Animal Feed Science and Technology 151 (2009) 32–43

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Evaluation of a stratified continuous rumen incubation system

Stefan Muetzel[a,b,*], Peter Lawrence[a], Ellen M. Hoffmann[a], Klaus Becker[a]

[a] *Institute for Animal Production in the Tropics and Subtropics. University of Hohenheim, 70593 Stuttgart, Germany*
[b] *Ruminant Nutrition & Greenhouse Gas Mitigation, AgResearch Limited, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand*

ARTICLE INFO

Article history:
Received 20 December 2004
Received in revised form 4 November 2008
Accepted 11 November 2008

Keywords:
Continuous culture
Rumen
rRNA
Microbial community

ABSTRACT

We present a continuous culture system based on one developed by [Teather, R.M., Sauer, F.D., 1988. A naturally compartmented rumen simulation system for the continuous culture of rumen bacteria and protozoa. J. Dairy Sci. 71, 666–673]. In which programmable stirring speeds allow a raft mat to form similar to that found in the rumen to produce a *de facto* dual flow system with different liquid and solid turnover rates that helps to maintain the protozoal population. Novel features include a non-clogging outflow, and a computer controlled, compact feeding system able to deliver ground substrates or pellets. The system comprises 12 thermostat controlled fermenter vessels with a working volume of 1 L each. The fermenters were primed with rumen fluid from two cows and continuously provided with the same diet as the donor animals (15 g/day for 3 days then 10 g/day) and artificial saliva (1 L/day). The concentration of short chain fatty acids (SCFA), microbial ribosomal RNA (rRNA) concentrations and total enzymatic activities were all lower in the fermenter than in the donor animals, but the proportions of SCFA remained the same. There was no difference in specific cellulase activity, but specific xylanase activity and total protozoal counts were significantly higher in the fermenters. The proportions of *Bacteria*, and *Eukaryota* (i.e., protozoa and fungi) were similar in the fermenters compared to the donor animal, but the proportion of the *Archaea* (methanogens) was increased.

Abbreviations: aNDF, neutral detergent fibre; CP, crude protein; DM, dry matter; rRNA, ribosomal ribonucleic acid; SCFA, short chain fatty acids.

* Corresponding author at: Ruminant Nutrition & Greenhouse Gas Mitigation, AgResearch Limited, Private Bag 11008, Palmerston North 4442, New Zealand. Tel.: +64 6 351 8320; fax: +64 6 351 8032.
E-mail address: Stefan.Muetzel@agresearch.co.nz (S. Muetzel).

0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2008.11.001

Total rRNA from cellulolytic organisms (*Chytridiomycetes*, *Fibrobacter* sp. *Ruminococcus albus* and *R. flavefaciens*) was similar to that in the donor animals. However, the composition of the cellulolytic community changed with the proportion of *R. albus* and the *Chytridiomycetes* increasing while the proportion of *R. flavefaciens* decreased, and the genus *Fibrobacter* was unchanged. Despite these differences, this *in vitro* system has potential to study effects of feed components on fermentation parameters. Whether the system will prove useful for modelling changes in the composition of the rumen microbial community needs to be evaluated further.

1. Introduction

Numerous attempts have been made to design continuous fermentation systems that mimic rumen fermentation and estimate the amounts of fermentation products that leave the rumen as nutrients for the host animal. Systems range from very simple apparatus with batch feeding (Weller and Pilgrim, 1974; Czerkawski and Breckenridge, 1977; Murphy and Lindgren, 1997) through more complex continuous systems (Slyter et al., 1964; Teather and Sauer, 1988; Fuchigami et al., 1989) to complicated systems where liquid and solid turnover rates can be set independently and end products are removed by filtering or dialysis of the fermenter contents (Slyter et al., 1964; Hoover et al., 1976; Merry et al., 1987; Hino et al., 1993).

One of the main problems with *in vitro* systems is maintaining a protozoal community. In simple homogeneous systems the high dilution rates needed for effective removal of end products leads to washout of protozoa. Therefore, the solid phase needs to have a lower turnover rate than the liquid phase, as it does in the rumen, so that protozoa attached to solid particles remain in the vessel longer. Teather and Sauer (1988) developed a simple and effective system in which different solid and liquid turnover rates were produced by allowing rumen contents to stratify in a rumen-like manner and overflow material was collected from the middle of the fermenter contents. Besides its simplicity, an advantage of the system is that material that leaves the system is 'selected' by gravity. Particles that are thoroughly digested tend to sink and are removed, as they are in the rumen. Hino et al. (1993) developed a similar apparatus and were able to maintain an active protozoal population, but their apparatus was rather complicated. Apart from counting protozoa, relatively little work has been completed to compare microbial populations maintained in these continuous systems.

We describe a modification of the system proposed by Teather and Sauer (1988) with a different outflow and a new feed dispensing system. Fermentation end products, enzymatic activities, microbial community composition at the domain level and four important cell wall degrading microorganisms within the fermenters are compared with corresponding values measured in donor animals.

2. Materials and methods

2.1. Incubation system

The system consists of two linear arrays of 6×2 L closed glass vessels with associated equipment for thermostatting, stirring, and dispensing feed and buffer. The fermentation products are removed through an airlocked overflow into flasks kept at 4 °C in a water-cooled bath to inhibit further fermentation (Fig. 1). Stirring rate and introduction of feed and buffer into each fermenter are controlled via circuits operated by a computer. pH and temperature of the medium in all vessels is recorded and data are logged at 10 min intervals.

Each fermenter consists of a standard double jacket glass jar (HWS Labortechnik, Mainz, Germany) with a customized outlet projecting upwards at an angle of 45° as the overflow. The effective volume of the fermenter is 950 mL. The top is covered by a standard 100 mm diameter glass lid with five inlets arranged in a quincunx; three 26 mm ground cones in line and two standard GL 25 screw