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Evaluation of techniques to detach particle-associated microorganisms from rumen contents

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Abstract

Effects of the most commonly used treatments to detach particle-associated microorganisms from rumen contents were investigated using rumen particles of different sizes. Particles were obtained before feeding from ruminally cannulated sheep. The extent of microorganism dissociation was determined using ^{15}N as an external marker. The first experiment studied the effect of anaerobiosis on efficiency of 1 g/l methylcellulose, pH 8, and chilling (4 °C). Due to poor detachment, the anaerobic procedure was discarded. The following factors, separately or in combination, were then examined in aerobic conditions on two classes of particles obtained from whole ruminal contents (large: >400 μm; small: 100–400 μm) being: (a) stomacher pummelling (5 min); (b) Waring blender homogenisation (3×1 min); (c) chilling (4 °C); (d) pH 8; (e) 1 g/l methylcellulose; (f) 10 ml/l methanol and 10 ml/l tertiary butanol. Samples were incubated for 5 h, except for treatments (a) and (b), and washed after treatment for 2 min under running tap water in a 25 μm gauze. Blending proved to be the most effective treatment (from 50 to 57% removal). Combinations of treatments did not improve detachment. N losses from treated samples were linearly related to ^{15}N removal. The percentage of

Abbreviations: ADS, anaerobic dilution solution; DAPA, diaminopimelic acid; DM, dry matter; FAB, fluid-associated bacteria; LRP, large rumen particles; M, methanol; N, nitrogen; NB, nucleic bases; OM, organic matter; PAB, particle-associated bacteria; SRP, small rumen particles; TB, tertiary butanol; WB, Waring blender; WRC, whole rumen contents

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particles removed was calculated using incubated and/or washed particles as the control, according to treatments. Results suggest that caution is needed when evaluating the effectiveness of treatments, because results are dependent on the type of particles chosen as the control.

Keywords: Rumen bacteria; Detachment; ^{15}N

1. Introduction

Obtaining representative samples of rumen microorganisms is important for accurate calculation of passage of nutrients to the intestine, and to estimate the quantity and composition of microbial amino acids. Rumen microbial populations attach to a variety of substrates, forming adherent multispecies biofilms that become stable and resistant to detachment (McAllister et al., 1994). Bacteria associated with rumen feed particles are usually described as being in one of three sub-populations as: (1) free-living bacteria associated with ruminal fluid; (2) bacteria loosely attached to feed particles; and (3) bacteria firmly adhered to feed particles (Czerkawski and Cheng, 1988; Miron et al., 2001).

Bacterial species that are associated with the rumen epithelium, and those attached to the surface of protozoa or fungal sporangia, have a minor role in rumen digestion (Miron et al., 2001). Loosely and firmly associated bacteria account for 70–80% of microbial matter, and microbial ATP production, in the rumen (Forsberg and Lam, 1977; Craig et al., 1987a; Miron et al., 2001). Adhesion to plant tissues is also pivotal to subsequent substrate degradation, which explains why most enzymatic activities (80% of the endoglucanase activity, 70% of the amylase activity and 75% of the protease activity) are associated with these bacterial sub-populations (McAllister et al., 1994). Microbial fibrolytic activity is also higher in solid-associated microorganisms (Williams and Strachan, 1984; Michalet-Doreau et al., 2001).

Particle-associated bacteria (PAB) differ in chemical composition from those recovered from ruminal fluid (Merry and McAllan, 1983; Olubobokun et al., 1988; Legay-Carmier and Bauchart, 1989; Martin et al., 1996; Volden et al., 1999a,b; Yang et al., 2001). Specifically, diaminopimelic acid (DAPA):N (Olubobokun et al., 1988; Legay-Carmier and Bauchart, 1989) nucleic acid:N or purine:N (Craig et al., 1987b; Poncet et al., 1988; Cecava et al., 1990; Martín-Orúe et al., 1997) and ^{15}N:N (Yang et al., 1989) of particle-associated populations is less than that for fluid-associated populations. Using only the fluid-associated population would lead to an underestimation of the microbial CP and AA flow to the small intestine (Yang and Poncet, 1993).

The techniques that have been proposed to detach microorganisms from rumen feed particles have used various combination of chemical and physical treatments which yield removal rates of 20–80% (Kudo et al., 1987; Legay-Carmier and Bauchart, 1989; Merry and McAllan, 1983; Whitehouse et al., 1994; Ranilla et al., 2001). In most cases, treatments were not separately evaluated and so their real effectiveness is unknown. In addition, results are not easily compared between studies since removal percentages often refer to samples of particles, chosen as a control, not subjected to standard procedures.

In this study, effects of several techniques, separately or in combination, were investigated using rumen particles of different sizes, and controls subjected to the same experimental