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Efficiency of phytase enzyme researched

IN little more than 10 years, enzymes have progressed from being an interesting curiosity to being an important, if not essential, ingredient.

At the same time, the questions have changed from whether they work to: How well do they work? How much response can be expected? Under what conditions? Is one enzyme more efficient than another?

Phytases were the first enzymes to achieve widespread use. Today, some form of phytase is incorporated into a majority of commercial poultry feed. Much has been learned about the activity of the various phytase enzymes, but there are still many questions that remain to be answered. Efficiency — in particular, relative efficiency — is an area where more information is needed.

C. Kwakernaak and J.D. Van der Klis of Schothorst Feed Research in Lelystad, Netherlands, and P. Plumstead of Danisco Animal Nutrition in Marlborough, U.K., presented a paper at the International Poultry Scientific Forum titled "*In Vivo* Efficacy of a Buttiauxella 6-Phytase Versus a Novel Citrobacter 6-phytase in Young Broilers" (abstract T115).

The objective of the research was to measure the relative biological response (relative efficiency) of two sources of phytase when each were added to a broiler diet to provide the same number of phytase units (FTU). The two sources of phytase were a six-phytase derived from *Buttiauxella* spp. and expressed in *Trichoderma* (BT) and a six-phytase derived from synthetic genes mimicking *Citrobacter braakii* and expressed in *Aspergillus* (CA).

One FTU is defined as the amount of enzyme needed for the release of 1 µmol of phosphate from sodium phytate solution (c = 0.0051 mol per liter) in one minute at pH 5.5 and 37°C.

Nine experimental diets were fed as pellets to Ross 308 male broilers housed in six replicate cages with 16 birds per cage. Birds were fed the diets from five to 21 days of age. A low-phosphorus negative control (NC) diet containing 2,900 kcal/kg, 21% crude protein, 0.44% phosphorus, 0.18% retainable phosphorus, 0.25% phytate phosphorus and 0.65% calcium was used.

The phytase products and diets were analyzed for *in vitro* phytase activity by LUFA in Oldenburg, Germany, according to the AOAC method. Both of the phytase products were added at levels of 250, 500, 750 and 1,000 FTU/kg on top of the NC diet. Feed and water were available *ad libitum*. Growth performance was measured, and at the end of the experiment, tibia were collected for the determination of ash content (four birds per replicate).

Statistical analyses of the phytase-supplemented diets showed significant ($P \leq 0.001$) differences between the phytase products for feed intake, bodyweight gain, feed conversion ratio and tibia ash content. For feed conversion ratio and tibia ash, there was a significant interaction ($P \leq 0.05$) between the phytase effect and dose level.

BT phytase resulted in, on average, a significantly higher bodyweight gain and feed intake compared with the CA phytase (7% and 5% higher, respectively). For tibia ash, the difference between the phytases was significant at each dose level (8% higher for BT), while for feed conversion, there was a significant difference at 500 and 1,000 FTU/kg (3-5% lower for BT). An exponential curve fitting showed that 309, 287 and 283 FTU of the BT phytase was equal to 500 FTU of the CA phytase based on bodyweight gain, feed conversion ratio and tibia ash, respectively.

The researchers concluded that, based on a standardized measure (AOAC) of *in vitro* activity, the *in vivo* efficiency of both of these six-phytases was significantly different.

Bottom Line

with
BILL DUDLEY-CASH*

Photo: Natural Resources Conservation Service.

*Dr. William A. Dudley-Cash is a poultry and fish nutritionist and has his own consulting firm in Modesto, Cal. To expedite answers to questions concerning this article, please direct inquiries to *Feedstuffs*, Bottom Line of Nutrition, 5810 W. 78th St., Suite 200, Bloomington, Minn. 55439, or email comments@feedstuffs.com.

Discussion

The results of this research clearly show that phytase products evaluated using a standardized measure of *in vitro* activity can result in markedly different efficiency when fed to chickens (*in vitro* activity). How can these results be so different?

First, let's look at the *in vitro* test. The substrate used in the *in vitro* test is sodium phytate, a chemically defined source of phytate phosphorus. The *in vitro* test is conducted at a pH of 5.5 and a temperature of 37°C.

The objective of the *in vitro* test is to provide a measure of phytase activity that, if the test is repeated with the same phytase product at different times and in different laboratories, will produce the same (or very similar) result. In short, it's a standardized method.

None of these standardized test conditions exist when a phytase product is mixed with feed ingredients and fed to a chicken.

The naturally occurring phytate present in feed ingredients is associated (complexed, chelated, etc.) with a wide variety of minerals, trace minerals, proteins, amino acids and carbohydrates. These associations alter the affinity of the phytase for the phytate complex and change the ability of the phytase to release phosphorus from phytate. The effect on various phytase enzymes is different.

These associations are dynamic through the length of the bird's intestinal tract, depending on pH and other conditions. The pH of the intestinal tract varies from less than 4.0 in the gizzard to more than 7.0 in the ileum. The average pH may be near 5.5, but the specific pH is hardly ever 5.5. Phytase enzymes have pH optima, and the optimum pH is frequently quite different for different enzymes.

While the *in vitro* test is conducted at a temperature of 37°C, the average body temperature of the chicken is about 39°C. This, too, may have an effect on the test results.

The formulation mix may also change the phytase response. Variations in the level or inclusion of ingredients (corn, wheat, sorghum, soybean meal, canola, dried distillers grains plus solubles, meat and bone meal, limestone and other ingredients) present the phytase enzyme with different levels of substrate (phytate) and different combinations of phytate complexes. All of these factors have an effect on the commercial response that can be achieved and likely vary with the different phytase products.

The commercial nutritionist needs relative efficiency information in order to select the product that will result in the best economic return. The best choice is not necessarily the product that is the cheapest per FTU or the product with the highest level of activity. The poultry industry needs an assay that reflects *in vivo* responses under a variety of commercial conditions.

The Bottom Line

Given the variables discussed, it should not be a surprise that the result of the accepted standardized test for FTU (or, perhaps, any standardized *in vitro* test) is not a good indicator of the relative *in vivo* efficiency of phytase products. In order to obtain a better measurement of the relative efficiency of phytase enzymes, we have to "ask the chicken."

The abstracts of the International Poultry Scientific Forum may be found on the U.S. Poultry & Egg Assn. website at www.ipe13.org/ipsf/docs/13AbstractBook.pdf.

Reference

AOAC. 4.10.06 Official Method 2000.12. Phytase Activity in Feed. ■