The gastrointestinal tract (GIT) is a complex ecosystem heavily influenced by different animal production practices (Celi et al., 2017). In turn, this directly affects the animal’s intestinal functionality, performance and health. Although ‘normal’ microbiota of production animals has yet to be clearly defined, we know that optimal gut function is characterized by a normal, stable, and diverse GIT microbiota and an intact GIT barrier (Celi et al., 2017).

Peptidoglycans – Non-ingredient Digestive Components
Research strives to elucidate meaningful relationships between the host mucosal immune system, the microbiome, and feed ingredients. As we search deeper and consider non-ingredient components, the peptidoglycan (PGN) content in the GIT lumen and subsequent effects on digestibility and absorption looms into view.

A mature broiler normally has $10^9$ bacteria per gram of ileal contents (Rehman et al., 2007). In the microbiome fraction of the gut, microbe replication and death release cell-wall components that become part of the intestinal contents. PGN is a primary one (Klausen and Ward, 2018), and makes up 30–90% of the dry weight of Gram-positive (Gram+) bacteria (Schumann, 2011) and about 10% of Gram-negative (Gram-) bacteria (Malanovic and Lohner, 2016). In total, PGN can be a significant part of intestinal contents of non-ingredient origin, especially when considering that dead cells comprise 30-35% of the total in the distal GIT, at least for humans (Apajalahti et al., 2003; Ben-Amor et al., 2005).

Consistent with this narrative, recent studies found that degradation of PGN from cell wall fragments by a novel microbial muramidase (Cohn et al., 2018) resulted in remarkable improvements on broiler performance (Lichtenberg et al., 2017; Yegani et al., 2018). Clearly, PGN needs to be a bigger part of the discussion on GIT functionality.

Peptidoglycans are Universal in Bacteria
PGN is common to all bacteria. It is a cell wall structural component that protects against internal osmotic pressure and is essential for cell survival. During bacterial growth, PGN fragments are released into the media due to cell elongation, expansion, and division. Each new generation of Gram+ bacteria adds 15 to 50% PGN to the outside environment. And with every replication of E. coli (Gram-), 6 to 8% more PGN accrues in the surroundings (Goodell and Schwarz, 1985).
Chemistry of Peptidoglycans

Several models highlight the basic features of PGN chemistry, which can be heavily influenced by species and growing conditions (Vollmer et al., 2008a). All PGN is comprised of a beta-1,4 polymer of N-acetylglucosamine (GlcNAc) that alternates with N-acetylmuramic acid (MurNAc) with short peptide cross-links to connect glycan polymers. Different bacterial PGNs vary in length of the glycan chain (Fig 1), the position of the peptide cross-links, and the amino acid composition of the peptides (Schleifer and Kandler, 1972). Bacteria also adjust these components and overall structure in response to environmental pressures such as growth phase, media, and presence of antibiotics (Vollmer et al., 2008a).

The PGN of Gram+ bacteria is at least 30 nm thick (Fig. 2a) and multilayered in remarkably different ways (Vollmer et al., 2008a). PGN can be purified mostly as an intact, bag-shaped ‘sacculus’ structure. Average lengths of the glycan chain in Gram+ bacteria range widely from 6 to 1000 sugars (Fig. 2b). Significant amounts of lipotechoic acids and capsular polysaccharides are also found (Vollmer et al., 2008a). Gram+ S. aureus displays a unique arrangement of glycan polymers and peptide cross-links. The peptides are usually 10-100 nm long, and crosslinked with short 12-sugar glycan strands (Vollmer, 2010).

Figure 1. Structure of peptidoglycan.

References: Matias et al., 2003, Matias et al., 2005, Matias et al., 2006.

On the other hand, the PGN sheet of Gram- bacteria is much thinner, being approximately 3 nm thick in *P. aeruginosa* and 6-7 nm thick in some *E. coli*. Only 3 or fewer layers of PGN may be present (Labischinski et al, 1991). Chain lengths of 18 to more than 100 have been found in Gram- bacteria, but most are between 40 and 80 sugars in length (Vollmer et al., 2008a; Fig. 2b). Each glycan strand also has a 1,6-anhydroMurNAc at the terminus.

The peptide crosslinks are always attached to the glycan strands at the lactic acid group of muramic acid. The peptides are most often composed of the 5 amino acids L-Ala-D-Glu-[L-Lys or DAP (diaminopimelic acid)]-D-Ala-D-Ala (Vollmer et al., 2008a), with some differences between Gram- and Gram+ that include L-Orn, meso-Lanthionine, L-2,4-Diaminobutyrate, L-Homoserine, or L-Hydroxylysine. Most Gram+ bacteria also contain up to 7 amino acids in interpeptide bridges that cross-link two adjacent PGN strands.

Common post-assembly chemical modifications appear in the glycan portion (Vollmer, 2008b). Modifications to the peptides and amino acids also exist which include PGN-anchored cell surface proteins. Interestingly, PGN is one of the most important sources of D-amino acids in nature.

**Enzymatic Removal of Cell Debris for Broiler Performance**

Muramidases cleave the beta-1,4 glycosidic bond between N-acetylmuramic acid and N-acetyl glucosamine in the glycan backbone. A novel microbial muramidase (EC 3.2.1.17) was recently selected by an iterative screening process for efficacy, stability and yield. In short, the successful candidate, the new product, Balancius, belongs to the glycoside hydrolase family 25 (GH25), and comes from the fungus *Acremonium alcalophilum* (Cohn et al., 2018).

In one of the first feed trials, Balancius significantly improved 42-day weight gain and feed conversion ratio (FCR) of broilers (Lichtenberg et al., 2017). No changes were detected in total anaerobic bacteria, total aerobic bacteria, enterobacteria and coliforms. Populations of Salmonella, Campylobacter and Clostridia were also unaffected (Lichtenberg et al., 2017).

In another study, Balancius improved broiler performance in a pelleted corn/SBM-based diet (Yegani et al., 2018). In this 42-day trial, 1,280 Cobb broilers were vaccinated with a commercial coccidiosis vaccine and raised in floor pens. Balancius supplementation improved (P < 0.05) body weight gain by 5.0% and FCR by 2.5%. Mortality was unaffected (P > 0.05) by the treatment.

Because Balancius specifically catalyzes the degradation of PGN – and because live bacterial populations were unaffected with this muramidase – these performance improvements are consistent with the destruction of PGN in the dead bacterial remnants in the intestinal contents. Taken with the abundance of PGN in the intestinal contents, it is possible that Balancius reduced any interferences in digestibility function or absorption caused by PGN.

**In the End**

Increasingly, PGN is recognized as a previously overlooked bacterial polymer with important ramifications to GIT microbiota and the digestion of nutrients. PGN is a common constituent in bacterial cell walls, especially in Gram+ bacteria. PGNs are degraded in nature by glycosyl hydrolytic enzymes called muramidases.

The novel product, Balancius, significantly improved body weight gain and feed conversion ratio in broilers fed corn/SBM diets without impacting intestinal microflora. Balancius benefits gastrointestinal functionality and improves performance.