0273-2289/91/3001—0009$02.00

Effect of the Addition of D-Xylose on Xylanase Activity and Digestibility of Fiber in an Artificial Rumen

RICHARD E. BRICE[1] AND IAN M. MORRISON*[,2]

The Hannah Research Institute, Ayr KA6 5HL, UK
[1]Present Address: Malaysian Rubber Producers Research Association, Tun Abdul Razak Lab., Brickendonbury, Herts SG13 8NL, UK and [2]Present Address: The Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Received December 18, 1989; Accepted April 23, 1990

ABSTRACT

The effect of adding D-xylose at two different levels as a continuous infusion to an artificial rumen was determined with respect to pH, methane production, volatile fatty acid production, fiber digestion, and xylanase activity. No changes in pH, methane production, or fiber digestion were observed as consequences of increased D-xylose concentrations. Although the propionic acid production was slightly raised on addition of D-xylose, the acetic acid production was lowered to the same extent. A low-xylose infusion increased the xylanase activity by 25%, whereas a high-xylose infusion lowered the activity by 17%. It was concluded that D-xylanase activity was not a rate-determining step in fiber degradation in the rumen.

Index Entries: Xylanase; D-xylose; fiber; digestibility; methane; volatile fatty acids.

INTRODUCTION

The plant cell wall, or what is frequently called "fiber" is an important source of energy to the ruminant (*1*). Although the carbohydrates of the cell contents pool of the grasses are considered to be fully utilized, the

*Author to whom all correspondence and reprint requests should be addressed.

carbohydrates in the cell wall, particularly cellulose and the hemicelluloses, are only partially utilized because of protective effects and inhibitory actions of the noncarbohydrate components of the wall such as lignin (*2*).

The cell wall carbohydrates are not hydrolyzed by endogenous animal enzymes but by the enzymic complement of the complex microflora present in the rumen. As the rumen is a virtually anaerobic environment, it is only recently that methods have been established to show that the appropriate cell wall-degrading enzymes are present. Therefore, little is known about the mechanisms involved in regulating the production and activity of polysaccharide depolymerases in mixed populations of rumen microorganisms. The artificial rumen technique (Rusitec) developed by Czerkawski and Breckenridge (*3*) has been adopted for the long-term experiments necessary to investigate these processes since it has been shown to be stable over long periods, is relatively simple to operate, and can readily provide material for analysis from both the liquid and solid phases of the system.

The hemicelluloses are far more complex polysaccharides than cellulose (*4*) in that there are a number of discrete types of polymer in the hemicellulosic fraction from a single plant and the different polymers are composed of a number of individual sugar residues. In grasses, xylans are by far the major components at all stages of growth and D-xylose itself is by far the major sugar residue present in the hemicellulosic fraction (*5*). Significant amounts of L-arabinofuranoside residues are also present as side chains. In addition, acetate groups are found in the native hemicelluloses and are reported to be ester-linked to some of the main chain D-xylose residues (*6*), whereas phenolic acid residues are also found but these are ester-linked at C-5 of some of the L-arabinose residues (*7*).

The major hydrolysis product of a xylan is D-xylose. In many enzymatic systems, the products of hydrolysis are inhibitors of the enzyme. Although xylanase (hemicellulase) activity has been shown to be present in cell-free rumen liquor (*8*), it is now known that fiber breakdown in the rumen occurs by the action of organisms in close contact with the fibers themselves. The enzyme preparations in these experiments were therefore obtained by washing them from the partially digested fiber contained in the bags that were recovered from Rusitec. It is recognized that α-L-arabinofuranosidase activity could be responsible, at least in part, for the reducing sugar produced in the enzyme assay used. However, other investigations using the artificial rumen (Brice, Morrison, and Kimberley, unpublished results) have shown that the activity of α-L-arabinofuranosidase in these fractions is very much lower than that of the β-(1→4)-D-xylanase.

In view of the large amounts of D-xylose that could be available from the hydrolysis of plant fibers, it was decided to investigate the potential of D-xylose as a rumen additive. In particular, the influence on xylanase activity and overall fiber digestion was investigated. It has been known for some time that the addition of D-glucose, either as the free sugar or in

a bound but easily degradable form (starch), initially inhibits cellulolysis in the rumen (e.g., *9*). It is only when the readily degradable D-glucose has been metabolized that cellulolysis rates are restored.

