Phytate Phosphorus Hydrolysis and Availability to Rumen Microorganisms

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Calcium phytate was found to be as available to rumen microorganisms as highly available inorganic phosphorus when tested by an artificial rumen technique involving cellulose digestion. Washed suspensions of rumen microorganisms were able to hydrolyze appreciable amounts of calcium phytate as measured by the presence of inorganic phosphorus following incubation. The optimum pH for phytase activity was found to be approximately 5.5. Additional factors influencing phytase production or activity were concentration of microorganisms in the medium, time of incubation, and substrate concentration.

ORGANICALLY BOUND PHOSPHORUS OR PHYTIC ACID is present in substantial quantities in a wide variety of plant feedstuffs consumed by farm animals. The nutrient availability of this form of phosphorus is poor in nonruminants such as swine and poultry (*3, 6*) but appears to be somewhat better in ruminants such as cattle and sheep, according to the studies of Reid, Franklin, and Hallsworth (*10*). It is well recognized that phytates must be hydrolyzed to free inorganic phosphorus before they can be absorbed by animals. The enzyme, phytase, has been shown to be present in the rumen. However, its origin, whether from rumen microorganisms or from ingested plant material, is not clear. The purpose of this study was to investigate the activity of phytase produced by rumen microorganisms, and to determine the availability of phytate phosphorus to rumen bacterial fermentation.

Experimental Methods

The method of Anderson, Cheng, and Burroughs (*1*) was used for the determination of phytate-phosphorus availability to rumen microorganisms. Briefly this method consists of measuring cellulolytic activity of a washed suspension of rumen microorganisms initially depleted of phosphorus. The depleted microorganisms were subsequently incubated with graded increments of phytate phosphorus and cellulose digestion was compared with digestion obtained with a phosphorus standard of known availability. The phosphorus-deficient medium used is presented in Table I.

In the present experiment rumen microorganisms were obtained from a fistulated Shorthorn steer fed a high-roughage diet. Calcium phytate was added to washed suspensions of rumen microorganisms at levels of 0, 5, 10, 20, and 40 γ of phytate phosphorus per ml. of medium. Cellulose digestion was measured at the end of a 24-hour incubation at 39° C.

Table I. Composition of Phosphorus-Deficient Basal Medium

Constituent	Amount, Grams/Liter
$NaHCO_3$	1.750
KCl	0.375
NaCl	0.375
$MgSO_4$	0.075
$CuSO_4.5\ H_2O$	0.001
$MnSO_4$	0.0002
$ZnSO_4$	0.00004
$FeSO_4.7\ H_2O$	0.0375
$CoCl_2.6\ H_2O$	0.001
Urea	1.000
Cellulose (Solka-floc)	5.000

Washed rumen microorganisms prepared according to the method of Cheng, Hall, and Burroughs (*4*) were used in studying the enzyme activity of phytase produced by the bacterial cells. The bacterial cells were placed in 20 ml. of nutrient suspension in 75-ml. tubes along with different amounts of calcium phytate, and incubated from 24 to 72 hours. At the end of the incubation period, 40 ml. of acetone was added to precipitate the bacterial protein. The precipitate was filtered, and the filtrate concentrated by evaporation of the acetone on a steam plate. The solution was then transferred to a 10-ml. volumetric flask and made to volume with distilled water. A second filtration was sometimes necessary prior to taking a 1-ml. aliquot for inorganic phosphorus analysis. Inorganic phosphorus was determined colorimetrically by the method of Fiske and Subbarow (*5*) using only slight modifications. Control tubes containing washed rumen microorganisms, which were boiled 10 minutes prior to incubation, were analyzed for inorganic phosphorus for comparative purposes. Any increase in inorganic phosphorus after incubation of viable microorganisms was assumed to represent the amount of phytate phosphorus hydrolyzed by the

