Animal Feed Science and Technology 183 (2013) 184–194

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Fecal sample preparation methods for gas chromatography analysis of fatty acids of ruminants fed different amounts of rumen protected conjugated linoleic acids (CLA)

Giacomo Cesaro[a,*], Franco Tagliapietra[a], Luca Grigoletto[a], Alessio Cecchinato[a], Dirk Dannenberger[b], Giovanni Bittante[a], Stefano Schiavon[a]

[a] *Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

[b] *Institute of Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany*

ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form 6 May 2013
Accepted 10 May 2013

Keywords:
Conjugated linoleic acids
Fatty acid methyl esters
Feces
Gas chromatography
Repeatability
Ruminants

ABSTRACT

Aim of this study was to compare three methods for determining fatty acid (FA) profiles in ruminant feces by GC. The first method (*J*) was based on a mild acid–base treatment directly performed on the dry fecal samples, completing in one step hydrolysis, extraction and methylation of FA. The second method (J_{EE}) was based on acid hydrolysis followed by an accelerated solvent extraction (ASE) of ether extract (EE) and by a mild acid–base catalyzed methylation of FA. The third method (C_{EE}) was based on an acid hydrolysis followed by ASE of EE and by an acid catalyzed methylation of FA (C_{EE}). The experimental design involved the fecal samples of 9 bulls fed a total mixed ration supplemented with 0, 8 or 80 g/d of rumen protected CLA (rpCLA; 3 bulls/dose). Feces collected from these bulls were analyzed, by GC, in triplicates by each method expressing FA contents as mg/g DM. The repeatability of FA and CLA measurements of each method was determined. For the content of CLA isomers, the methods presented heteroscedastic residual variances and, thus, were compared by linear regression. Within method, fecal contents of CLA were regressed against the rpCLA dose. The *F*-test was employed to test the significance of any slope that deviated from unity and any intercept that was different from zero. There were no differences among methods for the total amount of FA extracted, which averaged 24.55 mg/g DM. The *J* method was the most repeatable method for most single FA, and for the sums of SFA, MUFA and PUFA. The two EE-methods evidenced for C18:2*c*9,*t*11 CLA and C18:2*t*10,*c*12 CLA linear relationships with slopes and intercepts close to 1 and 0, respectively, whereas the relationships of J_{EE} and C_{EE} with *J* had slopes lower than unity. With increasing rpCLA dosage the EE-based methods provided lower increase of fecal contents of C18:2*c*9,*t*11 CLA and of C18:2*t*10,*c*12 CLA isomers and a higher C18:2*t*9,*t*11 CLA content than *J*, probably as a result of a modification of cis-trans isomerism caused by acid hydrolysis and ASE. The *J* method should be preferred as it provides more repeatable measures of the fecal FA profiles and because it causes a lower shift in CLA isomer composition with respect to procedures based on acid hydrolysis and ASE.

Abbreviations: ASE, accelerated solvent extraction; CLA, conjugated linoleic acid; CP, crude protein; DF, degrees of freedom; DM, dry matter; EE, ether extract; FA, fatty acids; FAME, fatty acids methyl esters; GC, gas chromatography; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; rpCLA, rumen protected CLA; RT, coefficient of repeatability; SFA, saturated fatty acids.

* Corresponding author. Tel.: +39 0498272777; fax: +39 0498272633.
E-mail address: giacomo.cesaro@studenti.unipd.it (G. Cesaro).

0377-8401/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anifeedsci.2013.05.007

1. Introduction

Ether extraction is the classical method for lipid determination of feeds and feces (Palmquist and Jenkins, 2003). Cold extraction methods, using different organic solvents to quantify the ether extract (EE) contents of feeds and digesta samples, are time consuming and often uneconomical (Sukhija and Palmquist, 1988). As feces are rich in soaps an acid hydrolysis need to be done before the solvent extraction step (Palmquist and Jenkins, 2003). To reduce the analytical times, the labor, the use of solvent and the analytical cost the solvent extraction can be automatically completed with an accelerated solvent extraction technique (ASE). Schafer (1998) evidenced as the content of FA extracted with ASE from muscle matrices was similar or better in comparison to the conventional extraction according to Folch et al. (1957).

Lipid content estimated by solvent extraction procedures is not precisely related to the nutritive value of the analyzed sample because non-nutritive waxes and pigments are also extracted, while soap are not extracted without an acid digestion, leading to inaccurate estimation of the nutrients content of feed and feces. Total and single FA contents, rather than the EE content of the sample are preferred information in studying the digestive utilization of lipids (Palmquist and Jenkins, 2003), particularly in the case of fat supplemented diets. With GC analysis the determination of the FA contents requires a methylation treatment to convert FA into fatty acid methyl esters (FAME), which can be conducted either on the collected EE or directly on the dried sample by in situ techniques (Sukhija and Palmquist, 1988; Carrapiso and Garcia, 2000).

