair nutrient intake, digestibility, or growth performance.

Studies on the effects of dietary castor oil on the lipid composition of ruminant derived products are scarce. Prado et al. (2016) and Valero et al. (2014) reported that inclusion of a blend of castor oil and cashew nutshell liquid (*Anacardium occidentale* L.) in the diet of feedlot finishing bulls greatly affected the muscle fatty acid (**FA**) composition. However, in both studies the FA profile was limited and none of these studies identified the ricinoleic acid or other hydroxy-FA derivatives in muscle. Only Maia et al. (2012b) identified low quantities of ricinoleic acid in muscle of goat kids fed diets

Received May 4, 2017.
Accepted July 3, 2017.
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supplemented with 3% of castor oil. More scarce are the studies on the effects of castor oil on milk FA composition. It was reported that feeding 2 g/d of ricinoleic acid to dairy cows increased milk yield and milk fat content (Gandra et al., 2014); in contrast, Pereira et al. (2010) found that inclusion of 3% of castor oil in goats diet reduced milk fat content and density compared with the control. However, neither study reported the milk FA composition. Only an older study found trace amounts of hydroxy-FA in milk fat of dairy cows fed a diet containing 0.5% of castor meal and castor oil (Robb et al., 1974), and in another study, only the ricinoleic acid was reported in cheese of goats fed diets containing 4% castor oil (Medeiros et al., 2014).

Because castor oil contains about 80 to 90% of ricinoleic acid, we expect that it is metabolized in the rumen and tissues, and thus a large number of hydroxy-FA derivatives, together with minor amounts of ricinoleic acid, will be found in milk of ruminants fed castor oil. However, the metabolism of ricinoleic acid in the rumen or tissues has not been largely studied. In vitro experiments with rumen fluid and rumen bacteria were conducted to study the influence of ricinoleic acid on the metabolism of linoleic acid (Wallace et al., 2007; Ramos Morales et al., 2012). These studies were focused on the hypothesis that ricinoleic acid was an intermediate of CLA synthesis in the rumen, but no relevant attention was made to the hydroxy or other FA intermediates formed from ricinoleic acid. Hence, this work aims to study the effects of the inclusion of 40 g of castor oil/kg of DM in the diet of dairy goats on the milk FA composition with particular emphasis on the potential FA intermediates derived from ricinoleic acid. In addition, we also aim to explore the possibility that the FA metabolites found in milk could result from the metabolism of ricinoleic acid in the rumen. To investigate this hypothesis, we incubated castor oil with ruminal digesta in vitro for 6 to 72 h.

MATERIALS AND METHODS

Animal Experiment and Diets

Animal experiment was performed at the Field Station for Small Ruminant Research of the Federal University of Paraiba (João Pessoa, Brazil). Ten Saanen × Alpine goats, at 39.7 ± 4.0 DIM and weighing 46.8 ± 6.1 kg, were used. Goats were individually penned and allocated at random to 2 experimental diets: nonsupplemented (control) and supplemented with castor oil (40 g/kg of DM). Feed was supplied as a TMR twice a day in excess (20%) to ensure ad libitum intake and goats had continuous access to drinking water. Experimental diets (Table 1) were formulated according NRC (2007) to meet the nutritional requirements of goats producing 2 kg of milk daily with 3% fat. The experiment lasted 7 d with an adaptation period of 15 d. Goats were manually milked twice a day. Before milking, the udder was cleaned and the strip-cup test was performed to detect mastitis. Post dipping was performed after milking using a commercial iodine solution. A composed milk sample per goat, representative of the last 2 d of the trial was prepared using proportional amounts of all milking samples. A composed sample per animal was collected using proportional amounts from morning and afternoon milking. Milk yield and DMI averaged 1.5 ± 0.32 kg/d and 1.5 ± 0.17 kg/d, respectively.

In Vitro Incubations

In vitro batch incubations were conducted at Faculty of Veterinary Medicine, University of Lisbon (Portugal). Ruminal fluid was collected from 2 fistulated sheep before the morning meal and immediately transferred to the laboratory in a thermostatic box at 39°C. Rumen fluid was immediately strained through 4 layers of cheesecloth and diluted (1:4, vol/vol) in the medium of Goering and Van Soest (1970) under CO_2 flux. The rumen buffered solution (6 mL) was distributed into Hungate tubes containing about 60 mg of a commercial TMR and 2.5 mg of castor oil (FJCampos, Pontinha, Portugal). The TMR contained dehydrated alfalfa (700 g/kg), wheat grain (105 g/kg), soybean meal (110 g/kg), and minerals and premix (25 g/kg). The chemical composition of the TMR was 902 g/kg of DM, 175 g of CP/kg of DM, 113 g of starch/kg of DM, 81 g of ether extract/kg of DM, and 213 g of crude fiber/kg of DM. For batch incubations, Hungate tubes were filled with CO_2 and closed with a butyl rubber stopper and screw cap, then tubes were incubated in a water bath (Unitronic, J.P. Selecta, Barcelona, Spain) at 39°C with gentle agitation for 0, 6, 12, 24, 48, and 72 h. After incubation, tubes were directly frozen and stored at −20°C. The allocation of tubes to the treatments, incubation time, order of filling with buffered ruminal fluid, and position in the water bath were randomized. The incubation procedure was replicated 3 times in 3 consecutive weeks with 2 tubes per treatment and time. Samples were freeze-dried (ScanVac CoolSafe, LaboGene ApS, Lynge, Denmark) and stored at −20°C until analysis.

Fatty Acid and Dimethyl Acetal Analysis

Fatty acid methyl esters were prepared from freeze-dried milk fat samples by direct transesterification using potassium hydroxide (2 *M*) in methanol according to Rego et al. (2009). Fatty acid methyl esters and