# Strategies to reduce antibiotics in swine production in China

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**Dec 17 2019 Bangkok**

# Briefing of Meritech and Founder-Dr Gene Jin

More than 30 years experiences in animal and feed industry worldwide; .

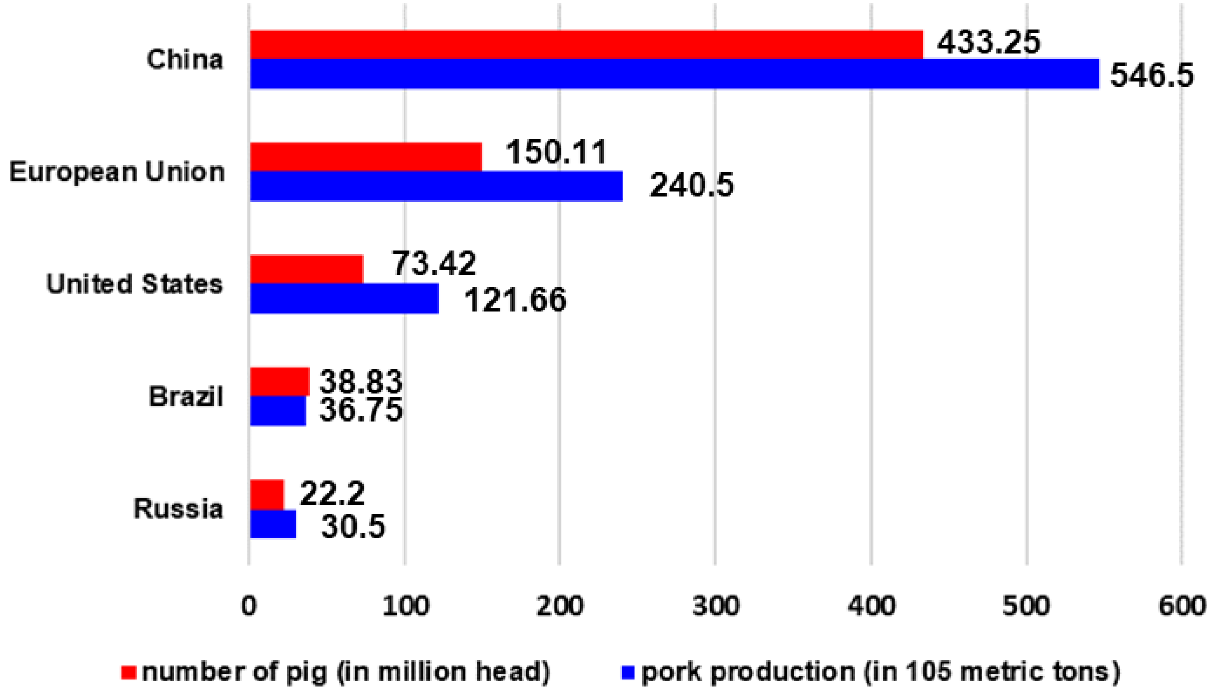
- Working Experience
  - Singapore: Danisco-Asia Pacific Tech Mgr;
  - Malaysia: Biomin-Asia Tech Mgr;
  - China: Lucta-Managing Director China;
- bEducation
  - Canada: Post-Doc of Mcgill/Manitoba Uni;
  - Malaysia: PhD of University Putra Malaysia;
  - China: MSc/BSc: Nanjing Agri Uni (top 2);
  - MBA-China-Europe Itnl Business School; Guelph Uni of Canada;
- From 2007 till now: co-founder and managing director of Meritech

Meritech Intl: Biotech-based company to developing green feed additives worldwide;

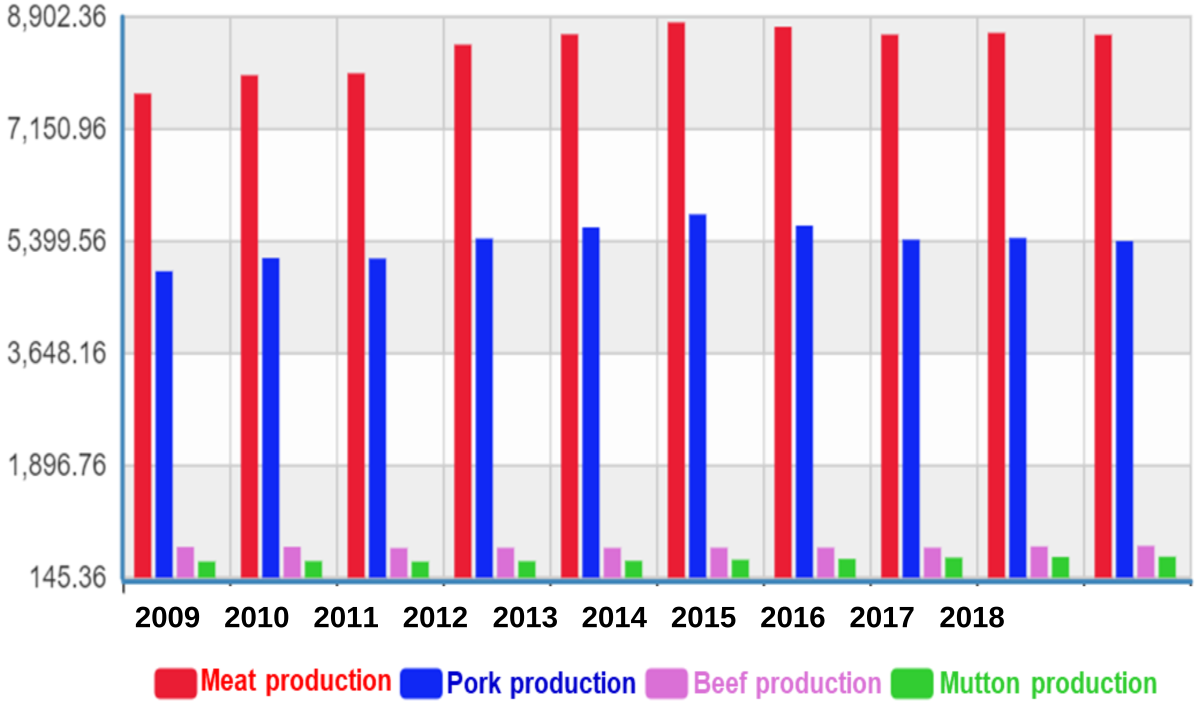
- Our operation offices are located in both Singapore and China.
- Meritech was set up in 2007 and market to China, Vietnam, Korea, Taiwan, Malaysia, The Philippines, Sri Lanka and so on.
- Our production is in American Industry Park, Guangzhou.

