Effect of linoleic acid oxidative compounds on superoxide dismutase activity and bacterial abundance in long-term ruminal cultures

Muhammad Kaleem, Francis Enjalbert, Annabelle Troegeler-Meynadier

Effect of linoleic acid oxidative compounds on superoxide dismutase activity and bacterial abundance in long-term ruminal cultures

M Kaleem[abc], F Enjalbert[abc], A Troegeler-Meynadier[abc]

[a]Université de Toulouse INPT-ENVT, UMR 1289TANDEM, F-31076 Toulouse, France
[b]INRA UMR 1289 TANDEM, F-31326 Castanet-Tolosan, France
[c]Université de Toulouse INPT-ENSAT, UMR 1289 TANDEM, F-31326 Castanet- Tolosan, France

BACKGROUND

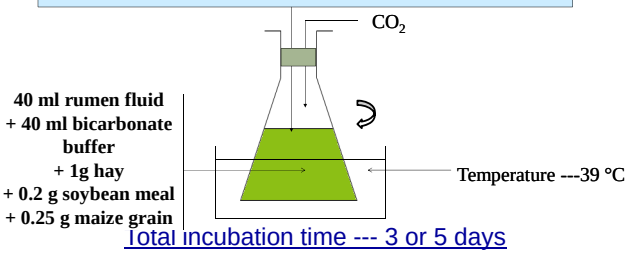
Heating sunflower oil both promotes peroxidation and leads to a modification of the ruminal biohydrogenation of polyunsaturated fatty acids *in vitro*[1]. Products generated during heating, like hydroperoxides and aldehydes could, at least in part, be responsible for the observed biohydrogenation modifications. One possible explanation for the effect of these oxidative compounds on biohydrogenation may be their action on rumen bacteria.

OBJECTIVES

The objectives of this long-term ruminal *in vitro* study were :
a) to explore the effect of two aldehydes and one hydroperoxide on ruminal bacterial population, using a quantitative approach by qPCR;
b) to investigate the response of rumen bacteria to this oxidative stress, by studying the effect of these compounds on rumen superoxide dismutase (SOD) activity.

MATERIALS AND METHODS

30 mg 13(OOH) *c*9,*t*11 octadecadienoate (HPOD)
OR
10 mg hexanal
OR
10 mg *t*2,*t*4 decadieanal (T2T4D)
OR
none



Total incubation time --- 3 or 5 days

•Each day, same quantities of respective oxidative product and fermentative substrates were added to each flask with 20 ml bicarbonate buffer.
•The last day of incubation, 60 mg *c*9,*c*12 C18:2 was added to each flask 5 h before the end of incubation.

Analyses:
In the laboratory, the samples were analysed for:
-SOD activity (UV spectrophotometry)
-Total ruminal bacteria qPCR quantification (kit QIAmp DNA®)
Statistics: General Linear Model + pairwise comparison (Tukey's Test)

RESULTS

Table 1: Effects of some lipid oxidation products on bacteria (log10(number of DNA copies) / ml) of ruminal cultures after 3 or 5

Log10(DNA copies) / ml of cultures	3-day cultures	5-day cultures
Control	10.85	11.32
HPOD	11.03	11.22
Hexanal	10.74	11.29
T2T4D	11.13	11.13
SEM	0.33	0.15
P	0.82	0.78

Initial inoculum contained : 11.17 log10(DNA copies) / ml of cultures. Ruminal bacterial density was not modified by duration of incubation nor tested lipid oxidative products (Table1).

Table 2: Effect of some lipid oxidation products on superoxide dismutase (SOD) activity (UI/ml) of ruminal cultures after 3 or 5 days of incubation ([a,b] $P < 0.05$).

SOD activity UI / ml of cultures	3-day cultures	5-day cultures
Control	7.27	9.61[a]
HPOD	7.18	9.70[a]
Hexanal	7.03	9.62[a]
T2T4D	5.92	7.69[b]
SEM	0.34	0.40
P	0.04	<0.01

SOD activity was significantly affected by tested oxidative products and duration of incubation. It increased with duration of incubation and decreased with addition of T2T4D, in particular a significant decrease of SOD activity in 5-day cultures was observed with T2T4D compared to other treatments.

CONCLUSIONS

The decreased bacterial SOD activity without diminution of bacterial population shows that T2T4D can affect microbial activity without modifying bacterial abundance Further analyses are going on to investigate the relationship between these observed changes and BH modulation.

REFERENCE

1. Privé, F., et al. (2010) Temperature and duration of heating of sunflower oil affect ruminal biohydrogenation of linoleic acid in vitro. *J. Dairy Sci.* 93:711-722.