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ATA 2019

3rd International Symposium on Alternatives to Antibiotics

Challenges and Solutions in Animal Health and Production

Bangkok, Thailand

December 16-18, 2019

Programme and Book of Abstracts

PROGRAMME & BOOK OF ABSTRACTS

3rd International Symposium on Alternatives to Antibiotics (ATA):

Challenges and Solutions in Animal Health and Production

The Berkeley Hotel, Bangkok, Thailand
16-18 December 2019

Organised by

USDA

Faculty of Veterinary Science · Chulalongkorn University

Department of Livestock Development

TCEB
THAILAND CONVENTION & EXHIBITION BUREAU

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IMPORTANT NOTES

Preregistration badge pick up

Date:	December 15, 2019
Time:	13:00-17:00
Venue:	The Berkeley Hotel Pratunam lobby

Main conference

Date:	December 16-18, 2019
Time:	December 16, 08:00 -17:00; December 17-18, 08:30 -17:00
Venue:	Mayfair Grand Ballroom, 11th floor

Registration

Date:	December 16-18, 2019
Time:	December 16, opens at 07:30
	December 17-18, opens at 08:00
Venue:	Mayfair Grand Ballroom, 11th floor

Opening ceremony

Date:	December 16, 2019
Time:	8:00
Venue:	Mayfair Grand Ballroom, 11th floor

Lunch

Date:	December 16-18, 2019
Time:	12:00-13:30
Venue:	The Palladium Hall, 10th floor

Welcome cocktail reception

Date:	December 16, 2019
Time:	18:00-20:00
Venue:	The Palladium Hall, 10th floor

The 3rd International Symposium on Alternatives to Antibiotics wishes to thank the following for their generous support

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WELCOME TO

The 3rd International Symposium on Alternatives to Antibiotics

Challenges and Solutions in Animal Health and Production

The Berkeley Hotel, Bangkok, Thailand

16-18 December 2019

In view of the continuing global concerns with antibiotic resistance, there is a pressing need to have a scientific forum to assess the scientific advancements made since the 2nd International Symposium on Alternatives to Antibiotics held at the World Organisation for Animal Health (OIE) in Paris, France, in 2016. The objectives of this 3rd International Symposium are to highlight promising research results and novel technologies that provide alternatives to antibiotics for use in animal health and production, assess challenges associated with their commercialization and use, and provide recommendations to support their development. The symposium will focus on the latest scientific breakthroughs and technologies that provide new options and alternative strategies for preventing and treating diseases of animals and reduce the use of medically important antibiotics in agriculture. Although some of these new technologies provide the means for implementing a One Health approach and have direct applications as medical interventions for human health, the focus of the symposium is on animal health and production and food safety.

The following six areas will be explored in detail through scientific presentations and expert panel discussions:

1. Vaccines that could reduce the use of medically important antibiotics
2. Microbial-derived products, such as probiotics and bacteriophage gene products
3. Non-nutritive phytochemicals, including prebiotics
4. Immune-related products, such as antibodies, microbial peptides and cytokines
5. Innovative drugs, chemicals, and enzymes
6. Regulatory pathways to enable the licensure of alternatives to antibiotics and incentives from stakeholders to support their development.

Rungtip Chuanchuen
Chair – organizing committee

Hyun Lillehoj
Chair – scientific committee

Cyril Gay
Chair – steering committee

GREETINGS FROM CU VET, THE LOCAL HOST

Dear Delegates of the 2019 ATA Symposium,

We warmly welcome you to Bangkok, the great city of angles. We are very happy that you decided to travel to this wonderful city to participate the great symposium devoted to alternatives to antibiotics. The ATA 2019 symposium is the result of the hard work of the exceptional team who has intended to increase knowledge of alternatives to antibiotics to veterinary fields. The scientific program has been carefully designed to provide topics that are directly useful to veterinary sectors as well as human and environmental sectors. We have been fortunate to recruit world-renown speakers who have agreed to share their expertise and professional knowledge to all of us. The oral and poster presentations were carefully reviewed and the presenters have been enthusiastic to share the results of their newest research. This symposium is also a platform for all delegates to communicate and learn from each other.

Thanks to all volunteers, speakers, the support of our partners from industry and in particular, participations of all delegates. This symposium will not be successful at this magnitude without all the contributions.

We are confident that you will enjoy the symposium and the scientific contents. Most importantly take your time to enjoy and experience the unique blend of traditional and modern cultures of Bangkok.

Prof. Roongroje Thanawongnuwech
Dean
Faculty of Veterinary Science, Chulalongkorn University

COMMITTEE

Scientific Committee

- Cyril Gay, USDA-ARS
- Hyun Lillehoj, USDA/ARS
- Rungtip Chuanchuen, Chulalongkorn University
- Elisabeth Erlacher-Vindel, OIE
- Dennis M. Dixon, NIAID, NIH
- Filip Van Immerseel, Ghent University
- Chengbo Yang, University of Manitoba
- Henk P. Haagsman, Utrecht University
- Peter M. H. Heegaard, Technical University of Denmark
- Hong Dong, Beijing University
- Jaap Wagenaar, Utrecht University

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- Cyril Gerard Gay, USDA, ARS
- Rungtip Chuanchuen, Chulalongkorn University
- Hyun Lillehoj, USDA, ARS
- Charles Li, USDA-ARS
- Chengbo Yang, University of Manitoba
- Ron Cravens, Cravens Life Sciences

Organizing Committee (Chulalongkorn University)

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- Kaywalee Chatdawong
- Sasi Jaroenpoj
- Saharuetai Jeamsripong
- Sayamon Srisuwatanasagul
- Pattrarat Chanchaithong
- Taradon Luangtongkum
- Anudep Rungsipipat
- Navapon Techakriengkrai
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- Rangsiya Prathan
- Suwanee Chalermchainukul
- Shabbir A Simjee, CLSI subcomittee

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INVITED SPEAKERS

Elisabeth Erlacher-Vindel

- OIE, France
- The global objectives for AMR and alternatives for the animals

Dennis Dixon

- NIH, United States
- Global strategies for developing alternatives to antibiotics for human health

Hyun Lillehoj

- USDA-ARS, United States
- Antibiotics, germs and antibiotic alternatives for animals

John Prescott

- University of Guelph, Canada
- Immunization of broiler chickens against necrotic enteritis: Progress and possibilities

Filip Van Immerseel

- University of Ghent, Belgium
- The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment

Tun, Hein Min

- University of Hong Kong, Hong Kong
- Microbiome for gut health: A modern tool and a target in the effort to address antimicrobial resistance

Todd Callaway

- University of Georgia, United States
- Non-antibiotic strategies to modify the microbial population of dairy cattle: impacts on milk production, animal health and food safety

Tom Rehberger

- Church & Dwight Co., Inc., United States
- Managing the gut microbial populations: From science to practice

Inkyung Park

- USDA-ARS, Korea
- Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives

Junjun Wang

- China Agricultural University , China
- Strategies to reduce antibiotics in swine production in China

Judy Chen

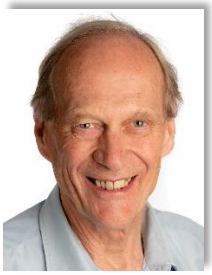
- USDA-ARS, United States
- Non-antibiotic treatments for honey bee diseases in the era of omics

Pietro Celi

- DSM, Switzerland
- Fighting AMR by optimizing gastrointestinal functionality: A holistic approach

Chengbo Yang

- University of Manitoba, Canada
- Organic acids as antibiotic alternatives in monogastric animals



John Furness

- University of Melbourne, Australia
- Sensing and reacting: micronutrients and phytochemicals in gut health

Hong Dong

- Beijing Key Laboratory of Traditional Chinese Veterinary Medicine, China
- Mechanisms of Baitouweng Decoction in the treatment of diarrhea caused by *Escherichia coli*

Emma Wall

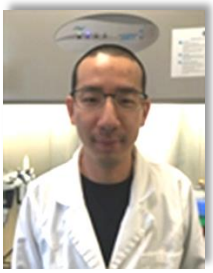
- Full circle science, USA
- Phytonutrients: The Next Generation

Prashant Mishra

- AVT Natural, Mexico
- Science-based use of plant extracts to improve animal health in post-antibiotic era: where are we?

Peter Heegaard

- Technical University of Denmark, Denmark
- Passive Immunity and IgG-like antibodies as an alternative to antibiotics



Woohyun Kim

- Gyeongsang National University, Korea
- Host defence peptides with anti-microbial and immunomodulatory activities as antibiotic alternatives

Douglas Korver

- University of Alberta, Canada
- Making the transition from research trials to field application

Henk Haagsman

- Utrecht University, The Netherland
- Reprogramming the innate immune system as an alternative

Joshua Hayes

- Center for Veterinary Medicine, Food and Drug Administration, United States
- US FDA's Regulatory Pathway for Alternatives to Veterinary Antimicrobials

Javier Pozo

- European Medicine Agency, Amsterdam, The Netherlands
- Promoting the authorization of alternatives to veterinary medicinal antimicrobials in the European Union

Takashi Kozasa

- Ministry of Agriculture, Forestry and Fisheries, the Government of Japan
- Legal framework for the approval designation of alternatives to antibiotics.

Erik De Ridder

- Health for Animals
- Industry perspective on the registration of alternatives to antibiotics.

SCIENTIFIC PROGRAM

Day 1: Monday 16 December 2019

07:30	**REGISTRATION BEGINS**	
08:00-08:05	Welcome address from Professor Dr. Roongroje Thanawongnuwech, Dean, Faculty of Veterinary Science, Chulalongkorn University, Thailand	
08:05-08:10	Objectives and expected outcomes for the 3rd International Symposium on Alternatives to Antibiotics - Dr. Cyril Gay, Senior National Program Leader, USDA-ARS, United States of America	
08:10-08:30	**Keynote Presentation:** The global objectives for AMR and alternatives for the animals • *Elisabeth Erlacher-Vindel*, The Antimicrobial Resistance and Veterinary Products Department, World Organisation of Animal Health (OIE), France	
08:30-09:00	**Keynote Presentation:** Global strategies for developing alternatives to antibiotics for human health • *Dennis Dixon*, NIH, United States of America	
09:00-09:30	**Keynote Presentation:** Antibiotics, germs and antibiotic alternatives for animals • *Hyun Lillehoj*, USDA-ARS, United States of America	
09:30-10:00	**COFFEE BREAK / POSTER SESSION**	
SESSION	**1. VACCINES**	
CHAIRS	*John Prescott*, Canada and *Cyril Gay*, United States of America	
10:00-10:25	Immunization of broiler chickens against necrotic enteritis: Progress and possibilities • *John Prescott*, University of Guelph, Canada	
10:25-10:50	The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment • *Filip Van Immerseel*, University of Ghent, Belgium	
10:50-11:05	Novel vaccine antigens identified by chicken monoclonal antibodies against apicomplexans • *Kazumi Sasai*, Osaka Prefecture University, Japan	
11:05-11:20	Development of a subunit vaccine targeting *Clostridium perfringens* enzymes for the control of necrotic enteritis in broilers • *Lisa Bielke*, The Ohio State University, United States of America	
11:20-11:35	Immunization with recombinant subunit vaccines from virulent *Clostridium perfringens* field strains confers partial protection against necrotic enteritis in broiler chickens • *Charles Li,* BARC/ARS/USDA, United States of America	
11:35-12:00	**Roundtable discussion**	
12:00-13:30	**LUNCH AT THE PALLADIUM HALL / POSTER SESSION**	
SESSION	**2. MICROBIAL-DERIVED PRODUCTS**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Tun, Hein Min, Hong Kong and *Todd Callaway*, United States of America	
13:30-13:55	Microbiome for gut health: A modern tool and a target in the effort to address antimicrobial resistance • *Tun, Hein Min*, University of Hong Kong, Hong Kong	
13:55-14:20	Non-antibiotic strategies to modify the microbial population of dairy cattle: impacts on milk production, animal health and food safety • *Todd Callaway*, University of Georgia, United States of America	
14:20-14:45	Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives • *Inkyung Park*, USDA-ARS, Korea	
14:45-15:00	Microbiological quality and possible role as a source of antimicrobial resistance genes of commercial probiotic products for livestock and aquatic animals • *Rungtip Chuanchuen,* Chulalongkorn University, Thailand	
15:00-15:15	Swine-derived probiotic *L. Plantarum* modulates porcine intestinal endogenous HDP synthesis • *Jing Wang,* Beijing Academy of Agriculture, China	
15:15-15:45	**COFFEE BREAK / POSTER SESSION**	
CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Tom Rehberger, United States and *Judy Chen*, United States of America	
15:45-16:10	Managing the gut microbial populations: From science to practice • *Tom Rehberger*, Church & Dwight Co., Inc., United States of America	
16:10-16:25	Responses of *Bacillus* amyloliquefaciens CECT 5940 supplementation in weaned pig diets • *Thammakit Thammathipborworn,* Nutrition & Care, Evonik (Thailand) LTD.	

16:25-16:40	Fermentate Bioactives Impact on SARA and a mastitis *Stretoccocus uberis* challenge to reduce AIF use in bovines • *Leon Samuel Barringer,* Diamond V, United States of America
16:40-17:00	**Roundtable discussion**
18:00-20:00	**WELCOME COCKTAIL RECEPTION THE PALLADIUM HALL**

Day 2: Tuesday 17 December 2019

SESSION	**3. INNOVATIVES DRUGS, CHEMICALS AND ENZYMES**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Chengbo Yang, Canada and *Junjun Wang*, China	
08:30-08:55	Strategies to reduce antibiotics in swine production in China • *Junjun Wang*, China	
08:55-09:20	Non-antibiotic treatments for honey bee diseases in the era of omics • *Judy Chen*, USDA-ARS, United States of America	
09:20-09:35	In vitro and in vivo characterization of a Gly-substituted DLP4 cationic peptide against *Staphylococcus aureus* CVCC 546 • *Bing Li,* Chinese Academy of Agricultural Sciences, China	
09:35-09:50	Afterlife of bacterial cell debris: Peptidoglycan in the gastrointestinal tract • *Christian Nyffenegger,* Novozymes A/S, Denmark	
09:50-10:20	**COFFEE BREAK / POSTER SESSION**	
CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Hyun Lillehoj, United States and *Pietro Celi*, Switzerland	
10:20-10:45	Combating AMR by optimizing gastrointestinal functionality: A holistic approach • *Pietro Celi*, DSM, Switzerland	
10:45-11:10	Organic acids as antibiotic alternatives in monogastric animals • *Chengbo Yang*, University of Manitoba, Canada	
11:10-11:25	25-OH-D3: An indispensable tool to managing antibiotic free feeding programs for commercial broilers • *Thau Kiong Chung,* DSM Nutritional Products Asia Pacific, Singapore	
11:25-11:40	Alternatives to Veterinary Antimicrobials (AVANT): A new EU project focused on diarrhoea in pigs • *Poul Jesper Baekbo,* SEGES, Denmark	
11:40-12:00	**Roundtable discussion**	
12:00-13:30	**LUNCH AT THE PALLADIUM HALL / POSTER SESSION**	
SESSION	**4. PHYTOCHEMICALS**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Hong Dong, China and *John Furness*, Australia	
13:30-13:55	Sensing and reacting: micronutrients and phytochemicals in gut health • *John Furness*, University of Melbourne, Australia	
13:55-14:20	Mechanisms of Baitouweng Decoction in the treatment of diarrhea caused by *Escherichia coli* • *Hong Dong*, Beijing Key Laboratory of Traditional Chinese Veterinary Medicine, China	
14:20-14:35	An anthocyanin-rich purple potato extracts reduce high fat diet and lipopolysaccharide (LPS) induced obesity and low-grade gut inflammation • *Hua Zhang,* Jiangxi University, China	
14:35-14:50	Dietary resistant potato starch alters immunological status and microbial populations in swine to limit *Salmonella* • *Crystal L Loving,* USDA-ARS, United States of America	
14:50-15:20	**COFFEE BREAK/ POSTER SESSION**	

CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Prashant Mishra, Mexico and *Emma Wall,* United States of America	
15:20-15:45	Phytonutrients: The Next Generation • *Emma Wall*, Full Circle Science, United States of America	
15:45-16:10	Science-based use of plant extracts to improve animal health in post-antibiotic era: where are we? • *Prashant Mishra*, AVT Natural, Mexico	
16:10-16:25	*In-vitro* antibacterial activity of phytobiotic against *Eschericia coli* and *Mycoplasma gallisepticum* • *Elvina Jonas Jahja,* PT. MEDION FARMA JAYA, Indonesia	
16:25-16:40	*In vitro* and *in vivo* evaluation of therapeutic effects of neutrapath™ against *Salmonella Typhimurium* • *Hongyu Xue,* Amlan International, United States of America	
16:40-17:00	**Roundtable discussion**	

Day 3: Wednesday 18 December 2019

SESSION	**5. IMMUNE-RELATED PRODUCTS**	
CHAIRS	**Advances in the research and development of alternatives to antibiotics**	**ACADEMIA**
	Peter Heegaard, Denmark and *Woohyun Kim*, Korea	
08:30-08:55	Passive Immunity and IgG-like antibodies as an alternative to antibiotics • *Peter Heegaard,* Technical University of Denmark, Denmark	
08:55-09:20	Host defence peptides with anti-microbial and immunomodulatory activities as antibiotic alternatives • *Woohyun Kim,* Gyeongsang National University, Korea	
09:20-09:35	Innovative enterobactin-specific egg yolk antibodies for controlling Gram-negative pathogens • *Jun Lin*, University of Tennessee, United States of America	
09:35-09:50	High throughput screening for natural host defense peptide-inducing compounds as alternatives to antibiotics • *Glenn Zhang,* Oklahoma State University, United States of America	
09:50-10:20	**COFFEE BREAK / POSTER SESSION**	
CHAIRS	**Research results from commercially available alternatives to antibiotics**	**INDUSTRY**
	Douglas Korver, Canada and *Henk Haagsman*, The Netherlands	
10:20-10:45	Making the transition from research trials to field application • *Douglas Korver,* University of Alberta, Canada	
10:45-11:10	Reprogramming the innate immune system as an alternative • *Henk Haagsman*, Utrecht University, The Netherland	
11:10-11:25	Efficacy of dried egg product administered to male broiler chickens during experimental necrotic enteritis • *Jeffery Escobar*, Elanco Animal Health, United States of America	
11:25-11:40	Yeast cell wall immunomodulatory and intestinal integrity effects on broilers challenged with *Salmonella* Enteritidis • *Ekachai Jenwitheesuk,* ICC Brazil, Brazil	
11:40-12:00	**Roundtable discussion**	
12:00-13:30	**LUNCH AT THE PALLADIUM HALL / POSTER SESSION**	
SESSION	**6. REGULATORY PATHWAYS TO ENABLE THE LICENSING OF ALTERNATIVES TO ANTIBIOTICS AND INCENTIVES FROM STAKEHOLDERS TO SUPPORT THEIR DEVELOPMENT**	
CHAIRS	*Joshua Hayes,* Food and Drug Administration, United States and *Peter Borriello,* Veterinary Medicine Directorate, United Kingdom	
13:30-13:55	US FDA's Regulatory Pathway for Alternatives to Veterinary Antimicrobials • *Joshua Hayes*, Center for Veterinary Medicine, Food and Drug Administration, United States of America	
13:55-14:20	EU approach to authorization of novel technologies with particular emphasis on alternatives to antibiotics • *Javier Pozo*, European Medicine Agency, Amsterdam, The Netherlands	
14:20-14:45	Legal framework for the approval/designation of alternatives to antibiotics. • *Takashi Kozasa*, Ministry of Agriculture, Forestry and Fisheries, the Government of Japan	

