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PECTIC ENZYMES OF RUMEN FLUID[1]

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SUMMARY

A glycosidic pectic enzyme was found in a number of rumen fluid samples and was characterized as γ-pectinglycosidase. It was exocellular and adaptive to the addition of pectic substances, with a high activity in the pH range of 7.0 to 9.5 (optimum 8.5). The rumen fluid was free of pectinesterase as determined at pH values of 4.5, 6.0, and 7.5. When a basal ration of four fistulated steers was supplemented with citrus pulp there was a tenfold increase in activity of γ-PG activity attributed to citrus pulp. There was no difference between the levels of citrus pulp used.

Pectic substances of forage crops and of pulp supplements used in ruminant feeding have taken on an added importance as a result of these substances being associated with the bloat syndrome (4, 13, 14). However, these reports are not conclusive and the problem of bloat appears to be more complicated than the levels of pectic substances found in the different forages (9). Forage crops do have an appreciable amount of pectic substances as a part of their total carbohydrate fraction. In a recent report (9), pectic substances in different forage crops ranged from as low as 1.5% of the dry matter in bromegrass to 7.9% in Ladino clover. Pectic substances in five varieties of alfalfa ranged from 4.7 to 6.7%.

Citrus and beet pulps, which are high in pectin, are also used extensively as supplemental feeds for dairy cattle and the production of citrus pulp alone was given in 1962 (3) as 350,000 T per year. In 1954 (7), the annual processing of citrus fruit wastes in the United States had reached two million tons, and thus indicated that more citrus pulp could be made available for feed use. It was estimated in this report that 40,000 T of pectin would be obtained from the annual production of citrus waste. Of the 22.02% total solids in citrus waste, 17.85% is pectin (7).

Pectin-digesting enzymes in rumen fluid have not been studied, although recently Wright (13) investigated rumen protozoa and, using washed suspensions, determined the presence of polygalacturonase and pectinesterase. In nonruminant animals such as dog and man, pectic enzymes have not been demonstrated in secretions taken from the digestive tract (5). Saliva and gastric juices were also free of pectin-digesting enzymes. Pectin passed through the stomach and part of the small intestine without loss, but was degraded in the large intestine, apparently by bacterial action (5).

Two general types of enzymes are involved in the breakdown of pectic substances: pectinesterase (PE), (syn. pectase, pectin methyl esterase), which catalyzes the hydrolysis of the ester bonds to yield methanol and pectic acid; and pectinglycosidases (syn. polygalacturonase, pectinase), which catalyze the hydrolysis of α-1,4-glycosidic linkages of pectic substances (6). At least three pectinglycosidases have been described (11). The enzyme most extensively studied and manufactured commercially is from higher fungi and, commonly called polygalacturonase, reacts only with de-esterified galacturonide residues of pectic substances and at pH optimum 4-5. The second enzyme, sometimes called depolymerase, is from bacterial source and reacts at pH 7 or above on pectic acid. The third enzyme is from *Neurospora* sp. (10) and reacts only with galacturonide residues of high methoxyl pectin and at pH 5.5. In this report, the nomenclature for the pectinglycosidases proposed by Smith (11) will be followed: β-pectinglycosidase (β-PG) for the fungal-type enzyme with pH optimum 4-5; α-pectinglycosidase (α-PG) for the Neurospora-type enzyme; and γ-pectinglycosidase (γ-PG) for the bacterial-type with pH optimum above 7.

This investigation was undertaken to test for the presence or absence of pectic enzymes in

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rumen fluid and then to subject the animal to increased levels of pectic substances by the addition of citrus pulp to the diet and follow the enzyme activity of the rumen fluid.

