In vitro fermentation parameters of equine cecal contents in a nitrogen deficient environment

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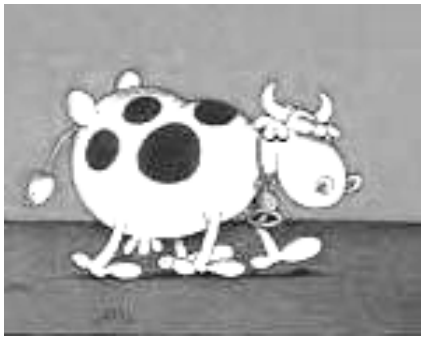
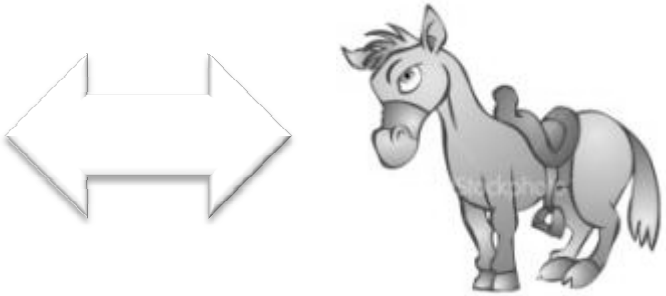
INTRODUCTION

The ruminal microbial ecosystem has been the most thoroughly studied gut system, particularly the quantitative aspects and the contribution of the rumen to the host's nutrition

Despite the anatomical and placement differences…

comparison between the rumen and the hindgut of horses is, some times, inevitable…





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INTRODUCTION

In the rumen, maximizing microbial growth in order to achieve an adequate relationship between energy (VFA) and protein is an objective.

Microbial growth and consequent fiber degradation rely on the energy and nitrogen availability.

ENERGY  **NITROGEN**

Santos et al., 2010

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ENERGY  **NITROGEN**

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OBJECTIVES

Evaluate the influence of an excess of energy in relation to nitrogen in equine cecal contents.

ENERGY  **NITROGEN**

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MATERIALS AND METHODS

Animals and diets

* 3 horses fitted with permanent cecal canulas
* Fed to maintenance level according to INRA recommendations with a standard diets of grass hay and concentrate feed (70:30)

* Cecal contents (CC) were collected 2 hours after the morning feed (9:00 a.m.)

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MATERIALS AND METHODS

Inoculum preparation

*** Cecal contents were:**

*** Passed through 6 layers of cheesecloth;**

*** Mixed with an N free mineral buffer solution (1:10);**

*** A mixture 10 g/l rapidly fermentable carbohydrates was added (glucose, 3.33 g/l; xylose, 3.33 g/l and soluble starch, 3.33 g/l) (CCB)**

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MATERIALS AND METHODS

Incubations

* In order to ensure that N was limiting in the incubation medium, a pre-incubation was performed for 2 hours (CCB2);

*After this period, 20 ml of inoculum were placed in incubation tubes, equipped with bunsen valves;

* Replicate tubes were collected at different incubation times:
*0h, 2h, 4h, 8h, 12h and 24 h

* Analyzed for pH, N-NH3, and VFA

Samples of CC, CCB and CCB2 were also analysed for pH, N-NH3 and VFA

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RESULTS AND DISCUSSION

pH, N-NH3 and VFA values for CC, CCB and CCB2

	pH	N-NH3	Total VFA
CC	7.35±0.1[a]	6.62±0.23***	3.75±0.48***
CCB	6.40±0.11[b]	3.16±0.19*	0.46±0.05*
CCB2	6.48±0.32[b]	2.86±0.23*	0.65±0.11**

Diferent letters indicate significant diferences ($p<0.05$); * *$p<0.05$ * *$p<0.01$; *** $p<0.001$

*** CC values for all parameters were significantly higher (p<0,001)**

***Values for NH3-N did not show any significant differences after the 2h pre-incubation period;**

***Total VFA were higher after the 2h pre-incubation period, indicating fermentative activity of the inoculum.**

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RESULTS AND DISCUSSION

Fermentation parameters evolution with time

	Total VFA	ppAc	ppPr	(Ac+But)/Pr
0	0.51±0.07^{a}	67.7±0.95^{a}	27.8±0.52^{ab}	2.62±0.06^{a}
2	0.55±0.07^{a}	68.3±1.06^{a}	27.3±0.58^{a}	2.67±0.06^{a}
4	0.59±0.06^{ab}	65.5±0.91^{ab}	29.8±0.50^{bc}	2.37±0.05^{b}
8	0.72±0.07^{ab}	65.6±0.92^{ab}	30.6±0.52^{cd}	2.27±0.06^{bc}
12	0.88±0.08^{bc}	61.8±1.06^{a}	32.5±0.52^{d}	2.08±0.07^{cd}
24	1.71±0.08^{c}	58.3±1.06^{c}	35.1±0.58^{e}	1.85±0.07^{d}

