DETERMINATION OF FATTY ACIDS AND CONJUGATED LINOLEIC ACIDS (CLA) CONTENTS IN RUMINANT FECES

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INTRODUCTION

Fecal FA profile is commonly determined by acid-ether extraction, also completed with ASE, and by a methylation prior to GC.

Many methods to determine the FA and CLA profiles are inadequate for matrix rich in soaps such as feces.

Jenkins (2010) proposed a method of FA analysis avoiding the isomerisation of CLA double bonds.

HYPOTESIS: acid-extraction and ASE could influence FA and CLA profile with respect to Jenkins (2010).

AIM

The aim of this study was to compare three analytical methods differing for the kind of FA extraction and the kind of methylation for measuring the faecal FA and CLA profile of bulls receiving increasing amount of a commercial rumen protected CLA product.

MATERIAL AND METHODS

- 54 crossbred bulls and heifers (5 to 16 months of age) were fed a total mixed ration supplemented with 0, 8 or 80 g/d of rumen protected CLA (rpCLA).
- Faeces were collected at 180 d on trial from all the bulls, oven dried (55°C), finely ground (1 mm) and stored at 4°C until analysis.
- After a prospective power analysis, faecal samples were processed with the following scheme: 3 methods × 3 rpCLA dose × 3 bulls × 3 replications = 81 FAs profiles.

METHODS COMPARED

1. **J_{EE}**

- Hydrolysis of the faeces with HCl (3N) (EC, 1998);
- ASE ether extraction (Schäfer 1988);
- Mild acid-base catalyzed methylation of FA (sodium methoxide, methanolic HCl and toluene as solvent) (Jenkins, 2010);

- Internal standard: Methyl 12-tridecenoate.

METHODS COMPARED

2. C_{EE}

- Hydrolysis of the faeces with HCl (3N) (EC, 1998);
- ASE ether extraction (Schäfer 1988);
- Acid catalyzed methylation of FA (Methanolic H_2SO_4 and *n*-Heptane as solvent) (Christie, 1993);

- Internal standard: Methyl 12-tridecenoate.

