AN *IN VITRO* ASSAY OF THE DEGRADATIVE ACTIVITY OF A FIBROLYTIC ENZYME AGAINST VARIOUS FEED SUBSTRATES

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The pre-feeding addition of fibrolytic enzyme products to improve digestibility or intake of ruminant diets is an emerging technology that has shown promising, although variable, production responses. This variability in response has been shown (Beauchemin *et al*. 1995) to be partly due to the specificity of the enzyme products for different feed types. An *in vitro* assay was developed to measure rate and extent of fibrolytic activity against a range of feed types using a *Trichoderma longibractiatum*-derived enzyme complex - Roxazyme® G2 Liquid (RG2; F.Hoffmann-La Roche Ltd, Basel, Switzerland).

The feed substrates used were Pangola grass, lucerne hay, barley grain and sorghum grain (69.2, 34.0, 18.9 and 8.8% neutral detergent fibre (NDF), respectively). In 10 mL tubes, 100 mg samples of dried (65^{o}C), 1 mm ground substrate were incubated for 5 min at 80-85^{o}C in 80% aqueous ethanol before centrifugation (10 min, 3000 rpm) and discarding of the supernatant. The pellet was resuspended in 10 mL ethanol, vortex mixed, centrifuged and the supernatant removed. These preparatory steps (based on the Megazyme Starch Assay) remove existing free reducing sugars and any active microbes.

The tubes containing washed substrates were allowed to equilibrate in an agitating waterbath (39^{o}C) and 1 mL of suitably diluted RG2 (1 mL:250 mL TDI H_2O) was added 15 min before the further addition of 9 mL of 0.1 M sodium acetate buffer (at 39^{o}C and pH 6.5). The incubation then proceeded for 0.5, 1, or 4 h, with gentle mixing every 0.5 h. Substrate and enzyme blanks were also included for each incubation time. Three separate incubation runs were carried out with duplicate samples for each substrate and time of incubation. The release of reducing sugars (RS) from the substrates was measured using the colorimetric Nelson/Somogyi procedure against a glucose standard curve (0 –200 μg) using a spectrophotometer set at 520 nm (Figure 1).

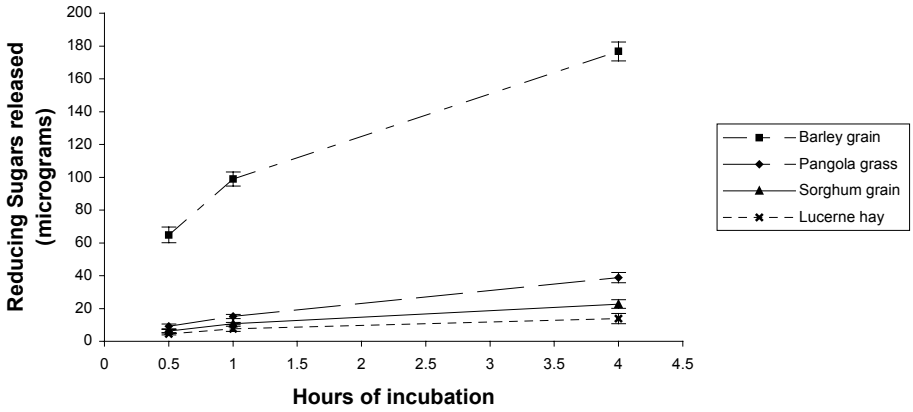

Figure 1. Reducing sugars (μg ± s.e.) released by Roxazyme G2 over a 4-hour incubation (pH 6.5, 39^{o}C).

The results reveal that RG2 showed enzymatic activity against all the substrates tested and enzyme/feed specificity was demonstrated by the significantly different ($P<0.0001$) RS release from the various substrates under the conditions of this assay. Activity was not directly related to NDF content alone and the rate of RS release was not different ($P=0.75$) across substrates. This assay is applicable when investigating enzyme/substrate specificity, however, interactions between the applied enzyme and microbes in the rumen prevent direct extrapolation to *in vivo* feeding situations.

BEAUCHEMIN, K.A., RODE, L.M. and SEWALT, V.J.H. (1995). *Can. J. Anim. Sci.* **75**, 641-644.

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