FREEING PHOSPHORUS

A newly designed enzyme unlocks a key nutrient, aiding animal nutrition and the environment

Phosphorus has been getting a bum rap. The mineral is a basic ingredient for life. For starters, it helps make up the DNA in all organisms and is needed for development of strong bones. But lately, it's been getting more attention as a polluter.

When excess phosphorus loads are allowed to drain from the land, they can tilt the fragile balance of life in rivers and oceans, causing numbers of some marine species to rocket and others to crash.

Now ARS scientists at the Southern Regional Research Center (SRRC) in New Orleans, Louisiana, have discovered a way to help animal producers rein in this runaway nutrient. Their logic: If livestock and poultry could retain more of the phosphorus in their plant-based feeds, less would be excreted. And that would mean less potential nutrient waste and pollution.

In 1986, the SRRC researchers—geneticist Edward Mullaney and biochemist Jaffor Ullah—were the first to characterize a natural enzyme that could accomplish this tall task. Called phytase, the enzyme sparks a chemical reaction in animals' stomachs, helping them better use the tied-up phosphorus in the plants they eat.

Now the two scientists are rebuilding this enzyme to make it even more effective—especially in the unique environments where it needs to perform.

It Takes Guts

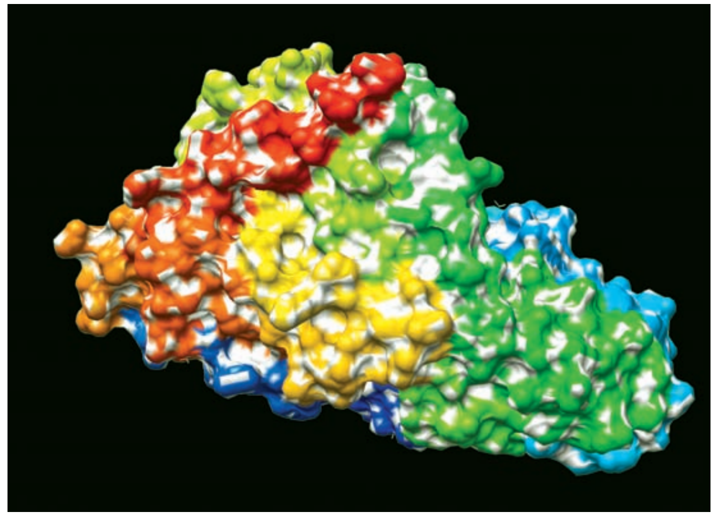
Phosphorus is tricky to deal with because of its multiple, naturally occurring forms. In rocks, the mineral occurs as phosphate; in plants, as phytic acid. But animals with simple stomachs—including pigs, chickens, and people—can't make use of these alternate forms. Our bodies just don't churn out the right enzymes.

"Think of lactose intolerance," says Mullaney. "Some people can't digest the sugar in milk because their bodies don't produce enough of the enzyme lactase."

So, in their search for a way to help livestock unlock tied-up phytic acid, the researchers turned to one of nature's most efficient degraders. The organism, a fungus called *Aspergillus niger,* is typically known for its food-spoiling ways, causing a black mold to grow on stored fruits, nuts, and seeds. But it does have a redeeming quality.

"*A. niger* produces phytase, which allows the fungus to break down the phytic acid in plants," says Mullaney. "Phytic acid exists across the plant world, and many organisms have evolved this enzymatic way to make use of it."

JAFFOR ULLAH (D550-4)



Powerful molecular-modeling software allows researchers to visualize the phytase molecule in various ways. This particular model fills in all the spaces between the structural components and presents an image of just the surface of the molecule.

Since this discovery, researchers have developed a phytase enzyme that can be added to livestock diets—to encourage better nutrition and reduce the costs associated with phosphorus supplementation. But despite its $500 million-per-year market, the enzyme has its shortcomings.

Its source, the *A. niger* fungus, is finicky—growing best in conditions that mimic its natural environment, which includes composting leaves and decaying plants. That means both the fungus and its enzyme are most vigorous at a pH of about 5 or 6. But the stomachs of chickens and livestock are much more acidic than that, closer to 3 or 3.5.

"The enzyme isn't nearly as effective at degrading phytic acid if it's not in the conditions it favors most," Ullah says.

Since researchers can't really alter the complex microenvironment found in an

REGIS LEFEBURE (K5657-3)

A newly designed phytase enzyme added to animal feed enables swine to use more phosphorus in the feed and excrete less of the phosphorus in their waste.