14:45-15:10	Industry perspective on the registration of alternatives to antibiotics. • *Erik De Ridder*, Health for Animals, Belgium
15:10-15:40	**COFFEE BREAK / POSTER SESSION**
15:40-16:30	**Roundtable discussion** • *Scientist perspective - Filip Van Immerseel*, University of Ghent, Belgium • *Regulatory perspective - Pete Borriello, Joshua Hayes, Javier Pozo, Takashi Kozasa* • *Industry perspective - Erik De Ridder*, Health for Animals, Belgium • *Policy perspective - Jomana F. Musmar,* U.S. Department of Health and Human Services, United States of America • *Funder perspective -Renée Larocque,* International Development Research Centre (IDRC), Canada **Roundtable questions** • What novel scientific data that are relevant to ATAs could be used to effectively support their licensing and registration with regulatory authorities? • What scientific information is critical for industry to know prior to sponsoring a product/idea for regulatory review? • What level of return must a product promise for it to be commercialized? • What are potential incentives to promote and maintain stakeholder interest in early to advanced R&D of alternatives to antibiotics? • Are there funding incentives for research to develop ATAs for livestock and aquaculture production in low- and middle-income countries?
16:30-17:00	**CONCLUSIONS AND NEXT STEP** Dr. Cyril Gerard Gay, Senior National Program Leader, USDA-ARS, United States of America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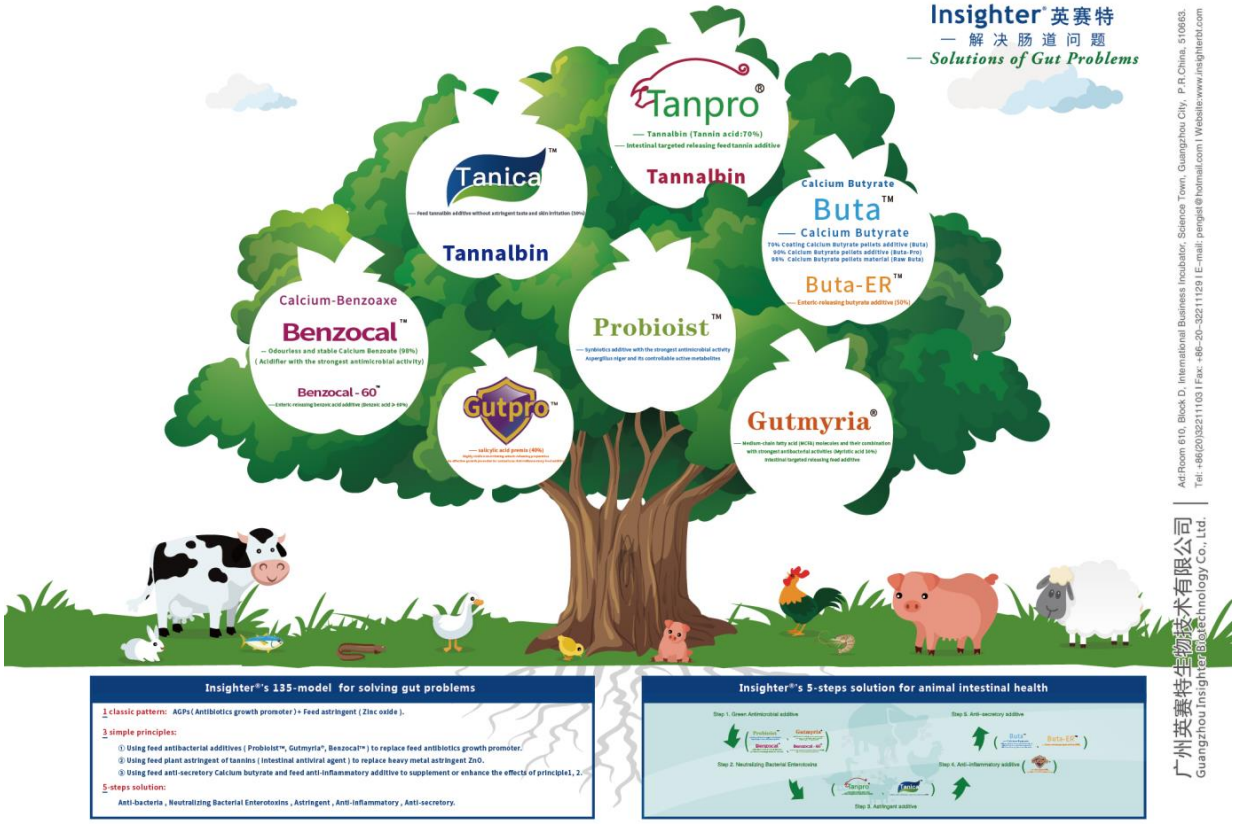

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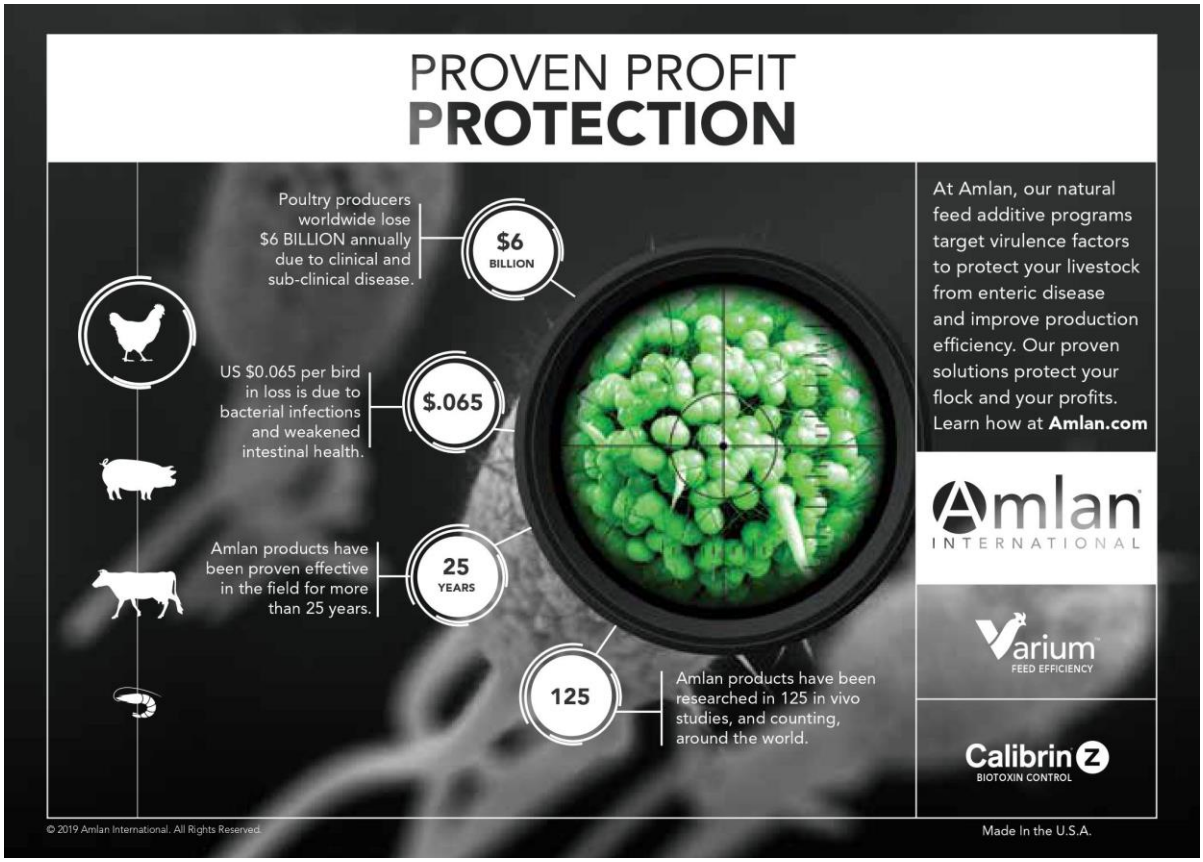
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LIST OF ABSTRACTS

Programme Overview

Objectives and expected outcomes

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In view of the continuing global concerns with the loss of medically important antibiotics, either due to regulatory restrictions or the emergence of antimicrobial resistance, this symposium provides a scientific forum to assess the scientific advancements made in the research and development of alternatives to antibiotics. The key objectives of this symposium are to highlight promising research results and novel technologies that provide alternatives to antibiotics for use in animal health and production, assess challenges associated with their commercialization and use, and provide actionable strategies to support their development. The symposium will focus on five product categories that could reduce the use of medically important antibiotics in animal health and production: 1) vaccines; 2) microbial-derived products; 3) phytochemicals; 4) immune-derived products; and 5) innovative drugs, chemicals, and enzymes. The issue of antimicrobial resistance is a priority 'One Health' issue with important ramifications for public health and agriculture. It is recognized that one of the fundamental challenges of the 21st Century will be to augment agricultural production to feed an increasing world population, which is wholly dependent on the availability of interventions to prevent and control animal and plant diseases. Importantly, the success of the global agricultural enterprise in preventing and controlling diseases will directly impact global food security and the Global Health Security Agenda, key initiatives identified by the World Organisation for Animal Health (OIE) and the United Nations Food Agriculture Organization (FAO) and the World Health Organization (WHO). To be clear, this symposium is not intended to be a venue to eliminate the use of antibiotics in animals as there is a specific need for antibiotics to treat diseases. Nor is this a venue to advocate strategies that use scientifically unproven approaches that will also eventually fail against documented pathogen adaptability and resistant strain development. Rather, the topics that have been selected for this symposium are the research of innovative products for the prevention and treatment of diseases, as well as the enhancement of animal production, that do not result in the creation of selection pressure favoring the development of antimicrobial resistance. As such, the research and development of innovative drugs and antibiotic alternatives are included as key strategic objectives in the United States National Action Plan for Combating Antimicrobial Resistant Bacteria (CARB). The global increase in antibiotic resistance among bacterial pathogens is believed to be due to the over- and misuse of antibiotics in human and animal health. One of the key public health concerns linked to agriculture is the potential development of antibiotic resistant strains within food animal production facilities and among food-borne bacteria that could seriously compromise therapeutic options and medical interventions. Thus, stewardship programs and alternatives to the continued reliance on antibiotics in agricultural production need to be developed. There is also increasing scientific evidence that implicates certain antibiotics with disrupting the normal flora of the gut, yielding negative consequence on the immune system, disease tolerance and health. As we move into the 21st Century and the demands for food products increase to meet the nutritional needs of a growing world population, finding alternative strategies to improve animal health and production has become a global issue, and a critical component of efforts to alleviate poverty and world hunger.

Keynote Presentation

The global objectives for AMR and alternatives for the animals

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Antimicrobial resistance (AMR) is a global human and animal health concern, which is influenced by the use of antimicrobial agents in human and veterinary medicine, and the plant sector as recognised at the highest political level by the UN General Assembly resolution 71/3, adopted in 2016. To combat antimicrobial resistance, the World Organisation for Animal health (OIE) develops science-based intergovernmental standards and guidelines covering terrestrial and aquatic animals.

The OIE contributed to the development of the WHO Global Action Plan on Antimicrobial Resistance, that was endorsed by the OIE's 180 Member Countries through a Resolution unanimously adopted in May 2015. On November 2016, following the request from its World Assembly of Delegates, the OIE compiled its AMR activities into a strategy.

The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials, outlines goals and activities to support Member Countries in their fight against AMR, and to encourage the national ownership and implementation of international Standards. The structure of the OIE Strategy supports the objectives established in the Global Action Plan, and reflects the mandate of the OIE, through four main objectives: i) Improve awareness and understanding; ii) Strengthen knowledge through surveillance and research; iii) Support good governance and capacity building; and iv) Encourage implementation of international standards.

Following these objectives, the OIE provides guidance and support to research into alternatives to antibiotics including vaccines, by working alongside partner organisations to encourage the development, uptake and registration of new tools, and validated products that will provide alternatives to the use of antibiotics and reduce the emergence and spread of AMR. In this framework, the OIE supported the United States Department of Agriculture (USDA) in the organisation of the first two "International Symposia on Alternatives to Antibiotics, Challenges and Solutions in Animal Production" (September 2012 and December 2016), that were held at the OIE Headquarters in Paris. In addition, the OIE supports the efforts of the STAR-IDAZ International Research Consortium (IRC) into improving the coordination of research programmes at international level to contribute to the development of new and improved animal health strategies for several priority diseases/issues, including on AMR and on the development of alternatives to antibiotics.

Keywords: global, objectives, AMR, alternatives, animals

Keynote Presentation

Global strategies for developing alternatives to antibiotics for human health

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Antimicrobial resistance is one of the top public health problems worldwide. Especially concerning is the loss of effective antibiotics for bacterial infections that were previously highly effective and led to major advances in human health. Continued exposure to existing classes of antibiotics with few new classes emerging, coupled with failure of pharmaceutical companies to recoup return on investment relative to other classes of drugs for use in modern medicine have created a complicated mix of challenges. The NIAID is a key component of the US national and international effort to address these challenges. Approaches include basic, translational and clinical research for better means of diagnosis, prevention and treatment, as well as preclinical and clinical resources to decrease the risks to antibiotic companies. NIAID antibiotic resistance research plans have included emphasis on exploring alternatives to traditional antibiotics. Examples of efforts to incentivize research in this area, including special funding announcements, along with selected examples of projects moving forward will be summarized.

Keywords: Antimicrobial, resistance, antibiotics, bacterial, infections

Keynote Presentation

Antibiotics, germs and antibiotic alternatives for animal

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Dietary antibiotics have been used in the food animal industry for more than 60 years, not only to control infectious diseases, but also to increase feed efficiency and improve growth performance. In chickens, subtherapeutic, in-feed antibiotics can increase body weight gain up to 8% and decrease the feed conversion ratio (feed intake/body weight gain) up to 5%, both compared with an antibiotic-free diet. Antibiotics overuse and abuse on a global scale have led to the emergence of multi-drug resistant "superbugs" from food animals and humans. The United States Food and Drug Administration has requested that agriculture producers discontinue sub-therapeutic dosing of antibiotics into animal feed, which for over 60 years, was the common practice to promote their economic value by increasing feed efficiency and growth. Therefore, development of novel antibiotic alternatives that can replace growth promoting drugs is timely and critical for sustainability of animal agriculture. At this third international symposium on alternatives to antibiotics, various strategies for developing novel alternatives to antibiotics for agriculture animal production will be discussed and we will learn more on the mode of action of novel antibiotic alternatives. In this talk, I will review our current knowledge on the underlying mechanisms of action of growth promoting drugs, the effects of antibiotics on gut microbiota and alternative alternatives to antibiotics. Antibiotics were originally thought to improve animal growth through reductions in the number and diversity of the normal bacterial flora present in the gut, which in turn, increased the bioavailability of nutrients available to the host and/or reduced the production of microbial metabolites deleterious to animal growth. Alternatively, antibiotics were suggested to improve growth performance through an anti-inflammatory effect directed toward the intestinal epithelium. With the advent of novel molecular biology and bioinformatics techniques, it is now clear that changes in the host intestinal inflammatory response, as well as the structure and diversity of the gut microbial community, occur when antibiotics are introduced into animal diets. Because there is a close cross talks that influence gut microbiota, immune system and brain function, understanding these interactions is critical to develop novel antibiotic alternatives. Current technological advances in "omics" technology is enabling global gene expression and metabolomic studies in commercial food animals to obtain better understanding of biochemical processes for the development of novel compounds for alternative ways of promoting growth and immunity. These approaches should provide the framework for future studies to identify natural chemical compounds to improve poultry growth performance without the use of in-feed antibiotics.

Keywords: Antimicrobial resistance, Microbiota, Antibiotics, Alternatives to antibiotics

SESSION 1

Vaccines

ORAL PRESENTATIONS

Immunization of broiler chickens against necrotic enteritis: Progress and possibilities

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Immunization of broiler chickens against necrotic enteritis (NE) caused by *Clostridium perfringens* is an important approach for control of this globally important disease. Although the last decade has seen considerable advances in understanding NE, including the 2008 discovery of NetB, a critically important pore-forming toxin, much remains to be discovered about the detailed pathogenesis and the basis of immunity to NE on which a scientifically designed immunization strategy must be based. The challenges include: 1. Understanding the basis and extent of immunity; 2. Induction of active immunity in newborn chicks in the face of maternal antibodies; 3. Identification and characterization of key protective antigens; 4. Defining experimental models to evaluate the protective efficacy of candidate antigens and the relevance of such models to naturally occurring NE; 5. Developing a vaccine that is safe, easy to deliver, robust under field conditions, and inexpensive; 6. Evaluating candidate vaccines under field conditions that do not compromise the welfare of the chicken.

Considerable progress has been made in identifying antigens of importance in immunity to NE using experimental immunization-challenge models. Passive immunity in chicks obtained through breeder vaccination using bacterial supernatant that includes the alpha toxin is short-lived; however, this vaccine has been currently withdrawn from the market. In inducing an active immunity, it is striking as to how many antigens of *C. perfringens* can provide some level of protection against experimental NE. These include (abbreviated names): Alpha toxin, EFtu, FBA, GPD, HP, NetB, PFOR, PGM and certain pilus proteins. It seems also clear that no one antigen can provide complete protection and that a combination of antigens or artificial hybrid protein constructs seem to offer superior protection against NE. It is clearly possible to immunize actively against NE but it is also clear that the level of protection by immunization depends on the severity of the experimental challenge.

How immunity to these antigens works is not defined. There is some evidence that antibodies may interfere with the growth of *C. perfringens* by binding to the bacterium itself and thus preventing their multiplication rather than, for example, by toxin neutralization or opsonization. The surprising protective efficacy of some "housekeeping" rather than just of virulence-associated proteins, suggests that bacterial growth inhibition might be a plausible explanation for their effect. Nevertheless, there seems to be a general agreement that oral immunization using recombinant vectors (example, *Eimeria*, *Lactobacillus*, *Salmonella*) can be a feasible option and that inducing an effective mucosal immunity is of great importance. An unresolved issue is whether NE evoked by coccidial infection requires that the *C. perfringens* strain be NetB-positive. If it does not, then the focus on NetB in immunization studies may be misleading, and research investment on the currently known most useful protective immunogens (alpha toxin, FBA, HP) would be more appropriate.

It is clear that immunization will be a useful but probably not perfect adjunct approach for controlling NE and that a search for the perfect antigen(s) and their delivery will likely delay progress in introducing immunization.

Keywords: Necrotic enteritis, immunization, review, progress, possibilities

The future of *Salmonella* vaccines in a geographically diverse and changing epidemiological environment

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Most important global *Salmonella* serotypes that cause food poisoning in humans are Enteritidis, derived mainly from eggs and egg-derived products, and Typhimurium, derived from porcine (and poultry) meat. *Salmonella* vaccination programs in laying hens, using inactivated and live vaccines, have been shown to be efficient in reducing egg contamination, while vaccination of broilers and pigs is still not commonly done, although there are some vaccines marketed and used in some regions. Apart from Enteritidis and Typhimurium, different serotypes can be of local and regional importance. Novel trends are the global emergence of *Salmonella* serogroup C strains in poultry, including multi-resistant clonal strains worldwide (e.g. *Salmonella* Infantis clones). Vaccines for poultry that are commercially available can be inactivated and live vaccines, the latter mostly based on spontaneous mutations, and mainly derived from Enteritidis and Typhimurium strains. Vaccines for the host-specific serotypes Gallinarum and Choleraesuis, that cause systemic infections in poultry and pigs, respectively, also are in use. In poultry, inactivated vaccines have been used for parent and layer flocks, and live vaccines mainly for layer flocks. Live vaccines are stimulating cell-mediated and humoral responses, and mucosal innate responses, as they mimic a natural infection, while inactivated vaccines mostly result in antibody responses. Both have been shown to be able to confer (at least partial) protection. Egg contamination in layers is well under control using vaccines. The most important challenges for *Salmonella* vaccines are the production of efficient vaccines for pigs and broilers, and the constant changes in serotype distribution, and thus the development of cross-protecting vaccines against a range of serotypes in broilers. For broilers, the difficulty is the build-up of active immunity in the short life span of the bird. An early bacteriological colonization-inhibition effect conferred by live vaccines has been described in the intestine but this is mainly efficient within the same serotype, so there is a lack of cross-protection using this method. For pigs, lymph node colonization seems difficult to control using vaccines. In addition, often serology is used for monitoring of *Salmonella* in pigs, and there can be interference with monitoring when piglets are vaccinated. Although there are no safety issues with current vaccines, the detailed knowledge on the molecular pathogenesis of *Salmonella* infections should result in attenuated and highly characterized deletion mutant vaccines, and add markers to differentiate vaccine and field strains and serological responses against vaccine and field strains. Regulatory aspects that are of importance in this regard are the faster acceptance of live attenuated mutant strains for emerging serotypes that contain identical gene deletions as one that are already marketed for other serotypes. Other challenges are early protection as chicks are highly susceptible during the post-hatch period, and extension of the duration of protection, considering the trend to extend the productive cycle in layers.

Keywords: *Salmonella*, poultry, vaccinaton, Enteritidis, Typhimurium

Novel vaccine antigens identified by chicken monoclonal antibodies against apicomplexans

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The phylum Apicomplexa comprises obligate intracellular parasites that infect vertebrates. All invasive forms of Apicomplexa possess an apical complex, a unique assembly of organelles localized to the anterior end of the parasite and involved in host cell invasion. The chicken antibodies have been demonstrated to be useful for immunochemical research and clinical applications. In contrast to mammals, chicken antibody diversity is mostly generated by somatic mechanisms. It may be possible to produce antibodies in chickens that are difficult or impossible to produce in mammals. Previously, we have developed chicken monoclonal antibodies (mAbs) raised against *Eimeria acervulina* (Protozoa, Apicomplexa) and demonstrated that the chicken mAb, 6D-12-G10, recognized the conoid of *E. acervulina* sporozoites as the apical cytoskeleton and significantly inhibited sporozoite invasions of T lymphocytes *in vitro*. This antigen was highly conserved among Apicomplexan parasites, including other *Eimeria* spp., *Toxoplasma*, and *Neospora*. In further analyses using this mAb, we identified the apical cytoskeletal antigen of *Cryptosporidium parvum,* a pathogen of increasing clinical significance in livestock, birds, and wildlife as well as humans. Here, we characterized this antigen in *C. parvum* to assess its potential as a vaccine against cryptosporidiosis. Indirect immunofluorescence demonstrated that the reactivity of 6D-12-G10 with *C. parvum* sporozoites was similar to those of anti-β- and anti-γ-tubulins antibodies. Immunoelectron microscopy with the 6D-12-G10 mAb detected the antigen both on the sporozoite surface and underneath the inner membrane at the apical region of zoites. The 6D-12-G10 mAb significantly inhibited *in vitro* host cell invasion by *C. parvum*. MALDI-TOF/MS and LC-MS/MS analysis of tryptic peptides revealed that the mAb 6D-12-G10 target antigen was elongation factor-1α (EF-1α). These results indicate that EF-1α plays an essential role in mediating host cell entry by the parasites and, as such, could be a candidate vaccine antigen against the apicomplexans.

