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Enzymatic approach of linoleic acid ruminal biohydrogenation

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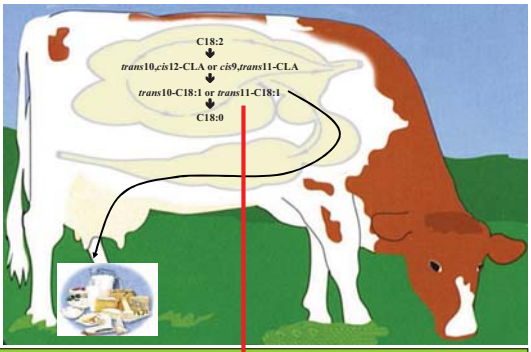
Ruminal biohydrogenation (BH) corresponds to a microbial reduction of dietary unsaturated fatty acid (Fig. 1). The control of BH reactions is of interest for researchers because BH directly affects the composition of fatty acids of milk and meat. In order to better understand C18:2 BH and its variations, the development of an enzymatic approach is necessary to ascertain if the action of modulators affects the bacterial enzyme activity or ruminal bacteria. The aim of this study was to investigate the C18:2 BH capacity of ruminal content after inactivation of bacteria by chloramphenicol, an inhibitor of protein synthesis in prokaryotes.

Figure 1: linoleic acid biohydrogenation



Materials and methods

CHLORAMPHENICOL 1mg/ml

Strained rumen fluid (1.6mm)

(adapted from Allison et al., 1962 and Rocha et al., 1996)

5h, 39℃

1 mL

Duration of incubations : 0, 1, 2, and 3 h

1 mL of bicarbonate buffer
1 mg of C18:2

39℃

Results and discussion

Table 1: Evolution of C18:0, C18:2, CLA and C18:1 isomers amounts (mg) during C18:2 BH.

Duration of incubation	0h	1h	2h	3h	SEM	P
C18:0	0.567^{a}	0.581^{a}	0.584^{a}	0.615^{b}	0.07	0.006
*cis*9-C18:1	0.044	0.04	0.042	0.043	0.001	0.631
*cis*11-C18:1	0.005	0.005	0.005	0.006	0.001	0.661
*cis*12-C18:1	0.002^{a}	0.004	0.006	0.007^{b}	0.001	0.031
*cis*15-C18:1	0.001	0.001	0.000	0.000	0.000	0.131
*trans*6+7+8-C18:1	0.004^{a}	0.004	0.005^{b}	0.005	0.000	0.013
*trans*9-C18:1	0.002	0.002	0.002	0.002	0.000	0.936
*trans*10-C18:1	0.003^{a}	0.000	0.025	0.032^{b}	0.006	0.047
*trans*11-C18:1	0.056^{a}	0.087	0.105^{b}	0.115^{b}	0.007	0.002
*trans*12-C18:1	0.009	0.007	0.005	0.007	0.001	0.560
*trans*13+14-C18:1	0.000	0.000	0.000	0.000	0.000	-
*trans*15-C18:1	0.004	0.004	0.004	0.004	0.000	0.712
*trans*16-C18:1	0.007	0.006	0.006^{a}	0.007^{b}	0.000	0.036
C18:2	1.074^{a}	0.749^{b}	0.592^{b,c}	0.508^{c}	0.043	<0.001
*trans*10,*cis*12-CLA	0.001^{a}	0.028^{b}	0.047^{c}	0.074^{d}	0.003	<0.001
*cis*9,*cis*11-CLA	0.000	0.000	0.000	0.000	0.000	-
*cis*9,*trans*11-CLA	0.003^{a}	0.034^{b}	0.024	0.019	0.006	0.029
*trans*9,*trans*11-CLA	0.000^{a}	0.004^{b}	0.005^{c}	0.008^{d}	0.000	<0.001

→ C18:0 was only significantly (P<0.05) increased after 3h of incubation

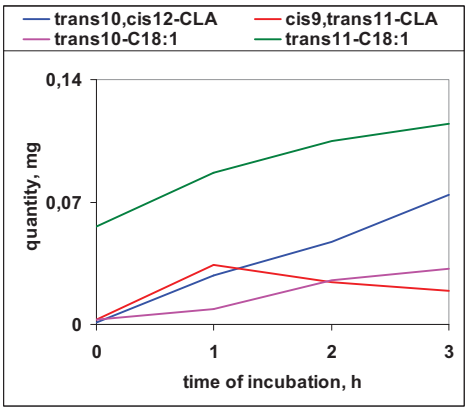
→ *cis*12-C18:1 and *trans*6+7+8-C18:1 weakly increased.

→ *trans*10-C18:1 and *trans*11-C18:1 were the most abundant trans-C18:1 isomers, and increased throughout incubation

→ C18:2 decreased during incubation

→ *trans*10,*cis*12-CLA and *cis*9,*trans*11-CLA were the most abundant CLA isomers. CLA increased throughout incubation, except *cis*9,*trans*11-CLA max after 1h

Figure 2: evolution of intermediates of linoleic acid biohydrogenation



- BH of C18:2 ⇨ *cis*9,*trans*11-CLA + *trans*10,*cis*12-CLA
  ⇨ *trans*11-C18:1 and *trans*10-C18:1
- *cis*12-C18:1 ⇦ trans10,cis12-CLA,
- *trans*6+7+8-C18:1 from the reduction of minor CLA isomers not quantified in this study (Shingfield *et al.*, 2008).
- *trans*11 pathway was rapid compared to *trans*10 pathway which was slow:
1. *trans*10,*cis*12-CLA accumulated *vs.* *cis*9,*trans*11-CLA max at 1h ⇨after 3h: *trans*10,*cis*12-CLA > *cis*9,*trans*11-CLA
2. *trans*10-C18:1 increased after 2h and < *trans*10,*cis*12-CLA.
-C18:0 began to increase in the media when *trans*11-C18:1 concentration was over 0.05 mg/mL.

Conclusion : Such evolution of fatty acids involved in C18:2 BH was similar to that reported in vitro with living ruminal microorganisms by Harfoot *et al.* (1973) and Jouany *et al.* (2007). This approach using Cm could be an interesting and valid method to study enzymes involved in C18:2 BH independently of bacteria, however 3h of incubation were not sufficient to study the final reduction.

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