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Effect of rumen-protected conjugated linoleic acid on ruminal biohydrogenation and transfer of fatty acids to milk in dairy goats

P. Schmidely[a,b,*], S. Ghazal[a,b], V. Berthelot[a,b]

[a] AgroParisTech, UMR791 Modélisation Systémique Appliquée aux Ruminants, 16 rue Claude Bernard, F-75005 Paris, France
[b] INRA, UMR791 Modélisation Systémique Appliquée aux Ruminants, 16 rue Claude Bernard, F-75005 Paris, France

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ABSTRACT

The objective of the study was to evaluate ruminal biohydrogenation (BH) and apparent transfer of long-chain (FA) from duodenum into milk in dairy goats fed Ca salts of palm oil (CaS, 45 g/d) or lipid-encapsulated conjugated linoleic acids (LE-CLA, 45 g/d providing 4.5 g trans-10, cis-12 C18:2/d and 4.5 g cis-9, trans-11 C18:2/d). Twelve lactating multiparous dairy Saanen or Alpine goats (65 ± 3 days in milk) were randomly allocated to two experimental treatments (CaS vs LE-CLA) for a 3 wks experiment. The goats were fitted with a ruminal cannula and a T-type duodenal cannula for the measurement of duodenal flow by supplying 2.4 g of Cr_2O_3/d through the rumen cannula during 3 wks: the first one was for reaching steady-state concentration of this marker in the digestive tract, the second wk was for measuring the daily flow of faecal chrome and nutrient faecal digestibility, and the third wk was for the duodenal sampling. LE-CLA did not affect intake, duodenal flow and apparent ruminal digestibility of DM, OM, NDF and starch. Compared to CaS, LE-CLA tended to decrease total digestibility of DM and NDF whereas total digestibility of starch was not affect by dietary treatments. The duodenal flow of FA was higher than FA intake in goats fed CaS or LE-CLA. LE-CLA increased the duodenal flows of C18:2 trans-10, cis-12, and C18:2 cis-9, trans-11, leading to a calculated 16% protection from BH for both FA. The LE-CLA treatment tended to decrease the duodenal flows of C18:2 cis-9, trans-12 and C18:2 trans-9, cis-12. The ruminal biohydrogenation of C18:1 cis-9, C18:2 cis-9, cis-12, and C18:3 cis-9, cis-12, cis-15 was not affected by the LE-CLA treatment. Average apparent transfer between the duodenum and milk fat was 22%, 44%, 36% and 22% for C18:2 trans-10, cis-12, C18:2 cis-9, trans-11 + C18:1 trans-11, C18:2 cis-9, cis-12 and C18:3 cis-9, cis-12, cis-15, respectively. Apparent transfer efficiency of long-chain FA was not affected by LE-CLA treatment.

1. Introduction

Conjugated linoleic acid (CLA) is a generic term used to describe positional and geometric isomers of linoleic acid (LA) produced during ruminal biohydrogenation (BH) of LA (Lourenco et al., 2010). The C18:2 trans-10,cis-12 isomer decreases fat yield and it alters milk fatty acid (FA) profile and secretion in cows (Bauman et al., 2011), in sheep (Weerasinghe et al., 2012) and in goats (Ghazal et al., 2014) by inducing a downregulation of the abundance of transcript of key genes involved in de novo synthesis of FA, and decreases in the uptake of long-chain FA and in the desaturation of FA in the mammary gland.

Transfer efficiency of C18:2 trans-10,cis-12 from diet to milk is low when it is not protected from ruminal microbes (Oliveira et al., 2012). Extensive BH of dietary polyunsaturated (PUFA) occurs in the rumen (Schmidely et al., 2008), which leads to an average 86% and 87% disappearance rate for both LA and linolenic acid (Glasser et al., 2008b). Consequently, protection of dietary lipids from ruminal biohydrogenation by Ca salts, formaldehyde treatment, amide bonds or lipid encapsulation (LE) has been developed (Moon et al., 2008) to prevent any change in microbial function and to increase the duodenal flows of PUFA. For example, estimated BH of C18:2 trans-10,cis-12 was 80% in a review of eight studies with Ca salts (de Veth et al., 2005) and around 65% with LE protection (Perfield et al., 2004). However, direct in vivo measurements of ruminal protection of LE-CLA from BH gave inconsistent results: duodenal flow of LE- C18:2 trans-10,cis-12 ranged between 5 to 16% of its intake in dairy cows (Pappritz et al., 2011), whereas it was around 65% in growing sheep (Wynn et al., 2006). To our knowledge, there is no data on CLA and nutrients duodenal flows in dairy goats fed LE- C18:2 trans-10,cis-12, a species that possibly differs from cows for the ruminal biohydrogenation of FA (Shingfield et al., 2010).