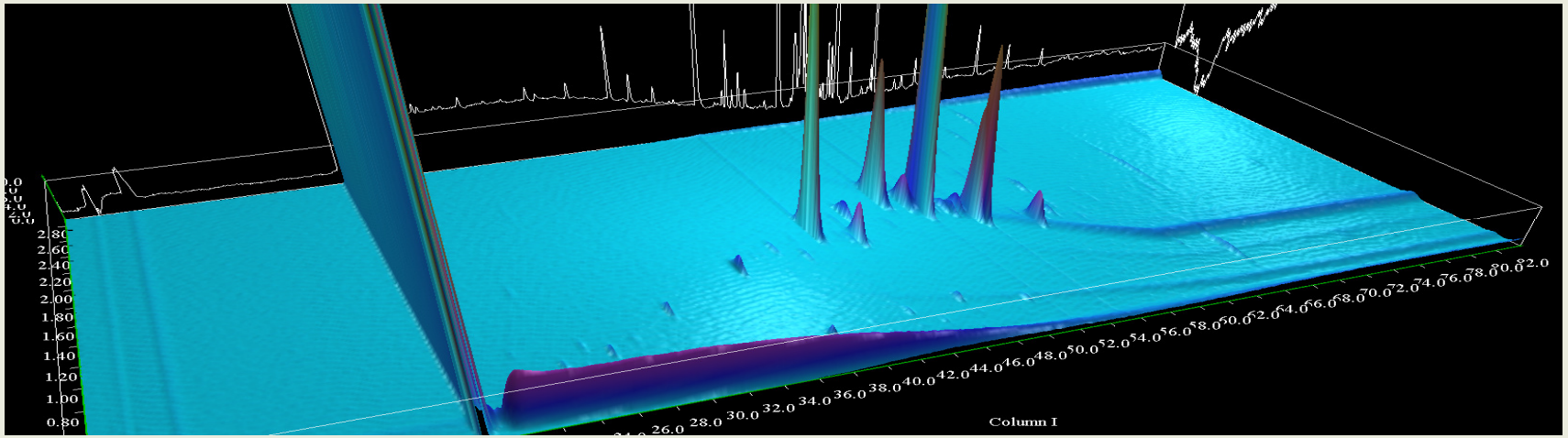
METHODS COMPARED

3. ***J***

- Direct acid-base esterification of FA performed on dry feces (Sodium Methoxide, Methanolic HCl and Toluene as solvent (Jenkins, 2010);

- Internal standard: Methyl 12-tridecenoate.

Gas chromatography analysis



Each sample was analysed using a double column GC×GC (modulator, auto-sampler, FID detector, chromatography data system software and Comprensive software). The three-dimensional chromatograms were processed, FA were identified by comparison of the peaks position in the samples with peaks position of FA presents in a GC reference standard.

Statistical analysis

Coefficient of Repeatability of each method: computed as ratio between variance components:

$$RT\% = [\sigma^2_{CLA} + \sigma^2_{Bull} / \sigma^2_{CLA} + \sigma^2_{Bull} + \sigma^2_{e}] \times 100$$

Source of variation: each FA and FA group were tested for variances homogeneity (Levene's test).

Homoscedastic FA were analysed with a hierarchical linear mixed model.

Heteroscedastic FA, CLA in particular, were compared by linear regression.

RESULTS

Coefficient of repeatability (%) of FA measurements (mg/g DM) and homogeneity of the variances among methods (Levene's test).

