# The prevention of leg disorders starts in the gut

Leg disorders are quite prevalent in poultry production. By supporting mineral use and controlling pathogenic bacteria, Bacillus sp. PB6 has the potential to reduce their prevalence and negative impact.

**by Eric N'guetta, Paulina Bukowska, and Mutasem Alhaddad, Kemin Europa. www.kemin.com**

## Intestinal health can support optimal mineral use

Multiple types of leg problems are encountered in poultry production. Regardless of their etiology, they typically not only affect growth performance, but also bird health and welfare.

A good way to illustrate their impact on welfare is that, in severe conditions, affected birds cannot move properly and become unable to meet their basic needs for feed and water.

At the same time, leg problems are also associated with increased incidence of slaughterhouse rejections due to more frequent breast blisters or cellulitis.

The optimal management of vitamin and mineral nutrition (for example vitamin D, calcium and phosphorus, trace elements) is often the first thing that comes to mind when discussing leg disorders.

This is of particular interest when issues are of nutritional origin. In such cases, maintaining intestinal health can play a crucial role for prevention, mainly by improving the composition of the intestinal microbiota, limiting intestinal inflammation, and promoting a healthy structure and function of the gastrointestinal tract.

## An example with Bacillus sp. PB6

Clostat is a probiotic that consists of spores of Bacillus sp. PB6 (ATCC PTA-6737), which originates from chickens that resisted an enteritis outbreak. It stimulates a well-developed and diverse intestinal microbiome, supports the growth of beneficial bacteria and has an antagonistic activity against specific pathogens found in the gastro-intestinal tract.

A study was conducted with female one-day-old Arbor Acres broiler chickens to evaluate to effect of Bacillus sp. PB6 (PB6) on tibia physical characteristics and serum parameters that are associated with calcium and phosphorus metabolism.

The Control group was fed with a basal corn soybean meal-based diet; the PB6 group received the same diet supplemented with Bacillus sp. PB6 at 1 x $10^8$ CFU/kg of feed until 42 days.

Each treatment consisted of 3 replicates of 20 birds. To analyse tibia characteristics and serum parameters, three birds per replicate

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**Fig. 1. Tibia weight and breaking strength at 21 days of age.**

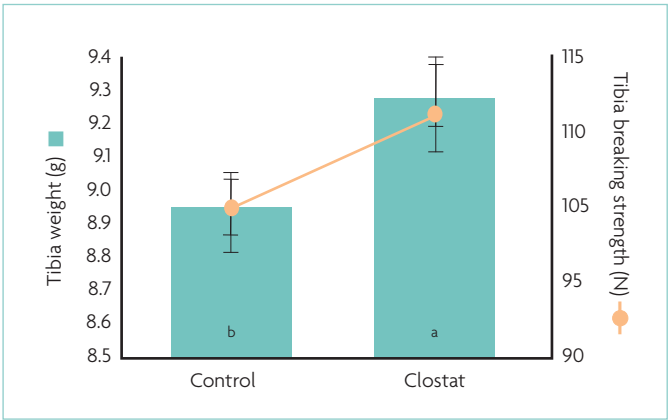
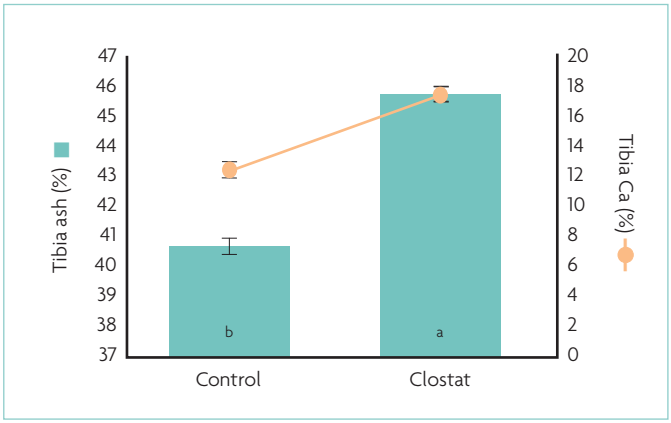



