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Ruminal digestion: development of medium-term cultures of ruminal content

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INTRODUCTION

Batch cultures are commonly used to study ruminal digestion because they are easy to implement. Nevertheless, most are short term studies (a few hours) that are unable to evaluate effects of dietary changes that affect the microbiota, which needs more than 24h to become apparent. So, this study aimed at evaluating persistency of microbial activities during medium-term (96h) cultures of ruminal contents.

MATERIALS AND METHODS

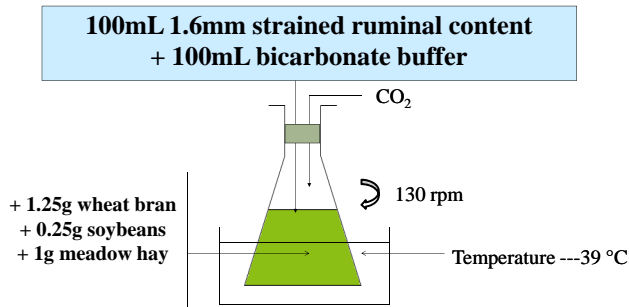


Fig 1: Start of 96h cultures – Day1

- Every 12h, the same quantities of substrates were added to each flask with 100 ml bicarbonate buffer, after removing half of the 200mL media.
- VFA, NH₃ and pH were recorded every 12h.
- Ruminal content at J1 and 96h cultures were used for measurement of microbial activities.

Measurement of microbial activities (carbohydrate and nitrogen disappearance and FA biohydrogénation):

- Inoculum was 100mL of rumen content or of 96h culture added with 100mL of bicarbonate buffer
- Substrates were: 2.5g of wheat bran + 0.5g of soybeans
- Duration of incubation was 8h at 39°C
- Incubates were assayed for NDF, nitrogen and starch content and fatty acids profile.

Statistical analysis:

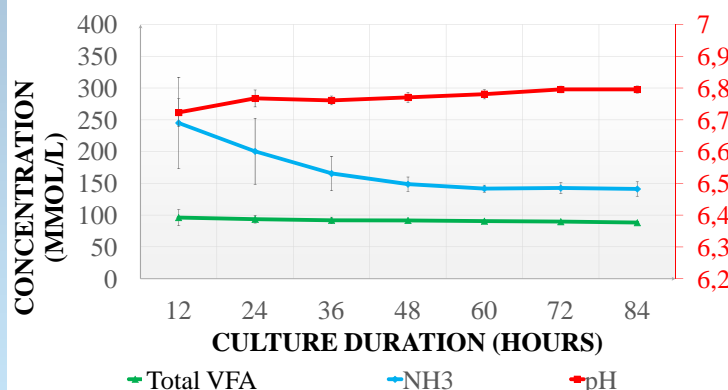
General Linear Model of SYSTAT

RESULTS

Fermentative activities were maintained during cultures (Fig 2):

- pH remained constant.
- Total VFA concentration remained constant but acetic acid proportion (C2) decreased by 11%, those of propionic acid proportion (C3) and butyric acid (C4) increased by 27% and 22%, respectively.
- NH₃ concentration decreased by about 50% during the first 48h and remained stable thereafter.

Fig 3: Evolution of fermentation parameters during the 96h cultures.



Compared to fresh ruminal contents, 96h cultures resulted in a twice lower NDF disappearance (16 vs. 28% in 96 h and fresh cultures, respectively), a similar nitrogen disappearance (14 vs. 13% in 96 h and fresh cultures, respectively) and a twice higher starch disappearance (91 vs. 43% in 96 h and fresh cultures, respectively). Moreover 96h cultures resulted in a 24% higher biohydrogenation extent of linoleic acid with an increase by 61% of trans-11 isomers production (Table1).

Table 1: Biohydrogenation activities of 96h ruminal cultures compared to that of fresh ruminal content used at J1 to start cultures.

		J1	J5	SEM	P
c9c12-C18:2	Disappearance (%)	55.7	69.2	1.7	<0.01
	Initial quantity (mg)	0.4	0.8	-	-
	Final quantity (mg)	1.8	2.8	0.3	0.04
t10 FA	Production (mg)	1.3	2.0	-	-
	Initial quantity (mg)	5.6	17.8	-	-
	Final quantity (mg)	21.6	43.7	0.9	<0.01
t11 FA	Production (mg)	16.1	25.9	-	-

CONCLUSIONS

The lower NH₃ concentration could have resulted from volatilization of aqueous ammonium solution (boiling temperature: 38°C). Fibrolytic activity producing mainly C2 were not favoured by the mid-term cultures, contrary to amylolytic activity producing mainly C3, possibly because of particle size of culture substrates which had been finely ground. The increase of C4 concentration was in agreement with the increase of linoleic acid biohydrogenation by trans-11 pathway, since this pathway is mainly due to *Butyrivibrio fibrisolvens*, a fibrolytic bacteria producing C4 and preferring hemicelluloses, provided by the wheat bran in our experiment.

This incubation procedure can be used to compare medium-term effects of dietary treatments on rumen microbial digestion in cultures but without quantification of these effects.