Phytase Feed Supplements from Transgenic Alfalfa

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Introduction

Buildup of phosphorus in the environment and the resulting degradation of water resources is of mounting concern. Much of this buildup is traceable to human activities. Important among these is livestock production. Monogastric animals, such as poultry and swine which can solubilize only a small fraction of the phosphorus in their grain-based rations, while excreting the remainder, have come under increased scrutiny. Supplementation of inorganic phosphorus into rations to meet animal nutritional requirements exacerbates the problem.

Much of the phosphorus in grain is in the form of insoluble phytates. Researchers have shown that supplementing poultry and swine rations with the enzyme phytase can lead to solubilization of the phosphorus, thus reducing the need for phosphorus supplementation and concurrently reducing the level of phosphorus in the excrement to approximately one-half of that normally experienced.

The enzyme phytase derived from *Aspergillus niger* has, to date, generally been produced in fermentation vats using genetically engineered microorganisms. Currently the cost of phytase supplementation is slightly higher then the cost of conventional supplementation with dicalcium phosphate.

As an approach to reducing the cost of phytase production, a multi-disciplinary ARS-UW team at Madison, Wisconsin has produced transgenic alfalfa with the capability of expressing phytase. This phytase can be recovered from juice extracted from the herbage. Other constituents of the juice including xanthophyll (used to pigment egg yolks and broiler skin), high levels of dietary protein, and various vitamins and minerals add to its value in rations. Leaf meal is another feedstuff which could be used to add phytase to monogastric rations. The use of whole alfalfa herbage, however, would not be desirable due to its high fiber content. Since phytase would potentially be needed in great quantities, but not in very pure or concentrated form, it is believed that the economic advantage of production in “plant bioreactors” such as alfalfa could be great. The advantage in capital costs is particularly great. Ideally, the cost of phytase supplementation should be competitive with the traditional dicalcium phosphate supplement, with the environmental benefits as an added incentive.

Methods

Sixteen phytase-producing alfalfa transformants were originally created. Early bioassays indicated that these transformants produced phytase at a range of levels. These transformants were vegetatively propagated in the greenhouse during the winter and spring of 1997 and approximately 7500 plants were set out into the field in mid-May in both replicated research plots as well as larger “production plots.” The plant densities in the two plot types were 9680 and 139396 per acre, respectively. Both sets of plots were harvested in late May and early June, with herbage from the research plots being dried and separated for production of leaf meal while the herbage of the highest phytase producers in the “production plots” was macerated and the juice expressed. Some of the juice was sprayed on ground corn at a 1:1 ratio (wet basis). Half of the total juice was sprayed on at a time with the resulting mix being sun-dried after each juice addition. The dried mix could then be stored. The remainder of the juice was stored in a freezer.

The leaves from approximately 1/3 of the research plots were detached by passing the herbage through an impacting rotor running at approximately 3450 RPM while the remaining herbage was passed between rubber rolls with differential surface speeds to strip the leaves. Phytase content, crude protein content, and fiber content of selected leaf meal samples were determined.

Results

Leaf Separation. The leaf to herbage ratios (d.b.) for three groups of herbage is shown in Table 1. The high ratio for the first group was due to the inclusion of many small stem fragments caused by the impact rotor and the inability to separate these from the true leaves. The ratio of .44 for the second group appears to be realistic based on previous work. Since this was first cutting herbage with a lower leaf:stem ratio than for subsequent cuttings, it would not be unrealistic to expect a ratio of 0.5 or greater for the season. The ratio for group 3 which was harvested one week later than group 2 was considerably lower. While the herbage mass (presumably non-leaves) increased by approximately 20% during the additional week, an absolute loss of leaves would have been necessary to explain the low ratio. If an average value for phytase activity of 180 units/g leaf meal is used along with a per acre herbage dry matter yield of 10,000 lb and a leaf yield of 0.5, the result is 400 x 10^6 phytase units/acre. At the rate of 400 phytase units/lb ration, 500 tons of ration could be dosed. If a cost of $3.00/ton for inorganic phosphorus is eliminated, the value of the leaf meal, for phytase content only, would be about $1500/acre. The protein and pigmenting (xanthophyll) value of the leaf meal would be in addition.

Wet Fractionation. Wet fractionation of five production plots containing the highest phytase producers resulted in average juice yields of 46.0% w.b. (s = 3.8) and 21.5% d.b. (s = 1.7). The phytase content of the juice averaged approximately 50 units/ml or 22,680 units/lb juice. At a herbage fresh weight of 50,000 lb/acre-yr, a juice yield of 0.46, a dosage of 400 units/lb feed, and potency of 22,000 units/lb juice, 630 tons of feed could be treated. If $3.00/ton for inorganic phosphorus supplementation is eliminated, the value of the juice would be $1890/acre-year plus the value of protein and pigmenting substances.

When juice was added to ground corn at a 1:1 ratio w.b. the activity calculated should have been 45 units/g mix, while the assayed value was around 33 units/g. It is not yet known whether this apparent activity loss of around 27% is real or a result of the assay method.

Conclusions

Economically significant phytase yields were achieved from transgenic alfalfa in its second year in field plots. In research reported elsewhere, the efficacy of this phytase was demonstrated in poultry and swine rations.

Table 1. Yields of leaf meal relative to herbage.

Group	Plants	Harv. Date	Rows*	Wt/plant (lb w.b.)	Separation Method	"Leaf" wt/ Herbage wt (d.b.)
1	Reps 1 & 2 (partial)	5/26/98	18	2.2	Impact Rotor, Screening	.65, s = .08
2	Reps 1 & 2 (partial)	5/26/98	19	2.2	Rubber Rolls, Screening	.44, s = .06
3	Rep 3	6/2/98	19	2.6	Rubber Rolls, Screening	.25, s = .04

*10 plants/row