Keywords: *Eimeria*, Cryptosporidium, Apical cytoskeletal antigen, Chicken monoclonal antibody, elongation factor-1α

Development of a subunit vaccine targeting Clostridium perfringens enzymes for the control of necrotic enteritis in broilers

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Necrotic enteritis (NE) is a pervasive enteric disease responsible for large scale economic losses within the global poultry industry. The etiologic agent of NE is *Clostridium perfringens* (CP), an opportunistic pathogen that utilizes numerous extracellular toxins and glycoside hydrolases (GH) as key virulence and nutrient acquisition factors. Notably, some GH, mucinases, degrade components of mucin in the gastrointestinal tract as an energy source. Targeting this mechanism may serve to reduce the incidence of disease associated with CP. Two experiments were completed that evaluated mucinase vaccine targets sourced from conserved peptide sequences of carbohydrate binding module 32 (CBM32) of CP mucinases. In experiment 1, 37 antigen peptides were synthetically generated and used to produce hyper-immune sera which was then evaluated for ability to obstruct CP growth *in vitro*. Total CFU of CP were measured at 4h, 6h, and 8h incubation to determine growth rate. Peptides 4, 5, 22, 24, and 30 were selected for further *in vivo* testing based on conservation or the ability to inhibit CP growth by over 50% at 6h and 8h. In experiment 2, the aforementioned peptides were conjugated to an agonistic, CD40-targetting antibody and evaluated *in vivo*. Broilers were given an *Eimeria maxima* (EM) and CP in order to induce NE and assess vaccine efficacy. Treatments included a non-vaccinated non-inoculated control (NVNC), non-vaccinated inoculated control (NVIC), vaccination with peptide 4, 5, 22, 24, or 30 (VP4-VP30), or a combination of all five peptides (MC). There was a significant increase ($p<0.05$) in the percent change in BWG (%ChangeBWG) relative to NVIC for VP22 and MC of 18.54% and 17.43%, respectively. MC vaccinated group had the lowest lesions with a mean score of 0.63±0.18. These results suggest the MC combination was the most successful in alleviating overall performance losses associated with NE-infected broilers and encourage future testing of MC in the development of an NE vaccine.

Keywords: vaccine, *Clostridium*, necrotic enteritis, *Eimeria*, subunit vaccine

Immunization with novel vaccine candidate recombinant antigens from virulent *Clostridium perfringens* field strains confers partial protection against necrotic enteritis in broiler chickens

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Necrotic enteritis (NE) is one of the top enteric infectious diseases in commercial broiler chickens that is caused primarily by *Clostridium perfringens* (CP) A/G strains and responsible for around $6 billion economical loss worldwide. Coccidiosis is the major predisposing factor for NE. With gradual reduction and eventual withdrawal of antibiotic growth promoters from animal feed due to public and regulatory pressures, alternatives to antibiotic approaches assume top- priority for global poultry industry. Vaccination should be an ideal approach for mass prevention. However, there is no effective vaccine commercially available for NE. In this study, the recombinant proteins: chimeric NetB and alpha-toxin (NA), chimeric Fructose-1,6-bisphosphate aldolase and a hypothetic protein (FBA/HP), truncated TpeL, and Collage adhesion protein (Cna) were evaluated for their vaccine efficacy against severe NE challenge with *netB*$^+$*tpeL*$^+$ CP strain using two different NE challenge models which were developed at ARS. Young broiler chicks were immunized twice subcutaneously with adjuvanted CP proteins on days 4, and 15 and various disease parameters were evaluated. Optimum protection was seen when CP proteins mixed with MONTANIDE™ ISA 71 VG (Seppic Inc., France) were given twice intramuscularly using a dual infection NE model (*E. maxima*/CP) and a CP alone NE model. Immunization with all pooled antigens provided better protection against virulent challenges in both models. Immunization with these immunogens merits further investigation in the future, especially in mucosal delivery route.

Keywords: Necrotic enteritis, vaccine, *Clostridium perfringens*, *ii* recombinant proteins

SESSION 1

Vaccines

POSTER PRESENTATIONS

VA1
Combination of multiple antigens are essential for the development of a novel vaccine against *Staphylococcus aureus* infection

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Staphylococcus aureus (S. aureus) is a common pathogen found in the community and in hospitals. Most notably, Methicillin-resistant *S. aureus* is resistant to many antibiotics, which is a growing public health concern. The emergence of drug-resistant strains has prompted the search for alternative treatments such as immunotherapeutic approaches. Prophylactic vaccination is the best approach to combat against MRSA since it can provide protection without any concerns regarding antibiotic resistance. To date, most clinical trials of vaccines or passive immunization against *S. aureus* have ended in failure. In this study, we investigated multiple proteins as possible targets for a vaccine. Mice vaccinated with these purified proteins elicited high titers of specific antibodies as well as Th1- and Th17-biased immune responses in mice. Animal test indicated a protection rate over 90% in several animal models against multiple strains of *S. aureus*. Interestingly, gdT cells transferred from the vaccinated mice to naïve mice can confer protection to the naïve mice against *S. aureus* challenge in skin infection models. These findings raise the hope that the candidate antigens could be developed into multivalent and serotype-independent vaccines against *S. aureus* infection.

Keywords: *Staphylococcus aureus,* Th17, Gamma-Delta T cells, MRSA

VA2
Montanide™ ISA 71 R VG for efficient vaccines against infectious coryza

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Infectious Coryza (IC) is a poultry disease caused by *Avibacterium paragallinarum* which affects respiratory system and causes economic losses (decrease of laying production and increase of culling rate in growing chickens). *Av. paragallinarum* is classified into three serovars with endemic presence in countries like India. Development of trivalent inactivated IC vaccines is a strategy to protect chickens. Adjuvants are required to improve their efficacy. MONTANIDE™ ISA 71 R VG (ISA 71R) is an adjuvant designed to resist to destabilizing antigenic media such as bacteria. In this study, we evaluated the performance of ISA 71 R formulated in a trivalent IC vaccine.

Firstly, 30 layers were injected at D0 and D21 with a trivalent vaccine based on ISA 71R. Vaccine stability was tested and efficacy was assessed by a virulent challenge at D35. The vaccine was stable and induced 100% protection against all three serovars.

Then approximately 100,000 layers distributed in 4 farms with high infectious coryza prevalence in Southern India were vaccinated with the same ISA 71R vaccine in a prime/boost protocol. Injection sites were inspected and mortality was monitored during 7 days post vaccination. Egg production and mortality were recorded weekly up to 17 months of age and compared to standard mortality and egg production. No untoward effects were observed after vaccination. No incidences of infectious coryza were observed during production period. Egg production and the mortality rate were similar to what is observed in a healthy flock.

The vaccine adjuvanted with ISA 71 R VG is efficacious in controlling IC in field conditions.

Keywords: Infectious Coryza, egg production, vaccine, adjuvant, Montanide ISA 71 R VG

VA3
DIVA vaccine provides cross-protection against *Salmonella* serovars in food animals

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Current limitations of available *Salmonella* vaccines include the lack of cross-protection due to serovar-specific protection and interference with *Salmonella* surveillance programs to identify *Salmonella*-positive herds or flocks. A major hurdle to overcoming these limitations is the highly variable, immunodominant lipopolysaccharide (LPS) that 1) differentiates the >2,600 *Salmonella* serovars by their differences in LPS (and flagella), thereby contributing to serovar specific immunity, and 2) is the antigenic focus of *Salmonella*-specific antibody detection ELISAs for herd level monitoring for *Salmonella* exposure. To address these limitations, a live attenuated *Salmonella enterica* serovar Typhimurium vaccine (BBS 866) was designed to dramatically reduce LPS by deleting the *rfaH* gene, thereby decreasing serovar specific immunity, generating an attenuated strain, and creating a DIVA vaccine (differentiate infected from vaccinated animals). Two vaccine trials were performed in swine. In the first vaccine trial, pigs were administered two doses of the vaccine (vaccination and booster) and challenged with wild-type *S.* Typhimurium UK1 that causes gastroenteritis. The swine rectal temperatures, plasma IFNγ levels, fecal shedding and tissue colonization with wild-type *S.* Typhimurium UK1 were significantly reduced in vaccinated pigs compared to mock-vaccinated swine. In the second vaccine trial, pigs were administered a single dose of the vaccine and challenged with virulent multi-drug resistant (MDR) *S.* Choleraesuis that causes systemic disease in swine. Compared to the mock-vaccinated group, the vaccinated pigs exhibited significantly reduced rectal temperatures, serum IFNγ levels, and tissue colonization. Furthermore, during the challenge period, the isolation of *S.* Choleraesuis from blood cultures was significantly greater in mock-vaccinated pigs compared to vaccinated swine. In both vaccine trials, the vaccine strain did not induce a serological response to *Salmonella* LPS and therefore an ELISA can be used to differentiate infected from vaccinated animals (DIVA). Because the *Salmonella* vaccine was designed to reduce serovar specificity to provide cross-protection against diverse *Salmonella* serovars, we evaluated its applicability in another food animal commodity, turkeys. The vaccine reduced systemic and intestinal colonization of vaccinated turkeys following challenge with MDR *Salmonella* Heidelberg. The data from these vaccine trials indicate that the live, attenuated *S.* Typhimurium vaccine can both protect food animals from *Salmonella* that cause systemic disease and also reduce the potential for transmission of *Salmonella* that cause foodborne disease in humans.

Keywords: *Salmonella*, vaccine, poultry, swine, DIVA

VA4
Survey of Avian Pathogenic *Escherichia coli* (APEC) in the Asia-Pacific Region

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Avian pathogenic *Escherichia coli* (APEC) is one of the leading pathogens economically affecting the poultry industry. It is commonly associated with the syndromic disease colibacillosis which can manifest itself in many different forms such as airsacculitis, cellulitis, pericarditis, perihepatitis and respiratory distress. Over the years, many virulence-associated genes (VAGs) have been discovered which contribute to this avian disease such as *hlyF*, *iroN*, *iss*, *iutA*, and *ompT*. Many of these VAGs are plasmid encoded and as a result, *E. coli* strains can acquire varying numbers and combinations. To gauge the prevalence of this poultry pathogen, we conducted a survey across the Asia-Pacific region over the span of two years to observe the APEC levels and profiles of selected VAGs. A total of 1,621 broiler gastrointestinal tracts were collected from 11 different countries: Australia, Bangladesh, Brazil, India, Japan, Malaysia, Myanmar, Philippines, Taiwan, Thailand, and Vietnam. Total *E. coli* was enumerated from the small intestine, and up to five colonies from each sample were isolated and cultured for down-stream characterization. A panel of 5 APEC VAGs (listed above) were used to distinguish APEC from non-APEC isolates, with any isolate possessing 2 or more of these VAGs considered to be APEC. Isolates were grouped by the number of VAGs possessed. In total, 9,625 *E. coli* isolates were isolated from all the samples, of which 4,012 (42%) were found to be APEC. APEC levels ranged from <1.0E+02 CFU/g to 1.8E+08 CFU/g. There was a wide range of counts in all countries, but Australia and Malaysia tended to have the lowest APEC level, while India and Taiwan had the highest. India and Taiwan also had the highest ratios of APEC to commensal *E. coli*, while Bangladesh and Australia had the lowest. In conclusion, we observed varying levels and distributions of APEC and the VAGs they carry across regions. Continued monitoring of the prevalence and severity of APEC worldwide, especially as producers continue to reduce antibiotic usage, will help assess the need for alternatives to antibiotics to combat this poultry pathogen.

Keywords: Avian Pathogenic *Escherichia coli*, colibacillosis, virulence-associated genes

VA5
Multi-drug resistance pump gene deletion strains cannot survive in egg white because of ovotransferrin-derived antimicrobial activity, and are safe and effective oral live attenuated vaccines

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Multi-drug resistance (MDR) pumps are of vital importance for microbial survival in a variety of environments as they can export antimicrobial molecules, including host-derived antimicrobial peptides. In this way bacteria can withstand the antimicrobial peptides at mucosal surfaces, and colonization in specific host niches can be achieved. MDR pumps thus constitute a virulence trait as a protection mechanism against innate host defenses. The outer membrane channel TolC, used by Salmonella MDR pumps to export antimicrobial compounds, was shown to be crucial for survival of *Salmonella* in egg white, using deletion mutants and agar spot and egg white survival assays. MDR pump mutants had an identical phenotype, indicating that strategies of *Salmonella* to survive in egg white are likely based on protection against egg white antimicrobial components. The *tolC* gene promoter was shown to be activated by egg white, pointing to an active upregulation of defense mechanisms when encountering harsh environments. Testing of egg white fractions, derived by chromatographic methods, showed that ovotransferrin was the main driver of the antimicrobial activity against the TolC deletion mutant, and not against the wild type. Either the TolC channel thus aids in pumping out siderophores to compete with ovotransferrin for iron, or TolC pumps out ovotransferrin-derived antimicrobial molecules. Triple oral vaccination of layer pullets (day 1, week 6, week 16) with a ΔTolC outer membrane channel mutant or a *ΔacrABacrEFmdtABC* MDR pump mutant was shown to protect against organ colonization and egg contamination by the wild type challenge strain. In the challenge control group, intravenous challenge with *Salmonella* Enteritidis at week 24 resulted in high colonization levels in the gut and internal organs, including the reproductive tract, and in high egg contamination levels, while vaccinated animals had significantly lower challenge strain bacteria in their organs. Egg contamination was completely prevented. These data show that the deletion specific genes, based on knowledge of the pathogenesis of the infection, can generate potential safe and effective vaccine strains for *Salmonella* in poultry.

Keywords: *Salmonella*, vaccines, MDR pumps

VA6
Lipopolysaccharide and lipopolysaccharide modification gene deletions affect TLR-4 mediated inflammatory signals in chicken oviduct cells and are potential safe live vaccines in production animals

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Salmonella Enteritidis is the world's most common cause of salmonellosis in part because it has the ability to colonize the oviduct and contaminate eggs, while *Salmonella* Typhimurium is mainly a contaminant of porc and poultry meat. One of the important aspects of vaccine development is differentiation of infected from vaccinated animals. This can be achieved either by differentiation with regard to the serological response (DIVA concept) or else by the phenotype of the strain on bacteriological media. LPS mutants have been described as potentially being of value as live oral vaccine strains for both poultry and pigs, because they can be attenuated and have defects in TLR-4 signaling and thus induction of inflammation. For chickens, using the *in vivo* expression technology, it was shown that the rfbH gene, involved in lipopolysaccharide O-antigen synthesis, is transcriptionally induced during growth in whole eggs at room temperature. A *S.* Enteritidis ΔrfbH strain was unable to multiply in eggs at room temperature and did not survive in egg white at 42 degrees C. The attenuation was most likely caused by an increased susceptibility of the ΔrfbH mutant to yet undefined antibacterial components of the egg albumen. Knock-outs of specific LPS modification genes, that alter the LPS structure by adding small molecules to the lipid A and LPS core structure, had an egg white survival defect and showed not to activate TLR-4 mediated downstream mediators (IL-1, IL-6, TNF-alpha). These gene deletions can thus be added to the list of potential targets when producing live attenuated vaccines. Gene deletions would also alter serologic responses against the LPS so that vaccinated birds can be differentiated from challenged birds. This was shown for piglets, in which immunization with ΔrfaJ and ΔrfaL mutants resulted in the induction of a serological response lacking detectable antibodies against LPS. The strains protected mice against *Salmonella* Typhimurium infection when orally administered.

Keywords: *Salmonella*, LPS, vaccines

VA7
Eimeria maxima vaccination via *Pichia pastoris* recombinant vector for coccidia protection in broiler chickens

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Coccidiosis remains one of the most devastating protozoal diseases faced by the global poultry industry, and multiple drug options for control are classified as antibiotics, which limits treatment options. Moreover, coccidiosis vaccines are known to contribute markedly to bacterial enteropathies such as necrotic enteritis. Many strategies, including vaccination, are used to control this illness, yet outcomes are variable. Here we describe the application of a novel recombinant vaccine targeting *Eimeria* spp. in *Eimeria maxima* (EM) inoculated broilers. A new *Pichia pastoris* vaccine-vector expressing thrombospondin-related adhesive protein (TRAP) family, rhomboid protease (ROM5) and high mobility group box 1 (HMGB1) protein has been previously developed. In experiment 1, we sought to compare live and killed forms of this vaccine. The comparison involved high (1x107 cell/mL) or low (1x106 cell/mL) doses using either oral or subcutaneous routes. Our previous vaccine with a Bacillus-vectored TRAP-ROM5-HMGB1 showed protection against EM M6 innoculation. Experiment 2 compared the *Bacillus* and *Pichia*-vector efficacy against EM M6 innoculation. In experiment 3, timing and the delivery of the vaccines were compared. However, this vaccine was carrying the zeocin gene as the marker. To evaluate the vaccine when this gene was removed, a fourth experiment was conducted. Results showed that there were no significant differences in body weight gain (BWG) or percent change in BWG (%ChangeBWG) relative to the positive control in experiments 1 or 2. In experiment 3, BWG was significantly higher in chickens that were vaccinated via drinking water at day-of-hatch or at d5 then boosted with the same vaccine via same route at d14. No differences were observed for lesion scores (LS) in any of the experiments. Most importantly, quantification of oocysts per gram (OPG) of feces was significantly lower in all groups vaccinated with a form of the *Pichia*-vectored vaccine especially at the level of accumulative oocysts shedding or oocysts shedding per bird in experiments 2 and 3. In experiment 4, the differences in BWG, %ChangeBWG, lesion scores and OPG were all non-significant. Overall, this approach to vaccination, or augmentation of live oocyst-based vaccines, appears promising.

Keywords: *Eimeria,* recombinant, avirulent, vaccine, *Pichia*

VA8
Evaluation of a Universal Subunit *Eimeria* spp. Vaccine

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Diseases associated with animal production and presently controlled by antibiotics represent a critical area for research and development. Coccidial infections in poultry have long been controlled by ionophores and/or coccidiostats and today these treatments have come under increased scrutiny by regulatory agencies and consumers. Additionally, traditional coccidia vaccines are limited to combining individual strains of attenuated oocysts; individual strains typically provide no cross-protection against other *Eimeria* strains. Therefore, there is a critical need for the development of new technologies to control *Eimeria* spp. Optimally, these new technologies should address and overcome concerns of both cross-protection and the one vaccine strain per species protection model. We have developed a novel vaccine platform, BTVCx, that incorporates a subunit/epitope sequence, common for all *Eimeria* spp. (broad spectrum), into an inactivated orally administered vaccine that protects poultry against coccidiosis by inducing mucosal immunity.BTVCx was evaluated in two separated mixed-*Eimeria* spp. challenge trials at Southern Poultry Research (Athens, GA). For each experiment, 1000 day of hatch chicks (Cobb 500) were randomly assigned to either the control non-treated group (Ctl) or the treated group (n=50/pen 10 replicate pens/group) that received BTVCx (0.2ml/bird/oral gavage) on d2 and 16 of life. On day 28 (exp1) or d21 (exp2), birds were challenged with a combination of *E. acervuline* (EA), *E. maxima* (EM), and *E. tenella* (ET). Six days post challenge, 5 birds/pen birds were sacrificed, group weighed, and coccidial lesion scored according to the Johnson-Reid scale wherein 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection. On d28 (experiment 1) or d27 (experiment 2), fresh fecal samples were collected from each pen determine the degree of oocysts shedding/cycling. Results showed significant reductions in lesions scores in both experiments 42% for experiment 1 and 45% for experiment 2 (36/39%EA, 43/39%EM, 60/66%ET, respectively) and total oocyst shedding was reduced 42% in experiment 1 and 65% in experiment 2 (40/75%EA, 68/85%EM, 40%ET, respectively). In a third challenge experiment (see experimental design above), BTVCx was compared to a Ctl group and commercial coccidia vaccine group (Vx): Productive parameters (Adjusted Feed Conversion Rate (FCR) and Average Weight Gain (DWG)) were measured throughout the course of the experiment. At the conclusion of the experiment (d42) statistical differences were observed in DWG and FCR when comparing the BTVCx group and the Vx group with the Ctl group; birds receiving BTVCx weighed an average of 87g/bird more than the Ctl group and feed conversion improved by 84 points. These data taken together indicate the potential of BTVCx as an alternative control strategy for coccidiosis.

Keywords: Vaccine, Coccidia, Alternative, *Eimeria*, Poultry

SESSION 2
Microbial-derived products

ORAL PRESENTATIONS

Microbiome for gut health: A modern tool and a target in the effort to address antimicrobial resistance

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The emergence and spread of antimicrobial resistance (AMR) is a global concern, and it has become a major political, social, and economic burden of our time. The use of antimicrobials in livestock agriculture has been a major focus of this issue since it is one of the potential contributing factors causing human AMR infections. Concerning AMR, projections suggest that by 2050, more people will die of bacterial infections than cancer due to the fact that currently available antimicrobials will no longer be as effective in treating bacterial infections. This will not only affect the health outcomes of humans, but it will also affect those of animals, including production yields of food-producing animals. Therefore, control and prevention of AMR require the exigent adoption of a "One Health" approach through the integration of human, animal, and environmental health. Research on AMR in both human and animals has focused mainly on pathogenic bacterial species which are readily cultured in the laboratory. Recent advances in next-generation sequencing of complex microbial communities (microbiomes) improved our understanding of the ecology of AMR in One Health. This cutting-edge technology enables to track the fate of AMR genes. On the other hand, our understanding is growing in the aspect of reciprocal, intimate relationships between microbiome and host immune system that are orchestrated by preceding microbial encounters and prepare the host for future ones. Antimicrobials alter the structure of the microbiota, expand the host-specific pool of AMR genes and bacteria, degrade the protective effects of the microbiota against invasion by pathogens, and may impair vaccine efficacy. Other strategies including manipulation of the gut microbiome to eliminate antimicrobial resistant bacteria or to boost host immune responses to vaccines may prove valuable in addressing antimicrobial resistance. In recent years, manipulation of the microbiome using microbial-derived products (including fecal transplantation) to improve gut health is becoming a promising alternative to antimicrobials in animal agriculture. Fecal microbiota transplant (FMT) has shown effectiveness in treating certain human diseases such as *Clostridium difficile* infection, however, the fundamental science behind the application of FMT is still not yet fully understood. With this notion, the application of FMT in livestock agriculture should be cautious and more research efforts are needed. Understanding the role of gut health in achieving optimal production is of essential to discover the most reliable and sustainable alternatives to replace antimicrobial compounds used in livestock.

