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Short communication

# Testing a stratified continuous rumen fermenter system

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ABSTRACT

The capability of maintaining protozoa populations and reduce the accumulation of undigested material was investigated in two experiments using a stratified continuous-culture rumen fermenter (CCF) at various setting.

The CCF consists of 8 × 2 L glass bottles, warmed at 39 °C and placed on a waterproof magnetic stirrer. Bottles are closed with a rubber stopper, with an insert for the inflow of artificial saliva and have the outflow at the base.

In *Experiment 1* the effect of two stirring frequencies (continuous vs intermittent, S1 and S2) and two dilution rates (*D*, 1.29 vs 1.04) on the pH values and on the counts of the protozoa in the fermentation fluid was evaluated. The pH reached a steady state after 4 d of fermentation and S1 bottles had lower pH than those S2 (6.50 vs 6.57, P<0.001). The protozoa in the fermentation fluid ($2.92 \times 10^5$/ml at the beginning) declined sharply in the first 2 d of fermentation and stabilized at about $13 \times 10^3$/ml in the S2 flasks, while the S1 flasks had lower protozoa concentrations (about $0.8 \times 10^3$/ml, P<0.001). The *D* had no effects on pH and protozoa.

*Experiment 2* aimed to test effects of varying the daily diet (*F*) amount (15, 20, 25, 30 g DM/d of a diet with 50:50 forage: concentrate ratio) on the organic matter (OM) digestibility (OMD) and on the fermentative patterns. In two fermentation runs of 8 d each, bottles were inoculated with 450 ml of rumen fluid and 1050 ml of artificial saliva, which was then pumped at a rate of 78 ml/h.

Lowering *F* reduced the OM accumulated inside the vessels (from 5.06 to 0.68 g/d; P<0.001) but did not affected the OMD for any of the dietary treatments (range between 0.460 and 0.510). The reduction of *F* led to a linear decrease (P<0.001) in volatile fatty acids concentration (from 65.8 to 45.3 mM) and to an increase of acetate:propionate ratio (from 2.91 to 3.42) and of pH (from 6.0–6.1 to 6.4–6.5). Lowering *F* tended to increase the number of protozoa (from 72 to $198 \times 10^3$/ml, P=0.082).

The tested CCF reaches stable conditions of fermentation after some days of adaptation, allows the survival of protozoa population and has a limited accumulation of undigested materials in the glass bottles.



*Abbreviations:* CCF, continuous-culture rumen fermenter; CP, crude protein; *D*, dilution rate; DM, dry matter; *F*, feeding level; *F*/*B*, feed to buffer ratio; NDF, neutral detergent fibre; $NH_3$–N, ammonia nitrogen; OM, organic matter; OMD, organic matter digestibility; *S*, stirring frequency; VFA, volatile fatty acids.

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## 1. Introduction

Continuous-culture rumen fermenters (CCF) are complex and elaborate artificial systems that try to reproduce what happens in the rumen in a most comprehensive way. They supply a continuous influx of mineral solution and provide a means for the continuous removal of fermentation liquid with a daily addition of substrate.

For many years scientists have attempted to simulate the complex rumen conditions and functions in vitro by employing different apparatus (Hoover et al., 1976; Czerkawski and Breckenridge, 1977; Teather and Sauer, 1988; Miettinen and Setälä, 1989). These systems should primarily reproduce rumen conditions (e.g. pH, turn-overs, temperature, etc.) but also be as simple as possible, designed using inexpensive components and requiring low management (Slyter et al., 1964; Vatthauer et al., 1970; Teather and Sauer, 1988; Muetzel et al., 2009). However, the need to mimic an in vivo very complex physiological function has revealed various limits of the available CCF systems.

Two of the main limitations are maintaining the protozoa population and avoiding a progressive accumulation of undigested materials in the fermentation flasks (Teather and Sauer, 1988; Muetzel et al., 2009). The latter problem can be solved by equipping the fermenter with an efficient mixing system of the flasks contents and a proper overflow outlet. However, this solution generally depresses the protozoa population, which cannot feed adequately if artificially stirred (Coleman, 1980) and require a space in the fermentation vessel "...where they can become sequestered with a removal rate considerably less than that of fluid turnover (Weller and Pilgrim, 1974)".

On these bases we developed a CCF, which is based on previous experience of Teather and Sauer (1988) and Muetzel et al. (2009) and allows the formation of a stratified rubber mat in the flasks by adopting a gentle system of mixing and an outflow at the bottom of the flasks. The apparatus is a tentative to optimize the conditions for the survival of an appreciable protozoa population and minimizing the accumulation of undigested material in fermentation flasks. Moreover, our system is easily replicable, because it has a simple design, uses inexpensive materials and has low management requirements. The description of this system and a discussion of its fermentative conditions would be helpful for researchers interested in organizing a similar rumen CCF. The aims of this work were to find the proper settings of this system capable of maintaining the protozoa population (stirring setting and dilution rate) and reduce the accumulation of undigested materials in the fermentation vessels (feeding level).

## 2. Material and methods

### 2.1. Incubation system

The system consists of 8 × 2 L glass bottles, immersed in a bain-marie warmed at 39 °C. Each bottle is placed on a waterproof magnetic stirrer (Variomag™), is closed with a rubber stopper with an insert for the inflow of artificial saliva, and equipped with a PE overflow tube. A peristaltic pump (PD 5201, ©Heidolph Instruments GMBH & CO KG) supplies the buffer solution from a reservoir to the fermenters (Fig.1).

The outflow at the base of the bottles allows a stratification of the feeding material: the less dense particles form a matter cap on the top of the fermentation fluid, useful for maintaining the anaerobic condition of the liquid and providing a

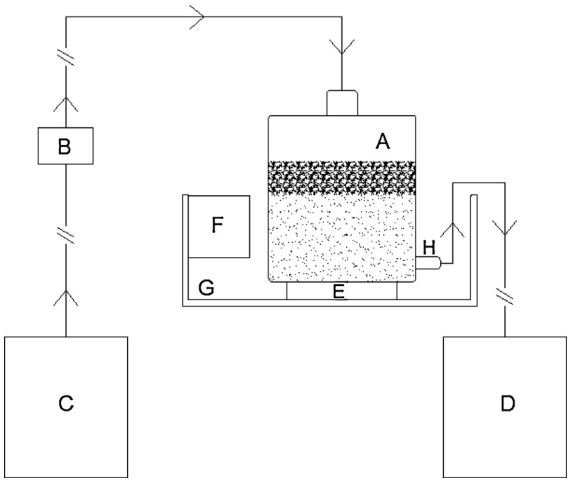


**Fig. 1.** Layout of the fermenter. A, glass bottle; B, peristaltic pump; C, buffer reservoir; D, effluent tank; E, magnetic stirrer; F, heater; G, waterbath; H, outflow. Fluids terminology: fermentation fluid (liquid contained in glass bottle A), ruminal liquid (original rumen inoculum inserted in the glass bottle A at the beginning of fermentation), buffer solution (mineral liquid inserted in the glass bottle A at the beginning of fermentation and continuously pumped in the glass bottle A from the reservoir C), effluent liquid (fermentation fluid outflowed from the glass bottle A in the tank D).