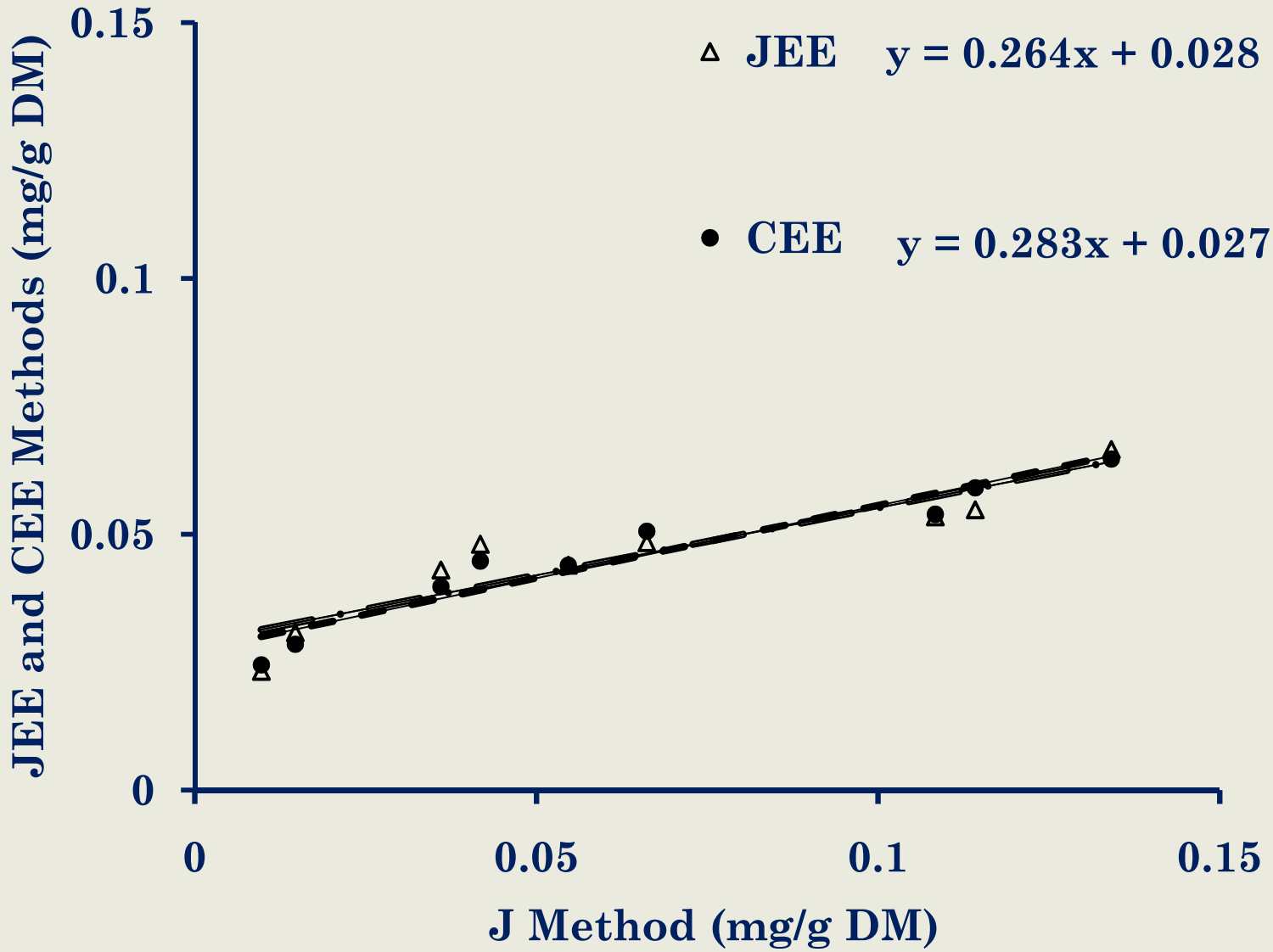
	Method				Levene Test
Fatty Acids (mg/g DM)	**J**	**J_{EE}**	**C_{EE}**		P
∑ FA	**98.3**	97.5	89.9		0.24
C14:0	**99.2**	98.8	95.5		0.40
C17:0	**99.5**	99.1	97.1		0.34
C18:0	**98.9**	98.4	94.6		0.23
C20:0	83.6	**91.9**	89.7		0.11
C22:0	69.6	**95.7**	89.4		<0.001
C24:0	73.9	**89.0**	78.2		0.25
C 18:1 (Vaccenic)	**98.2**	94.6	90.3		0.08
C18:1 (Oleic)	**91.3**	87.2	78.3		0.98
C18:2	**97.2**	**97.2**	90.3		0.42
C18:2,c9,t11 CLA	94.8	90.9	**96.8**		<0.001
C18:2,t10,c12 CLA	**99.3**	95.0	98.0		<0.001
C18:2,t9,t11 CLA	90.2	94.4	**94.7**		0.16
∑ CLA	**98.8**	97.0	98.7		<0.001
C18:3	43.6	**89.7**	78.2		0.24
SFA	**98.6**	97.9	91.9		0.24
MUFA	**96.4**	88.7	80.2		0.57
PUFA	**96.6**	96.3	87.3		0.87

Least square means and SEM due to the 3 different methods of FA analysis and to three increasing doses of rumen protected CLA (rpCLA).