Keywords: Microbiome, Gut Health, Antimicrobial Resistance

Non antibiotic strategies to modify the microbial population of dairy cattle: impacts on milk production, animal health, and food safety

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In the United States, the dairy industry includes approximately 9 million cattle that produce on average 19,000 pounds of milk a year, and comprise 50% of the ground beef supply. Cattle are ruminant animals that depend on a symbiotic relationship with the microbial population of their gastrointestinal tract to convert forage and grain to high quality meat and milk. The gastrointestinal microbial population of dairy cattle is extremely dense and diverse, and is a complex natural ecosystem that can be utilized to improve animal production efficiency, sustainability, animal and human health, as well as food safety. For example, a decreased ruminal microbiome diversity and increased lactate utilizing bacterial populations in beef and dairy cattle have been linked with increased milk production efficiency. While antibiotics have been used for many years to shift microbial populations to increase production efficiency, the mode of action of antibiotics on the gastrointestinal microbiome and host animal physiology (both positive and negative) remain largely unknown and unreplaceable. Non-antibiotic strategies have been devised to modify the microbial population of dairy cattle on the farm. A large number of approaches, including management practices, dietary changes, organic acid inclusion, probiotic and prebiotic feed additives, and vaccination have been widely used worldwide in the dairy industry to enhance milk production and feed efficiency, as well as to improve animal health. Many of these strategies rely upon harnessing the natural competitive nature of bacteria and specific microbial ecological factors to eliminate pathogens that negatively impact animal production, health, or food safety which may have unintended consequences of which we need to be aware. In this presentation we explore the ecology behind the efficacy of alternatives to antibiotics and how they may impact dairy production efficiency and can be used to improve both human and animal health.

Keywords: antibiotic alternatives, prebiotics, organic acids, microbiome, animal efficiency

Microbiological quality and possible role as a source of antimicrobial resistance genes of commercial probiotic products for livestock and aquatic animals

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The reduction of antimicrobial use is one of the most important action to combat antimicrobial resistance (AMR) crisis. Probiotic products are among alternatives to antibiotics that have been increasingly used in food animal production. Therefore, this study aims to determine the microbiological quality and the presence of resistance genes in some probiotics available for livestock and aquatic animals in Thailand. Nine commercial-probiotic products for livestock and aquatic animals were examined for the number of probiotic bacterial strains as indicated on the product labels. Confirmation of species was performed by multiplex PCR for *Lactobacillus* and *Enterococcus* and Amplified Ribosomal DNA Restriction Analysis (ARDRA) for *Bacillus*. The contamination of pathogenic bacteria (i.e. *Escherichia coli* and *Salmonella enterica*) and the presence of 56 genes that encode resistance to clinically-important antibiotics were determined. The results showed that none of the products tested were contaminated with *E. coli* and *Salmonella*. Inaccurate labelling in either numbers or species of bacteria was a common issue among the probiotic products tested. Some products did not contain the species as claimed on the label. *B. licheniformis* and *B. sphaericus* were commonly misidentified as *B. subtilis*. Of seven products claimed to contain *B. subtilis*, six were found to contain *B. subtilis* cluster consisting *B. pumilus*, *B. amyloliquefaciens* and *B. atropheus* and one was positive to *B. licheniformis*. Contamination of *L. rhamnosus* and *L. casei*-group was frequently found. *E. faecium* was mislabeled as *Streptococcus faecium*. Resistance genes encoding resistance to sulphonamides, streptomycin and tetracycline were observed in three products. One product contained both *sul1* and *aadA2* and one carried *tetA* and *tetM*. The study is currently being undertaken to find horizontal transfer of genes in probiotic samples. While the use of probiotics in food animals may generate beneficial effects, it can also pose risks as a source of resistance genes. The results highlight the need to regulate the production and the use of probiotics to assure their quality and reduce their potential contribution to the spread of AMR.

Swine-derived probiotic *L. Plantarum* modulates porcine intestinal endogenous HDP synthesis

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Weaning stress renders piglets susceptible to pathogen infection, which leads to post-weaning diarrhea, a severe condition characterized by heavy diarrhea and mortality in piglets. Probiotics exert beneficial health effects, mainly by reinforcing the intestinal barrier function and modulating the gut microbiota. However, the mechanisms of action, and especially, the specific immunomodulatory effects of probiotics on porcine have not yet been elucidated. Host defense peptides (HDPs) have antimicrobial as well as immunoregulatory activities and are involved in epithelial innate immune defense. Dietary modulation of endogenous HDP synthesis is an effective way to boost the host innate immune system. This study aimed to investigate the role of the swine derived probiotic *Lactobacillus plantarum* strain ZLP001 in porcine HDP induction and the underlying mechanism. To this end, we evaluated the stimulatory effect of *L. plantarum* ZLP001 on HDP expression in piglet intestinal tissue *in vivo* and porcine IPEC-J2 cells and 3D4/31 cells *in vitro*, and we examined the underlying intracellular signaling pathway in IPEC-J2 cells. Quantitative real time polymerase chain reaction (qPCR) analysis showed that *L. plantarum* ZLP001 treatment increased the mRNA expression of jejunal and ileal HDPs in weaned piglets. In IPEC-J2 and 3D4/31 cells, *L. plantarum* ZLP001 stimulated HDP expression, but different HDP induction patterns were observed, with the various HDPs exhibiting different relative mRNA levels in each cell line. In addition, *L. plantarum* ZLP001 induced HDP secretion, which enhanced the potential antimicrobial activity of IPEC-J2 cell-culture supernatant after incubation with *L. plantarum* ZLP001. *L. plantarum* ZLP001 induced porcine HDP expression through TLR2 recognition as evidenced by the fact that HDP expression was suppressed in TLR2-knockdown IPEC-J2 cells. Further, we found that *L. plantarum* ZLP001 activated the extracellular signal-regulated kinase (ERK)1/2 and c-jun N-terminal kinase (JNK) signaling pathways, as indicated by enhanced phosphorylation of both ERK1/2 and JNK and the fact that HDP expression was suppressed upon inhibition of ERK1/2 and JNK. Furthermore, *L. plantarum* ZLP001 activated c-fos and c-jun transcription factor phosphorylation and activity. We conclude that *L. plantarum* ZLP001 induces porcine HDP expression *in vivo* and in *vitro*, and the induction seems to be regulated via TLR2 as well as the ERK1/2/JNK and c-jun/c-fos signaling pathways. Modulation of endogenous HDPs mediated by *L. plantarum* ZLP001 might be a promising approach to improving intestinal health and enhancing diarrhea resistance in weaning piglets.

Keywords: *Lactobacillus plantarum* ZLP001, host defense peptide, weaning piglet, induction effect, underling mechanism

Small molecular weight metabolites regulating growth and immunity as postbiotic antibiotic alternatives

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Mounting regulatory pressure, consumer concerns about antibiotic resistant bacteria, and competition from alternative protein sources have prompted the poultry producers to search for novel antibiotic alternatives. Recently, *Bacillus* spp. as a direct fed microbial (DFM) is gaining in popularity as an antibiotic alternative. A metabolomic approach was used to characterize and identify host- and microbiome-derived biochemical compounds in the ileal content of broiler chickens which were fed dietary *Bacillus subtilis* as DFM. Fourteen-day-old broiler chickens (n = 196) were fed a basal diet or a diet supplemented with *Bacillus subtilis* 1781 or 747 as DFM. The chickens and the amount of feed that the broiler chickens consumed were weighed at 21 days of age for growth performance measurement. Eight chickens per group were euthanized and their ileal content harvested for metabolomic profiling. From 14 to 21 day of age, body weight gains of chickens fed diets supplemented *Bacillus subtilis* 1781 and 747 were significantly increased compared to those of chickens fed basal diet. Compared with unsupplemented controls, the levels of 83 biochemicals were significantly altered (25 increased, 58 decreased) in chickens given the *Bacillus subtilis* 1781 DFM-supplemented diet, while 50 were significantly altered (12 increased, 38 decreased) with the *Bacillus subtilis* 747 DFM-supplemented diet. The changes in the levels of intestinal biochemicals provided a distinctive biochemical signature unique to each *Bacillus subtilis*-supplemented group. These results provide the framework for future studies to identify natural chemical compounds that can be used for improving poultry growth performance.

Keywords: antibiotic alternative, *Bacillus subtilis*, gut metabolite

Managing the gut microbial populations: From science to practice

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Colonization and succession of the gastrointestinal microbiota in avian species is not well characterized; however as in other systems, it is recognized that the microbiota influences the health and production efficiency of poultry. Numerous studies have revealed that the gut microbiota of commercial poultry flocks is highly variable within and between flocks. We believe this is influenced by the initial colonizers of the gastrointestinal tract, as the small intestinal microbiota between producers differ significantly in poults and chicks at day-of-hatch. Our data suggests that horizontal transmission from the environment is greater than vertical transmission in poultry production due to common commercial management practices. We have shown that successional changes in the small intestinal microbiota start *in ovo* and are affected by management practices such as disinfection of the setter and the hatcher as well as administration of antibiotics and/or vaccines *in ovo* leading to hatchery-specific bacterial populations. The early establishment of lactic acid bacteria essential for stabilizing intestinal homeostasis, digestion and nutrient absorption, and nurturing mucosal conditions for immunological protection is disrupted in commercial birds. In addition, our research has documented significant populations of avian pathogenic *Escherichia coli* (APEC) in broiler chicks at the day of hatch. APEC is a causative agent for colibacillosis in birds in the form of airsacculitis, cellulitis, pericarditis, or perihepatitis. A high population of APEC can disrupt gastrointestinal homeostatis in the young bird and impact early growth and performance. If left unchecked, these isolates can translocate to the blood stream and cause colibacillosis.

In order to, promote colonization by beneficial bacteria and reduce the levels of APEC, without the use of antibiotics, a probiotic was developed comprised of two lactic acid bacteria selected for their immunomodulatory capabilities and two *Bacillus* strains that produced secondary metabolites inhibitory to APEC strains. This probiotic was administered to chicks in a single dose at the hatchery. A study comparing the probiotic against the antibiotic gentamycin, indicated that both the probiotic and the antibiotic reduced APEC levels in two-week old broilers compared to untreated birds. A second study showed a reduction in levels of APEC at day 7 as well as more uniform flocks as the coefficient of variability at harvest was reduced. The probiotic is therefore an effective alternative for antibiotics to establish a healthy microbiota and control APEC when applied at the hatchery.

Responses of *Bacillus amyloliquefaciens* CECT 5940 supplementation in weaned pig diets

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At weaning, piglets suffer from immunological, environmental, nutritional and social stress, consequently affecting the gut health and growth performance. Supplementation of probiotics is considered as one of the strategies to maintain the intestinal health and minimize the negative effects of the weaning stress in piglets. Probiotic, *Bacillus amyloliquefaciens* CECT 5940 is a natural spore - forming bacteria, which supports gut health and improves growth performance in poultry. However, there are limited studies conducted to delineate effects of dietary supplementation of probiotics *B. amyloliquefaciens* CECT 5940 in piglets. A study was therefore conducted to evaluate the effects of probiotic (*B. amyloliquefaciens* CECT 5940) supplementation on growth performance, plasma urea nitrogen and fecal microbiota in weaned piglets. A total of 180 mixed-sex pigs weaned at 28 d were randomly distributed to 3 dietary treatments consisting 6 replicate pens (10 pigs per replicate pens). The duration of the study was 62 d, which included pre-starter (28 to 60 d; 7 to 18 kg BW) and starter (61 to 90 d; 18 to 35 kg BW) phases. All the diets were corn-soybean meal-based and pigs were provided *ad libitum* access to feed and water throughout the study. The dietary treatments were i) control (without AGP and probiotic), ii) supplemented with AGP (Colistin sulphate at the inclusion of 20 ppm/kg of feed), iii) supplemented with probiotic (*B. amyloliquefaciens* CECT 5940 @ $1x10^9$ cfu per kg of feed). Data were analyzed as completely randomized design and each pen was considered as an experimental unit. Data on growth performance showed that during starter and overall periods, piglets fed probiotics had better (P<0.05) ADG compared with those fed AGP (481 g versus 468 g). The overall feed efficiency of piglets fed probiotics was improved (P<0.05) compared with AGP supplemented group (2.02 versus 2.07). The final BW of probiotics group (37.54 kg) was higher than AGP (36.75 kg) and control group (36.20 kg) pigs. There were no significant differences in plasma urea-nitrogen levels and microbial profiles between the dietary treatments. In conclusion, supplementation of probiotics (*B. amyloliquefaciens* CECT 5940) had better growth performance than AGP supplemented pigs, which implies that probiotics could be beneficial in improving performance of young pigs fed AGP-free diets.

Keywords: AGP, probiotics, piglets, performance

Fermentate Bioactives Impact on SARA and a Mastitis *Stretoccocus uberis* Challenge to Reduce AIF Use in Bovines

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The "normal" process for evaluating interventions for prevention of syndromes impacting production animals is to develop a molecule specific against the disorder. This is followed by controlled trials and finally large epidemiologic surveillance data to support widespread adoption. This presentation highlights the use of epidemiologic surveillance data to reverse engineer direct controlled trials. Following the controlled trials, proof of concept trials was designed and conducted to illustrate the impact of unique fermentate bioactives against sub-acute ruminal acidosis (SARA) and mastitis.

Data will be presented in two proofs of concept trials demonstrating the potential for reducing anti-infectives (AIF) used to prevent: 1. SARA, and 2. Bovine Mastitis.

1. Study: A 5x5 Latin square study was designed in which cannulated steers were fitted with indwelling pH monitors and placed on a high grain diet. Control animals were fed the same diet and received tylosin and rumensin whereas the treated animals received the high grain diet and only *Saccharomyces* bioactives. A negative control treatment received the high grain diet only. Results: The steers in the antibiotic and negative control treatments spent significantly more time below the SARA (< 5.6 pH for 180 minutes) threshold. The fermentate bioactive prevented induction of SARA and reduced the amount of rumen LPS.
2. Study: 20 multiparous Holsteins were randomized into a treatment and control group. A direct challenge of *Streptococcus uberis* was administered through the teat canal and data captured. Results: The treatment group demonstrated improved resistance within the mammary gland to mastitic events via: 1. Improved local, but not systemic, immune cell function; 2. Increased tissue response to the pathogen; 3. A priming effect on body tissues via HSPs (Heat Shock Proteins) activation; and 4. Gene up-regulation of key innate immune system compounds/cells.

Taken together, these studies demonstrate favorable shift in microbiome and up-regulation of the innate immune system as a result of the feeding of these novel bioactives, will reduce the dependence and need for AIF in bovine production settings.

Keywords: microbiome, fermentate bioactive, gene up-regulation, SARA, *Saccharomyces*

SESSION 2
Microbial-derived products

POSTER PRESENTATIONS

MI1
Effect of *Bacillus* spp. probiotic supplementation on performance, immune response and gut health of broilers challenged with *Salmonella* Enteritidis

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Inclusion of antibiotics in poultry diet as a growth promoters (AGPs) can reduce the prevalence of enteric pathogen such as *Salmonella* spp. With the emergence and dissemination of antibiotic resistance *Salmonella*, increasing consumer demand on use of antibiotics as a feed additive in U.S. Therefore, search for alternative strategies to replace antibiotics as a feed additive has gained interest in animal agriculture. In addition, continuing circulation of multiple serovars of *Salmonella* in poultry flocks, along with increasing reports of human Salmonellosis, warrants the necessity of developing control methods to decrease *Salmonella* load in poultry production. *Bacillus subtilis* and *Bacillus licheniformis* are potential probiotics and are currently used as a probiotic in poultry production. The overall objective in this proposal is to determine the effects of *B. subtilis* and *B. licheniformis* probiotic supplementation on performance, cecal *Salmonella* load, immune response, and gut morphology in broilers challenged with *Salmonella enterica* serovar Enteritidis.

A total of 360 one-day-old broiler chicks were randomly distributed to four experimental groups a 2 X 2 factorial set up of treatments; Control, Control + Challenge, Probiotics (10 mg of *B. Subtilis* strain HU58/kg of feed; HU58™ plus 100 mg of *B. licheniformis* SC307; Prepro™/kg of feed; Microbiome LABS, Saint Augustine, FL), and Probiotics + Challenge. Each treatment was replicated in 6 pens (n=6) with 15 chicks per pen. At 21 d of age, all birds in Challenge groups were inoculated orally with 250 µl of 1 X 10^9 CFU *S.* Enteritidis.

At 21 d post-*Salmonella* challenge, chickens challenged with *Salmonella* had the 11% lower ($P < 0.05$) BW gain compared to the control non- challenge groups. Chickens that were supplemented with probiotics in the *Salmonella* challenged groups had only 5.1% reduction in BW compared to the control group. At 5, 12, and 21 d post-*Salmonella* infection, chickens challenged with *Salmonella* had 1.99, 1.93 and 1.71 log Salmonella CFU/g of cecal contents while chickens supplemented with probiotics and challenged with *Salmonella* had 0.73, 1.59, and 1.32 log lower *Salmonella* CFU/g of cecal contents respectively. Chickens supplemented with probiotics and challenged with *Salmonella* had higher ($P < 0.05$) anti-*Salmonella* IgA compared to the control birds with *Salmonella* infection. At 21d post-*Salmonella* infection, chickens supplemented with probiotics and challenged with *Salmonella* had comparable villi height compared to the control non-challenge group, while control birds infected with *Salmonella* had the shortest villi height ($P < 0.05$). Increased villi height and crypt depth can improve nutrient digestibility and absorption and may explain the improved production performances in probiotic supplemented birds. It can be concluded that *B. subtilis* and *B. licheniformis* probiotic can be a tool to decrease *Salmonella* loads in the broiler intestine and *B. subtilis* and *B. licheniformis* supplementation can be expected to decrease broiler carcass contamination with *Salmonella*.

Keywords: *Bacillus* spp. probiotic, supplementation, *Salmonella* challenge, immune response, broiler chickens

MI2

Efficacy of Synbiotics to promote gut integrity and reduce *Salmonella* colonization in broilers

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The aim of this experiment was to study the efficacy of Synbiotics (a combination of *Saccharomyces cerevisiae, Bacillus subtilis, B. licheniformis, Lactobacillus acidiophilus, L. plantarum, L. lactis, Streptococcus thermophillus* and Fructooligosaccharides) to promote gut integrity and reduce *Salmonella* colonization. Two hundred and forty, one-day-old, female chicks were divided into 4 groups of 60 each. Each group was divided into 3 replicates of 20 each. Birds in-group 1-3 were served as treatment groups. Birds in-group 4 was a positive control group. Birds in-group 1 were provided Synbiotics 2 ml/L drinking water (DW) skip a day from 1-42 days of age (6 hr/day). Birds in-group 2 were provided synbiotics 2 ml/L DW for 3 consecutive days (6 hr/day) in each week. Birds in-group 3 were provided Synbiotics 1 ml/L DW every day from 1-42 days of age (6 hr/day). Birds in-group 4 were provided fresh water ad lib. At 21 and 42 day-old, three birds in each replicates were euthanized to collect intestine for histopathology analysis, and also collect rectal content to test for butyric acid activity. Body weight and feed conversion ratio was calculated at 21 and 42 day-old. At 21 day-old, all birds were challenged with 1ml of *Salmonella* Enteritidis Nalidixic acid resistance strain (108 cfu/ml). At 21 and 28 day-old, birds were cloacal swabbed for testing for *Salmonella* colonization. The results revealed that at 21 and 42-day-old birds in treatment groups showed better villi height and crypt depth than those birds in positive control group especially in the duodenum. At 21-day-old, birds in group 1 revealed significantly higher duodenum villi height and crypt depth (910.75±416.39; 77.78±48.70) than those of birds in group 4 (709.05±93.86; 39.52±9.74) ($P<0.05$). In addition, at 21-day-old, butyric acid activity of birds in treatment groups 1 (4.28±2.50), 2 (3.04±2.70) and 3 (4.07±2.20), showed significantly higher than birds in group 4 (1.91±1.51) ($P<0.05$). At 42-day-old, average body weight (kg) and FCR of birds in-group 1 (2.08±0.34; 2.27±0.09), 2 (2.17±0.15; 2.14±0.03), 3 (2.04±0.36; 2.23±0.06) showed better than birds in-group 4 (1.99±0.42; 2.37±0.18). At 21 day-old (before challenge), no *S.* Enteritis Nalidixic acid resistant strain was found in any group. However, at 28 day-old, *S.* Enteritis Nalidixic acid resistant strains were found lower in treatment group 1 (78%), 2 (78%), 3 (68%) than the positive control group 4 (100%). In conclusion, Synbiotics promoted the chicken performance and gut morphology and butyric acid activities.

Keywords: butyric acid activity, gut integrity, synbiotics, *Salmonella* reduction

MI3
Five years dynamic of *Salmonella enterica* in commercial poultry farms with and without probiotics application

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Probiotics application in commercial livestock serves for several needs such as improve production performance, promote animal health and support food safety in animal production. Numerous studies indicated the effect of probiotics on *Salmonella* reduction in chicken but most of publications imply only in experimental study scale. The insufficient information on real farm situation poses *Salmonella* control by probiotics use in commercial farms still obvious. To answer this question, we analyzed the data collection harbored from an ISO17025: 2005 service laboratory in Thailand, scope on probiotics application and *Salmonella* occurrence.