# China Animal Industry

## Pork production in numbers (2018)




## Meat production in China in the past decade (million tons)



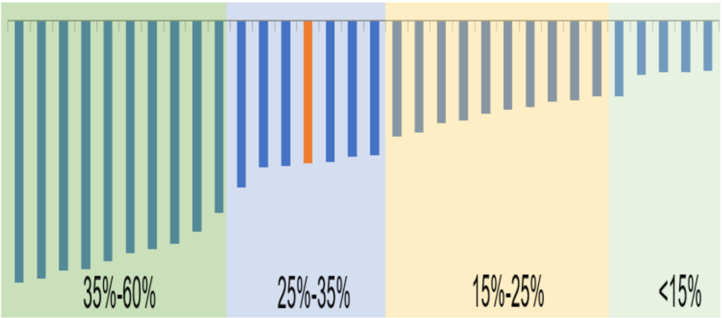

**Swine is the most important food animals in China!**

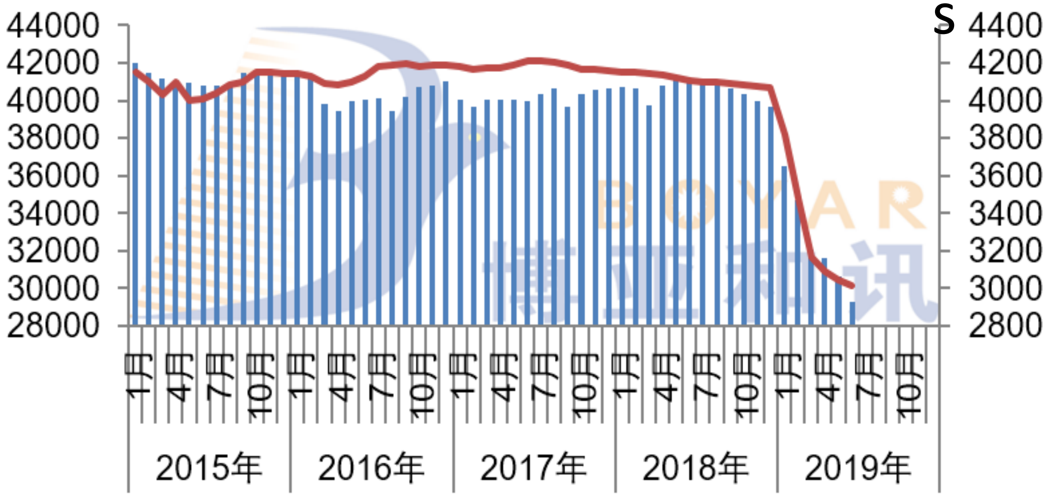
Data adapted from NBS by Prof Wang JJ

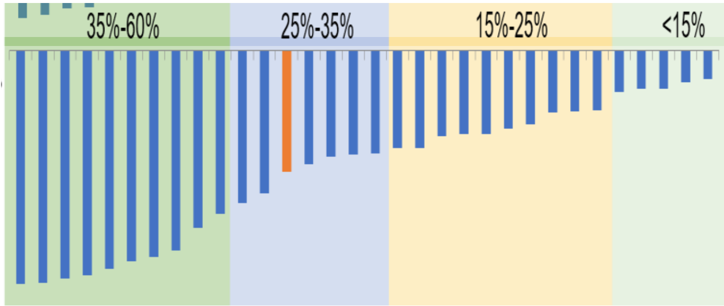
# Current Swine Production after ASF

Fattening

Sows

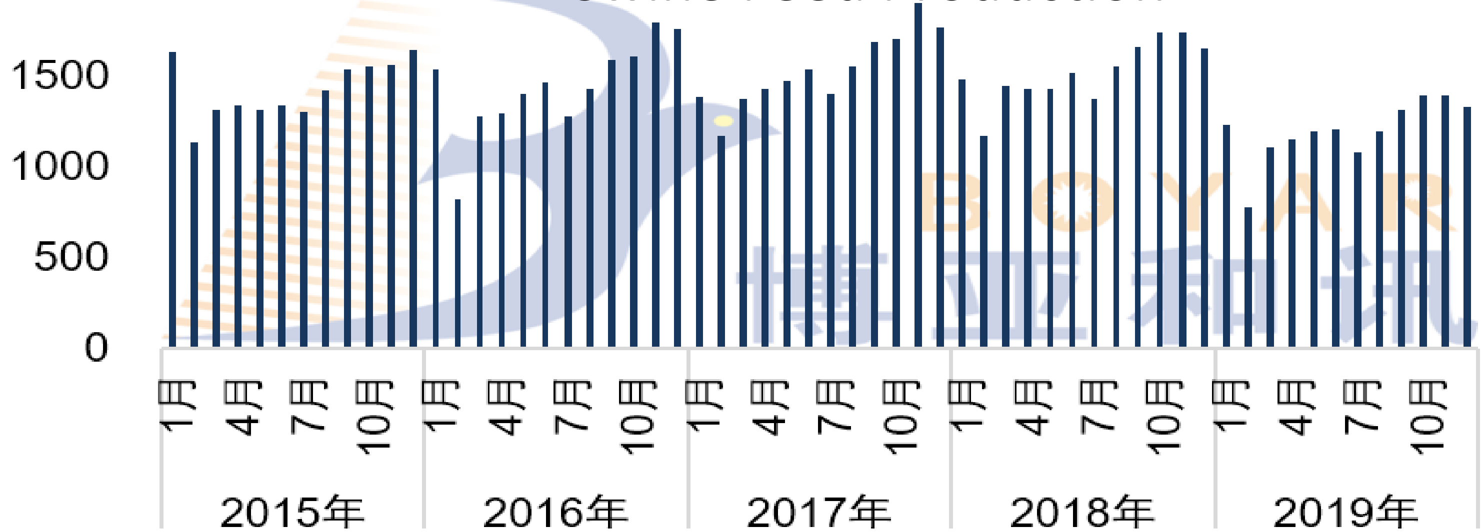



## Swine Feed Production

10k ton




Data: Boyar 2019

# Regulations limiting the use of AGPs: antibiotic resistance & resides

| | |
|---|---|
| Sweden | Complete ban, 1986 |
| European Union | Complete ban, 2006 |
| Australasia | Case -by- case |
| North America | Case -by- case, *in legislation* |
| Korea | Complete ban, 2012 |
| Vietnam | banned |
| Indonesia | banned |
| Thailand | ? |
| China | 2020 June 30；no AGP use |
| FAO/WHO | Monitor, risk assessment |

**China Regulations**

- **2017: stop liscensing Colistin GP**
- **2020 Jan 01: stop liscensing all AGPs**
- **2020 July 1st: stop AGPs use**
- **No liscense; no production no use no export?**

# Withdraw of antibiotics from animal feed

- **If no alternative technologies to be developed!!!**
  - **Reducing productivity** 
  - **Increasing cost** 
  - **Increasing disease incidence** 
  - **Economic loss by farmers** 
- **There is need to develop alternative technologies for the sustainable growth of animal industry!!!**

# Understanding gut health is key in AGP-free diets

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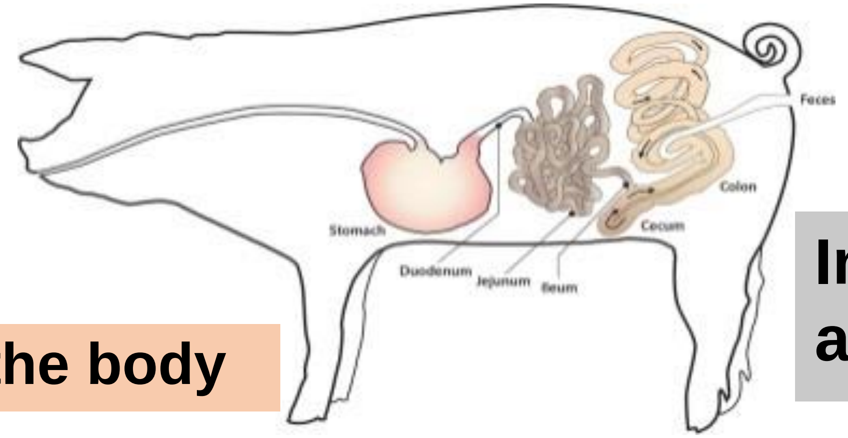
## The gastro-intestinal tract (GIT)

**Dictates health and well-being of an animal**




**Largest immune organ of the body**

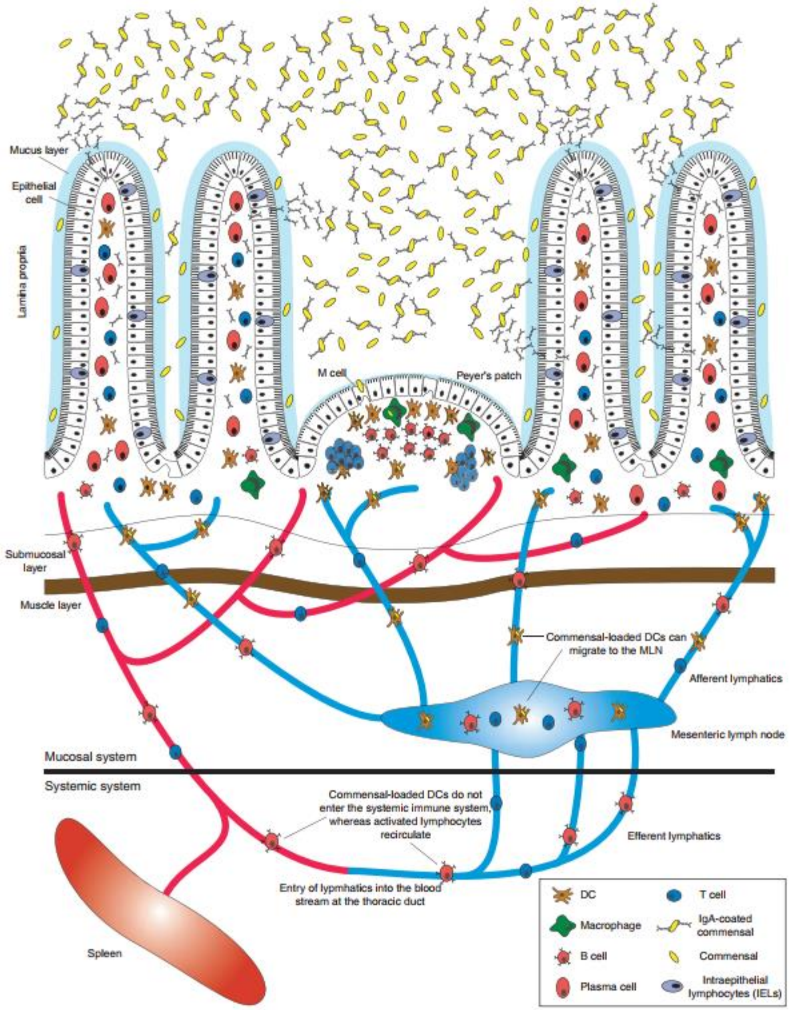
**Interface between non-sterile and sterile environments**

**Needs a balanced microflora to function efficiently**

**Bacteria, viruses, fungus, protozoa**

# Intestinal immune geography

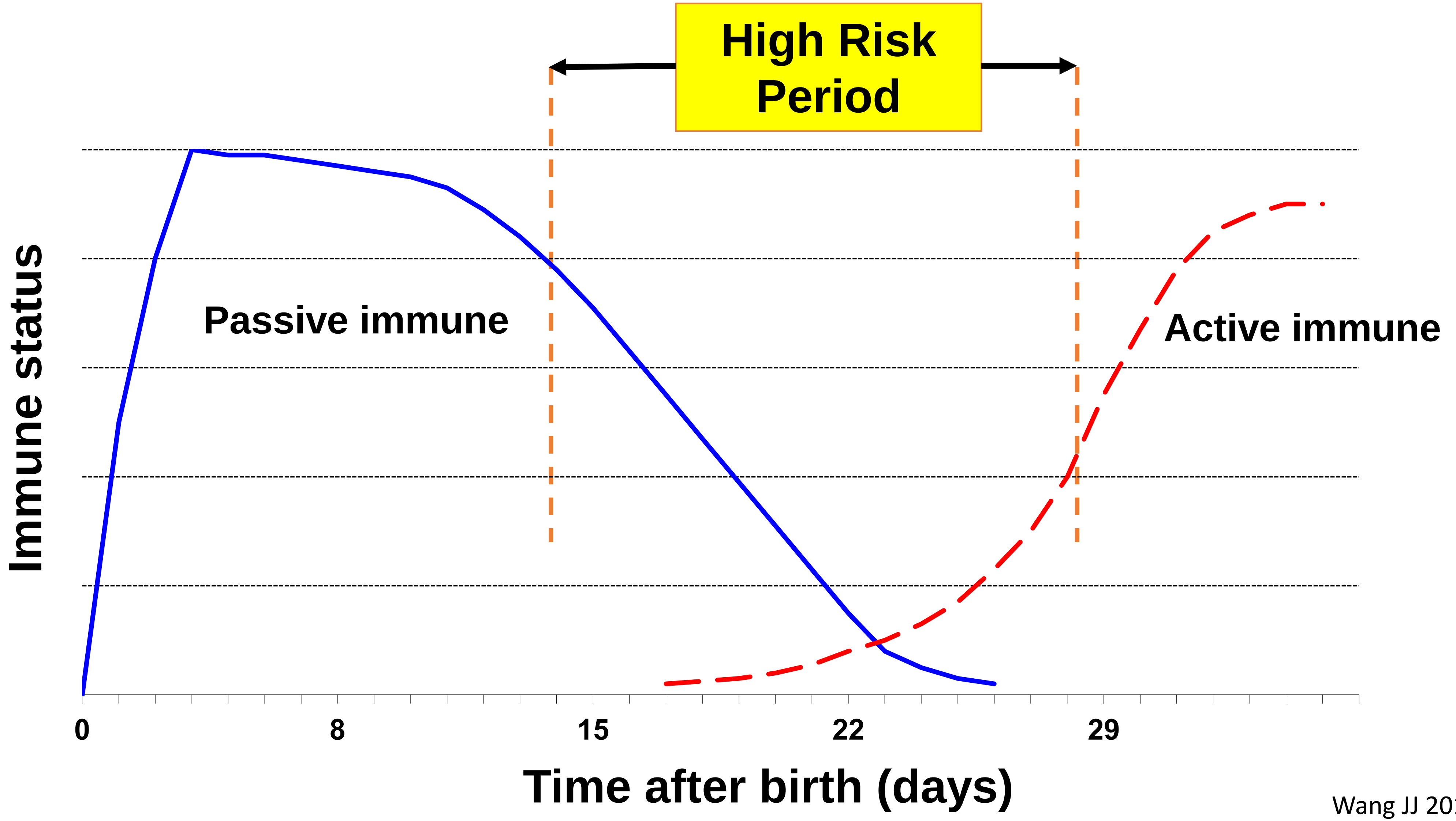
## The body’s second brain




**Commensal bacteria can mediate intestinal immunity**

**Macpherson *et al*., 2005**

# Changing immune systems

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# Strategies to formulate piglets AGP-free diets

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**Factors leading to PWS**

**LOW FEED INTAKE**
**Infections, Stress**

=> stimulate early feed intake and use highly digestible energy sources

**GUT WALL DAMAGE**

**absorption of antigens ↑**

=> use highly digestible protein and fat sources

**inflammation ↑**

**digestion and absorption ↓**

=> reduce Crude Protein and Fermentable carbohydrate content

**substrate ↑**

**microflora ↑**

=> feed restriction and increase retention time stomach

**colonisation resistance ↓**

=> functional feedstuffs and feed additives

**growth + adhesion of pathogens ↑**
**e.g. E.coli**

**toxine production ↑**

**DIARRHOEA, INFECTIONS**

# Suggestions to modify feed formulation for piglets

1. ZnO and antibiotic usage can be reduced via feed formulation (reduction crude protein, soluble fibers and increased u/s ratio) and feed additives (organic acids, phytogenics, MCFA)
2. Feedstuff choice can increase feed intake and improve gut health (fish meal, plasma protein, whey, highly digestible plant proteins & fats, synthetic amino acids)
3. Functional feed additives can improve nutrient digestion, promote gut development and/or reduce the growth of pathogenic bacteria
4. Feed processing improves digestion and feed efficiency i.e. pelleting/expanding grains, spray dried fats
5. Inert carbohydrate sources (rice/oat/sunflower seed hulls, straw) can be used in prestarter feeds to reduce the energy content, increase feed intake and increase gut development.

