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A batch incubation assay to screen plant samples and extracts for their ability to inhibit rumen protein degradation☆

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Abstract

A major objective in nutritional management of high yielding dairy cows is to reduce degradation of dietary protein by rumen microorganisms. The plant kingdom offers a huge diversity of complex compounds that may modify rumen proteolysis. To screen large sample collections and identify plants containing antiproteolytic compounds, rapid and efficient methods are required. Many assays to examine antiproteolytic effects are short term and address direct interferences with protease activities, but isolate the process of proteolysis from overall fermentation. This study aimed to develop a screening system based on a compound substrate, and adequate incubation times, to determine effects on carbohydrate fermentation and microbial growth as well. We describe a simple approach based on batch incubations with rumen fluid to monitor protein degradation over a 12 h incubation. To optimise the system, particular attention was given to the choice of the protein source in the mixed substrate. Casein, bovine serum albumin (BSA), RubisCo and soy protein were assessed with respect to solubility in the buffer medium, a well as detectability of single protein bands on polyacrylamide gels and degradation rates. The ionophore monensin was used as a positive control, due to its known efficacy, and effects were compared to those from *in vitro* and *in vivo* studies to validate results from

Abbreviations: BSA, bovine serum albumine; CP, crude protein; DM, dry matter; ME, metabolisable energy; OM, organic matter; PAGE, polyacrylamide gel electrophoresis; RUP, rumen undegradable protein; SCFA, short chain fatty acids; SFB, soybean flour; TMR, total mixed ration.

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our screening system. The rapid degradation of casein by rumen proteases impeded detection of monensin effects on proteolysis, thereby demonstrating that protein quality crucially impacts efficacy of additives in the assay. Finally, a mixture of soy protein and BSA at a total concentration of 169 g protein/kg was chosen as the standard. Reported effects of monensin on proteolysis and fibre degradation were demonstrated in the assay in a dose dependent manner and 3 μM monensin was chosen for routine application. The system has the advantage of distinguishing between substrate and microbial protein by a centrifugation step and directly monitoring disappearance of substrate protein or single protein bands. Concomitant measurement of gas production, SCFA, and proteolytic end products – ammonia and branched SCFA – provides additional information on overall fermentation as well as on amino acid deamination. The resulting assay was successfully applied for screening in the EC Framework V project, 'Rumen-up' to select antiproteolytic plants.

Keywords: Batch culture; Rumen proteolysis; Secondary compounds; Monensin

1. Introduction

Ruminant nutritionists have long recognized plants for their nutritional value and for their capability to modulate rumen fermentation. Plants produce a huge variety of complex chemicals not essential for their basal metabolism that are called secondary compounds. Their functions are as diverse as their chemical structures, as they serve as pigments, hormones and chemical defense against other plants, as well as exhibiting insecticidal, antimicrobial, and anti-predation activities against parasites, pathogens, and consumers. Many of these substances reduce palability and/or are toxic. However, many secondary compounds have beneficial medicinal or nutritional effects. Compounds with antimicrobial capacities may prove particularly useful to modulate rumen fermentation (Wallace, 2004; Busquet et al., 2006).

A major aim of manipulating ruminal protein degradation is to inhibit it in order to increase the amino acid flow to the small intestine. The protein supply to ruminants is largely microbial protein reaching the small intestine and dietary protein that escapes ruminal microbial degradation provided that the latter can be hydrolysed and absorbed post-ruminally (Klusmeyer et al., 1990; Noftsger and St-Pierre, 2003). However, a substantial proportion of dietary protein is degraded by symbiotic rumen microorganisms before it becomes available to the animal post-ruminally. High yielding dairy cows in particular need a substantial amount of rumen escape protein to meet their protein needs (Santos et al., 1998). Replacement of common protein supplements, such as soy protein, by proteins with high proportions of rumen undegradable protein (RUP) has not led to consistent beneficial results (Kung and Rode, 1996; Santos et al., 1998). Constraints on use of RUP rich proteins include decreased microbial biomass production, inappropriate amino acid profiles and a low digestibility of the RUP in the small intestine (Santos et al., 1998). Thus, instead of replacing high quality protein it may be more beneficial to protect these proteins from extensive degradation.

Monensin is one of a number of ionophores, which were included in ruminant diets to improve digestive efficiency. Their mode of action involves a protein-sparing effect that is due to the selective inhibition of protein fermenting bacteria (Van Nevel and Demeyer, 1977; Newbold et al., 1990; Yang and Russell, 1993; McAllister et al., 1994). Feeding of