MATERIALS AND METHODS

General Procedures

The gas composition was determined by the method of Czerkawski and Clapperton (*10*). The concentrations of volatile fatty acids (VFA) obtained on fermentation were determined by the method of Cottyn and Boucque (*11*) except that 3,3-dimethylbutanoic acid (*t*-butylacetic acid) was used as the internal standard. The dry matter (DM) content of the food and digested residues was obtained by washing the food and digested residues with water at 37°C, freezing in liquid nitrogen, and lyophilizing. The residues were finally dried at 105°C for 3 h and weighed. The xylanase activity was determined as follows: To the cell-free rumen fluid (1.0 mL: clarified by centrifugation at 2,000×g) was added the hemicellulose solution (0.1%: 1.0 mL), distilled water (1.0 mL), and dithiothreitol solution (0.01%: 1.0 mL). The incubation was carried out at 37°C for 18 h. The xylanase activity was calculated as the amount of reducing sugar [μg D-xylose: determined according to Lever (*12*)] produced per mL of enzyme solution per h. The hemicellulose was prepared in the laboratory by delignifying (*13*) a sample of perennial ryegrass and extracting the hemicelluloses with 1.0*M* sodium hydroxide. The hemicellulose solution was taken to pH 4 with acetic acid, filtered and precipitated with 4 vol of ethanol. The hemicellulose was dried by solvent exchange through ethanol, acetone, and diethyl ether. It contained residues of D-xylose (82%), L-arabinose (8%), D-galactose (3%), D-glucose (3%), and uronic acid (4%).

Rusitec Procedure

The apparatus used was the artificial rumen technique essentially as described by Czerkawski and Breckenridge (*3*). The inoculum of rumen liquor was obtained from sheep that had been fed a diet of medium-poor quality ryegrass hay similar to that being used in the experiment. The experiment consisted of continuously infusing three vessels of the Rusitec system with a soluble food that consisted of soluble starch (0.05% w/v), cellobiose (0.05%), and D-glucose (0.05%) as readily digestible carbohydrates, along with casein (0.1%) and casein hydrolysate (0.1%) as nitrogenous sources and minerals that were dissolved in artificial saliva (*14*). On the first day of the experiment, each vessel was charged with filtered rumen fluid (400 mL) and artificial saliva (400 mL). Each vessel also received one bag of rumen solids (25g) to supply a charge of the organisms in close contact with the fiber matrix and 2 bags of perennial ryegrass hay (3g:

D-value = 66), which had been chopped to ca. 2 cm length. After 24 h, the bag with the rumen solids was removed and replaced with another bag of hay. The hay used in the experiment had been extensively washed with water to remove any free D-glucose, D-fructose, sucrose, and fructosans and the water-extracted residue had been lyophilized. Apart from 24 and 48 h, the bag which had been present for 72 h was removed each day and replaced with a fresh bag.

When the bags of 72-h digested hay were removed from Rusitec, the bulk of the liquid phase [compartment 1 as described by Czerkawski (*15*)] was gently squeezed back into the vessel. Each bag was then placed in a small polythene bag and artificial saliva (50 mL: pH 6.9) was added. The bags were squeezed vigorously a number of times to dissociate the adhering bacteria and associated liquid phase and the solution collected. The washing was repeated once and a sample (10 mL) of the combined wash was taken, with the remainder being returned to the Rusitec vessel. The sample was centrifuged at 2,000 × g for 45 min at 4°C on an MSE Superspeed II centrifuge. The clear supernatant solution was removed and used for the assay of xylanase activity as described earlier. The first 9 d of the experiment were used to stabilize the three vessels. The first vessel was then maintained with the same infusion. The D-glucose component of the infusion into vessel 2 was replaced with a D-glucose/D-xylose mixture with each sugar representing 0.025% of the infusion. In vessel 3, the D-glucose was totally replaced with D-xylose at a concentration of 0.05% of the infusion. The infusion into vessel 2 was termed the "low xylose" food and that in vessel 3 was the "high xylose" food. These conditions were continued for a further 7 d with the xylanase activity in the digesta being determined each day.