Donpengberg 2019

# One objective; Two targets; Multiple nutritional tools

=> functional feedstuffs and feed additives (antibiotics)

gut microflora

objective: Good Gut Health

no diarrhoea
better feed intake
better FCR
better profit

2) substrate

=> reduce Crude Protein and Fermentable carbohydrate content
=> stimulate early feed intake and use highly digestible
=> use highly digestible protein and fat sources

=> feed restriction and increase retention time stomach

Jin 2017-2019

# Alternative to antibiotics in China

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- **Probiotics**: ***Lactobacillus, Bifidobacterium, Enterococcus…***
- **Antimicrobial peptides**
- **Organic acids: Benzoic acid, acetate, propionate, butyrate…**
- ***Phytogenics***
- **Prebiotics: FOS, isomalto-oligosaccharides...**
- **Synbiotics: Probiotics + prebiotics**
- **Feed enzymes: Specific enzymes, protease, amylase and lipase…**
- **Minerals: Copper sulfate, zinc oxide…**
- **Bacteriophage**
- **Therapeutic antibodies: egg-yolk antibodies**
- **….**

# Danish research on feed additives

Piglets 7-30 kg

| | No. of studies | % Change in daily gain |
|---|---|---|
| Antibiotics | 15 | +11 |
| Organic acids | 40 | +7.1 |
| **Phytogenics** | **19** | **+2.6** |
| Enzyme | 9 | +2.1 |
| Microbial culture | 14 | +1.0 |
| Oligosaccharides | 2 | ? |

De Lange et al., 2010； Kjeldsen et al., 2018

# Organic acids

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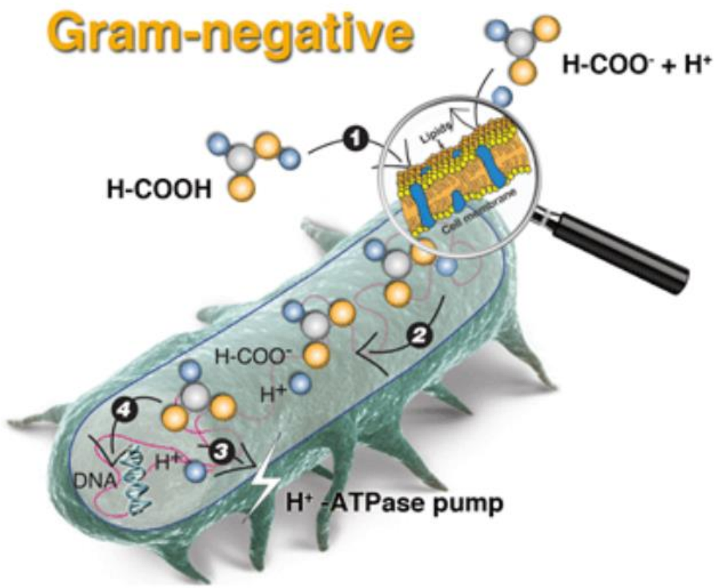
✦ **The first application of organic acids in monogastric animal production was aimed at the preservation of the feed against microbial spoilage.**

- ✓ propionic acid
- ✓ butyric acid
- ✓ sorbic acid
- ✓ acetic acid
- ✓ succinic acid
- ✓ **benzoic acid**
- ✓ lactic acid
- ✓ formic acid
- ✓ citric acid
- ✓ fumaric acid




✦ **But due to the broad antimicrobial activity of organic acids these compounds were recognized as particularly useful to improve performance *via* a modulation of the gut microflora.**

# Organic acids: antibacterial mechanisms

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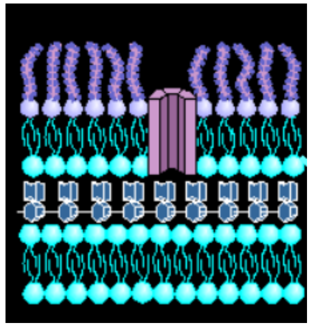
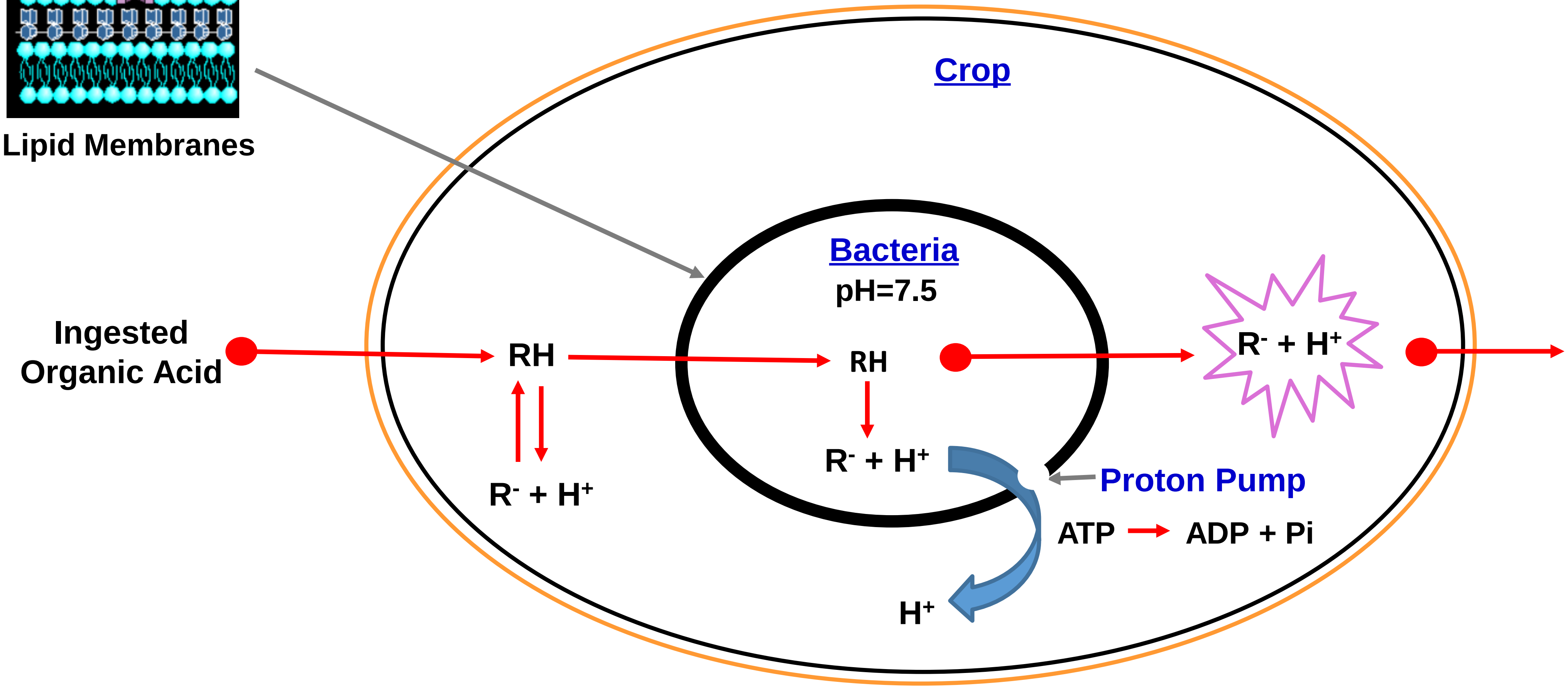
## Biocidal mode of action of organic acids

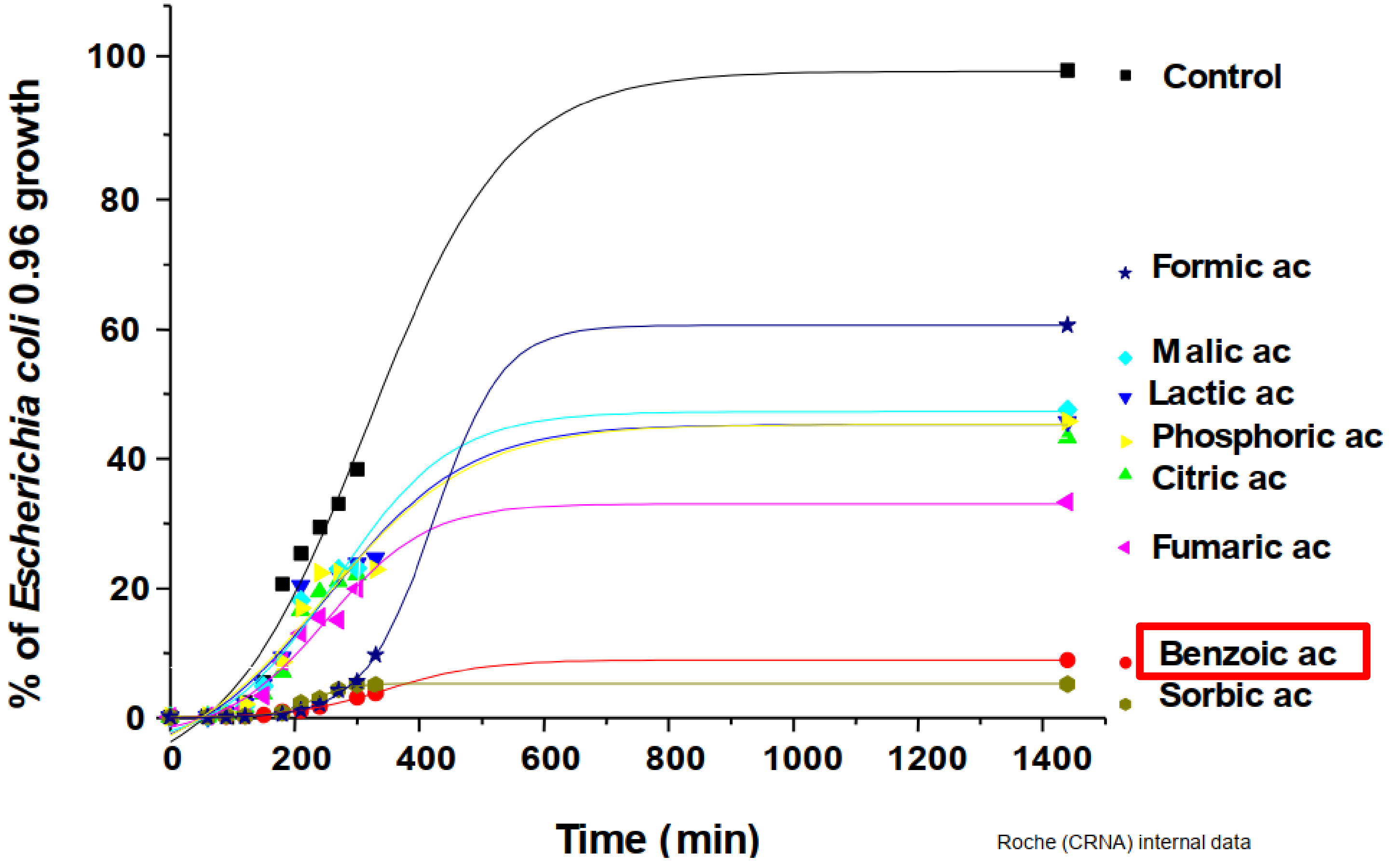


Lipid Membranes




Vinus *et al*., 2017

# MIC of most common organic acids




Roche (CRNA) internal data

**Antimicrobial activity of organic acids (1 mg/ml) on *Escherichia coli* 0.96**

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# Probiotics

✦ **Probiotics** have been defined by the **World Health Organization** as "microorganisms which, administered live and in adequate amounts, confer a benefit to the health of the host."