Diferent letters indicate significant diferences ($p<0.001$);

There was an increase in total VFA ($p<0.001$) from 0h to 24h, with a decrease ($p<0.001$) in [(C2 + C4)/C3] ratio

Excess of rapidly fermentable carbohydrates present in the incubations.

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RESULTS AND DISCUSSION

Fermentation parameters evolution with time

	Total VFA	ppAc	ppPr	(Ac+But)/Pr	pH
0	0.51 ± 0.07^{a}	67.7 ± 0.95^{a}	27.8 ± 0.52^{ab}	2.62 ± 0.06^{a}	6.60 ± 0.03^{a}
2	0.55 ± 0.07^{a}	8.37 ± 1.06^{a}	27.3 ± 0.58^{a}	2.67 ± 0.06^{a}	6.51 ± 0.03^{a}
4	0.59 ± 0.06^{ab}	65.5 ± 0.91^{ab}	29.8 ± 0.50^{bc}	2.37 ± 0.05^{b}	6.63 ± 0.03^{a}
8	0.72 ± 0.07^{ab}	65.6 ± 0.92^{ab}	30.6 ± 0.52^{cd}	2.27 ± 0.06^{bc}	6.61 ± 0.03^{a}
12	0.88 ± 0.08^{bc}	61.8 ± 1.06^{a}	32.5 ± 0.52^{d}	2.08 ± 0.07^{cd}	6.49 ± 0.03^{a}
24	1.71 ± 0.08^{c}	58.3 ± 1.06^{c}	35.1 ± 0.58^{e}	1.85 ± 0.07^{d}	6.33 ± 0.03^{b}

Diferent letters indicate significant diferences ($p<0.001$);

Results for pH were in accordance with VFA evolution, and decrease ($p<0.001$) from 0h to 24h.

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RESULTS AND DISCUSSION

Fermentation parameters evolution with time

	Total VFA	ppAc	ppPr	(Ac+But)/Pr	pH	N-NH3
0	0.51±0.07^{a}	67.7±0.95^{a}	27.8±0.52^{ab}	2.62±0.06^{a}	6.60±0.03^{a}	2.26±0.14^{a}
2	0.55±0.07^{a}	8.37±1.06^{a}	27.3±0.58^{a}	2.67±0.06^{a}	6.51±0.03^{a}	2.34±0.14^{a}
4	0.59±0.06^{ab}	65.5±0.91^{ab}	29.8±0.50^{bc}	2.37±0.05^{b}	6.63±0.03^{a}	2.68±0.14^{a}
8	0.72±0.07^{ab}	65.6±0.92^{ab}	30.6±0.52^{cd}	2.27±0.06^{bc}	6.61±0.03^{a}	2.67±0.15^{a}
12	0.88±0.08^{bc}	61.8±1.06^{a}	32.5±0.52^{d}	2.08±0.07^{cd}	6.49±0.03^{a}	2.88±0.14^{a}
24	1.71±0.08^{c}	58.3±1.06^{c}	35.1±0.58^{e}	1.85±0.07^{d}	6.33±0.03^{b}	2.76±0.15^{a}

Diferent letters indicate significant diferences ($p<0.001$);

Although without significant differences, values of N-NH3 tended to increase in the first 12 hours of fermentation, and then decrease until 24h.

***This can be due to microbial turn over in an N deficient environment, since there was an excess of carbohydrates**

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CONCLUSIONS

* The 2h pre-incubation period was sufficient to ensure that N was the limiting nutrient for bacterial growth in the incubations.

* VFA values increased during 24 hours of fermentation, indicating fermentative activity;

* N-NH3 showed an increase followed by a decrease, that can be explained with microbial turnover to overcome the lack of N in the environment;

*Data from the present study show that cecal contents continue to manifest fermentative activity in an N deficient environment;

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THANK YOU FOR YOUR ATTENTION!