

In vivo and In Vitro Measurements of Ruminal Redox Potential: a Comparative Study

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Background and Objective

Batch ruminal cultures are often considered as an interesting tool to study rumen metabolism. It is therefore necessary to verify if ruminal conditions offered by means of *in vitro* approach are similar to *in vivo* in terms of oxydo-reduction level assessed by the measurement of redox potential (E_h) .

Material and Design

A rumen fistulated dry dairy cow was adapted during 13 days to a hay-based diet supplemented with 43% of concentrates. Next 3 days were dedicated to sampling and measurements through two different approaches:

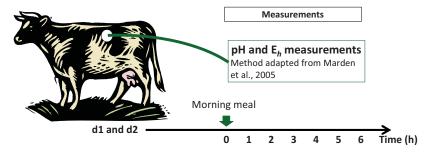
- IN VIVO APPROACH: On d1 and d2, ruminal pH and E_h were measured in vivo from feeding (0h) to 6 hours (6h) at 15 min interval
- IN VITRO APPROACH: On d3, pH and E_h were recorded from the start of incubation (0h) to 6 hours (6h) every 15 min.

For both methods, VFA and D+L-lactate contents were determined at 0h and 6h.

Methods

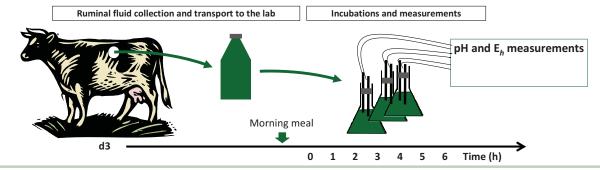
A method adapted from Marden et al. (2005) allowed continuous ruminal fluid sampling and physico-chemical parameters measurements in anaerobic conditions.

IN VIVO



IN VITRO

Ruminal fluid was sucked out the rumen just before morning meal, transferred to the laboratory under anaerobic conditions at 39° C and divided in 10 flasks (125 mL/flask). In each flask, substrates (starch, hay and urea) and a buffer solution (pH = 7, 125 mL/flask) were added. Flasks were bubbled with CO_2 and kept from light and air at 39° C in a waterbath rotary shaker. Two control flasks, not incubated and without any substrates were immediately frozen. Each incubated flask was equipped with a combined pH and a platinum electrode and closed to maintain anaerobiosis and enabling gas evacuation.



Results and Discussion

Fermentative parameters:

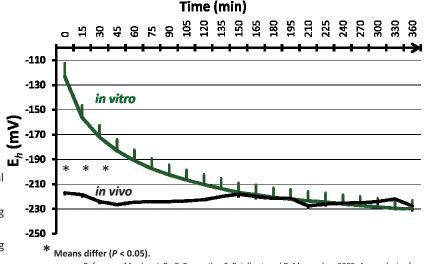
- At 0h, some differences between *in vitro* and *in vivo* values because of ruminal fluid aeration, transfer to the lab and dilution with buffer solution for in vitro incubations.
- At 6h, no more difference

Physico-chemical measurements:

- At 6h, different pH because of buffer solution used in vitro
- At 0h, *in vivo* E_h (– 217 mV) differed (P = 0.003) from *in vitro* value (– 123 mV) probably because of ruminal fluid contact with air outside the rumen.
- After 2 h, both methods yielded similar E_h values

Conclusions

- After the first 2 hours of incubation, the batch in vitro experimental method offered reducing conditions close to the rumen.
- After 6 hours of incubation, it offered a fermentative and reducing environment close to the rumen.
- Ruminal microbiota activity restored rapidly the strong reducing conditions *in vitro* after an exogenous perturbation.



		in vitro	in vivo	SEM	<i>P</i> -value
Total VFA (mM)					
	0h	36.9	60.1	1.76	0.01
	6h	67.3	74.8	4.04	0.12
Acetate (m <i>M</i>)					
	0h	26.5	43.0	1.43	0.02
	6h	46.0	52.2	2.75	0.07
Propionate (mM)					
	0h	4.65	7.79	0.11	< 0.01
	6h	10.3	10.5	0.72	0.81
Butyrate (mM)					
	0h	4.17	6.66	0.18	0.01
	6h	8.88	9.44	0.50	0.33
D + L Lactate (mM)					
	0h	0.62	0.03	0.01	0.01
	6h	0.07	0.05	0.04	0.68
οН					
	0h	6.98	6.83	0.07	0.08
	6h	6.80	6.61	0.06	0.01

Reference: Marden, J. P., C. Bayourthe, F. Enjalbert, and R. Moncoulon. 2005. A new device for measuring kinetics of ruminal pH and redox potential in dairy cow. J. Dairy Sci. 88:277-281