✦ **Mechanisms**

- destroy pathogenic microorganisms by producing antimicrobial compounds (bacteriocins and organic acids)
- improve gastrointestinal microbial environment by adherence to intestinal mucosa thereby preventing attachment of pathogens
- competing with pathogens for nutrients stimulate the intestinal immune responses
- improve the digestion and absorption of nutrients

# Common probiotics used in swine industry

| Genus | Species |
| --- | --- |
| *Lactobacillus* | *L. acidophilus; L. casei; L. delbrueckii sub sp. Bulgaricus; L. brevis; L. curvatus; L. cellobiosus; L. fermentum; L. plantarum; L. reuteri; L. salivarius sub sp. thermophilus; L. gasseri* |
| *Bifidobacterium* | *B. bifidum; B. adolescentis; B. animalis; B. infantis; B. longum; B. pseudolongum; B. thermophilum* |
| *Lactococus* | *L. cremori; L. lactis* |
| *Enterococcus* | *E. faeciu* |
| *Bacillus* | *subtilis; coagulans; cereus; licheniformis* |
| *Yeast* | *Saccharomyces cerevisiae; Aspergillus oryzae* |

**Dowarah *et al*., 2017**

# *In vitro* antibacterial activity of the most widely used probiotic bacteria

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| | *Lactobacillus acidophilus* | *Lactobacillus plantarum* | *Lactobacillus rhamnosus* | *Lactobacillus sakei* | *Lactobacillus casei* | *Lactobacillus gasseri* | *Lactobacillus johnsonii* |
|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | + | + | - | - | - | + | + |
| *Listeria monocytogenes* | + | + | - | + | - | - | + |
| *Salmonella spp* | + | + | - | - | + | + | + |
| *Shigella spp* | + | + | .. | - | .. | .. | + |
| *Klebsiella spp* | + | + | + | - | + | .. | .. |
| *Escherichia coli* | + | + | + | + | + | + | + |
| *Pseudomonas aeruginosa* | + | + | - | - | - | - | - |
| *Yersinia spp* | + | - | - | - | - | - | + |
| *Clostridia spp* | - | + | - | - | - | + | .. |

Angelakis *et al*., 2013

# Effect of feeding different *Lactobacillus spp.* in various categories of pigs

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| Application | Strain | Probiotic Effects | References |
| --- | --- | --- | --- |
| Neonatal piglets | *L. fermentum* I5007 | increase average weight gain, improve intestinal immunity | Liu *et al.*, 2014 |
| | *E. faecium* EF1 | induce a strong anti-inflammatory response in the small intestine | Huang *et al.*, 2012 |
| | *L. casei* | decrease the number of *E. coli* colonizing jejunal mucosa of gnotobiotic piglets | Bomba *et al.*, 1999 |
| Weaned piglets | *L. reuteri* BSA131 | improve weight gain and feed conversion, reduce the number of fecal coliforms | Chang *et al.*, 2000 |
| | LAB complexes | improve growth performance, increase apparent ileal digestibility of crude protein, crude fiber and organic matter | Giang *et al.*, 2010 |
| | *L. rhamnosus* GG | ameliorate diarrhea, increase sIgA concentrations and attenuate the elevation of serum IL-6 induced by *E. coli* K88 | Zhang *et al.*, 2010 |
| | *L. amylovorus* and *E. faecium* | increase monounsaturated and polyunsaturated fatty acids, modify and improve the fatty acid profile of pig meat | Ross *et al.*, 2012 |
| Growing-finishing pigs | *L. plantarum* ZJ316 | improve weight gain and feed conversion, reduce the incidence of diarrhea, improve meat quality | Suo *et al.*, 2012 |
| | LAB complexes | increase average dairy gain, improve feed conversion, increase digestibility of crude protein and organic matter | Giang *et al.*, 2011 |
| | *E. faecium* SF68 | increase nutrient digestibility and decrease fecal $NH_3$-N, $H_2S$ and volatile fatty acid concentrations | Chen *et al.*, 2006 |

# The scenario of using probiotics for swine production

**Probiotics**

**Intestinal morphology**
Longer villus (V) height, deeper crypt (C) depth and smaller V:C ratio

**Antioxidant status**
Diminish activity of reactive oxygen species (ROS) via production of superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione and thioredoxin

**Growth performance**
Improve weight gain, feed intake and feed efficiency.

**Immune status**
Enhance cell mediated immune (CMI) and humoral immunity (HI) with increase blood IgM, IgG, proinflammatory cytokines and CD4+ lymphocyte and reduce tumour necrosis factor-α

**Post weaning diarrhea (PWD)**
Reduce PWD by decrease in gut pH, $NH_3$-N and increase branch chain fatty acid (BCFA)

**Intestinal microflora and gut health**
Improve population of *Lactobacilli*, *Bifidobacterium* and reduce *E. coli*, *Enterobacteria* count in gastrointestinal tract (GIT).

Dowarah *et al*., 2017

# Antimicrobial peptides (AMPs)

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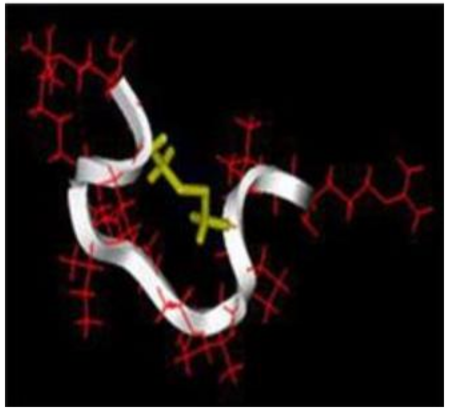
- AMPs are small biological molecules (<10 kDa, 12–50 amino acids) with a broad-spectrum of activity against bacteria, fungi, protozoa, and some viruses

- AMPs are widely regarded as the alternative to antibiotics



- 2012, in Paris, the 1st symposium "International symposium on alternatives to antibiotics" organized by WHO concluded "antimicrobial peptide is a potential antibiotic alternative".

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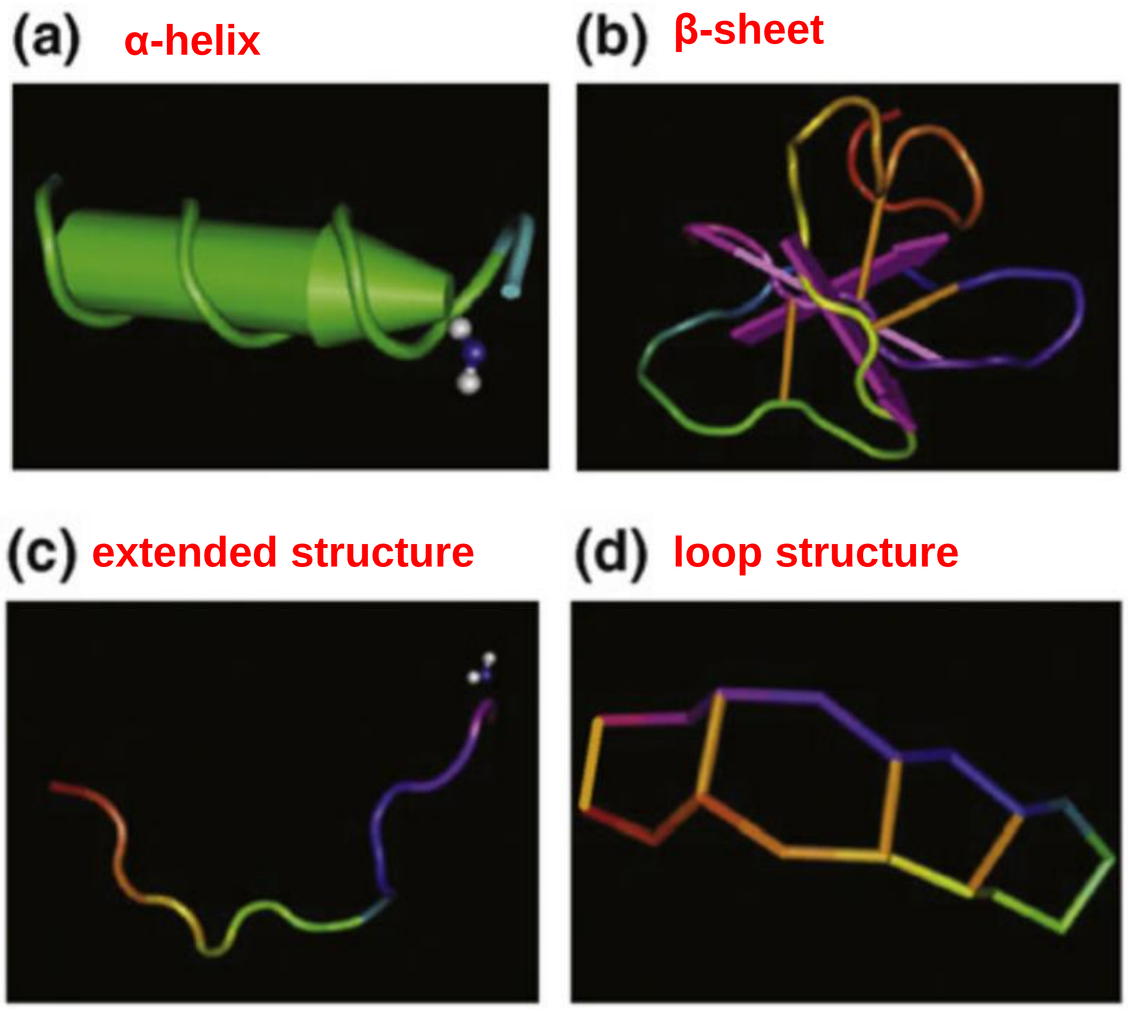
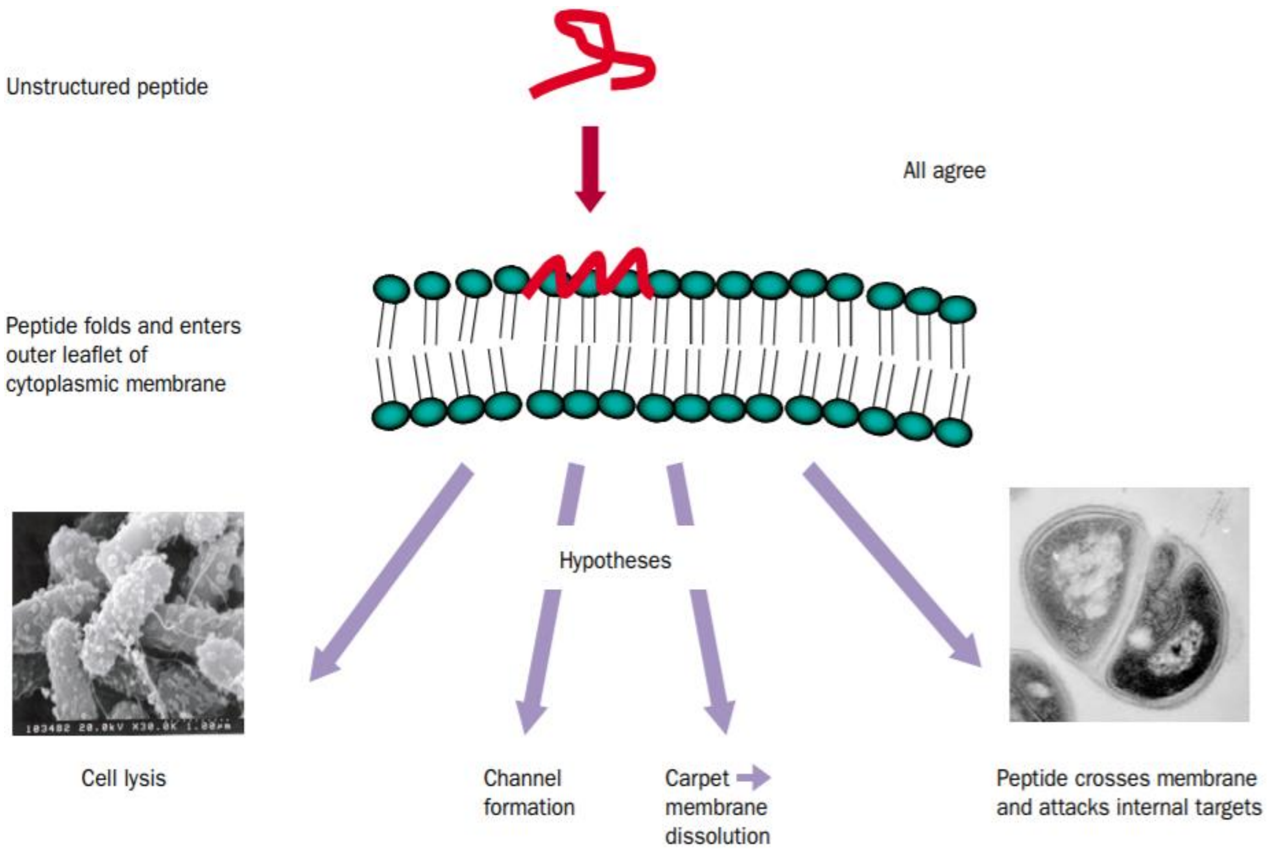
# AMPs: structure and mechanisms




