INHIBITION OF RUMEN CELLULASE BY AN EXTRACT FROM SERICEA FORAGE

The nutritive qualities of sericea (*Lespedeza cuneata*) forage for dairy animals are generally considered to be inferior to those of other crops, such as alfalfa (5). Although low intake and inferior quality of sericea forage have been associated with the high tannin and lignin content, chemical tests for these constituents are not entirely satisfactory as a means of predicting the value of the forage.

Recently it has been shown (1) that Muscadine grape leaves contain a water-soluble substance which inhibits the enzymatic hydrolysis of soluble cellulose. The substance was stable to heat, to weak acid or alkali, and was nondialyzable through cellophane against water. The cellulase inhibitor of grape leaves has been suggested to be a tannin-like substance, and such materials have been associated with the inhibition of certain hydrolytic enzyme systems such as pectinase (4). In view of the high tannin content of sericea, it was decided to test a water-soluble extract of this plant for a possible inhibitory effect on rumen cellulase activity.

EXPERIMENTAL PROCEDURE

Leaves were collected in October, 1960, from mature sericea (seed stage) grown at the Central Crops Research Station of North Carolina State College, Clayton, North Carolina. An extract was prepared by blending 15 g. of fresh sericea leaves with 300 ml. of distilled water for 10 min. The slurry was pressed through two thicknesses of cheese cloth and the aqueous extract was centrifuged for 15 min. at 3,000 r.p.m. The clear, straw-colored portion was removed and either tested immediately for effect on cellulase activity or frozen for later testing. Rumen fluid obtained from a fistulated bovine steer of the North Carolina State College herd was used as the source of cellulase. The rumen fluid was filtered through cheese cloth, centrifuged at a speed of 3,000 r.p.m., and mixed with distilled water to make a 20% solution. The prepared fluid was either used immediately or kept frozen until needed.

The viscometric method described by Bell *et al.* (1, 2) was used. Enzyme activity was expressed in cellulase units: 100 units equal 50% loss in viscosity of a 1.0% solution of sodium carboxymethylcellulose (CMC-7M, Hercules Powder Company, Wilmington, Delaware) buffered at pH 5.5, and held at 30.0° C. for 20 hr. Standard enzyme-reaction curves were used to convert percentage loss in viscosity to units of activity. Four milliliters of the 20% rumen fluid were mixed with 2 ml. of water extract of sericea. Distilled water was used in place of the inhibitor and/or enzyme solutions for the controls. The optimum pH for rumen cellulase was found to be slightly above 5.5, with 1% CMC at 30.0° C.

Fresh leaves of fescue (*Festuca arundinacea*), ryegrass (*Lolium multiflorum*), alfalfa (*Medicago sativa*), and soybean (*Glycine max*) were also extracted and tested as described for sericea.

RESULTS AND DISCUSSION

Sericea extracts had an inhibitory effect on rumen cellulase activity, as shown in Table 1,

TABLE 1
Effect of sericea extract and heat on 20% rumen cellulase

	Cellulase units [a]	Cellulase inactivation
		(%)
Rumen fluid	255	
Rumen fluid, heated	3	98
Rumen fluid plus sericea extract	3	98

[a] Each value is the mean of two replications.

whereas the other forages tested had no marked effect. Effects of various dilutions of the sericea extract were studied and are presented in Table 2.

TABLE 2
Effect of increasing levels of sericea extract on rumen cellulase activity [a]

Sericea extract	Cellulase inactivation
(%)	
5.00	96
1.00	87
0.50	75
0.10	42
0.05	30
0.001	18

[a] Each value is the mean of two replications.

These in vitro observations show that sericea water extracts contain a compound or compounds which will inhibit hydrolysis of carboxymethylcellulose. This compound has not been isolated or identified, but a color test for polyphenols and the suppression of the inhibition by polyphenol oxidase from the mushroom indicate that it is polyphenolic in nature. It is nondialyzable through a cellophane membrane

against water and is unstable in alkaline solutions. The effects of this compound (or compounds) in in vivo digestion have not been determined, but it has been suggested by Gill and King (3) that there may be rumen cellulase inhibitors and that information on their nature, mode of action, and origin might lead to improved digestive efficiency of ruminants. This will be tested in vivo with sericea breeding lines which have been shown to contain varying concentrations of inhibitor.

SUMMARY

(1) A water-soluble substance or substances in the leaves of sericea lespedeza, which inhibits the enzymatic hydrolysis of soluble cellulose, is reported. The reduction in cellulase activity obtained was proportional to the concentration of the inhibitor present.

(2) Extracts of fescue, ryegrass, alfalfa, and soybean forages were not inhibitory to rumen cellulase.

W. W. G. SMART, JR.
Animal Nutrition Section
North Carolina State College

T. A. BELL
Food Fermentation Laboratory
ARS, USDA
North Carolina State College

NANCY W. STANLEY
Animal Nutrition Section
North Carolina State College

AND

W. A. COPE
Crops Research Division
ARS, USDA
Raleigh, North Carolina

REFERENCES

(1) BELL, T. A., AURAND, L. W., AND ETCHELLS, J. L. Cellulase Inhibitor in Grape Leaves. Botan. Gaz., 122: 143. 1960.

(2) BELL, T. A., ETCHELLS, J. L., AND JONES, I. D. A Method for Testing Cucumber Salt-Stock Brine for Softening Activity. Publ. USDA, ARS-72-5. 1960.

(3) GILL, JAMES W., AND KING, KENDALL W. Characteristics of Free Rumen Cellulases. Agr. and Food Chem., 5: 363. 1957.

(4) HATHWAY, D. E., AND SEAKINS, J. W. T. The Influence of Tannins on the Degradation of Pectin. Biochem. J., 70: 158. 1958.

(5) HAWKINS, G. E. Nutritive Qualities of Sericea Forage. Bull. 318, Agr. Expt. Sta., Alabama Polytechnic Institute. 1959.