EXPERIMENTAL PROCEDURES

Citrus pulp feeding. Four rumen-fistulated Holstein steers were used in a trial of 4 weeks' duration. During the preliminary period all four received a basal diet of Coastal Bermuda grass hay plus a concentrate composed of 39.2% ground corn, 39.2% oats, 19.6% soybean oil meal, 1.0% salt, and 1.0% dicalcium phosphate. Two levels of citrus pulp [2] replaced a portion of the hay during the 3-wk experimental period. The feeding periods and amounts fed twice daily are outlined in Table 1.

TABLE 1

Diets fed

Citrus pulp level	Kind of feed	Period		
		Preliminary	Comparison	Terminal
		(lb)		
Low	Concentrate	2.0	1.0	2.0
	Hay	10.0	8.5	10.0
	Citrus pulp	0.0	2.0	0.0
High	Concentrate	2.0	1.0	2.0
	Hay	10.0	5.5	10.0
	Citrus pulp	0.0	4.0	0.0

Preparation of rumen fluid. Rumen fluid was collected 4 hr after the morning feeding. Collections were made eight times during the feeding trial. The ingesta were strained through gauze, centrifuged at 1,085 × *g* for 15 min, and stored in a refrigerator at 4 C, using ten drops of toluene per 100 ml of fluid as a preservative.

Measuring pectic enzyme activity. Pectinesterase (PE) was assayed essentially as described by Bell et al. (1). It was based on measurement of the rate of liberation of carboxyl groups as determined with standard sodium hydroxide at 30 C, and with 0.15 M sodium chloride. Determinations were made at three pH levels: 7.5 (usually optimum for plant PE), 6.0, and 4.5 (range for bacterial and fungal PE). Pectinglycosidase activity was measured by a viscometric method (2), using sodium polypectate as a substrate. Buffers used were citrate for below pH 7 and borate for above pH 7. The fungal enzyme with optimum activity at pH 4 to 5 is designated as β-PG and the enzyme activity with optimum above pH 7 is γ-PG. Standard enzyme reaction curves were used to convert percentage loss in viscosity to units of activity. Pectinglycosidase units were established which gave a value of 100 units to equal 50% loss in viscosity in 20 hr of an enzyme-substrate mixture at 30 C.

[2] Citrus pulp was Golden Isle, a Minute Maid product.

RESULTS AND DISCUSSION

Pectinglycosidase activity. To determine the type of pectic digesting enzymes in the rumen fluid, it was necessary to buffer the enzyme-substrate at a range from pH 5 to 8.5 and to compare the rumen enzyme action with enzymes of known pH characteristics. Fungal polygalacturonase (β-PG) of commercial source (46 AP) and bacterial macerans enzyme (γ-PG) from USDA source,[3] were used for comparison. As expected, the β-PG enzyme gave optimum activity at pH 4.9 and the γ-PG at pH 8.5. The rumen-fluid pectic enzyme responded more like the γ-PG enzyme, and gave little or no activity at pH 5 (Table 2). To establish the optimum pH value for use in further experiments, a series of rumen fluid samples taken on different days and at different times after feeding were analyzed for PG activity in a range of pH values from 4.9 to 10. As shown in Figure 1, and using an average of the observations, the pH optimum was 8.4. At pH 7.0 and 9.5, the relative activity was about 50%, and at pH 6 and 10, the activity was less than 20% of the optimum.

TABLE 2

Relative activity of fungal, bacterial, and rumen enzymes at three pH levels

pH of enzyme substrate mixture (30 C)	Relative polygalacturonase activity		
	Fungal (46 AP)	Bacterial (macerans)	Rumen 20% extract
	(units/milliliter)		
5.0	174	10	8
6.5	51	148	169
8.5	3	2,130	550

Decreasing rumen fluid concentrations, (20, 10, 5, 1, 0.5, and 0%) gave decreasing γ-PG activity as a straight-line function (Table 3). A heat-inactivated 20% rumen sample used as control showed no γ-PG activity.

[3] Supplied by Dr. E. F. Jansen, Western Utilization Research and Development Division, USDA, Albany, California.