RESULTS AND DISCUSSION

The methane production was about 150 mL per day. When D-xylose was added, a slight reduction (to about 120 mL per day) was observed in both the low- and high-xylose vessels but after 2 d the amounts produced in the two experimental vessels had returned to levels very similar to that in the control (Fig. 1). Although D-xylose is the major sugar residue present in the non-cellulosic portion of the fiber and, as such, contributes to the energy pool of the rumen, there is no information on the concentration of the free sugar in rumen fluid. The reduction in methane production may, therefore, be a result of a short time lag being required for some of the microbial population to become adapted to the new carbohydrate source. The VFA patterns also show that the proportion and amounts of acetic, propionic, and butyric acids were very similar prior to the change in infusion (Fig. 2). The steady state values for acetic, propionic, and butyric acids were about 17.5, 7.0, and 3.0 mmol per L. Thereafter, the propor-

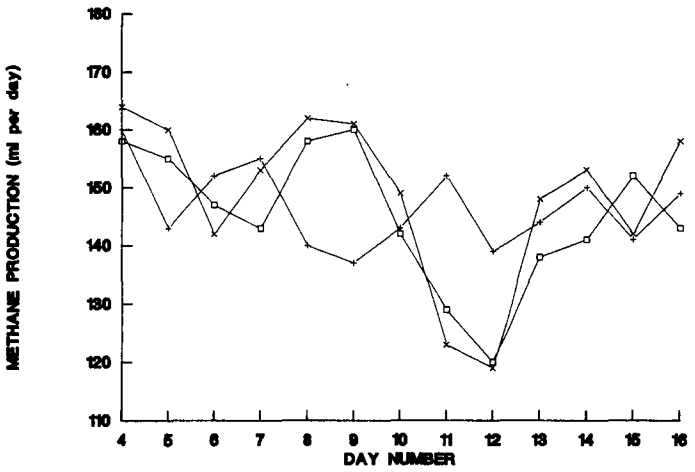

Fig. 1. Methane production from the three Rusitec vessels. +, Control; □, Low xylose; ×, High xylose.

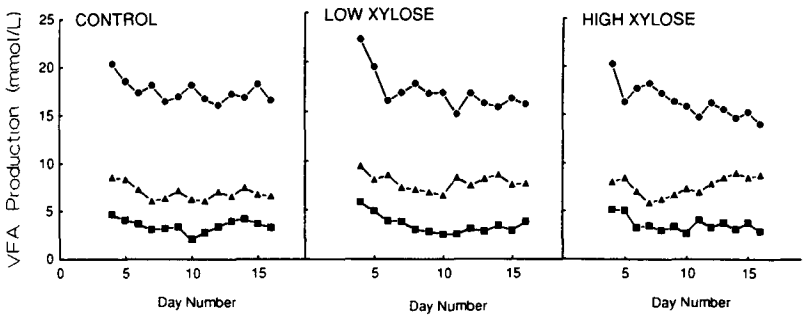

Fig. 2. Volatile fatty acid production from the three Rusitec vessels. ●, Acetate; ▲, Propionate; ■, Butyrate.

tion of propionic acid was increased slightly as a result of the inclusion of D-xylose in the diet mainly at the expense of acetic acid. For the "low xylose" and "high xylose" foods, respectively, the propionic acid concentrations rose to 8.0 and 8.5 mmol per L with the acetic acid concentrations falling to 16.0 and 15.0 mmol per L. The increase was greater with the higher level of xylose used. The concentrations of the minor acids, 2-methylpropionic (isobutyric), valeric and 2-methylbutyric (isovaleric), were very similar throughout the experiment and too low to observe any significant differences owed to the different carbohydrate sources used throughout the experiment. The pH in all vessels varied between 6.60 and 6.85 but there was no significant difference between vessels either before or after the administration of D-xylose.

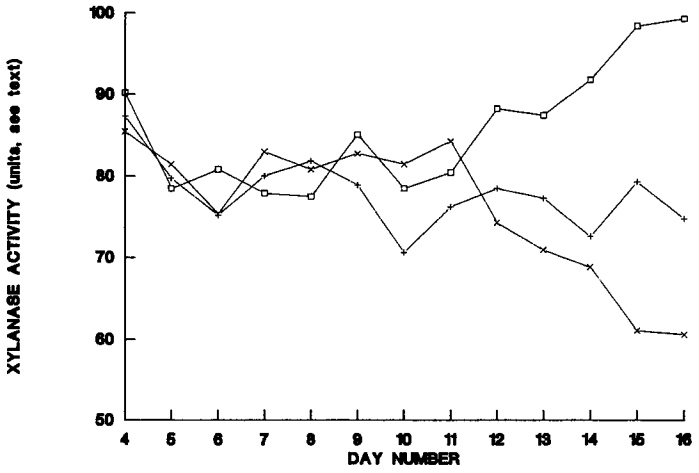

Fig. 3. Xylanase activity in the three Rusitec vessels. For definition of units, *see* the text. +, Control; □, Low xylose; ×, High xylose.