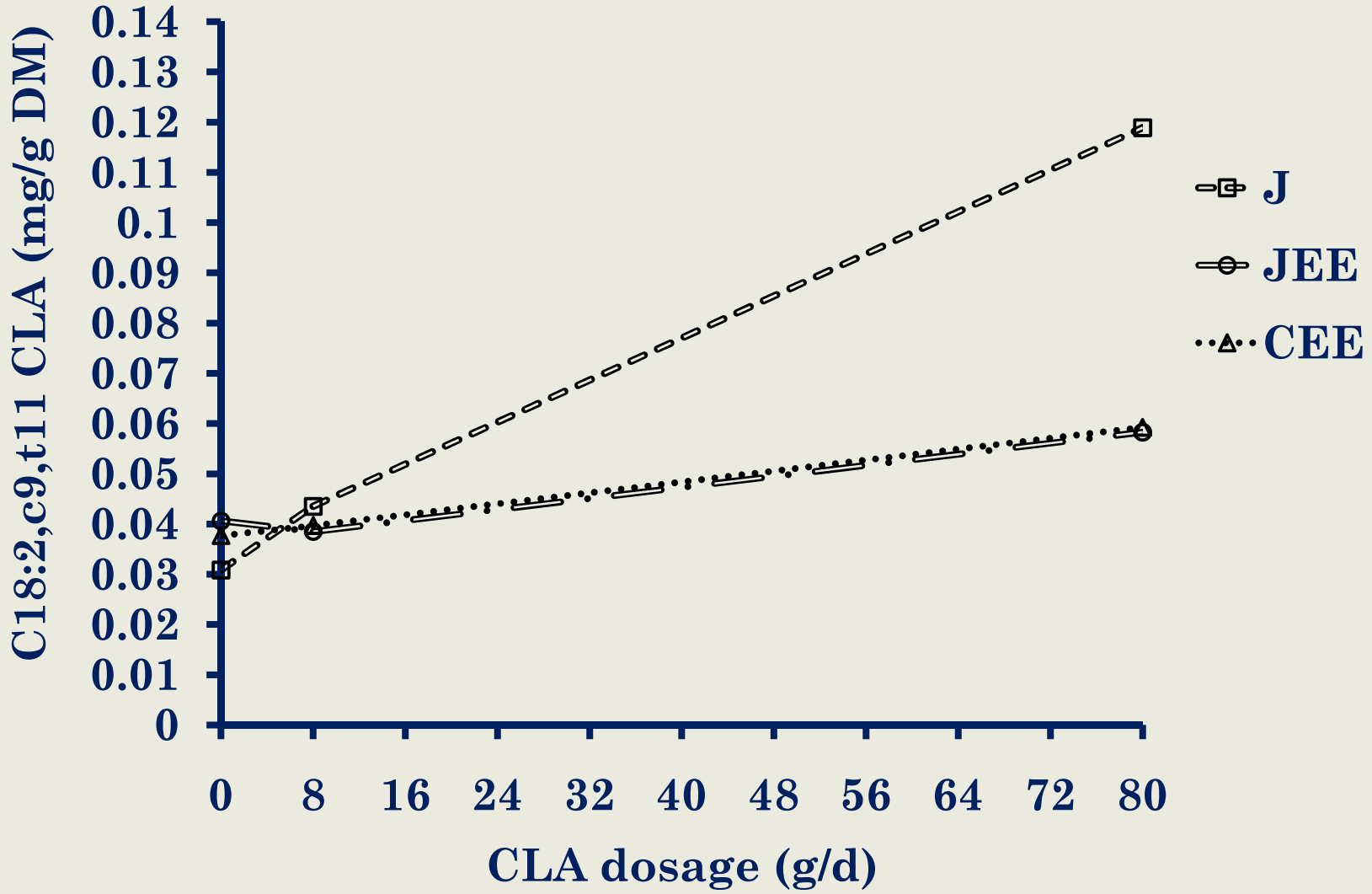
	Method			
Fatty Acids (mg/g DM)	J[1]	J_{EE}[2]	C_{EE}	SEM
∑ FA	24.490	25.130	24.050	2.500
SFA	20.012	20.456	19.312	2.383
MUFA	2.756	2.737	2.816	0.186
PUFA	1.721**	1.937	1.922	0.155
C18:2,c9,t11 CLA	0.064**	0.046	0.045	0.005
C18:2,t10,c12 CLA	0.048**	0.030	0.026	0.008
C18:2,t9,t11 CLA	0.031**	0.059	0.059	0.004
∑ CLA	0.143	0.135	0.131	0.015

1 *P <0.05 J vs. (JEE + CEE); **P <0.01 J vs. (JEE + CEE);
2 *P <0.05 JEE vs. CEE; **P <0.01 JEE vs. CEE.

