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Comparison of enzymatic activities of the reactions of linoleic and alpha-linolenic acids ruminal biohydrogenation



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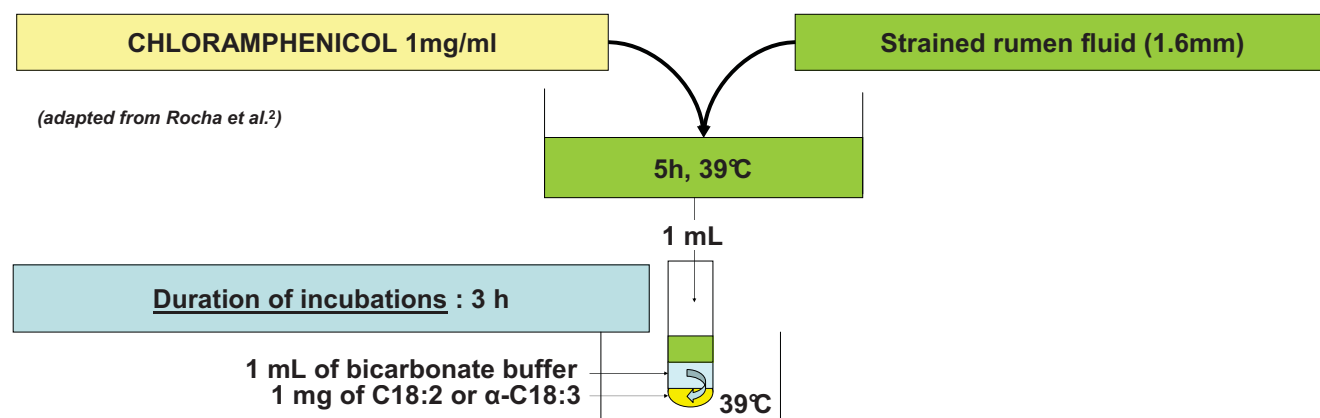
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Introduction Biohydrogenation (BH) is a microbial hydrogenation of dietary unsaturated fatty acids occurring in the rumen. BH is of interest because it directly affects the fatty acids composition of milk and meat. The aim of this study was to compare enzymatic activities of the reactions of C18:2 BH to those of α -C18:3 BH, using chloramphenicol, an inhibitor of protein synthesis in prokaryotes.

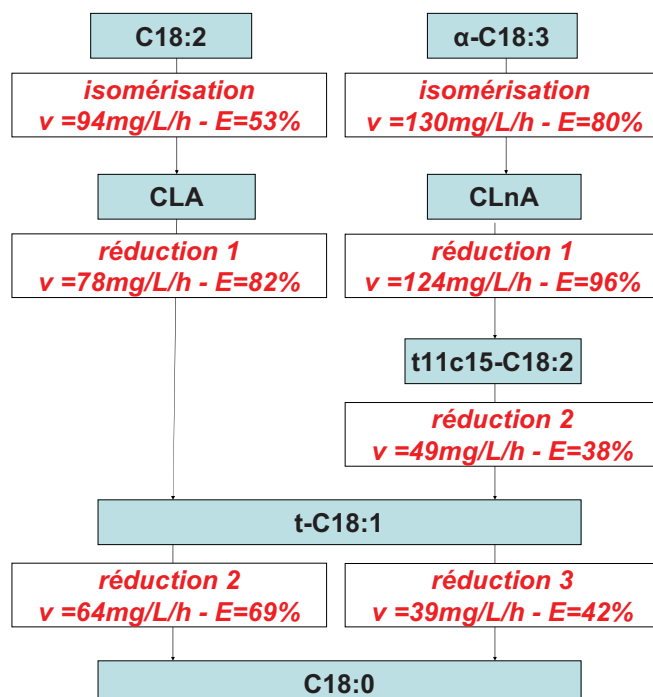
Materials and methods



Fatty acids were quantified by gas chromatography. Then rate (v , mg/L/h) and efficiency (E , %) of the reactions were calculated³.

Results and discussion

The isomerisation of α -C18:3 was quicker and more efficient than that of C18:2, which was probably saturated² ($v = 129.6$ vs. 94.4 mg/L/h; $E = 80.2$ vs. 52.7% , respectively). The reductions of conjugated isomers were rapid and efficient, mainly for CLnA ($v = 123.7$ mg/L/h; $E = 95.5\%$) compared to CLA ($v = 78.1$ mg/L/h; $E = 82.0\%$). However, for C18:2 BH, *cis*9,*trans*11-CLA disappeared faster than *trans*10,*cis*12-CLA so that their respective productions after 3h incubation was $+0.016$ mg vs. $+0.073$ mg. The last reduction of C18:2 BH was the slowest reaction ($v = 63.8$ mg/L/h; $E = 68.9\%$), and constituted the limiting step of C18:2 BH, resulting in *trans*-C18:1 accumulation. The second reduction of α -C18:3 BH was very slow and poorly efficient ($v = 48.8$ mg/L/h; $E = 38.2\%$), so that *trans*11,*cis*15-C18:2 highly accumulated ($+0.450$ mg produced). The last reduction of α -C18:3 BH was also a slow and poorly efficient reaction ($v = 38.8$ mg/L/h; $E = 41.9\%$), so that *trans*-C18:1 would probably have accumulated with a longer incubation.



Conclusion The BH of C18:2 and α -C18:3 were not exactly similar. C18:2 BH was slower, its isomerisation seemed to be rapidly saturable and the limiting step was the final reduction was the slowest reaction inducing an accumulation of *trans*-C18:1. For α -C18:3 BH, first and second reactions were rapid, so that few CLnA was present in the media. Contrarily, the third and fourth reactions were slow so that *trans*11,*cis*15-C18:2 firstly accumulated. Such an evolution was previously reported *in vitro* with live mixed ruminal bacteria^{1,3} indicating that the evaluation of BH does not require live bacteria, and confirming the validity and interest of this enzymatic approach.

¹Jouany, J.P., B. Lassalas, M. Doreau and F. Glasser, 2007. Dynamic features of the rumen metabolism of linoleic acid, linolenic acid and linseed oil measured *in vitro*. *Lipids*. 42: 351-360.

²Rocha, E.R., T. Selby, J.P. Coleman and C.J. Smith, 1996. Oxidative stress response in an anaerobe, *Bacteroides fragilis*: a role for catalase in protection against hydrogen peroxide. *J. Bacteriol.* 178: 6895-6903.

³Troegeler-Meynadier, A., L. Bret-Bennis and F. Enjalbert. 2006. Rates and efficiencies of reactions of ruminal biohydrogenation of linoleic acid according to pH and polyunsaturated fatty acids concentrations. *Repr. Nutr. Dev.* 46:713-724.