A Molecular Makeover

ARS researchers have developed new-and-improved enzymes to help farm animals digest more of the phosphorus in their diets so that less leaves their bodies in waste. To accomplish this, they used state-of-the-art software to create vibrant 3-D models of the phytase enzyme, which normally is too tiny to be seen even with an electron microscope. By assigning distinct shapes and vivid colors to all the enzyme's inner parts, the researchers were able to get a better view of how to best carry out their makeover.

animal's gut, Mullaney and Ullah set out to modify the enzyme itself.

More Shapely Proteins

An enzyme is a protein made up of a long, coiling rope of amino acids. The way those amino acids are folded and arranged dictates how the protein will perform.

In other words, if the shape of a protein changes, its function will too. The New Orleans researchers thought that with a slightly altered, more desirable shape, phytase could perform better under conditions it's not used to—like cold temperatures or extreme acidity, for instance.

So, by swapping some of the existing amino acids that make up phytase's coiling chain with alternate ones, SRRC scientists have been able to achieve a different breed of enzyme, with brand-new capabilities.

"All proteins have a three-dimensional structure," says Ullah. "Each one folds in its own unique way. We've been able to change our enzyme's natural folding by replacing some of its amino acids with other ones. Now the enzyme fits more snugly with the phytic acid from plants."

The Pig's the Proof

Since lab tests done with the redesigned enzyme showed such great promise, the next step was to test it on livestock.

Mullaney and Ullah collaborated with researchers at Cornell University in Ithaca, New York, to conduct feeding trials with the enzyme. Led by animal scientist Xin-Gen Lei, the Cornell team fed swine their typical diet of corn and soybean meal supplemented with the regular phytase. They fed other pigs the same meal, but substituted the newly altered phytase enzyme.

"Lei and the other Cornell researchers found that swine fed the phytase additive had a 13-percent weight gain during the 5 weeks of study," says Mullaney. "That's significant.

"From an environmental standpoint," he adds, "we should be able to assume that if more phosphorus is being used by an animal, less will be excreted in its manure."

Customized Enzymes Will Help Conserve

Mullaney and Ullah plan to develop tailor-made enzymes that can be used in a variety of applications. For example, they've already teamed up with a soil scientist in Australia, Alan Richardson, who's successfully expressed the novel phytase enzyme in the roots of the model plant *Arabidopsis*. Eventually, Richardson would like to introduce the valuable enzyme to a range of crop plants.

The project could have a staggering impact. If widely planted crops—such as soybeans, wheat, and corn—could more efficiently use phytic acid in soil, much less phosphorus fertilizer would have to be applied to produce a profitable yield.

While the SRRC researchers' specially built enzymes would certainly benefit farmers by helping them save on feed and fertilizer costs, the work is really part of a much greater mission: conserving future stores of phosphorus.

"This mineral is not a renewable resource," says Ullah. "Croplands can only absorb so much phosphorus in the form of applied fertilizers or manure. Whatever can't be soaked up is lost, often to our waterways. And there's currently no way to capture phosphorus that's leached into rivers and oceans. At some point—and some experts project it might be as little as 80 years from now—we could face a phosphorus shortfall."—By **Erin Peabody,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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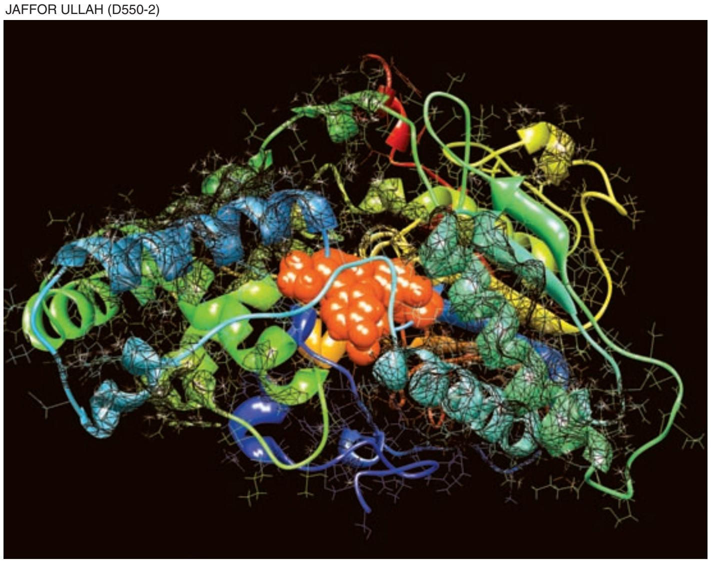

In this computer-generated image, the red beads highlight the important "active site" of the phytase molecule. The active site allows the enzymes to release phosphorus from the phytate in the feed and make it available to the animal. Changes to this site or neighboring region can drastically change how well phytase performs in an animal's digestive tract.