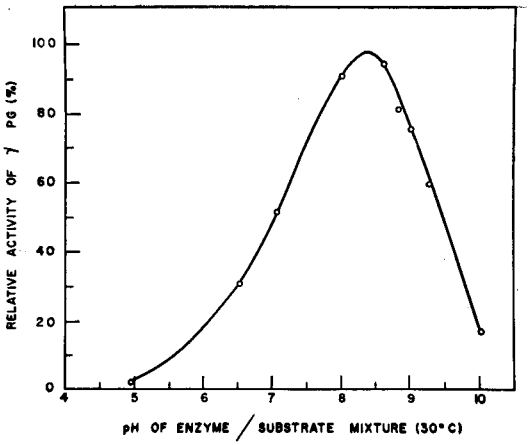

FIG. 1. Curve of pH and relative activity of γ-polygalacturonase activity.

TABLE 3

Decreasing concentrations of rumen fluid as related to PG activity

Rumen fluid concentration	γ-PG activity		
	Rep. 1	Rep. 2	Avg
(%)	(units)		
20	870	905	887
10	315	315	315
5	172	164	168
1	37	44	40
0.5	16	19	18
0	2	....	2
20[a]	0	0	0

[a] Heat-inactivated.

Pectinesterase activity. Rumen fluid samples, taken on different days and at different time intervals after feeding, were shown to be negative for pectinesterase. Activity was determined at three pH levels, 7.5, which is the optimum for plants, and at 4.5 and 6.0, which is the range of fungal and bacterial PE enzyme activity. Since pectic substances are deesterified to pectinic acids at pH 8 and above, exceeding this limit is unnecessary. Also, γ-PG will react with pectins and pectinic acids, so the rumen fluid would not require PE enzyme activity. On the other hand, the fungal enzyme, β-PG, operative at pH 4-5, will split only demethylated uronide bonds.

Influence of pectic substances (citrus pulp supplement) on γ-PG activity. The γ-PG activity of the fluid from the four steers prior to supplementation of citrus pulp averaged 220 and 217 units/milliliter at the initial and six-day period respectively (Figure 2). After one day of supplementation, the enzyme activity

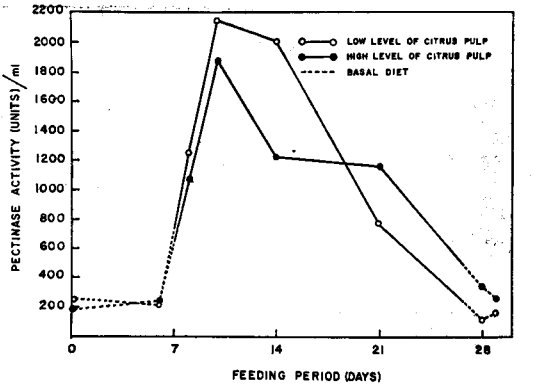

FIG. 2. Response of pectinase activity to a citrus pulp feeding.

increased to an average of 1,205 units/milliliter for the low level and 1,145 for the high level of supplementation. The peaks of the enzyme activity were reached after three days on the supplement and remained high until the steers were returned to the basal diet. After one and two days on the basal diet, the γ-PG activity of rumen fluid of all steers returned to the presupplement level of about 200 units/milliliter. There was no significant difference between the low and high level of citrus pulp. Both levels reached about the same maximum activity of 2,000 units/milliliter and returned to about 200 units on the basal diet. This tenfold increase in activity of γ-PG activity attributed to citrus pulp suggests that the formation of extracellular γ-PG enzyme was induced by the increase in total pectic substances in the diet. After 14 days of citrus pulp supplementation, the γ-PG enzyme activity decreased, but it did not approach presupplementation levels. The activity remained at least three times higher than either pre- or post-supplementation level. This study lends little support to the idea that pectin per se is involved in bloat. The authors are unaware of any reports linking high pectin concentrates (e.g., citrus pulp, beet pulp, apple pomace) to the etiology of bloat.

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