**Structure of antimicrobial peptides (AMPs)**




**Antibacterial mechanisms**

Hancock, 2001; Lai *et al*., 2008

# Effects of AMPs on the growth performance of weanling pigs

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| Antimicrobial Peptide (AMP) | Dose, mg/kg | Treatment Effects (%, Compared to Control) | | | References |
|---|---|---|---|---|---|
| | | ADG | ADFI | F:G | |
| AMP-A3 | 60<br>90 | 2<br>5 | 1<br>2 | 0<br>-5 | Yoon *et al.*, 2012 |
| AMP-P5 | 40<br>60 | 4<br>8 | 1<br>3 | -2<br>-5 | Yoon *et al.*, 2013 |
| AMP microcin J25 | 2 | 7 | 0 | -8 | Yu *et al.*, 2017 |
| Lactoferrin | 1000 | 34 | 17 | -15 | Wang *et al.*, 2007 |
| Lactoferrin | 1000 | 42 | 21 | -17 | Wang *et al.*, 2006 |
| Bovine lactoferrin | 1250<br>2500 | 16<br>13 | 15<br>13 | 0<br>0 | Wang *et al.*, 2008 |
| Bovine lactoferrin-lactoferrampin | 100 | 24 | 17 | -6 | Tang *et al.*, 2007 |
| Composite antimicrobial peptides | 4000 | -6 | -17 | -15 | Xiao *et al.*, 2013 |
| Composite antimicrobial peptides | 400 | 0 | 0 | -2 | Shi *et al.*, 2017 |
| Cecropin AD | 400 | 4 | 1 | -3 | Wu *et al.*, 2012 |
| Recombinant plectasin | 60 | 61 | 38 | -10 | Wan *et al.*, 2016 |

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# Effects of AMPs on the gut microbiota in weanling pigs

| Antimicrobial Peptide | Treatment Effects | References |
|---|---|---|
| Lactoferrin | Reduced total viable counts of small intestine *E. coli* and *Salmonella* in the small intestine | Wang *et al.*, 2007 |
| Bovine lactoferrin | Decreased the counts of *E. coli* in the ileum, cecum and colon and increased the counts of *Lactobacilli* and *Bifidobacteria* in the ileum, caecum and colon | Tang *et al.*, 2007 |
| AMP-A3 | Reduced total anaerobic bacteria, coliforms and *Clostridium spp*. in the ileum, cecum and feces | Yoon *et al.*, 2012 |
| AMP-A5 | Reduced fecal and intestinal coliforms and caecal *Clostridium spp* | Yoon *et al.*, 2013 |
| AMP microcin J25 | Decreased the counts of *E. coli* in the feces, increased the counts of *Lactobacilli* and *Bifidobacteria* in the feces | Yu *et al.*, 2017 |
| Potato protein | Decreased viable counts of total bacteria, coliforms and *Staphylococcus spp*. in cecum and rectum | Jin *et al.*, 2008 |
| Cecropin AD | Decreased total aerobes while increasing total anaerobes in the ileum and increased the numbers of *Lactobacillus* in the cecum | Wu *et al.*, 2012 |
| Recombinant plectasin | Increased the abundance of *Bifidobacterium* in the ileum | Wan *et al.*, 2016 |
| Composite AMP | Decreased the counts of *E. coli* in the feces | Shi *et al.*, 2017 |

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# Development of phytogenics prior to production

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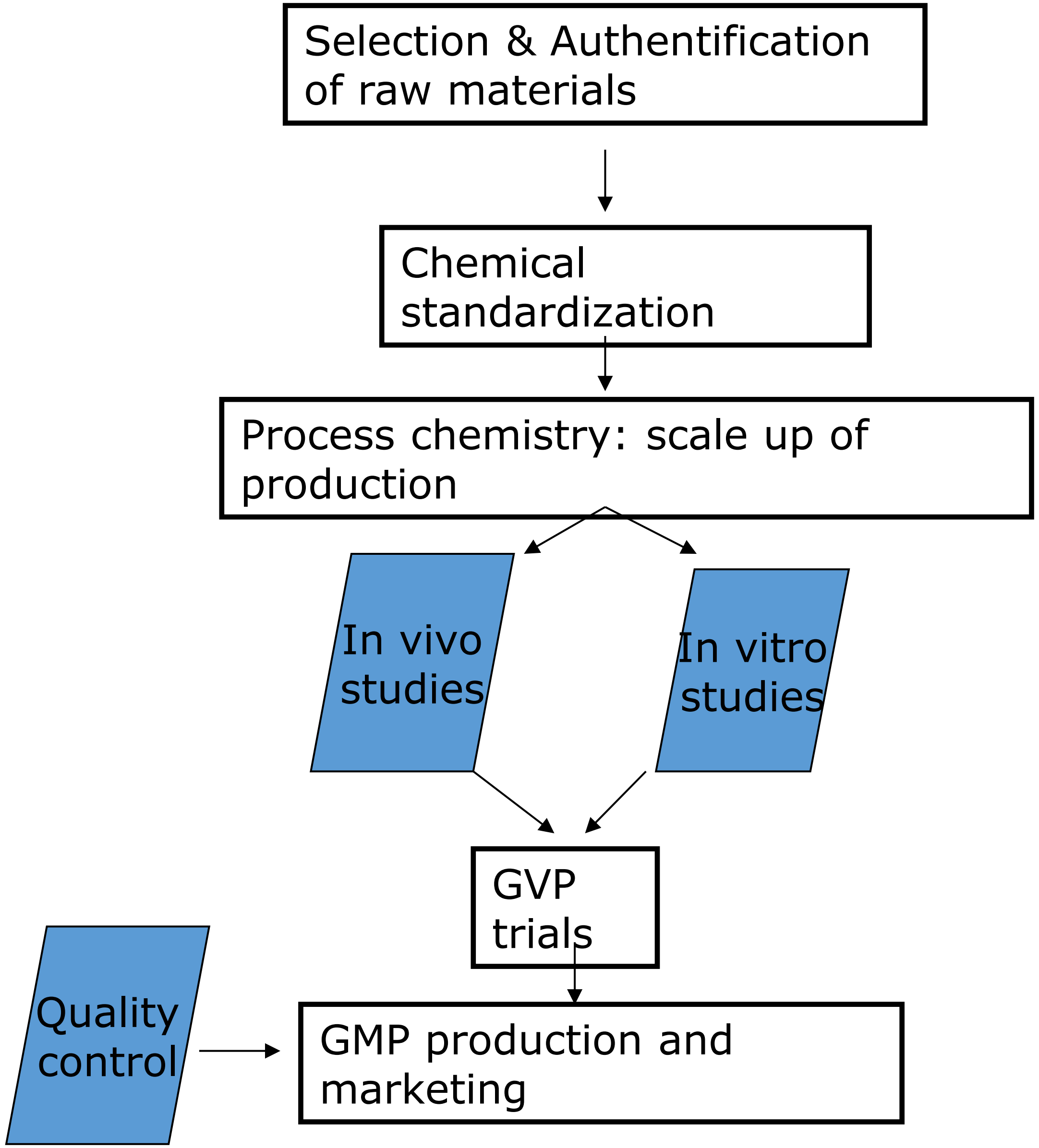
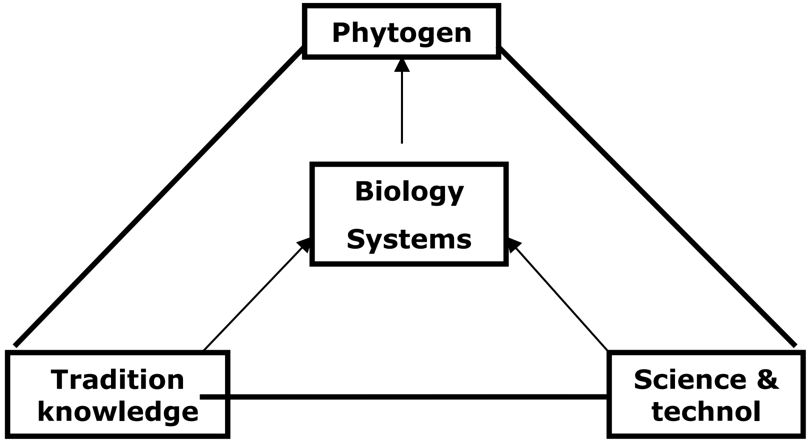
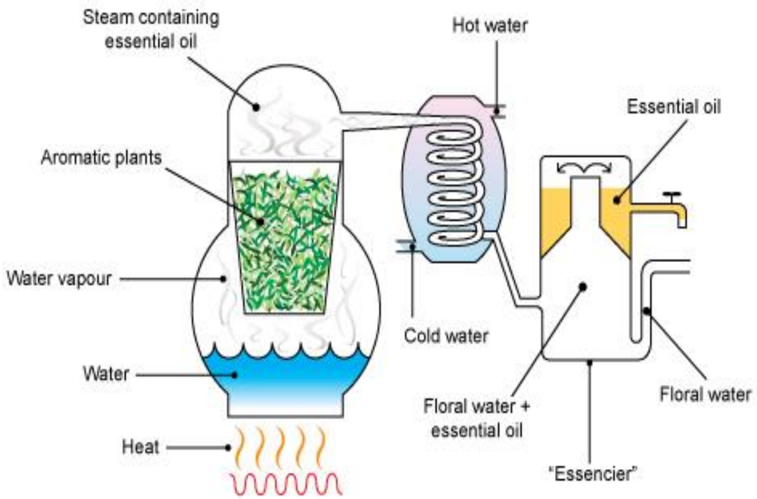
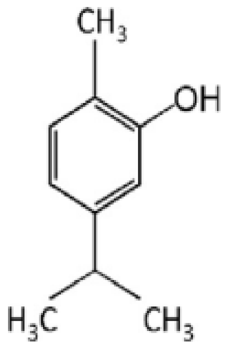
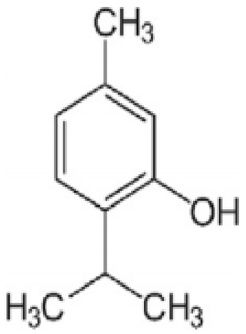
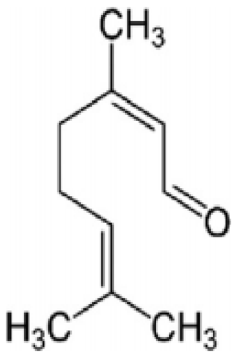
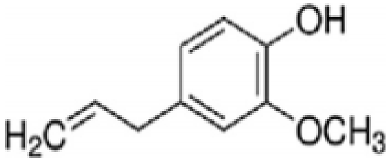
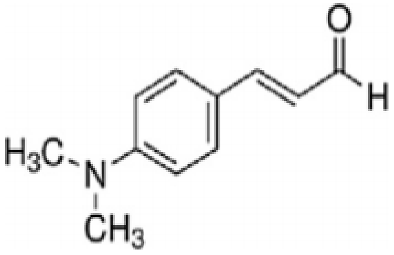