Data were analyzed from the collection of samples submitted from 4 commercial chicken breeder farms. Each farm submitted samples continuously for at least 5 consecutive years. In order to compare effect probiotics on *Salmonella* occurrence, 2 sets of data were generated in comparison of farms probiotics usage status; set 1) Early stage of probiotics application (EPA) farm and Continuous probiotics application (CPA1) farm (2007 - 2011), set 2) Continuous probiotics application (CPA-2) farm and non-probiotics application (NPA) farm (2014 - 2018).

A total of 902 samples were included in data set 1 and 4,222 samples in data set 2. To reduce the bias of data, environmental samples were excluded, only fecal samples (CPA 1, CPA-2 and EPA) and boot swab samples (NPA) were processed in the analysis. In data set 1, the percentage of *Salmonella* positive sample in EPA farm gradually decreased after the onset of the probiotics (average 34.18% to 18.82%). While the average percentage of positive sample during the identical 5 years in CPA-1 farm was 1.01% and the variation of positive sample is between minimum 0% to maximum 2.46%. In data set 2, the 5-years average occurrence in CPA-2 farm was 3.70% (minimum 2.40%, maximum 5.68%) and 5.33% (minimum 2.75%, maximum 8.66%) in NPA farm. Serovar information of samples also included in the analysis. The most present serovar was changed year by year and different between farms.

In conclusion, the information from data set 1 and 2 suggested that application of probiotics possible to reduce the *Salmonella* occurrence in commercial breeder farm and the continuous application of probiotics could stabilize the farm *Salmonella* status.

However, according to the data was obtained from the commercial laboratory service, multiple limitations are noted. The data in this study could not represent the chicken population in the farm since it was obtained from customer submission samples. The variation between the type of sample from each farm was depended on farm's *Salmonella* monitoring and management. This information may be affected by other *Salmonella* control procedures such as vaccination and farm management during the period of analysis.bioactives against sub-acute ruminal acidosis (SARA) and mastitis.

Keywords: probiotics, *Salmonella enterica*, commercial poultry farm

MI4
Fermented feed stuff increased orexin level associated with increased food intake and weight gain in weaning pigs

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Weaning exposes pigs to various stress factors, leading to growth retardation due to low feed intake, as well as disorders of gastrointestinal function. Fermentation of feed can be beneficial from a health standpoint, especially in the early stages of the pig lives. This study was conducted to investigate the effects of fermented feed stuff on the performance and gastrointestinal hormones involved in feed intake and growth in weaning pigs. A total of 320 Duroc × Landrace × Yorkshire weaning pigs (28 ± 2 d of age with body weight (BW) of 7.38 ± 0.24 kg) were divided into unfermented diet group (UFD) and fermented diet group (FD). Each group consisted of 8 replicates (pens), with 20 piglets per pen. The pigs from the UFD group were fed a basal diet, while the piglets from the FD group were fed a basal diet with 5% feed stuff, which was replaced by the fermented feed stuff for 21 days. Blood samples and tissue samples from the stomach, jejunum and ileum were obtained from six pigs from each group on day 10 of the trial for further analysis.

Pigs fed the fermented diet had higher average daily feed intake (ADFI) and average daily gain (ADG) during the first week, last 2-weeks, and over the entire 3-week period compared with pigs fed unfermented diet. Moreover, feed conversion was only improved by fermentation during the last 2-weeks (P<0.05). Pigs fed fermented diet had a higher serum orexin level and up-regulation in the expression of the prepro-orexin (PPOX) gene in the gastric fundus, jejunum and ileum mucosa (P<0.01), and the expression of IGF-1 (P<0.05) and IGFR (P<0.01) gene in jejunum was compared with pigs fed an unfermented diet. Results indicated that dietary supplementation with fermented feed stuff improved growth performance of weaning pigs by increased orexin, IGF-1 and IGFR levels.

Keywords: Fermented feed stuff, piglet, growth performance, orexin, IGF-1

MI5
Feeding pellets inoculated with *B. Amyloliquefaciens* strain H57 improves production parameters in sheep

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The live export of sheep is important to the Australian economy. There is a continuing need to improve the productivity and health of these sheep, and probiotics may be beneficial in this[1]. Previous reports showed that feed intake, feed conversion, live weight (LW) and body condition score (BCS) all improved when sheep were fed a diet containing probiotic *Bacillus amyloliquefaciens* strain H57 ('H57')[2]. Our study investigated the production responses when H57 is included in high-fibre pellets commonly fed to young sheep during live export.

Merino wethers (<1 year old, 35.1 kg LW & BCS 2.2) were fed high-fibre, 'export' pellets (control sheep, n=18), or the pellets inoculated with H57 (H57 sheep, n=18), for 28 days, followed by 14 days where all were fed the control pellets only. Pellet intake (PI) was recorded daily, with LW and BCS measured weekly, and feed conversion ratio (FCR) calculated weekly as PI/LW change. Samples of blood and rumen fluid were collected for analysis every 14 days. Data were analysed by a one-way ANOVA with repeated measures and significance at $P< 0.05$.

After 28 days in treatment, H57 sheep gained 25% more LW than control sheep (6.0 vs 4.8 kg). Compared to control sheep, H57 sheep were also of markedly higher BCS (2.94 vs 2.44), and although they consumed 6.9% more pellets (1429 vs 1337 g/day), they had a better FCR (6.8:1 vs 8.4:1). The H57 sheep also had higher concentrations of acetate and total volatile fatty acids (VFA) in rumen fluid than control sheep. All blood metabolites, leptin and insulin levels were not different and within the expected range for healthy sheep. Some of the responses persisted in H57 sheep even after the treatment, and over the 14 days when they were fed pellets without H57.

The marked differences in the production parameters measured are consistent with other studies[1]. While the energy-yielding products of rumen fermentation (VFA and acetate) were improved in H57 sheep, positive responses may have also occurred in the intestines of these sheep, all resulting in increases in tissue deposition measured as changes in LW and BCS.

In conclusion, young Merino wethers fed high-fibre, 'export' pellets inoculated with H57 ate more pellets, grew faster and converted the pellets into LW and body condition more efficiently than peers fed the same pellets without H57. The marked improvement in these production parameters could be expected to bode well for young Merino sheep prior to and during live shipment. The mechanisms by which these production responses occurred remain to be elucidated.

Keywords: probiotic, H57, *Bacillus*, sheep, export

MI6
Effect of microbial-derived and acid based feed additives on the antibiotic resistome in broilers

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Use of antibiotics results in the emergence of antibiotic resistance, which is a cause of global concern for human and animal health. The extent to which antibiotic resistance is associated with the use of chemical and biological agents used for the expressed purpose to control, deter, inhibit or kill harmful microorganisms is poorly understood, according to FAO, 2018. Three studies conducted in Thailand, USA and Austria evaluate the effect of antibiotics, probiotics and an acid-based feed additive on the prevalence of antibiotic-resistant bacterial population in broilers.

Study Thailand: Shotgun metagenomics was used to evaluate the effect of an antibiotic compound as well as a microbial derived product on the broiler caecal microbiota (microbiome), virulence factor abundance (virulome) as well as the antibiotic resistance genes (resistome). Taxonomical analysis revealed the positive influence of the product on the chicken gut microbiota, indicated by significantly increasing the abundance of the healthy microbiota, such as short chain fatty acid (SCFA) producing bacteria and by reducing the population of potentially pathogenic microorganisms (*Escherichia coli*, *Salmonella enterica*, *Campylobacter jejuni*, *Eimeria* spp., etc. This resulted in a significantly lower abundance of bacterial antibiotic resistance genes and virulence factors in the caecal microbiome of the chickens.

Study USA: The aim of this study was to evaluate the effect of a microbial derived product tested in the study in Thailand, an organic acid-based feed additive and ampicillin on the prevalence of antibiotic-resistant *E. coli* in the ceca of broilers. Administration of ampicillin in broilers for five days led to a significant increase in the abundance of *E. coli* strains resistant to ampicillin, amoxicillin-clavulanic acid, cefoxitin, and ceftriaxone. The effects of the microbial derived and acid-based feed supplementation on the prevalence of resistant *E. coli* are demonstrated by the significantly lower ceftriaxone minimal inhibitory concentration (MIC) values for this group than for the antibiotic group. Additionally, the group received microbial derived product exhibited lower MIC values than the ampicillin group.

Study Austria: The aim of this study was to evaluate the effect of an acids-based feed additive, as well as fluoroquinolone antibiotics, on the prevalence of antibiotic resistant *E. coli*. Treatment of broilers with enrofloxacin significanlty increased the number of *E. coli* resistant to ciprofloxacin, streptomycin, sulfamethoxazole and tetracycline; it also decreased the number of *E. coli* resistant to cefotaxime and extended spectrum beta-lactamase-(ESBL) producing *E. coli* in the ceca of broilers. The supplementation of feed with organic acids based product did not contribute to an increase of antibiotic resistant *E. coli*. The oposite was observed: significant decrease in *E. coli* resistant to ampicillin and tetracycline compared to the control and antibiotic group. The reason for such a decrease needs to be investigated further.
In summary, the findings from this experiment provided more evidence on the potential of microbial derived and organic acid-based feed additives as safe antibiotics' alternative in poultry farming.

Keywords: resistance, antibiotics, probiotics, acidifiers, microbials

MI7
Evaluation of a water applied biopromoter and feed administered MOS as antibiotic alternatives in Breeders and Broilers

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As the poultry industry discontinues the use of antibiotics, effective use of alternative strategies such as prebiotics are being investigated. The primary problems historically addressed by antibiotic use in the poultry industry are key parameters used to determine the effect of antibiotic alternatives. A measure of efficacy in broilers or meat-producing birds is growth promotion; where as in longer-lived, broiler breeder birds, disease prevention and egg production are important. A key benefit of antibiotics was the broad spectrum use in birds of different ages and purposes to improve bird production. In this study, we evaluate the effect of a multi-pronged prebiotic approach using a water additive biopromoter composed of inactivated fermented *Bacillus subtilis* fragments and yeast cell wall extracts, in addition to, a feed additive mannan oligosaccharide (MOS) composed of betaglucans from yeast cell wall extracts on a mineral carrier. Both of these prebiotics have demonstrated immunomodulatory capabilities, and we hypothesize that the combination will improve the production of both broilers and breeders as the use of antibiotics becomes less of an option.

A series of two trials were done to evaluate the effect of the biopromoter and MOS in both broilers and broiler breeders. A randomized controlled broiler study consisted of 215 broilers per treatment group raised in floor pens. The broiler trial compared birds administered a commercial diet, a diet with 0.5 kg/ton antibiotic (BMD) or a diet of MOS at 2kg/ton and biopromoter in the water at 0.2 mL/bird on days 3 and 17 of life. The broiler breeder study compared two 20,000 bird commercial houses of broiler breeders with a known farm history of colibacillosis. On this farm, one house was a control and received no treatment, and the second house received MOS at 2 kg/ton continuously during weeks 25-34 of life and 0.2 mL/bird biopromoter in the water weeks 24 and 26 of life.

In the broiler trial, MOS and biopromoter treated birds (2,388 g) had a 42-day body weight greater than untreated (2,243 g) or BMD treated (2,295 g) birds. Feed conversion ratio was also improved in broilers treated with MOS and biopromoter at 1.585 when compared to both untreated and BMD treated birds, 1.744 and 1.704, respectively.

Broiler breeders not administered the prebiotic combination experienced a spike in mortality and decreased production diagnostically associated with colibacillosis. MOS and biopromoter treated broiler breeders remained healthy and had an average egg production rate 23% higher than the control flock. Peak egg production was 84.48% in the treated flock and 67.03% in the control flock. The improvement in health, egg production and peak resulted in the treated flock producing a total of 899,230 eggs, which was 1.8X the total number of eggs produced by the control flock (497,425 eggs).

In conclusion, using a feed administered MOS product and a water applied biopromoter improved the beneficial production parameters of both broiler and broiler breeder production.

Keywords: mannan oligosaccharide, *Bacillus subtilis*, prebiotics, broilers, broiler breeders

MI8
Supplemental *Bacillus subtilis* DSM 32315 modulates intestinal structure, microbial composition and improve the performance in broiler chickens

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Probiotics offer one alternative to antibiotic growth promoters as they have been shown to improve the development and maintenance of a stable gut microbiome in poultry, which leads to reduced enteric disease and improved growth performance. Therefore, the objective of this study was to delineate the effects of *Bacillus subtilis* DSM 32315 (*BS* DSM32315) on intestinal morphology, microbial composition and performance in broiler chickens. A total of 240 Arbor Acre (AA) male birds were randomly assigned to 2 dietary treatments with 10 pens of 12 birds per pen. The dietary treatments included a basal diet (Control), a basal diet supplemented with 500g/MT of *BS* DSM32315, 1.0x10^6 CFU/g feed). Corn-soybean meal based basal diets were formulated on the recommendation which is normally used in china for starter (day 1-14), grower (day 15-28) and finisher (day 29-42) phases. Water and pelleted feed were provided ad libitum. Supplemental *BS* DSM32315 significantly increased (P<0.05) body weight, average daily gain, and feed intake of broilers at 28 and 42 d of age. Intestinal lesion scores were significantly reduced (P<0.05) in birds fed *BS* DSM32315, while there was tendency for higher (P=0.077) expression of Ileal tumor necrosis factor (TNF)-α. In the cecum, PCA plot defined groups from control and treatment groups occupied distinct positions. Birds supplemented with *BS* DSM32315 had higher abundance of *Firmicutes* and lower abundance of *Bacteroidetes* which also increased the abundances of *Christensenellaceae* and *Caulobacteraceae*, and simultaneously reduced the abundances of potentially harmful bacteria such as *Vampirovibrio*, *Escherichia/Shigella* and *Parabacteroides*. The villus height (VH) and VH to crypt depth ratio of ileum was signifcantly higher (P<0.05) in treatment group relative to control. *Clostridiales* and *Bacteroidales* accounted for the largest proportion in the community, which were respectively increased and decreased in treatment group as compared to control. Within *Clostridiales*, the majority belonged to the *Ruminococcaceae* and *Lachnospiraceae* families. Functional comparison based on KEGG orthologue groups demonstrated a decreasing trend (P<0.10) in the enrichment of the pathways for enzyme families, metabolism of cofactors and vitamins in birds fed *BS* DSM32315. Overall, supplementation of supplemental *B. subtilis* DSM 32315 altered microbial composition and intestinal morphology, thus improving the growth performance.

Keywords: *Bacillus subtilis* DSM32315, intestinal morphology, gut microbiome, performance

MI9
Bacillus subtilis DSM 32315 alters immunity, nutrient transporters and cecal microbiome of broiler chickens under necrotic enteritis challenge

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Poultry production programmes require elimination or reduction of the use of in-feed antibiotics growth promoters (AGP). This has led to an increase in the occurrence of necrotic enteritis, making it an economically significant poultry disease requiring an alternative nutritional interventions. Probiotics offer one alternative to AGP because they can elicit specific actions that promotes the development and maintenance of a stable gut microbiome, leading to a reduction in enteric disease and consequently improved performance. This study examined the effects of *Bacillus subtilis* DSM32315 (*BS* DSM32315) probiotic and antibiotic enramycin in broiler chickens challenged with pathogenic strains of *C. perfringens* on cecal microbial populations, functional diversity, nutrients transporters and mRNA expression of cytokines. Day-old Arbor Acre broiler chickens (n=360) were randomly assigned to three dietary groups; control, basal diet fed-group only; antibiotic, basal diet + enramycin 5mg/kg; and probiotic group, Basal diet + *BS* DSM32315, $1x10^6$ CFU/g of feed. Antibiotic and probiotic groups were challenged with *C. perfringens* at d 1 and from d 14 to d 21. Birds supplemented with enramycin and *BS* DSM32315 significantly ($P<0.05$) increased the species richness and the abundance of bacteroidetes by 6.8% at 35 days. Absolute qPCR method showed that at 14, 21 and 35 days of age, the bacterial abundance of *B. bifidum*, *Enterobacter*, and *L. salivarius* were significantly higher ($P<0.05$) in enramycin and *BS* DSM32315 group, while relative abundance of *E. coli* was significantly ($P<0.05$) higher in control group. The expression of anti-inflammatory cytokine of IL-10 and S-IgA were upregulated, while expression of pro-inflammatory cytokines of IL-6, TNF-α, and IFN-γ were downregulated. In addition, nutrient transporters of PepT1, LAT2 and CAT2 were upregulated in supplemented group and GLUT2, SGLT1, rBAT, carbohydrates and vitamins metabolism cofactor are enriched in *BS* DSM32315 fed-group. On the other hand, control group exhibited up-regulation in IL-6, TNF-α, and IFN-γ. Thus, it indicated that supplementation of *Bacillus subtilis* DMS 32315 reduced the effects of enteritis and enhanced the gut-microbial community and immune parameters in broiler chickens.

Keywords: Antibiotic growth promoter, *Bacillus subtilis* DSM32315, *Clostridium perfringens*, gut microbiome, immune response

MI10
Effect of *Bacillus*-based probiotics on improving the intestinal health and performance under enteritis challenge in broiler chickens

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This study investigated the effects of *Bacillus*-based probiotics on performance and intestinal health in broiler challenged with *Clostridium perfringens*-induced necrotic enteritis. One-day-old Arbor Acre (*n*=480) were randomly assigned to four treatments with 10 cages of 12 birds. Dietary treatments were; basal diet negative control (NC), with no probiotics nor antibiotics formulated to contain 2,930 and 3,060 kcal/kg with 24.1 and 16.0% CP, for starter and finisher diet, respectively; basal diet + enramycin (5 mg/kg), an antibiotic growth promoter (AGP); basal diet + *Bacillus subtilis* DSM 32315 (1×10^6 CFU per g of feed, BS) and basal diet + *Bacillus licheniformis* (1×10^6 CFU per g of feed, BL). Growth performance, intestinal morphology, intestinal lesion scores and short-chain fatty acids (SCFAs) were assessed. Average daily weight gain was significantly (P=0.01) higher in BS and AGP-fed groups. Feed conversion ratio was lowest in BS fed group compared to other dietary treatments (P=0.06). Similarly, mortality was lower in all probiotic fed groups compared to AGP-fed group (P=0.001). Intestinal lesion scores was not different among dietary treatments at d 21 (P=0.10) while it was significantly lower (P = 0.03) in birds fed AGP at d 35. In the duodenum and jejunum villus height to crypt depth (VH: CD) was higher compared with NC and BS. Probiotics-fed groups showed higher total SCFAs, acetic and butyric acid concentrations at d21 post-challenge than other groups. The present study indicated that Bacillus-based probiotics can ameliorate the enteritis conditions caused due to *Clostridium perfringens* challenge and the effects are similar to AGP-fed birds.

Keywords: Antibiotic growth promoter, *Bacillus licheniformis*, *Bacillus subtilis*, broiler, necrotic enteritis

MI11
Innate immunomodulation with BCG in porcine monocytes enhances responsiveness to heterologous agonists

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Immunomodulation engages the host's own immune system to fight against disease. The innate immune system recognizes and responds to a large range of foreign agonists, while the adaptive immune system recognizes very specific antigens. The vaccine strain of Mycobacterium bovis (*Bacillus Calmette-Guerin*; BCG) induces an alternative phenotype in monocytes that enhance their ability to respond to a range of heterologous agonists. This alternative phenotype is a form of innate memory and is characterized by long lasting epigenetic and metabolic changes in innate cells, such as monocytes and NK cells, that enhance the responsiveness of these cells to future heterologous agonists. As a method to improve animal health and reduce the use of antibiotics, we interrogated the ability of BCG to alter the innate memory phenotype in pig monocytes. Primary porcine monocytes were stimulated with either live or inactivated BCG (Danish strain). After 24h the supernatants were collected and the cells maintained in culture for 5d. Cells were restimulated with the heterologous agonist lipopolysaccharide (LPS; TLR4 agonist) or Pam3CSK4 (synthetic triacylated lipopeptide; TLR 2 agonist). Cells and supernatants were collected after restimulation for gene expression and cytokine production analysis. Enhanced innate memory (trained immunity) is characterized by increased cytokine production relative to non-stimulated controls. Priming with either live or inactivated BCG enhanced IL-1β and TNFα cytokine production when restimulated with either LPS or Pam3CSK4. Monocytes primed with BCG, but not restimulated with a heterologous agonist, did not produce measurable amounts of cytokine, indicating that the increased cytokine production observed with LPS or Pam3CSK4 restimulation was not due to residual cytokine production from the primary BCG stimulation. No significant differences were observed in IL1B or TNFA gene expression 6h after restimulation with LPS, but live BCG priming upregulated expression of caspase-1 and NLRP3 to LPS restimulation. Collectively, BCG can alter the monocyte innate memory phenotype and enhance responses to heterologous agonists. Innate memory may serve as a mechanism to enhance immune responses and reduce the use of antibiotics.