In dairy cows, transfer efficiency into milk (TEM) of C18:2 trans-10,cis-12 infused intravenously (Gervais et al., 2009) or in the duodenum (Maxin et al., 2010) was shown to be relatively constant

* Corresponding author at: AgroParisTech, UMR791 Modélisation Systémique Appliquée aux Ruminants, 16 rue Claude Bernard, F-75005 Paris, France.
E-mail address: philippe.schmidely@agroparistech.fr (P. Schmidely).

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with an average of 22% (de Veth et al., 2004). In dairy goats, TEM of C18:2 trans-10,cis-12 after its duodenal infusion was 18% (Andrade and Schmidely, 2006), and this may be affected by the type of lipid supplementation and the percentage of concentrate in the diet (Schmidely and Andrade, 2011). However, TEM of duodenal C18:2 trans-10,cis-12 after dietary supplementation or after dietary manipulation that increased its duodenal flow was shown to be highly variable. For example, TEM was 36% and 48% in cows fed increasing quantities of LE- C18:2 trans-10,cis-12 (Pappritz et al., 2011), suggesting that TEM may be affected by the dose used. Conversely, almost no C18:2 trans-10,cis-12 was detected in milk though its duodenal availability was increased by feeding linseed oil supplemented with long-chain PUFA (Sterk et al., 2012), whereas a TEM of 72% was reported in cows fed high-concentrate diet without buffer (Piperova et al., 2002). In dairy goats, there is no available data on the TEM of C18:2 trans-10, cis-12 after dietary supplementation.

In consequence, the first objective of the current study was to evaluate the effects of a LE-CLA (C18:2 trans-10,cis-12 + C18:2 ci-9,trans-11) supplementation on the duodenal flows of FA and nutrients (OM, fibers, starch) in dairy goats. A secondary objective was to determine the transfer efficiency of C18:2 trans-10,cis-12 from duodenum into milk.

2. Materials and methods

2.1. Animals and diets

The goats were cared and handled in accordance with the French legislation on animal experimentation in line with *the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes* (European Directive 86/609). Twelve mid-lactating multiparous Saanen or Alpine goats (BW = 61.8 ± 8.6 kg, mean ± SE) fitted with a ruminal cannula and a T-type duodenal cannula were used for a 6 wk trial. They were kept in individual pens with wooden floor for a first 3 wk period for progressive adaptation to experimental diets based on corn silage and to lipid supplementation (Ghazal et al., 2012). Then, the goats were housed in metabolism crate for collection of faecal and duodenal samples for the next 3 wks. At the start of the adaptation period, the goats were 65 ± 3 days in milk and the average milk yield was 3.91 ± 0.48 kg/d, with a milk fat content of 40.6 ± 6.29 g/L and a milk protein content of 31.8 ± 1.81 g/L.

The goats were assigned within breed to 2 treatments according to their milk production and milk fat content measured 30 days after parturition. Experimental treatments were either 45 g/day of Ca salts of palm oil (CaS, Saphir Intense, Eolia, 35172 BRUZ, France) or 45 g/d LE-CLA (Lutrell, BASF Animal Nutrition, CHATEAU-Gontier, France). The LE-CLA supplement had a lipid content of 65% and a fatty acid composition of 15% C18:2 trans-10,cis-12, 15% C18:2 cis-9,trans-11, 9% 16:0, 42% 18:0, 12% C18:1 cis-9%, and 1% C18:2 cis-9,cis-12, corresponding to a daily distribution of 4.5 g/d of C18:2 cis-9,trans-11 and 4.5 g/d of C18:2 trans-10,cis-12. The CaS supplement had a lipid content of 85% with 50% C16:0, 35% of C18:1 cis-9%, and 10% C18:2 cis-9, cis-12. Lipid supplements were top dressed on the feed and thoroughly hand-mixed before distribution of 15 g and 30 g in the morning and in the afternoon feed, respectively. Throughout wk 4–6, the goats were fed ad libitum a TMR based on corn silage (35% on a DM basis), beet pulp (20%), barley (15%) and a commercial concentrate (30%) (FLUVIA SELECTUNIC, Sanders, France). The CP, organic matter (OM), neutral detergent fibre (NDF) and starch contents (DM basis) of the basal diet without adding lipids supplements were 120, 922, 381, and 175 g/kg DM respectively. The net energy for lactation (NE_L) was 1500 kcal/kg DM. The composition of FA of the basal diet without adding lipids supplements is given in Table 1.