# Chemical properties of essential oils popularly used in pig feeds

| Compound | Carvacrol | Thymol | Citral | Eugenol | Cinnamaldehyde |
|---|---|---|---|---|---|
| Chemical structure |  |  |  |  |  |
| Formula | $C_{10}H_{14}O$ | $C_{10}H_{14}O$ | $C_{10}H_{16}O$ | $C_{10}H_{12}O_2$ | $C_9H_8O$ |
| Molecular mass, g/mol | 150.2 | 150.2 | 152.2 | 164.2 | 132.2 |
| Density, kg/m$^3$ | 976 | 969 | 893 | 1,067 | 1,050 |
| Melting point, °C | 0 to 2 | 49 to 52 | −10 | −12 to −10 | −7.5 |
| Boiling point, °C | 234 to 238 | 232 to 233 | 229 | 253 | 246 to 251 |
| Vapor pressure at 20 °C, Pa | 35 | 250 (50 °C) to 133 (64 °C) | 22 | 133 (78 °C) | 3.85 |
| Solubility in water, g/L | 0.83 to 1.10 | 0.85-1.01 to 1.4 (40 °C) | 0.59 | 0.80 to 2.41 | 1.42 to 1.45 |
| Solubility in ethanol, g/L | Good | 1,000 | Good | 500 (in 70%), good | 150 (in 60%), good |
| Octanol/water partition Coefficient, log $K_{ow}$ | 3.38 to 3.64 | 3.30 | 2.8 to 3.0 | 2.99 | 1.9 |
| pKa value | 10.4 | 10.4 | – | – | – |
| Physical appearance at room temperature | Colorless to pale yellow liquid | White crystalline powder or large colorless crystals | Pale yellow liquid | Colorless or pale yellow, thin liquid | Clear yellowish liquid |

**Omonijo *et al.*, 2017**

# Minimum inhibition concentration (MIC)

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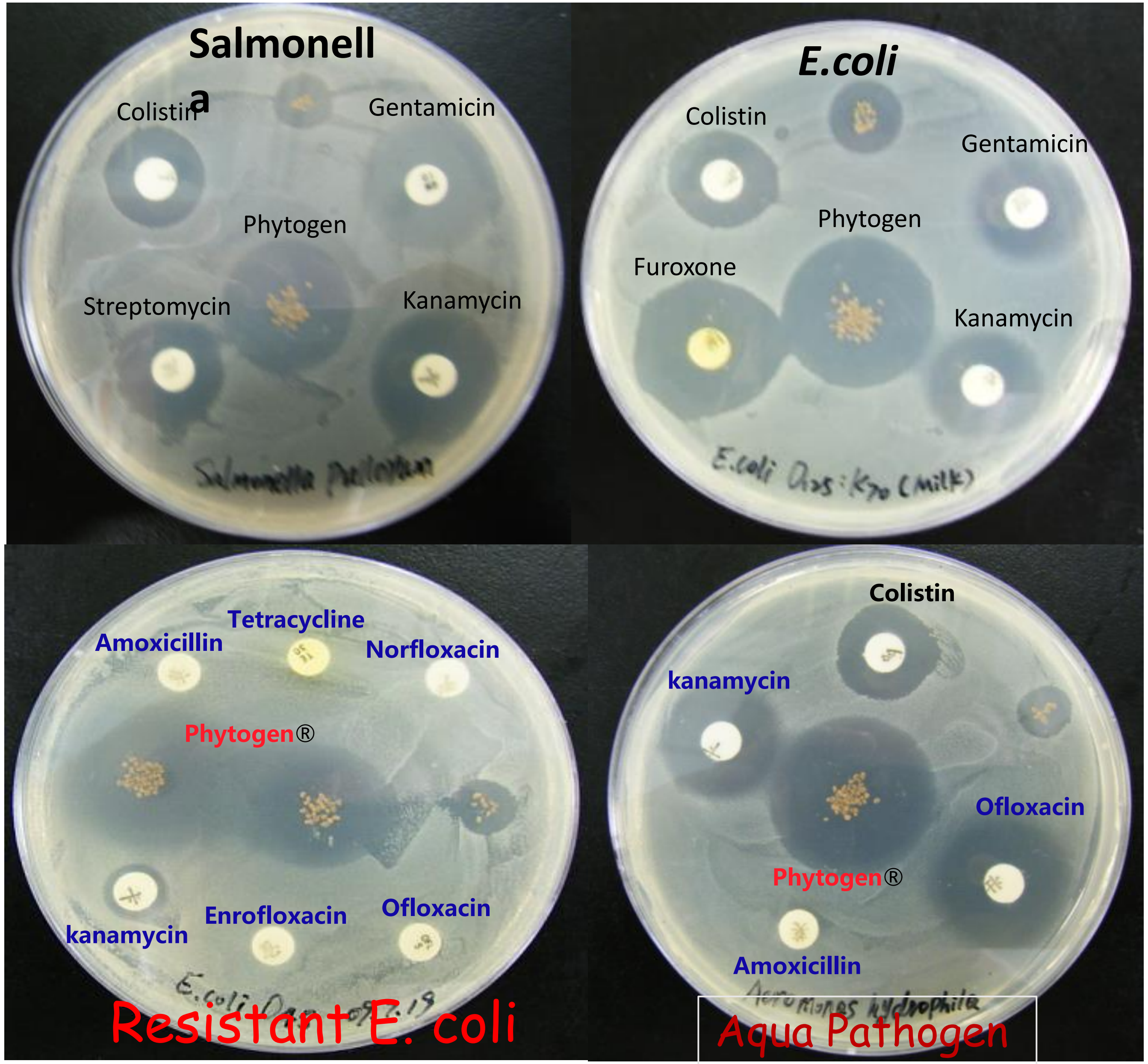
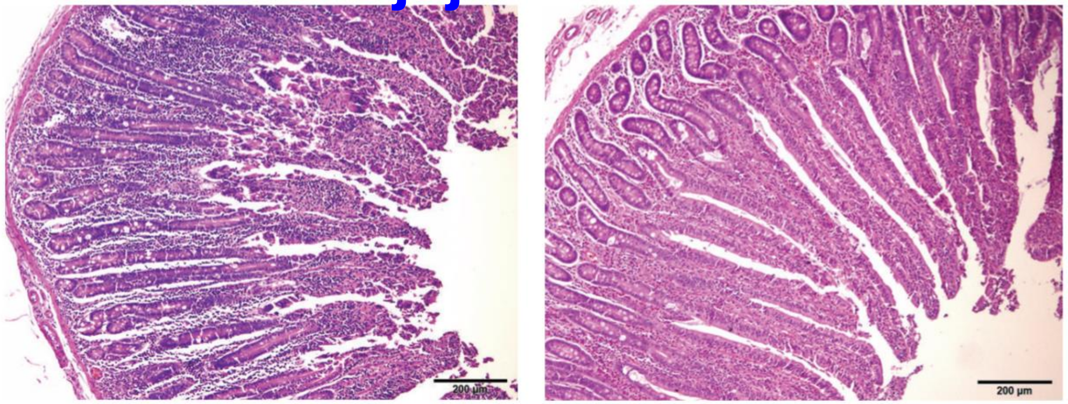
| Product | Pathogenic microbe | Gram | MIC (unit) | MIC (#) | Reference |
|---|---|---|---|---|---|
| Thymol | *Lactococcus piscicum* | + | mg/L | 320 | Navarrete et al., 2010 |
| | *Streptococcus phocae* | + | mg/L | 640 | Navarrete et al., 2010 |
| | *Flavobacteriaum psychrophilum* | – | mg/L | 320 | Navarrete et al., 2010 |
| | *Vibrio anguillarum* | – | mg/L | 80 | Navarrete et al., 2010 |
| | *Vibrio parahaemolyticus* | – | mg/L | 320 | Navarrete et al., 2010 |
| | *Pseudomonus* sp. | – | mg/L | 640 | Navarrete et al., 2010 |
| | *Brachyspira hyodysenteriae* | – | mmol/L | 1.25 | Vande Maele et al., 2016 |
| | *Escherichia coli* 0157:H7 | – | μg/mL | 166 | Si et al., 2006 |
| | *Salmonella typhimurium* DT104 | – | μg/mL | 233 | Si et al., 2006 |
| | *Escherichia coli* K88 | – | μg/mL | 100 | Si et al., 2006 |
| | *Lactococcus lactis* | + | mg/L | 1,280 | Navarrete et al., 2010 |
| Eugenol | *Vibrio* sp. | – | μg/mL | 156 | Seongwei et al., 2009 |
| | *Escherichia coli* | – | μg/mL | 625 | Seongwei et al., 2009 |
| | *Salmonella* | – | μg/mL | 156 | Seongwei et al., 2009 |
| | *Pseudomonas* sp. | – | μg/mL | 325 | Seongwei et al., 2009 |
| | *Edwardsiella tarda* | – | μg/mL | 56 to 125 | Seongwei et al., 2009 |
| | *Aeromonas hydrophilla* | – | μg/mL | 625 | Seongwei et al., 2009 |
| | *Brachyspira hyodysenteriae* | – | mmol/L | 2.5 | Vande Maele et al., 2016 |
| | *Escherichia coli* 0157:H7 | – | μg/mL | 466 | Si et al., 2006 |
| | *Salmonella typhimurium* DT104 | – | μg/mL | 400 | Si et al., 2006 |
| | *Escherichia coli* K88 | – | μg/mL | 300 | Si et al., 2006 |
| Carvacrol | *Listonella anguillarum* | – | μg/mL | 25 | Volpatti et al., 2013 |
| | *Brachyspira hyodysenteriae* | – | mmol/L | 1.25 | Vande Maele et al., 2016 |
| | *Escherichia coli* 0157:H7 | – | μg/mL | 283 | Si et al., 2006 |
| | *Salmonella typhimurium* DT104 | – | μg/mL | 167 | Si et al., 2006 |
| | *Escherichia coli* K88 | – | μg/mL | 100 | Si et al., 2006 |
| Cinnamaldehyde | *Brachyspira hyodysenteriae* | – | mmol/L | 0.31 | Vande Maele et al., 2016 |
| | *Escherichia coli* 0157:H7 | – | μg/mL | 133 | Si et al., 2006 |
| | *Salmonella typhimurium* DT104 | – | μg/mL | 100 | Si et al., 2006 |
| | *Escherichia coli* K88 | – | μg/mL | 133 | Si et al., 2006 |