Keywords: innate training, immunomodulation, pig, monocyte, heterologous protection

MI12
Phages for the Replacement of Antibiotics, and Reduction of *Salmonella*, in Poultry Farms in Kenya

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Globally, the poultry industry has increased by 5% every year for the past three decades, showing a higher growth rate than the pig (3%) and beef (1.5%) sectors. In Kenya, poultry farming represents about 30% of the total agriculture contribution to the Gross Domestic Product (GDP), with an estimated 75% of rural families keeping chicken. Infectious diseases associated with poultry farming and egg production pose high risks to the poultry industry, as well as to the farmers' and consumers' health. The most responsible species for enteric disease in humans transmitted by poultry products is *Salmonella enterica*. Moreover, pullorum disease and fowl typhoid, caused by *S. enterica* serovars Pullorum (*S.* Pullorum) and Gallinarum (*S.* Gallinarum) respectively, have been listed by the FAO as the most important bacterial diseases affecting chicken health and productivity in Kenya. Current methods of controlling or preventing *Salmonella* infections in poultry farms include the use of antibiotics. Furthermore, according to a recent FAO report, an estimated 75% of antibiotics administered to poultry are released in the environment and contribute to the emergence of antimicrobial resistance (AMR). Alternative strategies are being sought to curb the problem associated with antimicrobial resistance. As such, there is a growing interest to explore the use of bacterial viruses or bacteriophages (phages).

Due to the lack of knowledge and of prior reports on phage therapy in Kenya, the technology needs to be introduced and tested as viable, safe and effective. Therefore, the goals of the project are to optimize control measures to reduce antibiotic use as well as AMR *Salmonella* strains in Kenyan poultry farms by using available *Salmonella*-killing bacteriophages from the Félix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca) in Canada, as well as by using newly isolated phages from Kenya which have the capacity to kill Kenyan *Salmonella* strains. To that end, several naturally occurring phages with lytic activity against a range of *Salmonella* strains were isolated from chicken feces and water samples collected from various Kenyan poultry farms. Preliminary data suggest that phages able to infect and kill a strain of *S.* Pullorum could be successfully isolated. Currently, we are in the process of characterizing these newly isolated phages and the best candidates will be tested in a chicken model of pullorum disease in the near future.

Keywords: Bacteriophages, *Salmonella*, Pullorum disease, Poultry, Kenya

MI13
Transmissible antibiotic resistance genes present in *Escherichia coli* from USA and Thailand poultry

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Conventional poultry production has used sub-therapeutic levels of antibiotics in the diet to prevent disease and stimulate growth for many decades. Health concerns over the potential of antibiotic resistant bacteria in the food supply has resulted in consumer demand and regulatory changes limiting the use of antibiotics, thereby making alternatives to antibiotics part of the mainstream in most poultry markets today. One of the expected benefits of using antibiotic alternatives is a reduction in antibiotic resistance genes in the environment. In 2014, <5% of broilers in the USA were "Raised without Antibiotics" and this increased to >40% by 2018. To determine the prevalence and type of antibiotic resistance genes present in the USA poultry industry before the concerted push to raise poultry without antibiotics, we sequenced the genomes of 111 *Escherichia coli* strains from broilers and turkeys collected in 2014 and 2015 and analyzed them for acquired antibiotic resistance genes. Transmissible resistance genes to aminoglycosides, tetracyclines, sulfonamides, and beta-lactams were the most prevalent. A multiplex PCR was developed to detect seven of the antibiotic resistance genes. This assay was used to determine the presence of antibiotic resistance genes in poultry *E. coli* isolates from the USA and Thailand. The average number of antibiotic resistance genes detected per isolate was 1.7 in both countries with a maximum of five of the seven genes in any one isolate, but different genes were predominant in each county. Over 30% of the USA isolates possessed aminoglycoside resistance gene *aac3VIa*, compared to only 1% of the Thailand isolates. Beta-lactam resistance gene, *blaTEM1,* and tetracycline resistance gene, *tetA*, were the most prevalent in Thailand isolates at 70% and 60% compared to 25% and 27% in the USA isolates. Over time there was a significant decrease in the number of antibiotic genes per isolate in the USA. The average number was 2.2 genes in 2015 and 2016 which dropped significantly to 1.2, 1.3 and 1.4 in 2017, 2018 and 2019 respectively. This multiplex assay will be used for continuous monitoring of transmissible antibiotic resistance genes in avian *E. coli* across countries and time.

Keywords: transmissible antibiotic resistance genes, *Escherichia coli*

MI14
Potential of enzymatically hydrolyzed yeast (AVIATORTM) binding to enterotoxigenic *Escherichia coli in vitro*

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The use of *Saccharomyces cerevisiae* or enzymatically hydrolyzed yeast (EHY) as a nutritional feed supplement is commonly applied in many livestock productions. It improves animal production, promote health and also reduce the need for antibiotic use. The major component of EHY is mannan oligosaccharide which can improve gut health and act as a high-affinity ligand offering competitive binding site options for Gram negative bacteria.

The objective of this study is to evaluate the efficiency of AVIATOR™ bind to enterotoxigenic *E. coli*. The qualitative and quantitative EHY assays were performed *in vitro* with four enterotoxigenic *E. coli* strains. The qualitative assay was performed using overnight *E. coli* culture in TSB broth mix with various EHY concentrations and observed for the agglutination. The EHY-*E. coli* complex was agglutinated and easily observed in various concentration of EHY as clumping cell. Furthermore, the quantitative assay was also performed by counting the unbound *E. coli* after EHY reaction. The result showed that EHY has ability to reduce the number of *E. coli* approximately 3-4 log cells from original cell number. In conclusion, EHY showed the high potential agglutinate and reduce the number of enterotoxigenic *E. coli in vitro*. This positive phenomenon of EHY will associated with pathogen removal from the gastrointestinal tracts without attachment and colonization *in vivo*.

Keywords: Aviator, *Escherichia coli*, ETEC, enzymatically hydrolyzed yeast, agglutination

MI15
Potential of enzymatically hydrolyzed yeast (Aviator™) strongly agglutinate with *Salmonella* Typhimurium and *S.* Enteritidis

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The use of *Saccharomyces cerevisiae* or enzymatically hydrolyzed yeast (EHY) as a nutritional feed supplement is commonly applied in many livestock productions. It improves animal production, promote health and also reduce the need for antibiotic use. The major component of EHY is mannan oligosaccharide which can improve gut health and act as a high-affinity ligand offering competitive binding site options for Gram negative bacteria.

The objective of this study is to evaluate the efficiency of AVIATOR™ bind to *S.* Typhimurium (ST) and *S.* Enteritidis (SE) *in vitro*. The qualitative and quantitative EHY assays were performed *in vitro* with *S.* Typhimurium and *S.* Enteritidis strains. The qualitative assay was performed using overnight ST and SE culture in TSB broth mix with various EHY concentrations and observed for the agglutination. The EHY-*Salmonella* complex was agglutinated and easily observed in various concentration of EHY as clumping cell. Furthermore, the quantitative assay was also performed by counting the unbound *Salmonella* after EHY reaction. The result showed that EHY has ability to reduce the number of *Salmonella* approximately 3-4 log cells from original cell number. In conclusion, EHY exhibited the high agglutination and reduce the number of SE and ST *in vitro*. This positive phenomenon of EHY will associated with pathogen removal from the gastrointestinal tracts without attachment and colonization *in vivo*.

Keywords: Agglutination, Pig, Probiotic, *Salmonella*, Yeast

MI16
Evaluating the ability of probiotics to inhibit *Clostridium perfringens* cause diarrhea in pigs

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Using antibiotics in animal feed caused resistance in bacteria and was be forbidden in some countries. Thereby probiotics have evolved the alternatives to antibiotics in the pig industry. It is well documented that some probiotics produce bacteriocins that have the ability against pathogens in gastrointestinal tracts. There are many studies reported on the ability of probiotics in the inhibition growth of Gram-negative bacteria. However, Gram-positive pathogens are also one of the main causes of many diseases in pigs. Hence, our studies concerned the identification of probiotics against Gram-positive pathogens to enrich the application of probiotics and improve currently probiotic products. In this study, we carried out screening test whether our candidate probiotics are able to inhibit the growth of *C. perfringens in vitro*.
Twenty strains of probiotics either *Lactobacillus* spp. or *Bacillus* spp. and ten strains of pathogenic *C. perfringens* were isolated from healthy pigs and diarrheagenic piglets, respectively. The ability of probiotics to inhibit *C. perfringens* was tested by using the direct spot agar on nutrient agar in anaerobic conditions. The inhibition zone (clear zone) was measured as representative of probiotics inhibitory effect on pathogens. We found out 10 candidate strains of probiotics can inhibit *C. perfringens* with inhibiting zone diameter ranging from 6-18 mm. These strains will be used in the next experiments for selecting the best strains and the optimal growth condition on bacteriocins production.
Though this is a preliminary study with a small number of samples, this study showed potential probiotics to protect pigs against *C. perfringens*.

Keywords: Probiotic, *Clostridium perfringens, Lactobacillus* spp., *Bacillus* spp.

MI17
The inhibitory effect of *Bacillus* spp. to against the pathogenic *Escherichia coli* isolate from pig in Thailand

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E. coli infection is one of the important causes of diarrhea in piglets, resulting to economic loss due to increase piglet mortality, morbidity and decrease growth rate. The antibiotic is a common remedy for control *E. coli* spread in farm whether for prophylaxis, metaphylaxis or therapeutic purpose. Because of antibiotic using is popular in pig farm, the antibiotic resistance should be concerned. Thus, there is a need to study alternative antimicrobial agents for reduce or replace the using of conventional antibiotic. Probiotics are the interesting topic for developing the alternative antimicrobial agent since its well documented ability to produce the antimicrobial compound. The aim of this study was to investigate the inhibitory effect of *Bacillus* spp. against the growth of pathogenic *E. coli* on nutrient agar.

This study used *Bacillus* spp. to test the ability to inhibit growth of pathogenic *E. coli* isolate from nursery pig in Thailand by direct spot test. Enrofloxacin and gentamicin were used as control. The result showed the *Bacillus* spp. showed its ability to inhibit growth of pathogenic *E. coli* by showed the inhibition zone (clear zone) on nutrient agar comparable with the results from the control (enrofloxacin and gentamicin), however, with a small difference in diameter of inhibition zone.

In conclusion, the present study clearly showed the ability of *Bacillus* spp. to inhibit growth of pathogenic *E. coli*, therefore this *Bacillus* spp. could be a candidate for develop the alternative antimicrobial agent for the replacement of antibiotics using in pig farm. However, further study is needed for acid, bile tolerance properties and effect of its cell free supernatant (CFS).

Keywords: Probiotic, *Bacillus* spp., *Lactobacillus* spp., Enterotoxigenic *Escherichia coli*, ETEC

MI18
Effect of selected yeast fraction on the growth of *Clostridium perfringens*: Quantitative determination of growth inhibition and adsorption capacity

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To provide *in vitro* evidences on the antimicrobial effect of yeast cell wall (YCW), the effectiveness of YCW fractions in inhibiting the growth of several *C. perfringens* strains was quantitatively determined. The bacterium was grown in the presence of different YCW fractions at different concentration levels. The effect of YCW fractions on the growth parameters was analyzed. One product out of four materials was selected as the best candidate for *C. perfringens* inhibition. The selected product, at an optimal dosage of 1.25 mg/mL, increased the lag phase duration, and reduced the maximum growth rate and the final cell count in a significant manner with respect to the control. The adsorption of the pathogen to YCW was studied using the isotherm adsorption approach. The effect of YCW dosage, incubation time, and bacterial concentration on the adsorption was evaluated. The study proved that the product adsorbed C. perfringens cells in a dose and time dependent manner. Equilibrium isotherms showed that the cell adsorption onto the product was fast, stable over the time, and occurred with high affinity and capacity. The selected product sequestered up to 104 cells of *C. perfringens* per mg. To the best of our knowledge, this is the first report showing the in vitro efficacy of yeast fraction products to inhibit the growth of *C. perfringens,* and to reduce the culturable cells by an adsorption process. The *in vitro* approach proposed herein is as a powerful tool to study the adsorption of aerobic or anaerobic pathogens by eubiotics.

Keywords: yeast cell wall, antimicrobial, *Clostridium*, adsorption, feed additives

MI19
Evaluation and selection of Lactic acid bacteria based on inhibition capability against *Streptococcus suis* for probiotics product

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Streptococcus suis (*S. suis*) serotype 2 is one of the most important pathogens in pigs, which causes septicemia, meningitis, arthritis and pneumonia pigs. Moreover, this bacterium is considered an emerging zoonotic agent. Recently, increased antibiotic resistance of *S. suis* has been reported worldwide, and raises issues regarding food safety. There are several potential alternative methods to replace the use of antibiotics. One of them is to promote pig health by directly supplement probiotic bacteria and another is to use their antimicrobial substance, to inhibit the growth of pathogens. The objective of this study was to evaluate the potential of cell-free supernatant of selected lactic acid producing probiotic strains to inhibits the growth of 12 isolated *S. suis* (i.e. SS-01 to SS-12), in which isolated from infected nursery pig and its pathogenicity was confirmed by PCR technique. All the probiotic bacterial strains used in this study (*Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Pediococcus pentosaceus*) were selected mainly on the *in vitro* of inhibition ability on Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli* and the adhesion ability on swine intestinal mucus as shown in our previous study. The ability of different probiotic species and stains to inhibits *S. suis* serotype 2 was evaluated by zone of activity on agar well diffusion. The zone of activity was classified into four groups: no inhibition as follows: 0-7 mm; low inhibition: 8-14 mm.; medium inhibition: 15-21 mm., and high inhibition: >21 mm. The different lactic acid probiotics showed variety of capability to inhibit the growth of *S. suis* serotype 2. *Lactobacillus plantarum* KMP-F23-1 showed both medium and high inhibition zone of activity for SS-08, SS-03, SS-10 (i.e. 18.25, 26.00, and 21.75 mm., respectively) when compared with other lactic acid producing probiotics. *Lactobacillus plantarum* CU20 also showed medium and high inhibition zone of activity for SS-06, SS-07, SS-08, and SS-10 strains (i.e. 17.50, 17.50, 17.75, and 21.25 mm., respectively). *Lactobacillus acidophilus* KMP-TC001 showed high inhibition zone of activity with a diameter of 21.50 for SS-10. For *Pedicococcus* spp., *Pediococcus pentosaceus* PdAvPd02 showed high inhibition zone of activity for SS-10 (i.e. 20.00 mm.). According to inhibition zone of activity, we, therefore, included only *Lactobacillus* and *Pedicococcus* that showed medium to high inhibition zone of activity to become probiotics candidate in probiotic product, targeting this multi-strains probiotic product is able to minimize the growth of *S. suis* in pigs. In conclusion, different lactic acid producing probiotics such as *Lactobacillus* spp. and Pediococcus spp. showed variety of inhibition zone of activity for *S. suis*, thus we recommended that multi-strains of probiotics should be included in particular product in order to conquer the *S. suis* problem in pig industry.

Keywords: Probiotics, lactic acid bacteria, *Streptococcus suis*, inhibition, pig

MI20
Probiotic strain modulates gut microbiota and control the inflammatory response in chickens

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A probiotic strain (*bacillus subtilis* DSM 29784, BS29784) is capable of inducing beneficial effects on growth performance and could be therefore a reliable alternative to antibiotic growth promoters. The underlying mechanisms of probiotics, however, are often not fully understood. An *in vivo* investigation of microbiota profile aimed to observe a positive effect of BS29784 on butyrate producer bacterium such as Ruminococcus or *Lachnoclostridium*. An *in vitro* approach, using Caco-2 cells line, showed a decrease of pro-inflammatory compounds (IL8, iNOS) following the supplementation of BS29784. This was mainly explained by an activation of the NFkB pathway. Finally, we also demonstrated the positive correlation of tight junction gene expression with TransEpithelial Electrical Resistance, a sensitive indicator of barrier tissue integrity. In the present study, we used a two-step approach to identify major metabolites produced by BS29784 known to have beneficial effects on broiler performance and health. The first step consisted in cultures of the BS29784 grown in Luria-Bertani and Tryptic Soy Broth culture media. After 4h, 10h and 24h, the supernatant of cultures was analysed with UPLC/MS to identify the metabolites produced *in vitro*. The second step was an *in vivo* study, in which 1-day old broiler chicks were continuously administered BS29784 via the diet. At d13, intestinal samples from different locations were collected and analysed for a targeted metabolite analysis. A DNA extraction was performed on the intestinal samples to determine the relative abundance of *Bacillus* species in different intestinal locations (via qPCR). Nicotinic acid and hypoxanthine were the two main metabolites that were increased in the supernatant of BS29784 cultures. An increase in their concentrations was also measured in ileum and jejunum samples of 13-day old chickens to which the strain was administered. The wound healing assay confirmed the beneficial effect of these two metabolites on barrier function.

Keywords: *B. subtilis*, chicken, microbiota, inflammation, metabolite

MI21
Performance of broilers fed AGP free diets supplemented with a direct-fed microbial under hot climate

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This trial was done to determine broiler live performance to 42 days, under a hot climate in India. 2080 VenCobb 400 broilers were allocated in 4 groups with 26 birds per floor pen and 20 replicates per group: negative control group (NC), positive control group (PC), direct-fed microbial group (DFM) at Log 5 CFU/g of feed and Bacitracine group (BMD) at 500 g/T of feed. The DFM product was composed with 3 species of sporulated *Bacillus*: *Bacillus amyloliquefaciens, bacillus licheniformis* and *bacillus pumilus*. The room temperature reached a peak of 31.64 to 33.29°C from 4 to 6 weeks old. PC, DFM and BMD groups were fed with a challenging feed formula: 5 to 10% of DDGS, 8 to 10% of mustard cake extract and 5 % of medium quality meat and bone meal (which is still usually used in India). NC group was with a classical and non-challenging corn and soybean-based diet.

The body weight at 42 days was 2.707 kg in NC group, but only 2.144 kg in PC group, due to the feed challenge. It reached 2.205 kg in BMD group and 2.254 kg in DFM group, significantly higher than PC group (p=0.001).

The broilers were vaccinated against Newcastle disease during the trial at 7 and 21 days. The humoral vaccine response was evaluated by HI-test at 42 days. The response was 5.05 Log2 in NC group, 4.75 Log2 in PC group, 4.10 Log2 in BMD group and 5.85 Log 2 in DFM group, significantly higher with DFM (p=0.001). The cell-mediated immunity was also evaluated by basophilic hypersensitivity test. The response was respectively 70.05, 72.65, 65.65 and 95.20 significantly stronger with DFM (p=0.004).

The ileal microbial count in CFU/g was evaluated at 42 days. The quantity of *E. coli* was respectively 3.942, 3,849, 3.968 and 3.510, significantly lower with DFM (p=0.019). The quantity of *Clostridium perfringens* was respectively 3.515, 3.748, 3.510 and 3.158, also significantly lower with DFM (p=0.015).
This direct-fed microbial enabled to improve the growth performances under a natural heat stress, helped to improve the vaccine response and reduce the pressure of potentially pathogenic bacteria like *E. coli* and *Clostridium* in the gut.

Keywords: broiler, direct-fed microbial, hot climate, feed challenge, performances

MI22
Different from antibiotics: Improving gut health additives by understanding their mode of action

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Over the last two decades, several types of gut health supporting additives have been shown to have at least the potential to improve gut health and animal performance and as such, to be applied as alternatives to antibiotics. Initial research on these products was largely focused on their direct 'antimicrobial' effect, as it was tempting to hypothesize that any successful alternative for antibiotics would need to have a similar (antimicrobial) effect. However, it becomes clearer that gut health additives have working mechanisms that are very different from those of antimicrobials.

I will argue that direct antibacterial effects most likely do not underlie the main working mechanisms of additives, and that improvement of the development and application of these additives will depend on the investigation of their mode of action at the microbial and cell-biological level. This will be exemplified with recent research data on different classes of feed supplements, including butyrates, probiotics and phytogenics.

Butyrate, for instance, is a molecule that is well known for its ability to elicit numerous effects in the digestive tract. While most publication describe positive effects of butyrate supplementation, I will present data demonstrating that the effects of supplementing livestock animals with commercial butyrate products are heavily dependent on the enteric location where butyrate is delivered in the digestive tract. For example, in at least some conditions, elevated butyrate concentrations in the fore- and midgut of broilers may induce negative effects on caecal microbiota diversity and/or inflammation, as opposed to increased butyrate concentrations in the hindgut.

In addition, monoglycerides of short-chain fatty acids, the metabolites of probiotics and the phytochemicals in botanical products are unlikely to have substantial specific bacteriostatic effects, as exemplified by EU-funded study evaluating their effectiveness against *Campylobacter*. More likely, they exert their function by modulating effects that can be trigger at low intestinal concentration, such as the inhibition of bacterial quorum sensing.

Lastly, results will be shown from a trials demonstrating that further improvement in gut health and economic profitability of the producer can be achieved, by implementing research based programs, combining different classes of additives.