Effect of methods on the faecal contents of C18:2,c9,t11 CLA



C18:2,c9,t11 CLA content in the feces of bulls fed rpCLA (0, 8, 80 g/d) determined with different methods.



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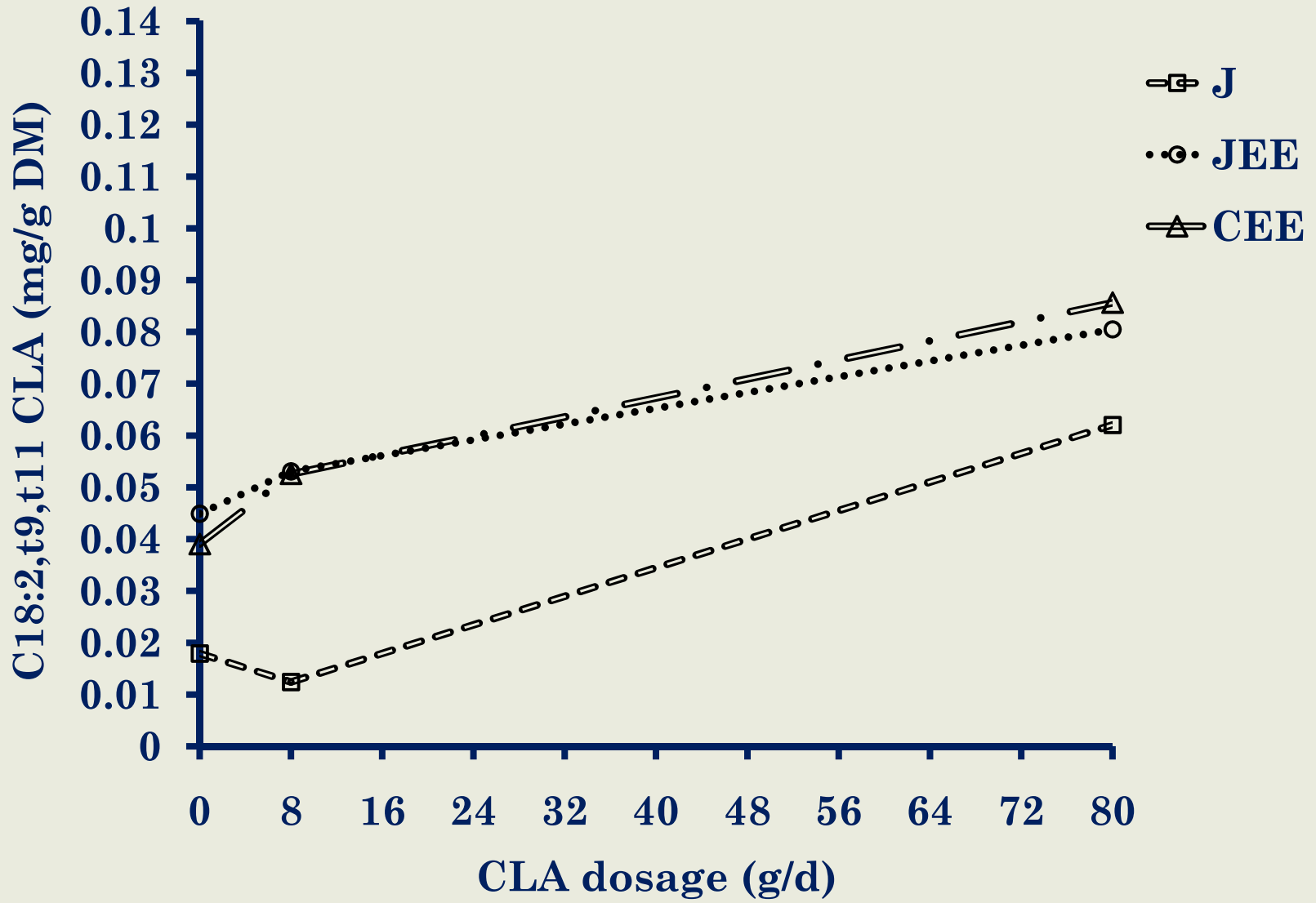
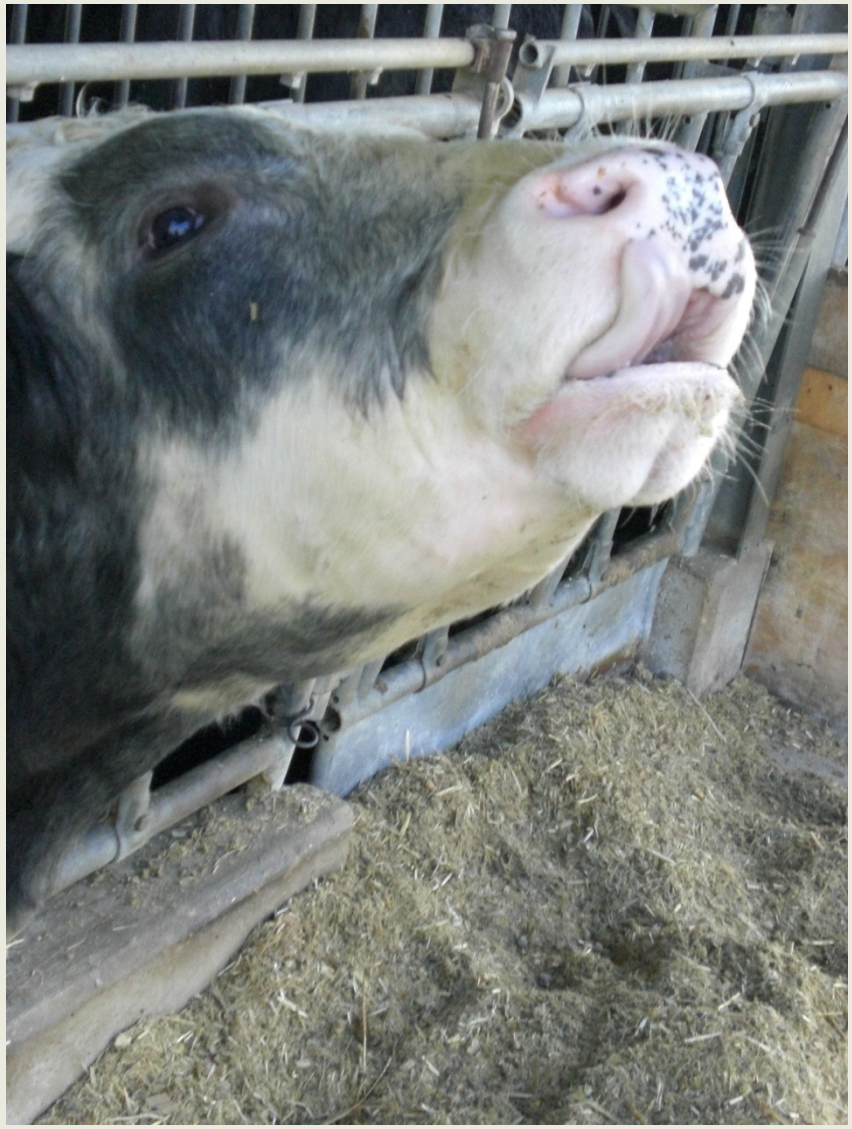


CONCLUSIONS

- The total amount of FA extracted with the three methods was similar. The J method did not impaired the total FA extraction compared to the other two more aggressive methods.
- The two methods based on the acid hydrolysed-ASE underestimate the faecal *c,t* and *t,c* CLA isomers and overestimate the *t,t CLA* forms. The acid-hydrolysis with ASE induced isomerisation of CLA.
- Jenkins's mild acid-base treatment should be recommended for a contextual determination of FA and CLA profile of faeces by GLC.



Thanks for your attention.