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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 i*n 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:

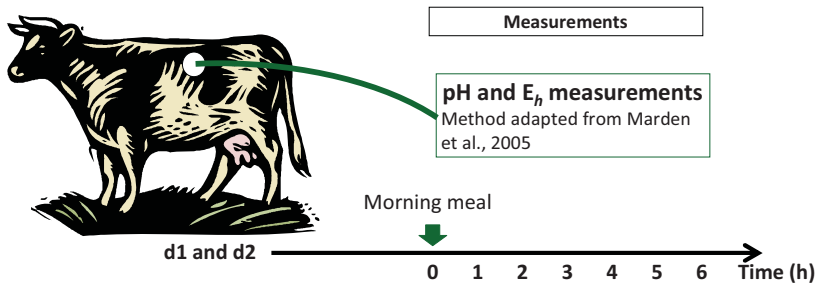
- ***IN VIVO* APPROACH**: On d1 and d2, ruminal pH and E_h were measured *in vivo* from feeding (0h) to 6 hours (6h) at 15 min interval
- ***IN VITRO* APPROACH**: On d3, pH and E_h were recorded from the start of incubation (0h) to 6 hours (6h) every 15 min.

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

Methods

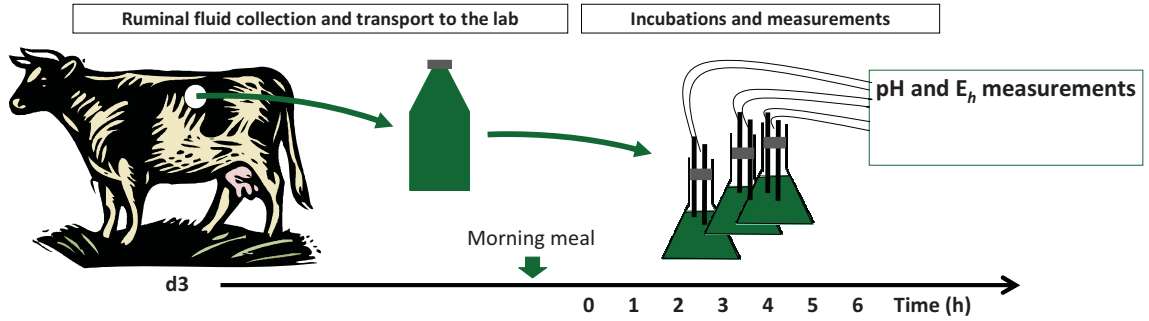
IN VIVO

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



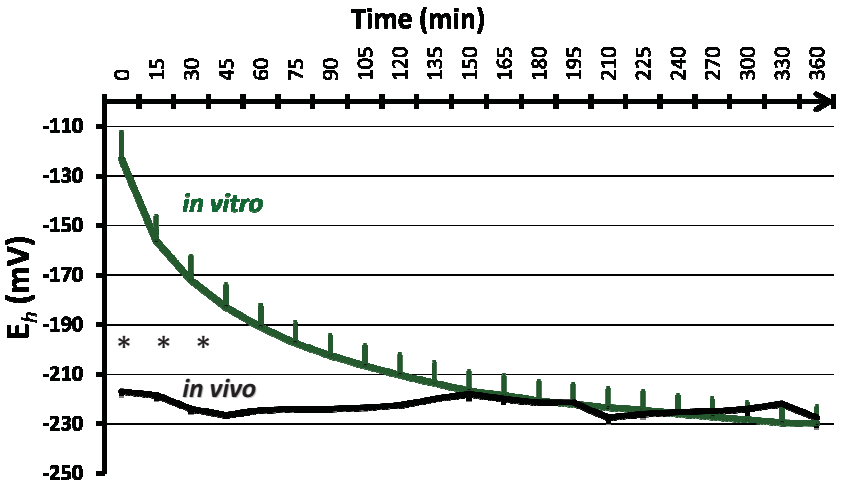
IN VITRO

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



* Means differ ($P < 0.05$).

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

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.

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.