Keywords: butyrate, phytogenics, probiotics, working mechanism

MI23
Antimicrobial Effect of *Bacillus* Probiotics against Foodborne Pathogens

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Foodborne illness is still a major health problem worldwide, according to a report released from the Center for Disease Control and Prevention (CDC). Nowadays, antimicrobial resistance is a major threat to human and animal health which is also related to the multidrug resistance of foodborne pathogens in food-producing animals such as poultry and pigs. The aim of this study was to evaluate the antimicrobial activity of 3 species of *Bacillus*; *B. subtilis* 16AvBa10, *B. licheniformis* KMP9 and *B. amyloliquefaciens* 16AvBa18 against 3 species of foodborne pathogen including *E. coli* ATCC 25922, *S.* aureus ATCC 6538 and *Salmonella* for reference strains (*S.*Typhimurium DMST 15674, *S.* Enteritidis DMST 15676) and field isolated (*S.* Typhimurium 001, *S.* Enteritidis 022, *S.* Virchow 001, *S.* Hadar 002) by delayed antagonism in solid nutrient medium method. It was found that the 3 species of *Bacillus* were able to inhibit the growth of all foodborne pathogens. And the ability of *Bacillus* on pathogenic inhibition varies among the difference species and strains. *B. licheniformis* KMP9 showed the highest effectiveness against field isolated *Salmonella* for ST 001, SE 002 and *S.* Virchow 001 was 20.7 $\pm$ 3.7 mm, 22.8 $\pm$ 1.7 mm, 27.4 $\pm$ 2.5 mm, respectively while *B. subtilis* 16AvBa10 showed the highest antibacterial ability on both reference strain of S. Typhimurium DMST 15674, *S.* Enteritidis DMST 15676 and *S.* Hadar 002 was i.e. 17.5 $\pm$ 3.3 mm, 8.8 $\pm$ 1.7 mm and 26 $\pm$ 5.7 mm, respectively. For the other foodborne pathogens such as *E. coli* ATCC 25922 and *S. aureus* ATCC 6538, the *B. licheniformis* showed a highest inhibition zone of 28.0 $\pm$ 3.5 mm and 30.8 $\pm$ 1.2 mm, respectively. However, *B. amyloliquefaciens* 16AvBa18 also showed the potential on antibacterial activity of *E. coli* and *S. aureus* and *Salmonella* many serovars too. Although Lactic acid bacteria always inhibited gram negative bacteria such as *Salmonella* and *E. coli*, some species of *Bacillus* showed the ability on it too. These studies suggested that the *Bacillus* are one alternative to use for foodborne pathogen control in Livestock.

Keywords: *Bacillus* spp., Antibacterial activity, Foodborne pathogen

MI24
Characterization of virulent bacteriophages infected multidrug-resistant *Aeromonas hydrophila*

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In the context of protein deficiency due to the rapid increase of the world's population and the decrease in production, freshwater fishes provide a high source and quality of nutrition with affordable price for consumers. Nevertheless, the disease outbreak in fish, particularly Motile Aeromonas Septicemia Disease (MAS) caused by multidrug-resistant (MDR) *Aeromonas hydrophila* was widespread and gave rise to high economic losses for farmers. The MDR *A. hydrophila* can not be treated with antibiotics using in aquaculture. The alternative methods should be applied for the eradication of MDR *A. hydrophila* such as herbs and bacteriophages. Therefore, we aimed to isolate and characterize *A. hydrophila* specific bacteriophages. The MDR *A. hydrophila* BT09 isolated from diseased striped catfish (*Pangasianodon hypophthalmus*) was screened and used for bacteriophage isolation. Fifty-four bacteriophage strains were isolated from 120 water samples and three strains namely pAh5.5BT, pAh6.2BT and pAh6.2TG were selected based on the criteria of plaque diameter, un-adsorption rate, and lytic cycle time. The results showed that the selected bacteriophages exhibited the largest plaque diameter of 1.19±0.27 mm, 1.17±0.09 mm, 1.50±0.02 mm and the lowest percentage of free phage with 16.3%, 13.2%, and 35.5%, respectively. Besides, all of them determined the shortest lytic cycle of 15 minutes. The TEM images showed that all of the bacteriophages had an icosahedral head, long contractile tail, particularly with the collar and belonged to the *Myoviridae* family. The selected bacteriophages exhibited wide host range with and high specificity with the isolated *A. hydrophila*. Single strain or combination of the bacteriophages offered the widest host range with 81.8%. The bacteriophages can be used as a potential alternative to antibiotics to control MAS in striped catfish and other freshwater fish species.

Keywords: *Aeromonas hydrophila*, antibiotic resistance, bacteriophage, Motile *Aeromonas*, Septicemia

MI25
Control of Mycotoxins in Farm Animals: a Key Step in Antibiotic Free Production

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Mycotoxins are toxic secondary metabolites produced by certain fungi such as *Penicillium*, *Fusarium* and *Aspergillus*. Mycotoxin producing fungi damage crops, causing severe economic losses to food and feed production. Moreover, several mycotoxins impair immunity and organ functions, causing loss of productivity, diseases and, in extreme cases, death in animals that consume contaminated feed. Several studies have highlighted the significance of interactions between mycotoxins and intestinal diseases such as clostridial necrotic enteritis and coccidiosis in poultry. In swine, mycotoxins are known to cause a severe immune suppression and increase the severity of pathogens such as *Salmonella* spp. and *Pasteurella multocida*.

In farm animals, mycotoxins can impair gut health via three main routes:

1. They promote the opening of tight junctions in the intestine, by actively suppressing the production of tight junction proteins such as claudins and occludins.
2. The promote villi fusion and atrophy. Furthermore, some mycotoxins such as deoxynivalenol and fumonisins are capable of inhibiting the production of intestinal transporter proteins such as GLUT2 and SGLT1.
3. They promote immunosuppression, by directly triggering apoptosis of immune cells; or immune overstimulation by modulating the expression of several cytokines. Immune overstimulation is not beneficial to production animals, due to the high metabolic cost and because it is an extra stress factor.

When it comes to estimating the mycotoxin contamination risk, it is important to keep in mind that mycotoxins always co-occur and often lead to synergistic interactions, where the toxicity of one mycotoxin is increased by the presence of others. As reported by several studies, the likelihood of synergistic interactions are higher at subclinical levels. Furthermore, grains do not only contain mycotoxins, but they might be contaminated by different types of bacteria and other toxic substances, such as pesticides and heavy metals, that increase the overall risk. As shown by several studies, co-occurrence of mycotoxins often results in greater immunosuppression, reduced feed intake, decreased weight gain and nutrient utilization. In this context, it is important to mention that mycotoxin risk thresholds that issued by international agencies, such as EFSA and FDA, only take into account data based on *in vitro* studies on single mycotoxin contamination, thus presenting huge limitations when it comes to estimating the real toxicity of mycotoxins in the field.

Because of the broad variety of negative effects on the gut and immune system, control of mycotoxins should be the first step in antibiotic free production.

Keywords: mycotoxins, immune suppression, gastrointestinal health

MI26
Improved growth performance and reduced mortality in broiler chickens supplemented with two novel strains of *Bacillus subtilis*

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The use of probiotics can be a natural and sustainable alternative to the practice of including sub-therapeutic levels of antibiotics in livestock and poultry feed to improve intestinal health. Probiotics have the potential to reduce presence of certain deleterious or pathogenic microorganism, reducing efficient growth in food animals. A randomized complete block design was used to evaluate the effects of two novel *Bacillus subtilis* strains, Correlink™ ABS-747 and ABS-1781 on broiler chicken performance. Cobb 500 males were housed for 42 d in a single environmentally controlled room with 78 floor pens (26 pens/treatment) at 50 birds/pen. Diets were administered in 4 feeding phases 0-11 (ST), 11-25 (GR), and 25-35 d (FR) and 35-43 (WD). An antibiotic-free basal diet was mixed for each phase and *Bacillus subtilis* was added to each basal diet to make treatment diets, which were identical in ingredient and nutrient composition. Study treatments were: Control, 747 (Control + 1.5×10^5 CFU of ABS-747/g feed), and 1781 (Control + 1.5×10^5 CFU of ABS-1781/g feed). During each study phase, birds had *ad libitum* access to feed and water. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), unadjusted and adjusted for mortality (MA), and production efficiency index (PEI) were determined for each feeding phase and overall. Data were analyzed with SAS (v. 9.4, SAS Institute, Cary, NC) using pen as the experimental unit, treatment as a fixed effect, and block as a random effect. No differences (P=0.11 to P=0.90) among treatments were found for BW, ADG, MA_ADG, ADFI, MA_ADFI, FCR, MA_FCR, and PEI during ST, GR, or FR phases. Mortality was reduced with the inclusion of ABS-747 (4.2%) and ABS-1781 (4.1%) during GR (P<0.06) compared to Control (5.1%). Inclusion of ABS-1781 reduced (P<0.01) mortality during FR compared to Control (3.1 vs 3.8, respectively). Inclusion of ABS-747 and ABS-1781 during WD improved (P<0.0001) ADG, MA_ADG, FCR, MA_FCR, PEI and mortality compared to Control. During the 42-d trial, and compared to Control, birds supplemented with ABS-747 and ABS-1781 improved final BW by 4.7% (2.07, 2.16, and 2.17 kg, respectively; P<0.0001), ADG by 4.8% (48.3, 50.6, and 50.7 g/d, respectively; P<0.0001), MA_ADG by 4.9% (49.2, 51.3, and 51.9 g/d, respectively; P<0.0001), FCR by 8.9 points (1.881, 1.778, and 1.806, respectively; P<0.0001), MA_FCR by 8.6 points (1.787, 1.703, 1.699, respectively; P=0.0003), and PEI by 8.2% (248, 273, and 264, respectively; P=0.0006). Inclusion of ABS-1781 reduced (P=0.03) 42-d mortality by 13% from 2.7% in Control to 2.4%. Overall, broiler chickens supplemented with Correlink™ ABS-747 and ABS-1781 demonstrated superior performance to birds fed the control diets.

Keywords: *Bacillus subtilis*, probiotic, broiler, growth performance

MI27
Occurrence of antimicrobial resistance in different swine farm management systems using TaqMan array cards

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Antimicrobial agents contribute to the emergence of antimicrobial resistance (AMR) by selection of resistant bacteria. For veterinary medicine, most antimicrobial agents are used for treatment and/or as feed additives for prophylaxis. Recently, antimicrobial agent-free farms have increased, but whether AMR is reduced in these farms is unclear. We previously developed a TaqMan array card (TAC) which included 79 sequence specific PCR targets for detection of antimicrobial resistance associated genes for ten important antimicrobial classes used in human and veterinary medicine. In this study, TAC was used to detect AMR directly in stool samples to determine the occurrence of AMR across different swine farm management systems.

We studied in three different fattening pig farm categories including 1) extensive-antimicrobial-use - both treatment and feed additive 2) limited-antimicrobial-use - treatment only, and 3) no-antimicrobial-use. Eighty pig farms located in Thailand were enrolled and five stool samples were randomly collected from healthy 20-25 weeks old fattening pigs from each farm, yielding 400 stool samples. Two hundred milligrams of stool underwent DNA extraction and 20 µl was used as DNA template. Resistance gene copy number was normalized with bacterial 16S copy number of each sample for comparable across sample.

The number of resistance genes in extensive-antimicrobial-use farms (28.5 ± 4.3) was higher than limited-antimicrobial-use farms (23.3 ± 3.7) and no-antimicrobial-use farms (22.5 ± 3.3; $p<0.05$, one-way ANOVA). The prevalence of resistance genes in extensive-antimicrobial-use farms was higher than the other 2 groups for 20 resistance genes, including *bla*$_{SHV}$, *bla*$_{CTX\text{-}M1\ group}$, *bla*$_{CTX\text{-}M9\ group}$, *bla*$_{GES}$, *bla*$_{VEB}$, *bla*$_{CMY2\text{-}LAT}$, 23S rRNA 2075G-*Campylobacter* spp., *mphA*, *qnrB1*, *gyrA*83L-*E.coli*, *aacC2*, *aac(6')-lb*, *armA*, *rmtB*, *mcr-1*, *mcr-2*, *dfrA5-14*, *dfrA17*, *catA1*, and *floR* ($p<0.05$, Chi-square test). We then generated a logistic regression model to predict antimicrobial exposure by using resistance genes as predictor. The prediction model showed 75% and 83% sensitivity, 87% and 83% specificity, 81% and 83% accuracy for training and tested data set respectively.

Our study indicates that the conventional system of extensive-antimicrobial-use influences the occurrence of AMR. TAC is a rapid high throughput tool which may be useful for large scale surveillance. The prediction model may use for monitoring of farm management systems.

Keywords: Antimicrobial resistance, AMR, TaqMan array card, Swine

MI28
MRSA in the nasal microbiome in neonatal pigs – a pilot study for developing competitive exclusion

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Livestock Associated Methicillin-resistant *Staphylococcus aureus* (MRSA) is a good colonizer in pigs and occupationally exposed people become LA-MRSA positive. As this is considered to be a public health risk, measures to reduce LA-MRSA colonization in pigs should be explored, such as competitive exclusion. We studied the porcine nasal microbiome shortly after birth and the association with colonization of *S. aureus* and MRSA. Nasal swab samples (n=104) were obtained from eight neonatal pigs, from two litters, directly after birth until six weeks that were processed for Illumina MiSeq using the V3-V4 region of the 16SRNA and *tuf* gene for *staphylococcal* spp. identification. *S. aureus* and MRSA were quantified by real-time PCR (CFUeq) and culture. Error-free, non-chimeric Sequence Variants (SV) were identified using dada2 pipeline followed by its compositional analysis using CoDaSeq (Github: ggloor/CoDaSeq). We accounted for compositional effect of sequencing data by CLR (Centered Log Ratio) transformation and repeated measurements of animals via rmcorr and mixed effect model. RSV/TSV present in less than 10% of samples and relative abundance below 0.001 were filtered-out. Contaminant RSV/TVS were identified by Frequency based algorithm from decontam r package. A total of 2764 16S RSVs and 1239 *tuf* TSVs were obtained. With 16S sequencing, species belonging to the genera *Corynebacterium*, *Streptococcus* and *Acinetobacter* were correlated with a low number of MRSA (CFUeq) and cultured *S. aureus*. Notably, *tuf* sequencing could resolute up to 22 different *Staphylococcal* species, of which *S. microti*, *S. haemolyticus* and *S. hyicus* were the most abundant. Only, *S. microti, S. simulans, Macrococcus canis* were negatively correlated with a low number of MRSA (CFUeq) and cultured *S. aureus*. The identified bacterial species in this study can be further explored for development of competitive exclusion to control LA-MRSA in pigs.

Keywords: microbiome, MRSA, competition, colonisation

MI29
Antioxidant potential of *Pediococcus pentosacceus* strains isolated from porcine milk

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Oxidative stress occurs throughout the life of mammals. Early weaning stress syndrome of piglets is a bottleneck problem restricting the development of intensive pig breeding, which often leads to severe oxidative stress in piglets and intestinal barrier dysfunction. Porcine milk not only provides rich nutrition and immunoglobulin, but also contains diversified microorganisms which are an important but often overlooked source of probiotics for piglets. Here, eighty isolates of *Pediococcus pentosacceus* from porcine milk were screened to evaluate antibacterial activity *in vitro* and the property to protect hosts from oxidative injury *in vivo* by drosophila paraquat resistance assays. The 21 suckling piglets were orally administrated with *P. pentosacceus* Q82 at different doses and all animals were sampling on the seventh day after weaning.

Our results reveal that *P. pentosacceus* Q82 was susceptible to 19 antibiotics and against several enteropathogens including *Staphylococcus aureus, Salmonella* and ETEC. Compared with the control, the mRNA expression of CAT in ileum and liver were significantly increased and the concentration of GSH-PX and CAT in liver were significantly also increased. In the nucleus, the expression of Nrf2 is increased in the rectum, lactobacillus was higher in the treatment group by 16S RNA sequence. This study aims to decipher systematically the probiotic effect of *P. pentosacceus* from porcine milk and sheds new light on new probiotics source and rapid drosophila screening probiotics model for prevention and relieving severe oxidative stress of piglets.

Keywords: *Pediococcus pentosacceus*, oxidative stress, Drosophila melanogaster, porcine milk, Nrf2

MI30
Developing a global dynamic dashboard as a one-stop shop for AMR related research and development in One Health Sectors

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Antimicrobial resistance (AMR) poses a worldwide threat for both human and animal health. Global efforts to address AMR have grown steadily in the last couple of years, but require more cross-sectoral coordination. The increasing emergence and spread of AMR have been discussed in many countries and at a range of international fora including the UN General Assembly, the World Health Assembly, the G7, the G20, the OIE and the FAO, resulting in high political interest and commitment. In July 2017, the G20 called for a new international research and development (R&D) Collaboration Hub to maximise the impact of existing and new basic and clinical antimicrobial research initiatives, which led to the establishment of the Global AMR R&D Hub.

The Hub, launched in May 2018, supports global priority setting and evidence-based decision-making on allocation of resources for AMR R&D through the identification of gaps, overlaps and potential for cross-sectoral collaboration and leverage in AMR R&D. Currently the global partnership consists of 16 countries, the European Commission and two philanthropic foundations and four observers (WHO, OECD, FAO, OIE).

One of the key activities for the Global AMR R&D Hub is the development of a dynamic dashboard (the dashboard) that will present close to real-time data on all AMR R&D investments globally. The dashboard will present high level (categorized) information on research projects throughout the research and innovation value chain on therapeutics, preventives, diagnostics, surveillance, policy and interventions across all One Health sectors. Data is being collected in a staged approach, beginning with new products against human bacterial infections. The first stage of the dashboard will be launched in the first quarter of 2020.

Following the launch, the dashboard will expand to present AMR R&D investments into animal health followed by plant and environmental health. To ensure the applicability and utility of the dashboard to the animal health sector, the Global AMR R&D Hub has begun additional work and extensive consultation in order to:

- implement any lessons learnt from collecting human AMR R&D investments when searching for and categorizing animal health-related projects and investments
- develop standard key words that will be used to search databases containing public and charitable funding information to ensure relevant animal health projects and investments are captured
- identify additional sources of information on animal health AMR R&D projects and investments
- define categories that will ensure there is meaningful representation of animal heath projects and investments on the dashboard, and
- collaborate with similar initiatives to ensure there is no duplication of effort.

Addressing AMR requires global action with active participation from all world regions and all One Health sectors. Through the development of the dashboard, the Global AMR R&D Hub will be the global knowledge centre on all AMR R&D activities and investments. The dashboard will support global priority setting and decision making and lead to more efficient use of international resources through the identification of gaps, overlaps and potential for cross sectoral collaboration and leveraging in AMR R&D.

Keywords: dynamic dashboard, One Health, research projects, funding

MI31
Improving research coordination to focus efforts to reduce AMR in animal production

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STAR-IDAZ International Research Consortium on Animal health (IRC) is a global initiative to coordinate research programs at international level to contribute to the development of new and improved animal health strategies for at least 30 priority diseases/infections/issues, including antimicrobial resistance and the development of innovative alternatives to antibiotics. The Consortium is supported by the OIE, that also hosts its scientific secretariat. Target deliverables include candidate vaccines, diagnostics, therapeutics, other animal health products and procedures, and key scientific information/tools to support risk analysis and disease control.

STAR-IDAZ IRC is establishing a Working Group (WG) of experts to identify research gaps for the development of alternatives to antibiotics, focusing in particular on mechanisms of immunomodulation, compartmentalization of resources, and influence of the microbiome. The WG will also investigate the mode of action of antibiotics in growth promotion. STAR-IDAZ IRC members will use the identified research gaps to guide future research funded by their organizations.

The aim is that new research will improve understanding of the relationship between control of sub-clinical infection and growth on the role of the microbiome in maintenance of health and on the identity and mode of action of putative new antimicrobial products– targeting the pathogen and/or the host. This will ultimately help in the development of new non antibiotic-based antimicrobial products and approaches for controlling infections and enhancing productivity, while maximizing the life of existing and new therapeutics.

Keywords: AMR, animal, STAR-IDAZ, IRT, production

MI32
Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Racehorses and Horsemen in Northeastern Thailand

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Food-borne disease caused by *Salmonella* spp. is one of important public health problems. The antimicrobial resistance causes failure of regular therapy and increases the cost of treatment. The objectives of this study were to determine prevalence and antimicrobial resistance of *Salmonella* spp. isolated from racehorses and horsemen in Northeastern Thailand. A total of 63 samples from racehorses and horsemen were collected (30 and 33 samples, respectively) at farms in Northeastern Thailand. The samplings were collected during April – December 2018. All samples were examined for *Salmonella* spp. isolates and identification by ISO 6597:2002. The prevalence of antimicrobial resistance patterns was assessed using disk diffusion technique among 7 antimicrobials. *Salmonella* spp. contaminated to racehorses and horsemen were 4.86% and 3.03%, respectively. The identified serovars from racehorses were *S.* Abony (23%) and *S.* Inganda (15%); from horsemen was *S.* Tumodi II. Penicillin was high resistance of *Salmonella* spp. isolated from racehorses. The prevention and control of *Salmonella* spp. transmitted between racehorses and horsemen were very important such as hygiene, sanitation and standard farm management.

MI33
Use of *Clostridium perfringens*-specific bacteriophage to control necrotic enteritis in broiler chickens

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The present study was aimed to determine the dietary supplementation of *Clostridium perfringens*-specific bacteriophage (CPBA) in broiler chickens infected with experimental necrotic enteritis (NE). Total of seven-hundred-eighty day-old feather-sexed male broiler chicks were individually weighed and randomly allocated into 5 treatments (12 replicates with 13 birds per replicate): 1) non-challenged control group (NC); 2) NE-challenged control group (PC); 3) PC with CPBA supplementation at 10^5 cfu/kg (low CPBA); 4) PC with CPBA supplementation at 10^6 cfu/kg (medium CPBA); and 5) PC with CPBA supplementation at 10^7 cfu/kg (high CPBA).

The chickens were orally gavaged with 10-dose coccidiosis vaccine on d 9 and *C. perfringens* on d 14, 15, and 16 to experimentally induce NE. Each pen was used as an experimental unit and all data were analyzed by one-way ANOVA using General linear model (GLM) procedure of SAS 9.4. Body weight gain at 21 d were lower in the PC group compared with the NC group (P<0.001). Dietary CPBA increased body weight gain (P=0.001) compared with the PC group. Mortality due to NE infection was highest in the PC group (P=0.002) and compared with PC group overall mortality was decreased (P=0.038) with the medium and the high CPBA treatments.