Table 1
Fatty acids (FA) content of the basal diet.

Item	Basal diet
Fatty acids, mg/g DM	FA ± SD
C14:0	0.05 ± 0.02
C16:0	3.16 ± 0.66
C18:0	0.49 ± 0.11
C20:0	0.51 ± 0.05
C16:1cis-9	0.49 ± 0.19
C18:1 cis-9	5.11 ± 0.06
C18:1 cis-11	2.51 ± 0.31
C18:2 cis-9,cis-12	6.18 ± 0.07
C18:3 cis-9,cis-12,cis-15	0.82 ± 0.03
Total fatty acids	16.8 ± 1.10

2.2. Experimental measurements and sampling

During the whole trial, feed intake was recorded daily by weighing feed offered and refused by the goats. Two diet samples were collected at wk4 and 6 for feed composition analyses and total FA determination. Goats were weighted once a week in the middle of the day. At wk 6, two milk samples of 30 mL (one at the evening milking and one at the following morning milking) were individually collected with a preservative (Bronopol; Grosseron SA, St Herblain, France) and stored at 4 °C until milk composition analysis (protein content, fat content and lactose). Two additional milk samples were collected in the same conditions but without preservative, and stored at −18 °C for milk FA composition.

Chromium oxide (Cr_2O_3, Prolabo, Paris, France) packaged in gelatin capsule (Société LGA, Bandol, France) was used as a marker to determine the digesta flow at duodenum as described in cows (Pappritz et al., 2011; Brask et al., 2013). Gelatin capsules containing the marker were inserted into the rumen twice daily before every meal for 21 days (wk 4–6), supplying 2.4 g of Cr_2O_3/d. Before distribution of the marker, faeces and duodenal contents were sampled at wk 5 to have a standard sample for Cr_2O_3 analysis. Daily distribution of the marker at wk 4 allowed reaching marker equilibrium in the digestive tract, and daily total collection of faeces was realized at wk 5 to measure the daily faecal Cr_2O_3 flow. During the last 3 days of wk 6, 12 samples (60 mL each) of duodenal content for each goat were timetabled to provide an overall sample representative of duodenal content throughout the day and night (each 6 h). Collection times were advanced 2 h on day 2 and 3 of duodenal sampling period to provide one sample for every 2-h interval in a theoretical 24-h period. The duodenal samples and the faeces samples were frozen at −20 ∘C and pooled at the end of the period until freeze-drying and determination of Cr_2O_3, DM, starch, OM, NDF, and FA contents.

2.3. Laboratory analysis

The TMR, the duodenal samples and the faecal samples were dried in air-oven at 87 °C for 24 h, and ground through a 1-mm sieve before analyses. They were then analyzed for DM, OM, and NDF (Van Soest et al., 1991) and starch (Gluco-sequant® Glucose/HK, Roche/Hitach Diagnostic, D-68298 Mannheim, Germany) contents. Dry matter, ash, and enzymatic starch in feed were determined following the ISO standards 6496 (ISO, 1999), 5984 (ISO, 2002), 15914 (ISO, 2004), respectively. The FA content and profile of the TMR, refusals and duodenal contents were determined according to a modification of the procedure of Bas et al. (2003), using nonadecanoic acid (0.25 mg/mL) as internal standard on much lower quantities of samples (150 mg of samples). The FA were double-methylated with sodium methanolate and Boron trifluoride as described by Bas et al. (2007). GC chromatography was performed with a Varian Chrompack CP 3800 system (Varian S.A, Les Ulis, France) equipped with a flame ionization detector. FAME were separated on a 100 m*0.25 mm ID fused silica