The use of rumen protected conjugated linoleic acids (rpCLA) as additive for ruminant and the analytical quantification of CLA in biological samples gained interest in the last decade (Medeiros et al., 2010). In dairy cattle a supplementation of rpCLA markedly reduces milk fat and increase milk CLA contents (Bauman et al., 2008). von Soosten et al. (2012) also suggested that rpCLA supplementation could exert a protective effect against excessive use of protein and fat body reserves of dairy cows within 42 d in milk. Feeding of CLA supplements during early lactation may also improve the reproductive performance of dairy cows (de Veth et al., 2009). In beef cattle a supplementation of rpCLA was found to increase meat CLA content (Gillis et al., 2007; Schiavon et al., 2010, 2011), feed efficiency (Dal Maso et al., 2009; Schiavon et al., 2010; Schiavon and Bittante, 2012) and N efficiency (Schiavon et al., 2012).

The CLA isomers have considerable effects in biological systems at low concentrations (Pariza et al., 2001; Pariza, 2004; Bhattacharya et al., 2006) and the quantification of CLA isomers is also complicated by their unstable nature, due to the presence of unsaturated double bonds which makes these isomers easily subjected to epimerization and isomerization (Park et al., 2002; Jenkins and Lee, 2007; Nuernberg et al., 2007). Methylation was found to be a critical step for a contextual determination of FA and CLA contents in various lipid samples (Park et al., 2002). Base catalysts avoid migration and isomerization of double bonds but do not esterify free FA (Kramer et al., 1997). On the opposite, acid catalysis esterifies all complex and simple forms of FA but may cause isomerization of conjugated double bonds (Kramer et al., 1997; Christie et al., 2007; Jenkins, 2010).

A number of comparisons among GC methods for measuring FA profiles have been published, but these very rarely regarded feces (Outen et al., 1976; Sukhija and Palmquist, 1988; Cesaro et al., 2011). To date, most comparisons performed on tissues, blood, milk and meat products (Kramer et al., 1997; Yamasaki et al., 1999; Fritsche et al., 2000; Park et al., 2002; Aldai et al., 2005; Ficarra et al., 2010) were focused to evaluate the effects of different methylation procedures and reagents, but these treatments were done after that fat fractions were extracted from the matrixes. Thus, a major emphasis was put on methylation process and less on the extraction step. Fecal matrix differs substantially from other biological matrixes. As feces contains soaps, the acid hydrolysis commonly used for the extraction of FA, and physical conditions applied to accelerate the extraction such as ASE, would be responsible of an alteration of the FA profile even before the methylation step, irrespectively of the acid or alkaline nature of the reagents involved in the methylation step. This hypothesis could be tested against the method recently proposed by Jenkins (2010) that was specifically developed to operate a contextual hydrolysis extraction and methylation of FA with a mild acid–base treatment that prevent the isomerization of CLA.

Thus, the aims of this work were: (i) to evaluate if and to what extent, an extraction of FA from feces conducted under acid–ASE conditions influences the FA profiles with respect to the one step mild acid–base treatment proposed by Jenkins (2010); (ii) to verify if after the acid-ASE extraction of EE from the fecal matrix the acid or the mild acid–base methylation treatment influence the resulting FA profiles of feces; and (iii) to compare these procedures in terms of repeatability.

2. Materials and methods

All experimental procedures were approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padova (CEASA, Legnaro, Italy).

2.1. Animals, diets and fecal collection

Fifty-four crossbred young bulls and heifers were fed ad libitum a total mixed ration containing 108 g/kg DM of CP, 35 g/kg of FA and supplemented with 0, 8 or 80 g/d of rumen protected CLA (rpCLA) from 5 to 16 months of age (18 animals for each rpCLA dose) and they consumed 9.3 kg/d of DM on average. The total mixed ration was composed, on DM basis, of corn meal (400 g/kg), corn silage (276 g/kg), soybean meal (33 g/kg), dried sugar beet pulp (113 g/kg), wheat bran (70 g/kg), wheat straw (66 g/kg), vitamin and mineral mixture (26 g/kg), calcium soap (9 g/kg), and hydrogenated soybean oil (7 g/kg). The rpCLA supplement consisted of methyl esters of CLA bound to a silica matrix and coated with hydrogenated soybean