Host defense peptide with anti-microbial and immunomodulatory activities as antibiotic alternatives

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Topics to be discussed

- Antimicrobial and host defense peptides
- Chicken coccidiosis
- Chicken-derived synthetic peptide: cNK-2
- Nanoencapsulation of cNK-2
Issues in poultry production

- AGPs: Supplemented in the feed more than 60 years
- Issues on resistance to antibiotics and consumer’s awareness
- Decreasing in use of AGPs
- Increasing poultry health problem and therapeutic antibiotics use
- Increasing demand for alternatives
Alternatives to antibiotics

Antimicrobial peptides

Probiotics

Prebiotics

Organic acids

Hyperimmune IgY

Others (Bacteriophages, Clay)

Enzymes

Phytogenics (Essential oils, Oleoresins)

Antimicrobial peptides (AMPs) and host defense peptides (HDPs)

- Oligopeptides produced by all known species
- Part of innate immune response
- Broad spectrum antimicrobial activity
- More than 4,000 natural AMPs

Extracted from AMR Centre
Increasing interest and limitations AMPs

- High sensitivity of antimicrobial activities to environment
- Poor bioavailability
- High production cost
- Regulatory hurdles

Mahlapuu et al, 2016. Frontiers in Cellular and Infection Microbiology
Chicken coccidiosis

Necrotic enteritis

> $3.2 billion

> $2.0 billion

2019 Research Priorities of the American Association of Avian Pathologists

Natalie Armour*, Mark Burleson*, Eric Gingerich*, Seiche Genger*, Travis Schaal*, John Gilsson*, Naola Ferguson-Noel* and John Smith*

<table>
<thead>
<tr>
<th>Rank</th>
<th>Score</th>
<th>Subcategory</th>
<th>Research Needs Statement</th>
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<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>Intestinal Health</td>
<td>Develop non-antibiotic strategies to optimize gut health, increase resistance to intestinal pathogens and improve feed conversion</td>
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<tr>
<td>1</td>
<td>4.1</td>
<td>Clostridial Diseases</td>
<td>Investigate risk factors contributing to the development of Necrotic Enteritis, including the role of feed ingredients</td>
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<td>2</td>
<td>4.0</td>
<td>Coccidiosis</td>
<td>Determine the most effective non-ionophore rotation strategies for the control of coccidiosis and subsequent Necrotic Enteritis, for preserving the long-term efficacy of the drugs, and for ameliorating resistance.</td>
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<tr>
<td>3</td>
<td>3.9</td>
<td>Reovirus</td>
<td>Investigate the epidemiology of reoviruses, and the emergence of novel reovirus strains</td>
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<tr>
<td>3</td>
<td>3.9</td>
<td>Histomoniasis</td>
<td>Determine risk factors for the development of Histomoniasis. Determine whether early coccidiosis, breed/strain and sex impact the risk of developing Histomoniasis</td>
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<tr>
<td>3</td>
<td>3.9</td>
<td>Infectious Bronchitis</td>
<td>Determine the epidemiology, risk factors and effective control strategies for nephropathogenic Infectious Bronchitis</td>
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<td>4</td>
<td>3.8</td>
<td>Intestinal Health</td>
<td>Conduct research to understand the intestinal microbiome and immunity, host-pathogen interactions and how various feed ingredients and additives modulate these functions to affect intestinal health</td>
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**NK-lysin: homologue of human granulysin**

NK-lys: homologue of human granulysin

Porcine NK-lysin

Human granulysin

NK-2: Cationic core region of NK-lysin (27 a.a.)

pNK-2 effects on parasites

(Trypanosoma cruzi, Plasmodium falciparum)

NK-lysin homologues have paraticidal effect
### Clusters That Contain More Than 14 ESTs

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<th>Contig ID</th>
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<th>Organism</th>
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Min, Lillevold et al., 2005. Molecular Biotechnology

**Chicken NK-lysin is the most expressed in* Eimeria*-infected intestine**
Chicken NK-lysin: cloning

Chicken NK-lysin expressed in cytotoxic T cells and the intestine

Hong and Lillehoj et al., 2006. Veterinary Immunology and Immunopathology
Antimicrobial activity of cNK-lysin

Chicken NK-lysin effects on *Eimeria* spp.
Chicken NK-lysin-derived peptides

A

B

\[ \text{Number of Sporozoites (X 10^9)} \]

\[ \text{Cont} \quad \text{cNK-1} \quad \text{cNK-2} \quad \text{cNK-3} \quad \text{cNK-4} \]

\[ \mu g/mL \]

\[ \text{24} \quad 49 \quad 15 \quad 31 \quad 19 \quad 38 \]

\[ \mu M \]

\[ \text{25} \quad 25 \]

\[ \text{Viability (%)} \]

\[ E. \text{tenella} \quad E. \text{acervulina} \]

\[ \text{Number of Sporozoites (X 10^6)} \]

\[ \text{Number of Tachyzoites (X 10^6)} \]

\[ \text{1 hr incubation} \quad \text{3 hr incubation} \]

Lee and Lilheiroj et al., 2013. Veterinary Parasitology
Somewhat attenuated antimicrobial activity in physiological salt condition
No cytotoxicity on chicken cells
Immunomodulation of cNK-2

Chicken NK-lys in modulates immune responses

Kim, Lillehoj and Min, 2017. Scientific Report
Immunomodulation of cNK-2 is regulated through MAPK pathways.
Internalization of cNK-2

cNK-2 internalized into host cells

Kim, Lillehoj and Min, 2017. Scientific Report
Endocytosis of cNK-2 is regulated by actin polymerization.
Applications of chicken NK-lysin derived peptide

- Direct killing
- Toward pathogen
- Toward host
- DELIVERY?
- Immuno modulation
  - Chemokine induction
  - Anti-inflammation
  - Signaling pathway activation

DeLivery
Nanoencapsulation of cNK-2

Characterization of NPs
- efficiency
- size
- ζ-potential
- release profile
- stability
- bioactivity
- toxicity

Encapsulated NPs
PLA-PEG

Treatment into chicken intestinal epithelial cells

in-vitro Eimeria infection

Assessment
- antimicrobial activity against sporozoites
- sporozoite invasion
- NPs tracking

Oral delivery to chickens

in-vivo Eimeria infection

Assessment
- body weight gain
- oocyst shedding
- intestinal lesion
- immune response
- NPs tracking
Nanoencapsulation of cNK-2

Chaudhari, Kim, and Lillehoj, unpublished data

Shifted pattern in functional groups
Size ranged 85-120 nm and spherical in shape with smooth surface

Chaudhari, Kim, and Lillehoj, unpublished data
Nanoencapsulation of cNK-2

Retaining immunomodulatory property and slow release

Chaudhari, Kim, and Lillehoj, unpublished data
Nanoencapsulation of cNK-2

active cNK-2
- Antimicrobial
- Immunomodulatory
- Unstable in the body

Encapsulation in nanoparticles

Encapsulated cNK-2
- Retaining functional properties
- Slow release
- Stability
Nanoencapsulation of cNK-2

Encapsulated cNK-2

Chaudhari, Kim, and Lillehoj, unpublished data
Summary

- Chicken NK-lysin has been identified as the most expressed gene in *Eimeria*-infected intestinal lymphocytes.
- Chicken NK-lysin derived peptide, cNK-2 has strong anti-coccidial effects as well as immunomodulation.
- Oral administration of nanoencapsulated cNK-2 increased growth performance and reduced intestinal lesion and oocyst shedding in coccidiosis.
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