Fig. 3 shows the xylanase activity extracted from the solid digesta from the three vessels. When D-glucose was infused into each vessel, there was a mean xylanase activity of 80 U. After the change-over, the control vessel remained fairly constant with a mean value of 77 U. The vessel with the "low xylose" infusion gave an increase in activity of about 25% up to 97 U at the end of the experiment, whereas the activity in the vessel with the "high xylose" fell to 64 U, a decline of 17%. The mean values for the digestibility of the hay, prior to addition of D-xylose, were 61.3 (±2.2), 62.4 (±2.1), and 62.3 (±1.4). After addition of D-xylose, the mean values were 62.5 (±2.0), 61.4 (±1.8), and 60.8 (±2.0) for control, low-xylose, and high-xylose, respectively. During the first 9 d, there was no significant differences between the three vessels, as would be expected. However there were also no significant differences between the vessels during the second stage of the experiment when the total concentration of carbohydrate in the infusion remained the same but the proportion of xylose in the infusion was increased.

These results indicate that, at a concentration of 0.025%, the presence of free D-xylose in the infusion medium can significantly increase the xylanase activity in the rumen liquor, which is in intimate contact with the fiber matrix [compartment 2 as described by Czerkawski (*15*)], whereas higher concentrations ($\geq 0.05\%$) of D-xylose will significantly reduce the xylanase activity. However, these changes in xylanase activity did not affect the digestibility of the chopped hay used as substrate in the artificial rumen. These results indicate that the level of xylanase activity in the rumen is not a limiting factor in the digestion of plant cell wall material.

In the intact cell wall, a high proportion of the D-xylose residues in xylans are esterified at C-3 with acetyl groups. It has recently been shown that acetyl esterases are found in many cell wall-degrading systems and Biely et al. (*16*) have demonstrated the cooperativity of acetyl esterase and xylanase in maximizing the degradation of acetyl xylan. A ferulic acid esterase has also been reported although it did not appear to be able to attack polymeric substrates (*17*). Esterase activity was detectable in the enzyme preparations reported in this experiment, but it was not possible to determine if it was acetyl xylan esterase or other types of esterase present in the rumen liquor. It is possible that similar noncarbohydrase activities are involved in fiber degradation in the rumen and may be the rate-determining steps.

REFERENCES

1. Morrison, I. M. (1979), *Proc. Nutr. Soc.* **38,** 269–274.
2. Morrison, I. M. (1988), *J. Sci. Food Agric.* **42,** 295–304.
3. Czerkawski, J. W. and Breckenridge, G. (1977), *Br. J. Nutr.* **38,** 371–384.
4. Wilkie, K. C. B. (1979), *Advances in Carbohydrate Chemistry and Biochemistry,* Wolfrom, M. L., Tipson, R. S., and Horton, D. **36,** 215–264.
5. Bacon, J. S. D., Gordon, A. H., Morris, E. J., and Farmer, V. C. (1975), *Biochem J.* **149,** 485–487.
6. Mueller-Harvey, I., Hartley, R. D., Harris, P. J., and Curzon, E. H. (1986), *Carbohydr. Res.* **148,** 71–85.
7. Morrison, I. M. (1980), *Grass Forage Sci.* **35,** 287–293.
8. Morrison, I. M. (1976), *Carbohydr. Res.* **47,** 129–139.
9. Mould, F. L., Orskov, E. R., and Mann, S. O. (1983), *Anim. Feed Sci. Technol.* **10,** 15–30.
10. Czerkawski, J. W. and Clapperton, J. L. (1968), *Lab. Pract.* **17,** 994–996.
11. Cottyn, R. G. and Boucque, C. F. (1968), *J. Agric. Food. Chem.* **16,** 105–107.
12. Lever, C. M. (1972), *Analyt. Biochem.* **47,** 273–279.
13. Wise, L. E., Murphy, M., and D'Addieco, A. A. (1946), *Paper Trade J.* **122,** 35–43.
14. McDougal, E. I. (1948), *Biochem. J.* **43,** 99–109.
15. Czerkawski, J. W. (1979), *Hannah Res. Inst. Ann. Rep.*, HRI, Ayr, UK, pp. 69–85.
16. Biely, P., MacKenzie, C. R., Puls, J., and Schneider, H. (1986), *Biotechnol.* **4,** 731–733.
17. MacKenzie, C. R. and Bilous, D. (1988), *Appl. Environ. Microbiol.* **54,** 1170–1173.