Ensuring animal feed formulas meet all nutritional requirements is particularly challenging for vital mineral elements such as calcium. Essential for bone development and a key player in several metabolic paths, calcium is often added to diets with large safety margins to avoid deficiencies. But if calcium deficit poses a threat to animal growth, high calcium concentrations that are highly soluble can also severely impair productivity.

Animal production strongly depends on healthy animal growth. In turn, growth and maturation rely on the normal development of the skeleton.

Bone mineralisation is dependent on a variety of elements with phosphorus and calcium being the two most important. In poultry, for instance, calcium is the highest concentration mineral in the body, representing more than one third of the total mineral body content of adult birds and one third of eggshell components.

Although mostly found in the skeleton (almost 99%), calcium also catalyses several metabolic and physiological processes including contracting heart and muscle fibres and transmitting nerve impulses.

Calcium supplementation: a tricky balance

To fulfil the nutritional needs required by the expanding livestock production sector, diets have been formulated using different types of cereal-based ingredients. Although providing high amounts of energy, cereals are poor in several elements including calcium, most presenting concentrations as low as 0.02% and 0.06%.

To meet calcium requirements, diet formulations usually include inorganic sources of this nutrient such as limestone and dicalcium phosphate.

As an example, in a typical corn-soy based starter diet for broilers, more than 80% of the calcium comes from inorganic sources.

Unlike phosphorus addition, calcium supplementation is low-cost, and limestone is easily accessible. In addition, excretion of excess calcium does not explicitly cause environmental concerns. Taken together these factors often lead to over-supplementation.

An unexpected anti-nutrient

High calcium concentrations can be as harmful as low ones: calcium deficiencies have a negative impact on growth performance, while increased concentrations have anti-nutritional effects on other important key nutrients. Of major importance is the negative effects of excessive calcium on phosphorous availability and digestibility.

Calcium has the ability to form complexes with phosphate groups in the gut, which interfere with phosphorus availability. Phosphorus is crucial for energy mobilisation and protein synthesis but also bone development and overall maintenance. Its primary storage form in plant tissues is phytate, which is broken down by phytase to release phosphorus.

For animals that lack phytase, like poultry and swine, the enzyme is added to feed. Continued on page 23

Fig. 1. Calcium pH-dependent effect on protein-phytate binding (adapted from Prattley et al. 1983).

Fig. 2. Comparative pH profile with five different phytases, including the novel Axtra PHY.
**Digestibility**

*Fig. 3. The increasing proportion of highly soluble calcium in the diet decreases phosphorus digestibility*

Continued from page 21

Formulas. High dietary calcium content affects phytase effectiveness due to the formation of calcium-phytate complexes. The high prevalence of calcium also triggers phytate to bind with protein molecules more easily, further reducing phytate availability to be hydrolysed by phytase.

Proteins usually form complexes with phytate at the low pH (~4) of the upper gastrointestinal tract but not at higher pH levels. This process is challenged in the presence of calcium that promotes protein-phytate chelation at pH ~4, thereby acting as a cation bridge to promote the indirect binding of proteins and phytate (Fig. 1).

This anti-nutritional effect of calcium depends largely on its solubility, which is very much influenced by the calcium source and particle size. Limestone, the major inorganic calcium source used in poultry diets, is marketed under different particle sizes that influence calcium’s solubility. Particle size impacts the calcium-phytate binding and its consequences. Research has shown that the smaller the limestone particles, the faster they solubilise in the upper part of the digestive tract, making it unavailable to form the anti-nutritional calcium-phytate complexes. Axtra PHY is a Buttiiauxella phytase with high bioefficacy and activity at low pH. This phytase acts fast in the intestinal tract, hydrolysing phytate during the early stages of digestion. The enzyme revealed an improved pH profile when compared to its competitors, performing even at very low pH levels (~2.5) (Fig. 2).

By cleaving phosphate groups rapidly, phytase decreases calcium-phytate binding and thereby mitigates its anti-nutritional effect on both proteins and minerals. But phytase efficiency largely depends on the size of the calcium particles found in different calcium supplementation sources. For instance, phytase addition may occur at different doses when overcoming the negative impact of highly soluble calcium (HSC) on nutrient digestibility or the effects of limestone. The increasing proportion of HSC in the diet decreases phosphorus digestibility in groups not supplemented with phytase because the precipitation of phytate complexes is larger. To compete with the phosphorus digestibility of limestone-supplemented diets, HSC diets, therefore, require higher doses of phytase (Fig. 3).

High dietary calcium levels affect phosphorus metabolism and, therefore, animal growth performance and average weight gain. To examine the impact of phytase on counteracting the negative effects of high dietary calcium levels, Axtra PHY was tested in an in vivo trial using young birds. An inclusion of 1000 FTU/kg of phytase slowed down the excess calcium-related decrease in performance in relation to the control feed. The results also demonstrated that higher doses of phytase may be needed to restore performance for birds fed diets containing fine limestone than for birds fed diets containing coarse limestone.

At only 500 FTU/kg, the same enzyme restored body weight gain to numbers close to that found in the positive control. However, for diets containing fine limestone, performance was only restored with the enzyme addition at 1,000 FTU/kg (Fig. 4). This is in line with the previously mentioned results, thereby showing that calcium particle size does influence the amount of phytase needed to counteract the calcium negative effects.

The benefits of a fast-acting phytase and the right calcium source

A dual-action strategy can be employed to alleviate the detrimental effects of calcium over-supplementation on nutrient digestibility and growth performance. First, the addition of a fast-acting phytase promotes a timely and successful hydrolysis of phytate and avoids chelation. What is important to note here is that a strong pH profile phytase ensures the phytate is hydrolysed swiftly in the very acidic first portion of the gut. Secondly, adjusting the amount of calcium included in diets (better calcium-to-phosphorous rates) and observing the solubility of the selected calcium source will aid improving feed quality and animal health.

One central consideration here is the grind size of the calcium supplementation. Diets containing higher calcium inclusion levels and/or more soluble calcium sources, such as HSC or coarse limestone, benefit from using a tailored phytase dose to completely remove phytate and avoid the anti-nutritional effect of phytate-calcium complexes.

**Fighting the anti-nutritional battle with phytase**

Although all marketed phytase enzymes break down phytate, not all phytases are the same. Enzymes with a superior pH profile will hydrolyse phytate more quickly in the upper part of the digestive tract, making it unavailable to form the anti-nutritional calcium-phytate complexes. Axtra PHY is a Buttiiauxella phytase with high bioefficacy and activity at low pH. This phytase acts fast in the intestinal tract, hydrolysing phytate during the early stages of digestion. The enzyme revealed an improved pH profile when compared to its competitors, performing even at very low pH levels (~2.5) (Fig. 2).

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**Fig. 4. Axtra PHY phytase counteracts the negative impact of limestone in starter phase broiler (1-21 days) growth performance.**

<table>
<thead>
<tr>
<th>Diet Description</th>
<th>PC1</th>
<th>NCI + Axtra PHY (500 FTU/kg)</th>
<th>NCI + Axtra PHY (1000 FTU/kg)</th>
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<td>Body weight gain (g)/bird/day</td>
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<td>1000</td>
<td>1040</td>
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<tr>
<td>PC2</td>
<td>NCI2 + Axtra PHY (500 FTU/kg)</td>
<td>NCI2 + Axtra PHY (1000 FTU/kg)</td>
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</tr>
<tr>
<td>Coarse</td>
<td>a</td>
<td>a</td>
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