**Omonijo *et al.*, 2017**

# Antimicrobial effects and gut development of piglets

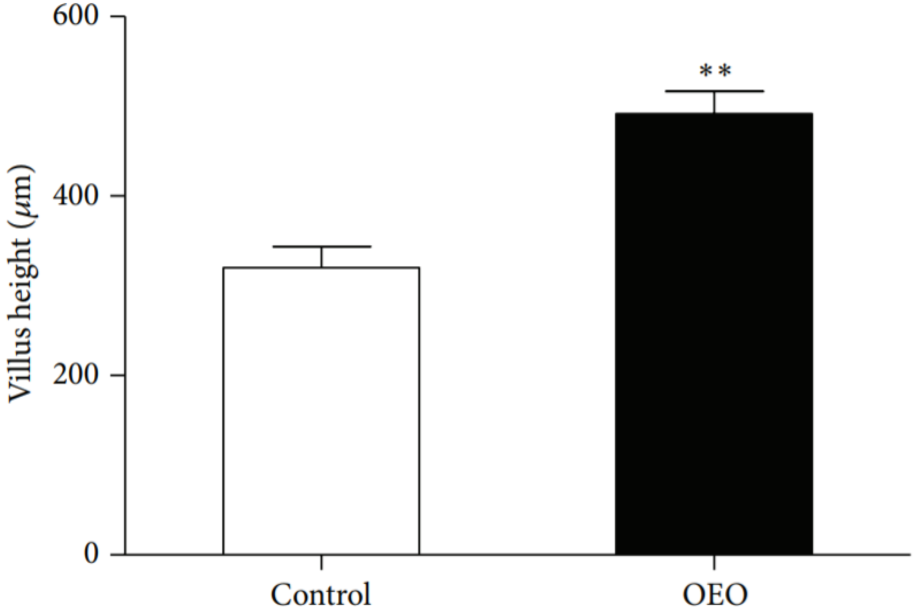



jejunum



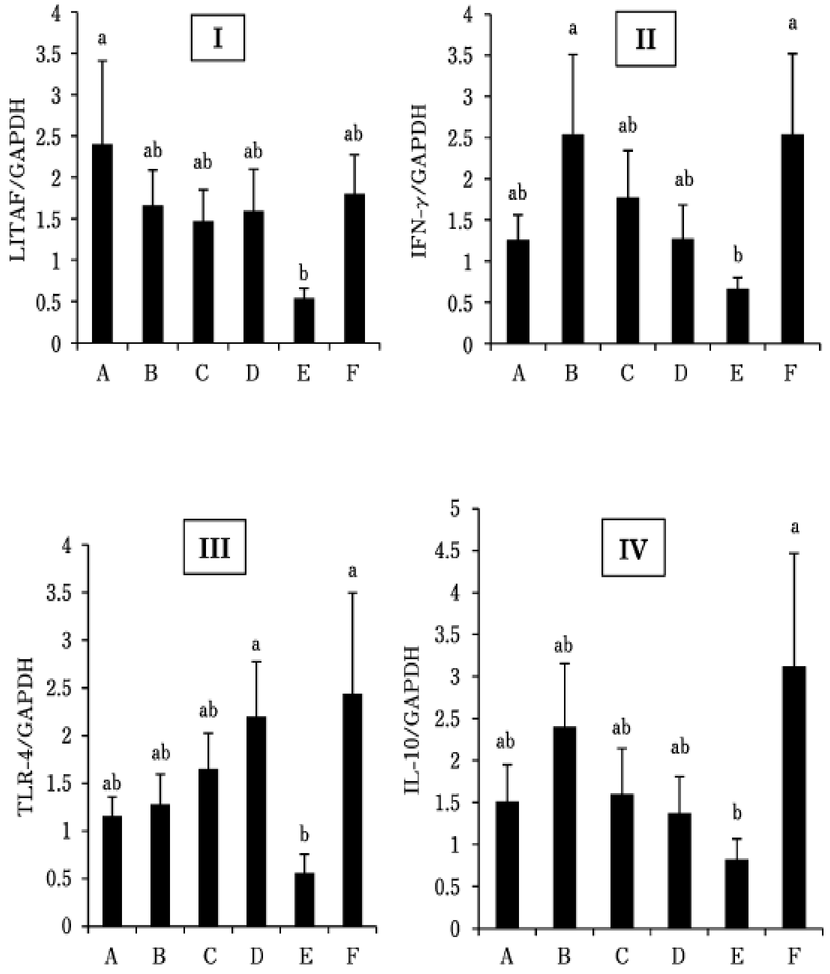
Control

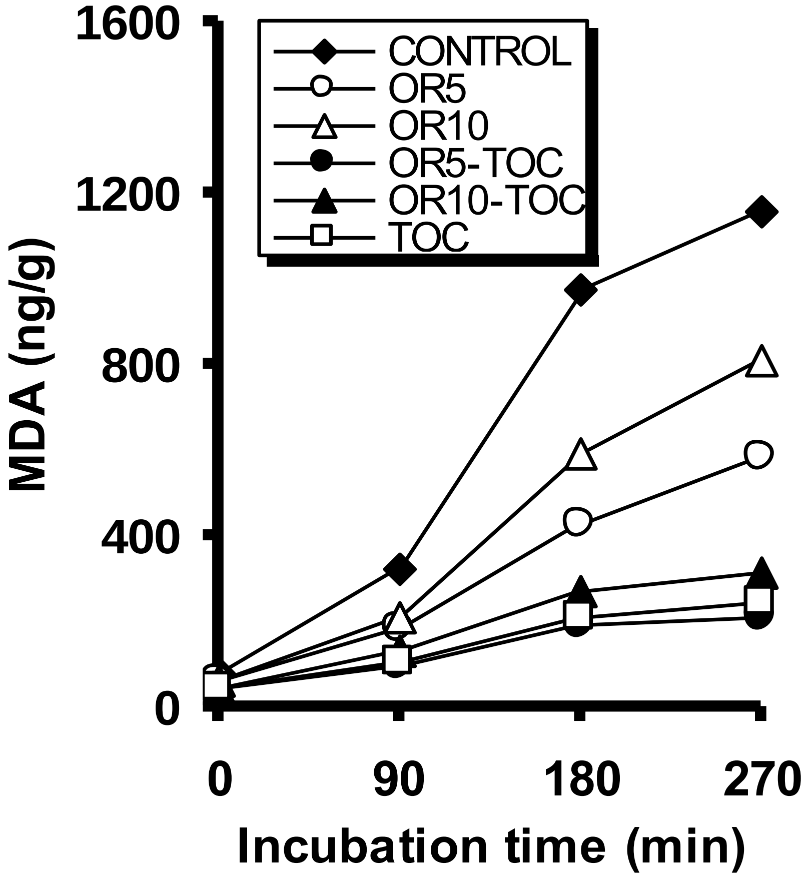
Phytogen

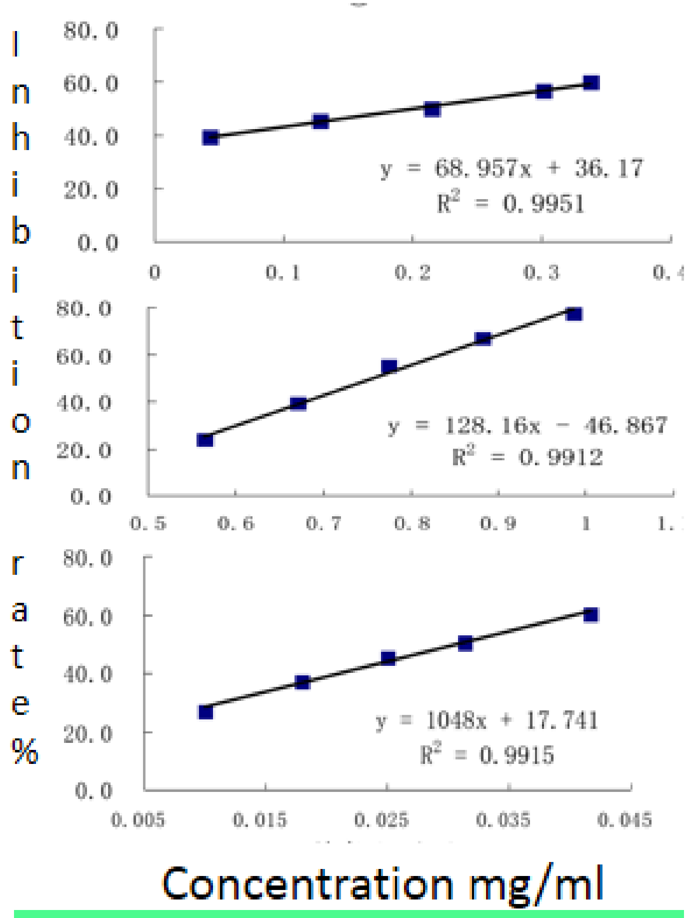



Meritech R&D, and Zou *et al*., 2016

# Oregano downregulated ileal IL-6 and tonsil LITAF, IFN-γ, TLR4 and IL-10 gene expression & exerted a significant anti-inflammatory effect



