IN VIVO REGULATION OF GLUTAMINE SYNTHETASE ACTIVITY IN RUMEN BACTERIA FROM TWO EXTREME DIETS

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Ammonia is central to the nutrition of the ruminant animal, since 60-80 % of microbial nitrogen passes through the rumen ammonia "pool". This ammonia is used for the synthesis of microbial protein, which is ultimately available to the animal after degradation of the rumen microflora in the abomasum (Hobson and Wallace 1982). The major enzymes responsible for assimilating ammonia, namely glutamine synthetase (GS:EC 6.3.1.2), glutamate synthase (GOGAT:EC 1.4.1.13) and glutamate dehydrogenase (GDH:EC 1.4.1.2, 3 and 4), have been detected in rumen bacteria, however a comprehensive study of the factors influencing the presence and activities of these enzymes in vivo has not been performed. Some novel findings from such a study are reported.

Two rumen fistulated Merino wethers were housed individually in metabolism pens with unrestricted access to water. In addition to basal diets of chopped wheaten straw fed ad libitum at 0845 and 1645 hrs daily, 2 litres per day of "synthetic saliva" (SS) as a buffer (McDougall 1948) was continuously infused into the rumen of each animal. One animal recieved only the SS infusate, resulting in a low nitrogen/low energy diet, whereas the daily infusate for the second animal was supplemented with a suspension of mixed carbohydrates, equivalent to 200 g starch, and 1 mole NH_4Cl (high nitrogen/high energy). At 1145 hrs, 300 ml of SS plus 0.5 moles NH_4Cl was added to the rumen of each animal through the fistula, and rumen fluid samples were taken at 0, 30, 60, 120 and 240 min. "post-pulse". GS γ-glutamyl transferase (γ-GT) activities (Patterson and Hespell 1985) were assayed in cell-free extracts prepared from the bacterial portion of the rumen samples.

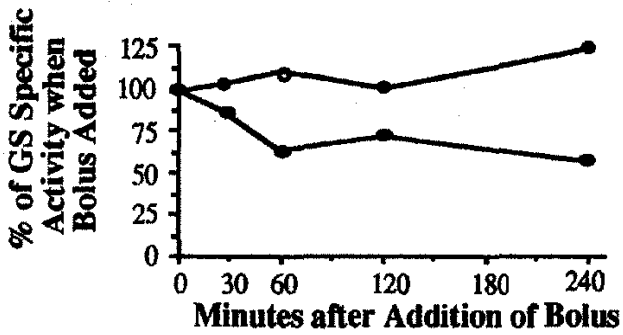

Figure 1 : Effect of a bolus of SS only (o) or SS plus 0.5 moles NH_4Cl (•) on GS γ-GT activity in bacteria from the rumen of an animal on a low nitrogen/low energy diet.

The 0.5 moles NH_4Cl bolus resulted in a large increase in rumen ammonia concentration in both diets (280-fold in the low/low diet, and 7 to 10-fold in the high/high diet). Fig. 1 shows that this bolus caused a concomitant decrease in GS activity in the low/low diet, compared to a control bolus of SS only, which had no effect on GS activity or rumen ammonia concentration. A bolus of 0.005 moles NH_4Cl added to the low/low diet resulted in a six-fold increase in rumen ammonia concentration, and a 17 % decrease in GS activity, 30 min. "post-pulse" in the low/low diet (not shown). Thus, GS activity decreased in a dose dependent manner, suggesting rapid regulation of activity in response to ammonia. No effects were observed on GS activity for the 0.5 moles NH_4Cl bolus in the animal fed the high/high diet and the 0.005 moles bolus was not administered to this animal. Normal GS activities in bacteria from this diet were five-fold lower than in the low/low diet, whereas the ammonia concentration was 14-fold higher (4.15 cf. 0.294 mM for the low/low diet). GOGAT and GDH activities were not affected by any of the bolus additions in either animal.

It is concluded that ruminal GS activity is sensitive to rapid increases in ammonia concentration, but only when GS activity in the bacteria is high, usually as a consequence of very low ammonia levels. The other major assimilatory enzymes did not appear to be sensitive to increases in ammonia and/or glucose levels in the rumen.

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