Afterlife of bacterial cell debris: Peptidoglycan in the gastrointestinal tract

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1. The problem: dead bacteria and cell debris in the gut
2. Solving the problem: using a novel microbial muramidase
3. How the solution works
Nikolai Fedorovich Gamaleia (1859-1949)

- Worked in Pasteur's lab in 1886
- Later joined Ilya Mechnikov
- studied the effect of injection of dead bacteria in rabbits and sheep
Afterlife of bacterial cell debris

Live bacteria + Dead bacteria (whole cells + fragments)
A holistic view of microbiota: includes dead bacteria and cell debris

Live bacteria + Dead bacteria (whole cells + fragments)
The problem: dead bacteria and bacterial cell debris in the gut
All parts interact with the host cells in the GIT

Feedstuffs Sep 26, 2018 Understanding threats to poultry performance, Nature Metabolism Jan 2019, Vol 1 34-46
Peptidoglycan (PGN): a major component of all bacteria

- PGN dry weight:
  - Gram- 10%
  - Gram+ 80-90%

- Unique biopolymer only found in bacterial cell wall

- Provides structure, shape and counteracts osmotic pressure

- Abundant in the gut
Peptidoglycan: a complex polymer forms the bacterial cell wall

Gramp+ bacterium

Plasma membrane

Thick peptidoglycan

Structure
Peptidoglycan: a complex polymer forms the bacterial cell wall

Gram+ bacterium

Plasma membrane

Thick peptidoglycan

Structure

Glycan strand

N-acetyl-glucosamine

N-acetyl-muramic acid

L-alanine
D-glutamate
L-lysine
D-alanine

Peptide bridge: can vary depending on bacterial species
Solving the problem
Using a novel microbial muramidase to cleave PGN

- **Gram+ bacterium**
  - Plasma membrane
  - Thick peptidoglycan

**Structure**

- Site of muramidase catalysed hydrolytic cleavage
- Glycan strand
- N-acetyl-glucosamine
- N-acetyl-muramic acid
- L-alanine
- D-glutamate
- L-lysine
- D-alanine

Peptide bridge: can vary depending on bacterial species
A novel microbial muramidase
A novel microbial muramidase

- Hydrolyses peptidoglycan (PGN)
- $\beta$-1,4-N-acetylmuramidase activity
- Lack of apparent antimicrobial potency
- The only solution targeting dead and decomposing bacteria
How the solution works
The novel microbial muramidase degrades peptidoglycan from relevant gut bacteria

* Significant p < 0.05

- **B. gallinarum**
- **E. gallinarum**
- **L. aviarus**
- **L. gallinarum**
- **L. kitasatonis**

Reducing ends (Abs 405 nm)

Origin of purified peptidoglycan

**no enzyme**

**with enzyme**
Microscopy of intact and hydrolyzed peptidoglycan

No enzyme

With enzyme

Enterococcus gallinarum
The novel microbial muramidase depolymerizes peptidoglycan into smaller fragments.
Novel microbial muramidase: Impact on animal physiology

PGNs accumulate in the intestinal tract

“Good” bacteria feeds on smaller PGN debris

Nutrients are now able to be absorbed

Performance improves from better nutrient absorption
In vivo data of a novel microbial muramidase

Muramidase supplementation in broiler chicken diet
- Delivers consistent effects in more than 50 in vivo studies performed globally.
- Increases body weight gain
- Improved utilization of feed (improved FCR)
- Increased digestibility of key nutrients
- A range of other beneficial effects
- Can save globally 9 million tons of CO2 equivalents.
Effects of the novel microbial muramidase can be measured throughout the GIT

- Male Cobb 500, 35 days old
- Muramidase dose: 45 000 LSU(F)/kg
- Diet type: Corn-SBM- wheat (15%) + ionophore as coccidiostat

Source: ME-08/18 VN E. Perez Calvo & R. Aureli
The novel microbial muramidase - first of its kind

- Muramidase enhances gut functionality by cleaning up bacterial debris from GIT
- Muramidase only degrades cell fragments, leaving live bacteria unaffected
- Muramidase catalyzed PGN degradation can be measured in vivo
Thank you
Questions
Further informations about the novel microbial muramidase
Lack of anti-microbial potency confirmed *in vivo* and *in vitro*

**In vitro**
With 8X dose

**In vivo**
With 10X dose

**No detection of antimicrobial potency in MIC assay**
- MIC (Minimal Inhibitory Concentration) assay is the industry-standard measurement of antimicrobial potency
- 7 reference strains recommended by the European Food Safety Authority
- 30 field strains isolated from poultry

**No significant reduction in caecal bacterial counts**
- Total caecal aerobes and anaerobes (CFU/g)
- Enterobacteria, Coliforms, Lactobacilli (CFU/g)
Conformational selection: “Smart” muramidase distinguishes between peptidoglycan conformations

Live cells

Stretched due to pressure inside live cell
(Turgor pressure: up to 20 atm)

Dead cells

Relaxed due to lack of pressure from live cell
Monomer quantification

Chemical hydrolysis to single muramic acid sugars

5 M hydrochloric acid (pH<0) for 24 hours at 100°C

Quantification of muramic acid
Novel method to quantify PGN degradation in digesta samples through muramic acid analysis

- **Ratio** = \(rac{\text{Soluble muramic acid}}{\text{Total muramic acid}}\)
Muramidase vs hen egg white lysozyme
**Chicken type lysozyme = antimicrobial**

Hen egg white lysozyme and other higher animals
GH22
C-like lysozyme (c for chicken)
Hydrolysis of 1→4 beta linkage
Found in milk, saliva, mucus, tears, egg-white
First enzyme, structure was solved in 1960ies
Classical Koshland retaining mechanism containing covalent glycosyl intermediate
Side activity on chitin (also 1→4 beta linkage)
Glu35=cat residue, Asp52 catalytic nucleophile in large cleft
127 aa, 4 disulphide bonds, 5 helical regions (40% of aa), five regions of beta sheet with rc and beta turns
Structure shard with GH19 (chitinases), GH23(lysozymes (goose)), GH124 (cellulases), GH134 (mannanases)
~130 aa

**Novel microbial mura-Midase = not antimicrobial**

Fungal muramidase
GH25
“Chalaropsis”-like lysozyme
Hydrolysis of 1→4 beta linkage
β-1,4-N-acetylmuramidase activities
Likely retaining
Mechanism=neighboring group participation
Structure unrelated to GH22 etc.
TIM-barrel (eight-stranded beta barrel flanked by 6 (normally 8) alpha helices
Long groove in C-terminal face, culminating in deep whole of highly neg electrostatic potential = cat site
DIE motif (D and E cat residues, around pos 100)
~200 aa
Mechanism

Retaining mechanism for a β-glycosidase:

HEWL-koshland mechanism

Novel microbial muramidase: neighbouring group participation