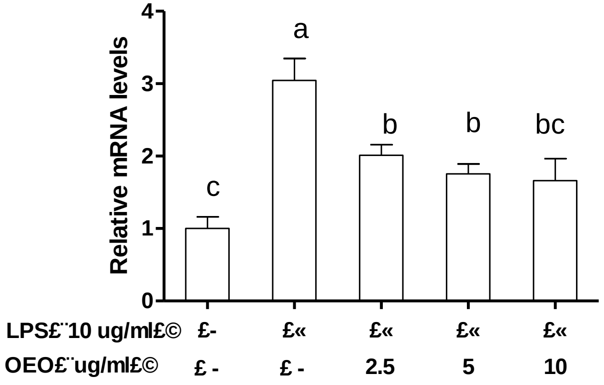




Inhibtion (IC50) of lipid oxidation of rat liver were:
VC=0.76 mg/ml; VE=0.21 mg/ml;
OEO=0.03 mg/ml.
Therefore, OEO>VE>VC

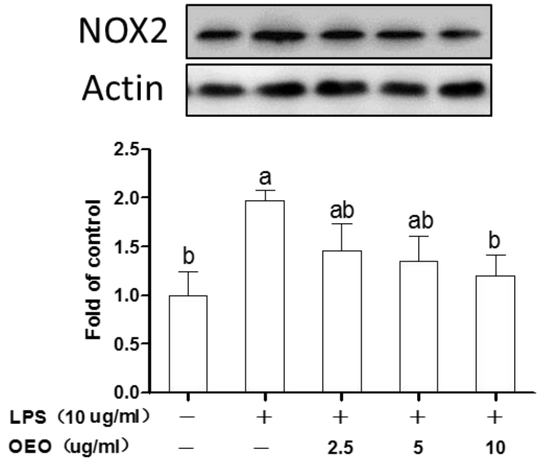
Meritech R&D, and Hang L. *et al.*, 2012

# Mode of Action of Anti-oxidative effects of Phytogen

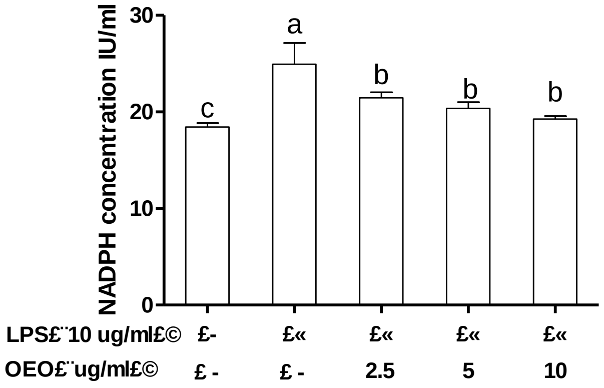
## NOX2 gene expression




## NOX2 protein expression




## NOX2 Enzyme activity




*Research Article*

**Oregano Essential Oil Induces SOD1 and GSH Expression through Nrf2 Activation and Alleviates Hydrogen Peroxide-Induced Oxidative Damage in IPEC-J2 Cells**

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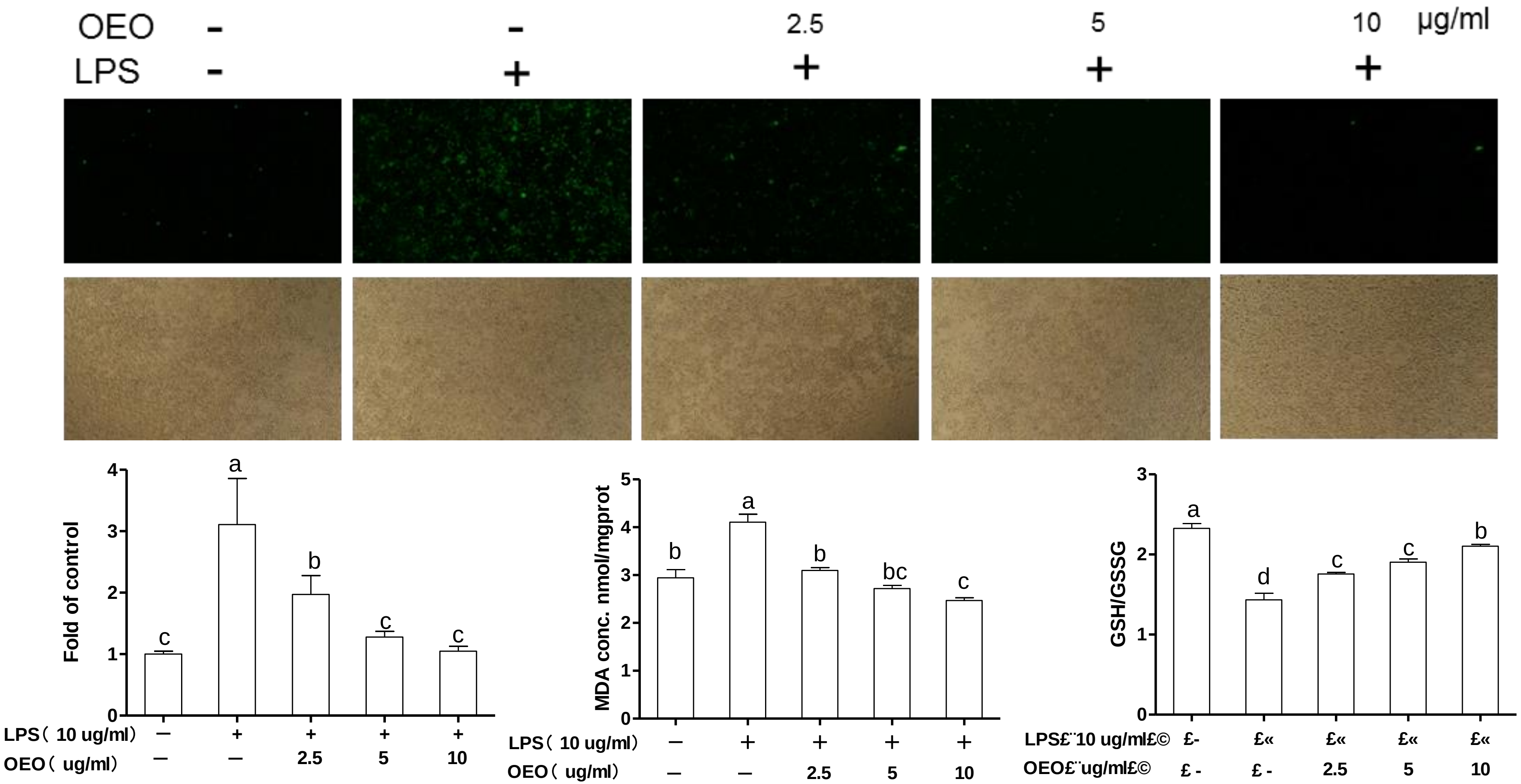
note：Phytogen supplied from Meirtech

# Mode of Action of Anti-oxidative effects of Phytogen




- OEO can reduce cellular anti-oxidative stress, through reducing ROS and MDA and enhancing the ratio of GSH：GSSG

note：OEO=Phytogen from Meritech

# Use of Phytogen in grower-finisher pigs with AGP-free diet

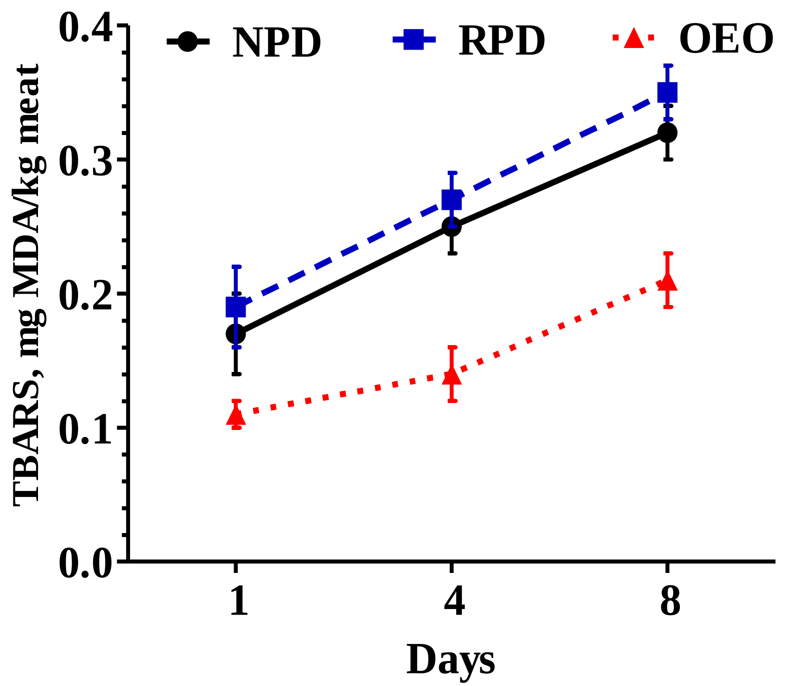
MERITECH

- improve BW/FCR
- reduce cost of pork

Trransport effect to finishers

| | Control | Phytogen | SEM |
|---|---|---|---|
| **Live wt loss (%)** | **3.32[a]** | **2.10[b]** | **0.11** |
| **Carcass wt (kg)** | **68.42[b]** | **73.39[a]** | **0.71** |

Meat Science, 2017




4°C Storage in refrigeritor

EU-CN joint project 2011-2015

# Multiple active ingredients in Phytogenics

MERITECH

| Plants | Antioxidant | Antiviral | Bactericide |
|---|---|---|---|
| *Bay* | 3 | 5 | 5 |
| *Cassia* | 3 | 3 | 3 |
| *Cayenne* | 9 | 6 | 8 |
| *Cumin* | 5 | 7 | 11 |
| *Garlic* | 9 | 5 | 13 |
| *Ginger* | 6 | 6 | 17 |
| ***Oregano*** | **14** | **11** | **19** |
| *Rosemary* | 12 | 10 | 19 |
| *Sage* | 7 | -- | 6 |
| *Thyme* | 4 | 3 | 5 |

From Duke （1994）; Bonos 2019

# Summary: Strategies to AGP-free diets for piglets

MERITECH

- Bio-security: tight and strict control
- Reducing pathogens and microbial load thru improving protein disgestibility and using higher inert fiber
- Alternatives to AGP
  - Acidifiers
  - Phytogenics
  - MSFA
  - Probiotics/Prebiotics
  - Anti-Microbilal Peptide (not approved by MOA China)
  - Other magics in the market

# Thanks for your attention

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