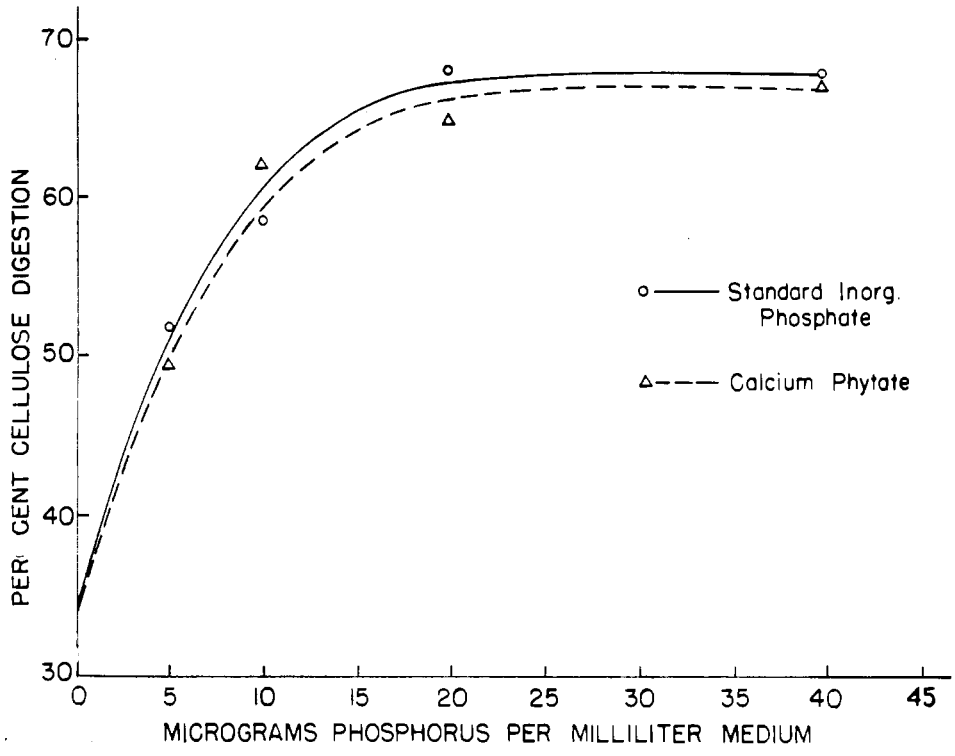

Figure 1. Availability of phytate phosphorus to rumen microorganisms

phytase produced by the rumen microorganisms.

The optimum pH of phytase activity was determined by incubation of 20-ml. suspensions of rumen microorganisms, prepared from 80 ml. of rumen liquid, with calcium phytate containing 18 mg. of phytate phosphorus, at 39° C. for 24 hours. In this experiment, potassium acid phthalate buffer was used to maintain the pH between 4.5 and 6.5, and boric acid buffer was used in maintaining the pH from 7.0 to 8.5.

Results and Discussions

The availability of phytate phosphorus to rumen microorganisms appeared to be close to 100%. The addition of 5, 10, 20, and 40 γ of phytate phosphorus per ml. of washed suspension of rumen microorganisms resulted in cellulose digestion comparable to cellulose digestion obtained when corresponding levels of standard inorganic phosphorus were added to similar tubes. These results are presented graphically in Figure 1. That the increase in cellulose digestion is due to the phosphorus liberated from phytate rather than inositol, which is also a product of phytate hydrolysis, appears evident on the basis of earlier experiments by Hall, Cheng, and Burroughs (*7*), which showed no enhancement in cellulose digestion when inositol alone was added to a washed suspension of rumen microorganisms containing sufficient inorganic phosphorus in the fermentation medium.

Measurements of inorganic phosphorus present in the fermentation medium revealed substantial phytase activity of the viable rumen microorganisms and essentially no activity of microorganisms which were boiled prior to incubation. Table II reports the amount of inorganic phosphorus released from calcium phytate when added in different amounts to the fermentation medium, and incubated 72 hours at pH 5.5.

