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Fibrolysis in rumen mediated by a mixture of exogenous polysaccharidases

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ABSTRACT

There is an increasing interest in the addition of fibrolytic enzymes to the rumen as a way of improving the utilization of the fibrous components of the diet. However, the mechanisms involved in such effects remain uncertain; direct enzymatic hydrolysis has been suggested, but indirect benefits through a modified rumen environment for cellulolytic bacteria have also been reported. In the work reported here, different carbohydrases and their interactions with ruminal cellulolytic activity were evaluated. Pure substrates for standard activity measurement were carboxymethyl-cellulose, birchwood xylans and citrus pectin; forage samples were alfalfa (*Medicago sativa* L.), berseem trefoil (*Trifolium alexandrinum* L.), oat fodder (*Avena sativa* L.) and annual ryegrass (*Lolium multiflorum* L.). Eleven commercial products, covering different polysaccharidase activities, were evaluated as single sources of enzymes and further pooled together and their individual activity assessed. The results indicated that no single enzyme could account for the overall fibre breakdown observed with the complete pool of enzymes. When six enzymes were selected according to their contribution towards fibre hydrolysis in the pool, essentially the same activity was observed as when all 11 were present. We concluded that the greatest effect was obtained when a mixture of cellulase, xylanase and pectinase was added, preferentially dosed according to the relative proportions of different polysaccharides in the substrate. Microscopic observations confirmed the hydrolytic capacity of the enzymes used. The incubation medium was modified with citric acid to obtain pH values of 6.8, 6.4, 6.0 and 5.6. The polysaccharidase activity of commercial enzymes was not affected by pH in a microbial free medium but *in vitro* rumen fluid breakdown was severely depressed as acidity increased. However, when commercial enzymes were added to the rumen fluid, hydrolysis was improved as the medium became more acidic. We concluded that exogenous and microbial enzymes compete for the same substrate.

Abbreviations: CEP, commercial enzyme product; CMcellulase, carboxymethylcellulase; DM, dry matter; M, molar concentration; NDF, neutral detergent fibre; RF, rumen fluid; E, enzyme; VFA, volatile fatty acids.

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1. Introduction

Improving the fibrolytic activity in the rumen by feeding exogenous enzymes has been a goal for nutritionists for many years (Bowden and Church, 1959; Leatherwood et al., 1960). It has been observed that the glycolytic state of fungal enzymes gives them more stability in the rumen (Plou et al., 1999) and this may increase their potential benefits. Various *in vitro* (Feng et al., 1996), *in situ* (Lewis et al., 1996) and *in vivo* (Rode et al., 1999) studies have shown that exogenous polysaccharidases increase fibre breakdown in the rumen; however, the mode of action of these enzymes is still uncertain (Beauchemin et al., 2004). Direct hydrolysis of the polysaccharides would be an unlikely mode of action as the small amount of enzymes added to the diet would be insufficient to explain the increases in the rumen fibrolysis (Dawson and Tricarico, 1999; Morgavi et al., 2000a; Rode et al., 2000). As a result, most of the studies have addressed the hypothesis that there is a positive interaction between exogenous enzymes and rumen microorganisms.

It has been reported that the fibrolytic enzymes improve the attachment of rumen microorganisms to feed particles (Morgavi et al., 2000b), and that their colonization (Newbold, 1997) explains the increase in fibre digestion. It has also been suggested that the fungal xylanases may expose the more protected areas of plant tissue to the enzymatic activity of rumen bacteria (Dawson and Tricarico, 1999). Howes et al. (1998) described changes in the rumen microorganism population and in the final products of the fermentation as a result of the addition of xylanases in the diet. These authors found an increase in the VFA production and in the C2:C3 fatty acid ratio in the rumen. There is also evidence that sterilized extracts of *Aspergillus oryzae* and *Saccharomyces cerevisiae*, rich in xylanases, increase the population of *Selenomonas rumiantium* and the utilization of lactate in the rumen (Martin and Nisbet, 1992), which improve the rumen environment for cellulolytic microorganisms due to the higher pH associated with a lower lactate concentration in the rumen fluid.

The results from studies which use enzymes obtained from a single organism, or with a low diversity of saccharidases, or which use purified substrates rather than native plant sources, should be interpreted with caution as they do not represent the various interactions that occur within the rumen. They may also have limited application in terms of the hydrolytic capacity on more complex substrates, such as the cell walls of forages (Rode et al., 2000). The natural affinity of pure enzymes for their specific substrates may also limit the possibility of explaining the complex enzymatic system of the rumen. Therefore, there is a need to develop new methodologies that allow the assessment of the hydrolytic capacity of groups of enzymes on different forage species.

The aim of the present study was to evaluate the individual and combined effect of various polysaccharidases and their interaction with the rumen microorganisms on fibre digestion. In addition, a new methodology for the evaluation of the hydrolytic capacity of mixed fibrolytic enzymes on native substrates is proposed.

2. Material and methods

2.1. Substrates

Alfalfa (*Medicago sativa* L.) in prebloom state; berseem trefoil (*Trifolium alexandrinum* L.) and oat fodder (*Avena sativa* L.), both in vegetative stage; and annual ryegrass (*Lolium multiflorum* L.) in heading stage were harvested fresh in the field and leaf blades were used as substrates. In order to achieve a final particle size of approximately 2 mm the forage samples were chopped with dry ice in a blender until 80% of the particles were between 1.9 and 2.1 mm as measured with a calibre. Samples were then kept at −20 °C. Prior to incubation, the samples were oven heated at 110° C for 10 min in order to stop any microbial or plant enzymatic activity which might have a confounding effect on the results of the experiment.

2.2. In vitro incubation

The incubations with rumen fluid were conducted following the procedure outlined by Pichard (1977) based on a bicarbonate buffer, reducing agents, macro and micro mineral solutions and continuous CO_2 gassing. When rumen fluid was not used, a mineral phosphate buffer (0.02 M and pH 6.8) was used as incubation media for enzymes, at the same temperature as *in vitro* rumen systems. The enzymatic solutions (5 mg/ml) were added to the substrates 1 h before the incubation started, being allowed to for 30 min at room temperature and 30 min at 39 °C. Rumen fluid was collected from a mature dry cow fed chopped alfalfa hay and ground corn twice daily at maintenance level at a ratio of 70:30. Neutral-detergent fibre (NDF) was determined by the method of Van Soest et al. (1991), assayed without a heat stable amylase, expressed inclusive of residual ash and presented on a DM basis.

2.3. Fibrolytic enzymes and their activity on pure substrates

Eleven commercial enzyme products (CEPs) covering different polysaccharidase activities were used (Table 1). Pure substrates of carboxymethylcellulose (Signa C-4888), birchwood xylans (Signa X-0502) and citrus pectin (Signa P-9135) were used to determine the cellulase, xylanase and pectinase activity of the CEPs, respectively. The substrates were dissolved at 20 g/L in 0.1 M buffered phosphate pH 6.8 and 0.1 M sodium citrate. Aliquots of 100 μl of substrate solution and 50 μl of enzyme solution (5 mg/ml) were added to a tube and incubated in triplicate at 39° C for 30 min to determine cellulase and pectinase activities and for 10 min for xylanase activity. One batch run per enzymatic activity was conducted with a total