**Fig. 2. Tibia ash and calcium percentage at 42 days of age.**



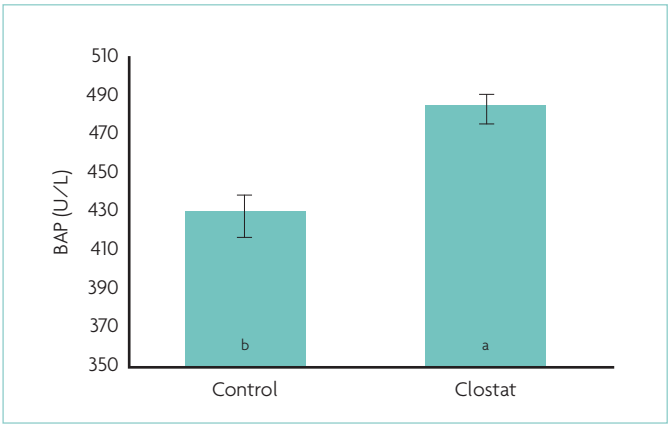



**Fig. 3. Serum level of bone-specific alkaline phosphatase at 42 days of age.**

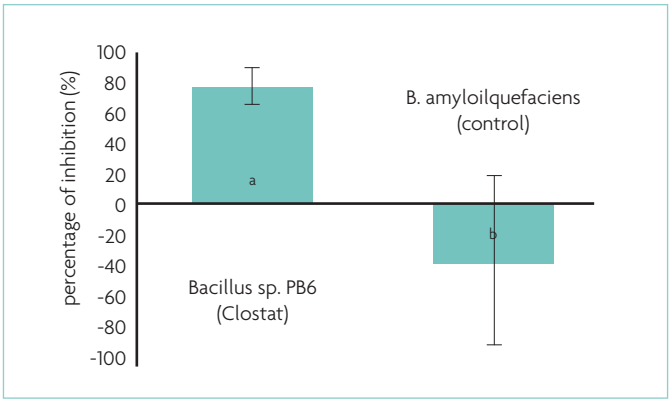



**Fig. 4. Average in vitro inhibition of 9 isolates of avian pathogenic E. cecorum with of Bacillus sp. PB6 or B. amyloliquefaciens (type strain).**

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were randomly selected. Blood samples were taken from the brachial vein, and tibias were removed (d21 and d42). PB6 significantly improved tibia weight and tibia breaking strength on day 21 ($P<0.05$) (Fig. 1).

On day 42, tibia ash and calcium content were significantly improved by PB6 ($P<0.05$) (Fig. 2). Regarding serum parameters, bone-specific alkaline phosphatase (BAP, an enzyme that correlates with osteoblast proliferation and bone formation), was also significantly improved by PB6 (Fig. 3).

These results indicate that microbiota management through the supplementation of PB6 is a valuable tool to support optimal mineral use and strengthen bone health, which is instrumental to prevent leg disorders from non-pathogenic origin.

## Broiler leg disorders are not always from nutritional origin

Beside the suboptimal use of minerals, it must be noted that important emerging leg disorders have a non-nutritional origin.

They are often related to infections of either the nervous system, the joints, or other soft tissues. Infections caused by Enterococcus cecorum fall into this category. This second category of leg disorders also highlights the decisive role bacterial homeostasis in the intestinal tract plays to maintain leg health.

## Early microbiota management is a potential tool to prevent Enterococcus infections

Enterococcus spp. are normally commensal inhabitants of the broiler gastro-intestinal tract.

They are gram-positive cocci that belong to the group of lactic acid bacteria. However, some Enterococcus strains differ from their commensal counterpart by showing increased pathogenicity due to specific virulence factors.

These characteristics give avian pathogenic E. cecorum the ability to escape the bird's immune system and colonise different tissues outside of the gut. As a result, E. cecorum infections in broilers cause a musculoskeletal disease called enterococcal spondylitis (ES).

The disease leads to increased mortality and to crippling leg disorders starting at around 3-4 weeks of age (or sometimes before) due to hind limb weakness or paralysis.

Very interestingly, it has been demonstrated that early colonisation of the bird's gastro-intestinal tract by pathogenic E. cecorum occurs during ES, whereas unaffected birds are colonised by commensal E. cecorum only after two weeks.

Associated with the more permeable gut of young birds, this early colonisation can shortly lead to bacteremia, and to the colonisation of other organs including the spinal cord.

The leg weakness observed during ES is the results of the compression of spinal cord, occurring subsequently to the infection of the free thoracic vertebrae. Mortality typically ranges between 5-15% during outbreaks.

Considering the pathogenesis of ES, enhancing the microbiota and the intestinal health at placement could be a strategy of choice to prevent outbreaks by preventing early colonisation of the birds by pathogenic E. cecorum.

## Previous prevention strategies have relied on the use of antibiotics

In many countries, successful preventive strategies in the field have mostly relied on starting birds with lincomycin and spectinomycin.

However, it must be noted that regulation EU 2019/6 further restricted the preventive use of antibiotics in groups of animals in the European Union as of January 28th 2022, which increases the need to have more sustainable alternatives for the prevention of E. cecorum infections.

In addition, antibiotic resistance in Enterococcus spp. is unfortunately not rare and interventions after disease onset do not always stop mortality.

## The early use of Bacillus sp. PB6 as a preventive strategy

As early colonisation of the bird's intestine by pathogenic E. cecorum is an important predisposing factor, our approach aims at supporting the establishment of a diverse and resilient microbial environment as early as possible to prevent undesired colonisation.

The effect of PB6 was evaluated in an in vitro study carried out using nine clinical E. cecorum isolates from confirmed outbreaks of ES.

The cell free supernatant obtained after the culture of PB6 was incubated with the avian pathogenic E. cecorum strains and growth reduction was measured, in comparison to the E. cecorum cultures incubated alone. Different type strains were used as a negative control (results shown for B. amyloliquefaciens type strain). PB6 consistently inhibited or delayed the growth of all E. cecorum strains tested during this trial (Fig. 4).

Combined with its beneficial effects on the intestinal microbiome, these in vitro results suggest that PB6 is a promising tool to contribute to the early prevention of E. cecorum infections in broiler production and to limit antibiotic use.

## Conclusion

Pathogenic as well as non-pathogenic leg problems often start in the gut, either due to poor use of minerals or colonisation of the gastro-intestinal tract by undesired bacteria.

Altogether, these results suggest that microbiota management with Bacillus sp. PB6 (Clostat) has the potential to prevent both types of leg problems by promoting bacterial homeostasis. ■

**References are available from the author on request**