As much as 27% of phytate phosphorus was hydrolyzed by the microrganisms. When the amount of phytate phosphorus in the medium was increased to 32 mg., the amount of hydrolysis increased to 1.58 mg.; however, the percentage of hydrolysis at this high concentration of phytate was relatively poor (4.9%). These values underestimate the total amount of phytate hydrolyzed, as the amount of phytate phosphorus hydrolyzed and absorbed in the growth of the bacterial cells cannot be measured by the procedure employed. On the other hand, the fact that not all of the phytate phosphorus was hydrolyzed during the 72-hour incubation period suggests that this enzyme system acts in this respect like cellulolytic enzymes produced by rumen microorganisms. This is in contrast to the general behavior of urease and amylase produced during fermentation, which usually brings about more nearly complete hydrolysis of these substances in a few hours. The slow rate of phytate hydrolysis was shown by Andrews and Herrarte (*2*), who found that certain bacterial suspensions hydrolyzed only 12% of the sodium phytate in 24 hours and 16% in 72 hours. Recent experiments by Perlès (*9*) showed that plant phytase was inhibited by a large excess of substrate. This inhibition

Table II. Hydrolysis of Calcium Phytate by Washed Suspensions of Rumen Microorganisms

Phytate P Added, Mg./20 Ml.	*Inorganic P Released, Mg./20 Ml.*	*Phytate P Hydrolyzed, %*
1.0	0.27	27.0
2.0	0.26	13.0
4.0	0.43	10.7
8.0	0.46	5.8
12.0	0.63	5.2
16.0	1.15	7.2
20.0	1.15	5.7
24.0	0.87	3.6
28.0	1.06	3.8
32.0	1.58	4.9

Table III. Influence of Concentration of Rumen Microorganisms upon Hydrolysis of Phytate Phosphorus[a]

Concentration, Microorganisms in Suspension[a], Ml.	*Inorganic P Released, Mg./20 Ml.*	*Phytate P Hydrolyzed, %*
40	0.16	0.9
80	0.16	0.9
120	0.26	1.5
160	0.42	2.3
200	0.70	3.9
240	0.83	4.6
280	1.08	6.0
320	0.93	5.2

[a] Ml. of rumen liquid used in preparation of 20 ml. of washed suspension.

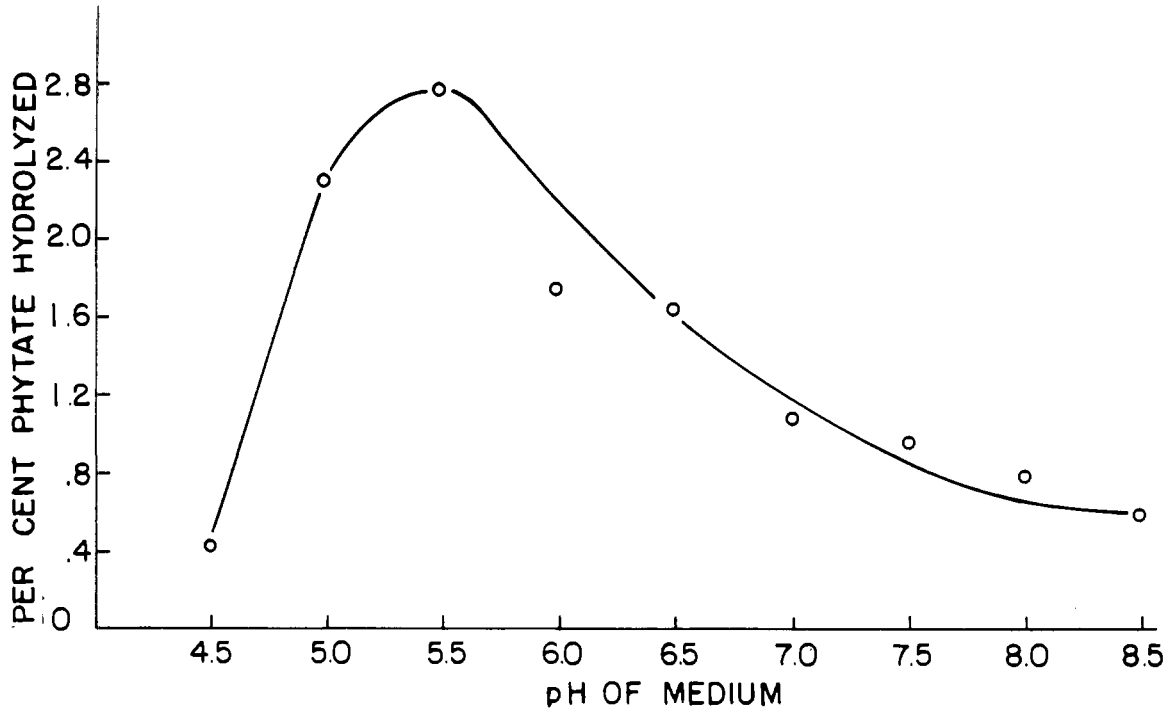

Figure 2. Effect of pH on hydrolysis of calcium phytate by rumen microorganisms

Table IV. Influence of Time of Incubation on Phytate Hydrolysis

Time of Incubation, Hr.	Inorganic P Released, Mg./20 Ml.	Phytate P Hydrolyzed, %
1	0.00	0.0
24	0.16	0.9
48	0.26	1.5
72	0.65	3.6
96	0.95	4.7

may also be true in the case of phytase produced by rumen microorganisms.

The influence of pH on phytase activity of rumen microorganisms is shown in Figure 2, which indicates that the optimum pH of phytase activity is about 5.5 (used in experiments reported in Tables III and IV). This pH appears to be similar to that of wheat phytase found by Peers (*8*), who reported an optimum pH of 5.15. In the bovine rumen the pH is usually between 6.0 and 7.0, which allows hydrolysis to proceed but not at an optimum rate. Reid, Franklin, and Hallsworth (*10*) suggested that the hydrolysis of phytate in their experiments must have been affected by phytase originating from rumen microorganisms with an optimum pH similar to that found in the rumen rather than the optimum pH required by plant phytases. However, they failed to offer any data to support their view on the optimum pH of phytase activity of rumen microorganisms.

The concentration of microorganisms in the preparation of suspensions used in the fermentation tubes exhibited some effect on the amount of phytate hydrolyzed. Table III presents results with 20-ml. suspensions prepared from quantities of rumen liquid varying from 40 to 320 ml. Calcium phytate supplying 18 mg. of phytate phosphorus was added to each tube at the beginning of the 24-hour fermentation period.

The amount of phytate phosphorus hydrolyzed increased from 0.9 to 6.0% as the concentration of rumen microorganisms increased. The duration of incubation also increased the amount of phytate phosphorus hydrolyzed, as shown in Table IV.

In all of these enzyme hydrolysis experiments, control tubes containing boiled suspensions of rumen microorganisms and medium were found to yield insignificant amounts of inorganic phosphate. It thus appears that rumen microorganisms produce the enzyme, phytase, which is capable of hydrolyzing phytate present in plant feedstuffs. Factors such as pH, time of incubation, concentration of substrate, or concentration of active cells which alter fermentation rate, also alter the activity or the amounts of phytase produced. Since rumen microorganisms produce ample supplies of phytase capable of supplying their needs for inorganic phosphorus from phytate, it seems likely that the majority of phytase present in the rumen is from bacterial origin. Such a conclusion has broad implications in the nutrition of ruminants, suggesting not only that the availability of phosphorus of plant sources is high, but also that its usage is not dependent upon the absence or presence of accessory enzymes in the plant.

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