Jejunal NE lesion scores were lower in the all CPBA groups regardless of CPBA doses (P<0.001) compared with the PC group at 2 days post *C. perfringens* challenge. The concentration of acetate was increased in the PC group compared with NC group (P=0.034). Also the concentration of Lactate was highest in PC group compared with NC and CPBA groups.

In conclusion, *C. perfringens*-specific CPBA supplementation ameliorated growth performance of broiler chickens with NE infection and can be considered as effective feed to lessen production additive and gut health losses due to NE infection in broiler chickens.

Keywords: necrotic enteritis, growth performance, gut health, *Clostridium perfringens*, broiler chickens

MI34
Antimicrobial resistance in *Salmonella enterica* isolated from meat-type ducks in Nakornpathom province Thailand

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The emergence of antimicrobial resistance (AMR) in *Salmonella enterica* isolated from meat-type ducks has become important public health issues because duck meat is widely consumed in many countries. As seen in other food animals, study of AMR in ducks will elucidate root cause of AMR and provide data for alternative to antibiotics (ATA) development in the future. Nakorn Pathom province located in Central region of Thailand and there is the high density of duck population. Due to intensive duck-raising system in this area, antimicrobial agents have been used to treatment and control the spread of infectious bacterial pathogens. It is possible that extensive or indiscriminate use of antimicrobial in duck farms might be led to increased level of antimicrobial resistance (AMR) in *Salmonella* in this area. Therefore, the objective of this project was to characterize of antimicrobial resistance in *Salmonella* isolates from meat-type ducks in Nakorn Pathom province Thailand. A total of 705 fecal samples were obtained from 3 meat-type duck farms. Fecal samples from the same flock at different ages (one-day old duckling, 40-42 days old, 60-70 days old) were collected at each farm. All fecal samples were subjected for isolation and identification of *Salmonella spp*. All *Salmonella*-positive samples were tested for serotyped by the Kauffmann-White scheme. Antimicrobial susceptibility was determined in all *Salmonella* isolates. The presence of resistance genes in AMR *Salmonella* isolates were detected based on their resistance phenotype using PCR. A total of 89 of 705 (12.6%) were positive for *Salmonella*. One hundred and thirty-two *Salmonella* isolates were identified. Serovar Altona was the predominant serotype in this study (36.4%). Most of the isolates showed highly resistant to sulfamethoxazolem (100%), tetreacycline (24.2%), streptomycin (21.2%), spectinomycin (18.2%), ampicillin (15.9%). None of the isolates was resistance to cefoperazone, gentamicin, ciprofloxacin. The bla_{TEM}, *aadA2*, *strA, dfrA12* genes were detected in *Salmonella* resistant strains to ampicillin (52.4%), streptomycin (57.1%), streptomycin (21.4%), trimethoprim (25%), respectively. The plasmid-borne *qnr* genes in fluoroquinolone-susceptible *Salmonella* strains were *qnrB* (3.8%) and *qnrS* (1.5%). Overall, meat-type ducks production is an important sector in poultry industry. The highlight of our study revealed that ducks are potential source of AMR in food-producing animals but there is still a lack of knowledge of AMR in duck population. Therefore, the data from the research will provide more complete picture of trend and situation of AMR in poultry production in Thailand.

Keywords: Antimicrobial resistance, *Salmonella,* ducks

MI35
Organic produce as a potential alternative source to reduce the spread of antimicrobial resistance bacteria

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Antimicrobial resistance (AMR) is now a serious public health issue worldwide. In response to reduction of antimicrobial use, organic agricultural products have been a new trend for consumers due to the reputation of being environmental-friendly and the limited use of artificial chemical substances, especially antimicrobials. However, the main concern of organic products is the contamination of antimicrobial resistant organisms derived from animal manure used in organic farms. The objectives of this study are to evaluate the concentrations of fecal coliforms and *Escherichia coli*, to determine the association between environmental factors affecting on bacterial loads in conventional and organic vegetables and fruits sold in fresh markets and supermarket, and to determine antimicrobial resistant pattern of *E. coli*.

Seven types of samples consisting of sweet basil, spring onion, coriander, cabbage, lettuce, cucumber and tomato were selected since they are commonly consumed raw or minimally cooked in Thai cuisine. A total of 335 conventional produce were collected from six fresh markets (n=168) and 24 supermarkets (n=167), while organic samples (n=168) were collected from 26 supermarkets. Enumeration method was used to determine the level of fecal coliforms and *E. coli* according to the United States Food and Drug Administration (U.S.-FDA).

The average concentrations of fecal coliforms in conventional samples from fresh markets and supermarkets, and organic samples from supermarkets were $3.52x10^4$, 507, and 51 MPN/g. The average concentrations of *E. coli* in conventional from fresh markets and supermarkets and organic samples from supermarkets were $1.88x10^4$, 44.6, and 26 MPN/g. Season, types of markets and types of vegetables were statistically associated with the concentrations of *E. coli* based on negative binomial regression model. The highest indicator bacterial contamination was observed in sweet basil followed by lettuce, coriander, spring onion, cabbage, cucumber and tomato, respectively.

The concentrations of *E. coli* in organic samples were statistically significant lower compared to conventional samples. Thus, the contamination of antimicrobial resistant organisms might be lesser. High concentrations of *E. coli* observed in fresh markets may occur due to cross contamination from direct contact and less processing before selling. Furthermore, the elevated concentrations of *E. coli* are observed in rainy season maybe because rain can facilitate *E. coli* contamination in produce by splashing the bacteria and aerosol formation. Antimicrobial susceptibility test of *E. coli* will be carried out and the results are expected to support organic produce as an option of antimicrobial resistance reduction.

Keywords: *E. coli*, organic produce, vegetables and fruits

MI36
Typing of resistance plasmid *Escherichia coli* for future development of conjugative inhibitors

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Plasmid is a mobile genetic element that could carry multiple resistance determinants. Horizontal transfer of resistance plasmid occurs between intra- and inter-species and is the major route for emergence and spread of antimicrobial resistance (AMR). Inhibition of R-plasmid transfer is a choice to reduce dissemination of AMR. Research for development of new molecules as conjugate inhibitor(s) should be encouraged. However, data on epidemiology and evolution of R-plasmid is still limited. Commensal *Escherichia coli* resides intestinal tracts of humans and animals and their resistance phenotype and genotype reflect antimicrobial use, while *E. coli* is ubiquitous. Therefore, this study aimed to perform plasmid replicon typing to investigate epidemiology and evolution of R-plasmid in *E. coli* along the food chain. It is to produce molecules used for plasmid curing in the future.

A total of 1,338 *E. coli* were obtained from rectal swab from clinically healthy pigs (n=839), pork (n=396), and humans (n=103) during 2008-2017. These samples come from thirteen provinces across Thailand including Chiang Rai, Nongkhai, Mukdaharn, Udonthani, Nakornratchsrima, Buriram, Suphan Buri, Aung Thong, Kanchana Buri, Ratchaburi, Chachoengsao, Sakaew and Chon Buri. Plasmid replicons were typed by using multiplex PCR for 18 incompatibility (Inc.) groups including A/C, B/O, F, FIA, FIB, FIC, FIIA, HI1, HI2, I1, K, L/M, N, P, T, W, X, and Y.

The result shows that sixteen of eighteen Inc. groups were found except Inc L/M and T. The highest three Inc. groups, i.e. Inc. F, FIB and K were identified in every region of Thailand and possessed more than 60% of each sample type i.e. human, 64.55%; pork, 65.85% and pig, 63.39%. Inc. W, X and HI2 were detected only in *E. coli* isolated from pigs. From geographic data, four Inc. types (i.e. F, FIB, K and N) were identified across Thailand. The Inc. HI1 and I1 groups found in the isolates from every part of Thailand, except the Central part.

The results indicated that plasmids in a variety of Inc. groups can be identified in *E. coli* in Thailand. Particular plasmid patterns were related to particular sources of the isolates (i.e. humans, animals and food). Transferability of plasmid to another bacterium is now under progress. The plasmids should be characterized in the further. The similar research should be conducted in other bacterial species and along food chain. The observations also suggest that novel molecules for curing and inhibiting plasmid should be developed. The molecules must be additionally tested in *in vivo* due to the existence of variable plasmid in clinical strains.

MI37
Multidrug efflux systems as potential targets for new drug development in *F. columnare* isolated from Asian sea bass (*Lates calcarifer*)

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Flavobacterium columnare is a Gram-negative long rod bacterium that causes columnaris disease. This disease leads to economic loss in freshwater fish production in Thailand including Asian sea bass (*Lates calcacrifer*). Antibiotics in many classes have been used for therapeutic objectives such as quinolones, tetracyclines and sulfonamides. Imprudent usage of antibiotics has been evident. However, antimicrobial resistance (AMR) strains have been increasingly reported in *F. columnare,* especially resistance to quinolones. Besides, comprehensive study of quinolone resistance genes and mechanisms is still very limited. This study aimed to investigate quinolone resistance-associated genes of *F. columnare* using genome analysis. The quinolone resistant *F. columnare* isolates exhibited the highest MIC values to oxolinic acid and enrofloxacin (>64 µg/mL and 1 µg/mL, respectively) were submitted to whole genome sequencing and resistome analysis were performed. The complete genome of *F. columnare* consists 3.1 Mb and 2,941 protein coding sequence were predicted. Resistome analysis revealed that 169 genes were predicted as AMR genes and 45 genes were responsible for quinolone resistance. Most of them belong to efflux pump gene family including resistance-nodulation-cell division (RND), major facilitator superfamily (MFS), ATP-binding cassette (ABC) and multidrug and toxic compound extrusion (MATE) transporter efflux pump. Genes relevant to RND efflux pump family, which have been recognized as a major mechanism involved in multidrug resistance phenotype were found at the highest number. This is the first comprehensive study of quinolone-associated genes in *F. columnare* in Thailand and the results also highlight multidrug efflux mechanisms as potential targets for novel drug development against *F. columnare*.

Keywords: antimicrobials, Asian sea bass, characterization, quinolones, *Flavobacterium columnare*

MI38
Resistome analysis of *Aeromonas veronii* NK02 isolated from Nile tilapia (*Oreochromis niloticus)* by focusing in aminoglycoside resistance associated genes

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Thailand is one of the major producers and exporters of freshwater fish in the in the world, of which the most important species is Tilapia (*Oreochromis niloticus*). The production is threatening by bacterial diseases especially *Aeromonas veronii*. The fish farmers usually use antibiotics for treatment of the infections, of which gentamicin is a commonly used antibiotic. Non-prudent use of antibiotics may lead to resistance in aquaculture. This study aimed to determine the susceptibility of *A. veronii* to gentamicin and analyze the resistome based on whole genome sequencing. *A. veronii* NK02, the clinical isolate that is common is used. The *gyrB* gene was first sequence and the MICs of five antimicrobials including amoxicilin sulfamethoxazole/trimethoprim, gentamicin, enrofloxacin, and oxytetracycline were determined by broth microdilution method. The NK02 was resistant to amoxicillin and gentamicin with the MIC value over than 256 µg/ml. The whole genome of *A. veronii* NK02 was sequenced with illumine Hiseq platform. Subsequently, the genome assemble data was submitted to CARD and Resfinder database for resistome analysis, respectively. The results showed the presence of ten antimicrobial resistance genes (ARGs) associated with resistance to aminoglycoside, beta-lactams, quinolone, sulfonamide and tetracycline. In comparison to ARGs database, two of ARGS, *aac(3)-IIb* and *aac(6')-Ib-cr*, were detected. The genome of *A. veronii* NK02 was blasted against the existing database by Blast2GO. The results showed that the nucleotide identity of genes was higher than 85% and related to the phenotypic resistance. Additionally, these genes can be also detected in genome assembly of *A. veronii* isolated from freshwater fish from India, Spain and Korea retrieved from NCBI genome database. However, the nucleotide identity to aminoglycoside resistance genes from those strains was lower than *A. veronii* NK02 (≤60%). However, the percentage of identity to aminoglycoside associated genes is not associated with the MIC value. In conclusion, the aminoglycoside associated genes found in *A. veronii* NK02 may acquire from other resistant bacteria circulated in the aquatic system or selective pressure from non-prudent use of antibiotics. Thus, alternative to antibiotics should be developed for treatment of *A. veronii* either in aquaculture to avoid selection of ARGS and other antimicrobial resistance gene.

MI39
Antimicrobials Susceptibility of *Vibrio* spp. infected Marine Asian sea bass (*Lates calcarifer*) Cultured in Krabi, Thailand

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Asian sea bass (*Lates calcarifer*) is one of the cultured marine fish that has been widely cultivated in South and Southeast Asia, including Thailand. The fish is known for its rapid growth rate, and high tolerance to environmental conditions. One of the major problems in raising this fish is a disease challenge caused by *Vibrio* spp. and antibiotics have been used as a tool to prevent the outbreak. This could result in emergence and spread of antimicrobial resistance, particularly in the aquatic environment. This study was conducted to examine antimicrobial susceptibility of *Vibrio* spp. from infected marine cultured Asian sea bass in Ko Lanta, Krabi Province, Thailand. It is a case study with purposive sampling from the farm post-outbreak of scale drop, fin and tail rot disease which associated with Vibriosis. A total of 40 *Vibrio* isolates were collected from the skin lesions, liver, spleen, and kidney of 12 Asian sea bass. Phenotype identification showed three species of *Vibrio*, namely *V. campbellii* (KR05), *V. parahaemolyticus* (KR08; 20; 21; 30) and *V. harveyi* (KR16; 26; 31; 37; 38; 39; 40). Five antibiotics, enrofloxacin, norfloxacin, oxytetracycline, sulfamethoxazole/trimethoprim and oxolinic acid were tested by Kirby Bauer disk diffusion susceptibility test. Nine strains of *Vibrio* spp. showed inhibition zone ≥ 20 mm and three strains (KR05; KR08; KR26) exhibited undetermined zone of inhibition. The observations showed that most (75%) *Vibrio* spp. strains still are still susceptible to antibiotics tested. However, in order to prevent the emergence and spread of antimicrobial resistance associated with fish farming, prudent use of antibiotics in aquaculture needs to be encouraged. Alternative to antibiotics should be researched for disease prevention and treatment of diseases, especially caused by *Vibrio* spp.in fish farming, not limited to marine cultured Asian sea bass.

MI40
Sex pilus specific bacteriophage to drive bacterial population towards antibiotic sensitivity

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We used plasmid-mediate pilus-specific phages to demonstrate that they not only infect and lyse bacteria harbouring self-transmissible plasmids but also select for mutants which had lost the plasmids and were therefore resistant to the phage, but also a smaller number of mutants with mutations in the plasmid *tra* region so that pili were not produced. The percentage of plasmid-minus mutants increased with prolonger passage in the presence of phage. In groups of young chickens infected with *Salmonella* possessing a highly self-transmissible AMR plasmid pilus phage reduced colonisation and spread between chickens but also selected for massive plasmid loss. These phages also increased plasmid loss in more repressed plasmids but this was less marked. The phages also eliminated conjugation ability.

MI41
Mutations of Streptomycin Resistance Genes in *Mycobacterium tuberculosis* Thai Isolates

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Drug-resistant TB is an alarming issue in Thailand which is one of the 22 high TB burden countries and molecular characterization of anti-TB drugs resistance for this region is necessary. Streptomycin (SM) is recommended by World Health Organization (WHO) as a part of the standard regimens for the retreatment of multidrug-resistant tuberculosis (MDR-TB). To date, the information on SM resistance (SMR) gene mutations correlated to the SMR of *Mycobacterium tuberculosis* (*Mtb*) Thai clinical isolates is limited.

In this study, by using PCR amplification and DNA sequencing analysis, SMR associated mutations of *rpsL*, *rrs*, *gidB* and *whiB7* genes were examined in 101 *Mycobacterium tuberculosis* clinical isolates with various drug susceptibility profiles from Thailand. Their mutation patterns, the frequency related to SMR and the subsequent utility for diagnostic value were determined.

The *rpsL* mutations, Lys43Arg, Lys88Arg and Lys88Thr, and the *gidB* mutations, Trp45Ter and Gly6Asp, were found to be correlated with SMR. The Lys43Arg was the most predominant *rpsL* mutations (94.1%) and found in 69.6% of the SM resistant isolates. Among them, mixed *rpsL* WT and Lys43Arg sequences were found in a SM mono-resistant isolate. The *rpsL* mutations had the highest sensitivity (73.9%) and specificity (96.4%) for the detection of SMR in *Mtb* Thai isolates. Surprisingly, *rrs* mutations associated to SMR were absent in this study. The combination of *rpsL* and *gidB* mutations exhibited 76.1% sensitivity and 96.4% specificity for the identification of SMR. *whiB7* was not responsible for the resistance in SM resistant isolates lacking *rpsL* and *rrs* mutations. Our study suggested that the majority of SMR in *Mtb* Thai isolates were responsible by *rpsL* and *gidB* polymorphisms.

MI42
Antagonistic activity of *Bacillus* Probiotics against Enterotoxigenic *Escherichia coli* (ETEC) and colistin resistant *E. coli* from Pigs in Thailand

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In Thailand, the livestock development department has announced the acts for antibiotics controlling in animal feed since 2018. Many medicated feed including colistin has been controlled and will be used for treatment only. Probiotics are an alternative to reduce antibiotic usage in animals. The aim of this study was to evaluate the antagonistic activity of 21 strains of 4 *Bacillus* species including *B. subtilis,* 13 strains; *B. licheniformis,* 3 strains; *B. pumilus,* 1 strain and *B. amyloliquefaciens,* 4 strains against 8 strains of pathogenic *Escherichia coli* including *E. coli* ATCC 25922, 5 strains of enterotoxigenic *E. coli* (ETEC) and 2 strains of colistin resistant *E. coli* containing *mcr-1* by delayed antagonism in solid nutrient medium method. It was found that 19 strains of 3 *Bacillus* species including *B. subtilis, B. licheniformis and B. amyloliquefaciens* were able to inhibit the growth of *E. coli* reference strain and 7 strains of enterotoxigenic *E. coli*. The inhibitory ability of *Bacillus* on varies among the difference species and strains. *B. subtilis* KMPN008 showed the highest effectiveness against *E. coli* reference strain and 2 colistin resistant *E. coli* strains of which the inhibitory zone was 25.7 $\pm$ 3.1 mm, 27.0 $\pm$ 4.1 mm and 35.8 $\pm$ 4.7 mm, respectively. B. *amyloliquefaciens* 16AvBa17 showed the highest antibacterial ability on both enterotoxigenic *E. coli* 02 and 03. The other 3 strains of enterotoxigenic *E. coli* (i.e. strains 01, 04 and 05) were inhibited by *B. subtilis* 3 strains. The inhibitory zone of KMP- BCI-1, KMP CU 4 and KMP-BCI-2 was 29.8 $\pm$ 8.3 mm, 36.2 $\pm$ 4.8 mm, and 35.8 $\pm$ 3.8 mm, respectively. These studies suggested that use of multiple species and strains of *Bacillus* will generate better inhibitory effects on enterotoxigenic *E. coli* from pigs.

Keywords: *Bacillus* spp., Antibacterial activity, Enterotoxigenic *E. coli*

SESSION 3
Innovative drugs, chemicals and enzymes

ORAL PRESENTATIONS

Strategies to reduce antibiotics in swine production in China

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Antibiotics have been widely used in a prophylactic way in piglet diets to promote growth performance and reduce diarrhea incidence. However, the resistance of pathogens to antibiotics and the risk of residues of antibiotics in animal products induced a growing interest in the use of alternatives to in-feed antibiotics. According to a recent announcement from Chinese government, the antibiotic growth promoter (AGP) except Chinese herbal extracts will be forbidden from production starting from January 1st, from production in animal feed from July 1st and totally out of feed products in circulation from end of next year (2020). Since the in-feed antibiotics are mainly used for relieving the weanling stress and promoting the growth of piglets, different efforts for manipulation of weanling piglet's diets from scientific and industrial community have been inspired by following this new legislation, which including development and extension of antibiotics alternatives, low protein diets, well-chosen feed ingredients, as well as its pre-digestion or pre-processing. On the other hand, environmental control and management measures have also been adopted to improve the health and growth of the pigs, especially the piglets during weanling and nursery stages. Generally speaking, antibiotics alternatives are the first to be extensively investigated and adopted, including the commonly used acidifier, essential oils, medium-chain fatty acids, zinc oxide, probiotics, prebiotics, oligosaccharides, plant extracts and the newly developed antimicrobial peptides, which are mainly functioning through regulation of the intestinal micro-environments including epithelium barriers and commensal microbiota. At the same time, by considering the immature status of intestinal development and enzyme secretion in weanling piglets, selection of feed ingredients which can be easily digested, or with higher digestibility and lower anti-nutritional factors, a diet with lower protein level but more balanced amino acids provision, or a diet supplemented with beneficial fiber, are now been extensively tested and widely applied in the weanling piglets diet. To decrease the burden of intestine for digestion, the pre-digestion or pre-treatment of individual feed ingredient or whole diet by using enzymes, fermentation, their combination or heat treatment are becoming more and more popular, which are considered to produce more nutrients with lower molecular weights, increase digestibility and promote absorption, as well as eliminate the anti-nutritional factors and reduce pathogenic bacteria in the feed. To the last but not least, providing a warm and clean environment has been approved to be efficient for controlling the diarrhea incidence in weanling piglets, and the timely and reasonable manure management has also been suggested to be a contribution of clean space for swine growth. In summary, the strategies mentioned in this review are the current efforts in China aiming to promote the reduction of in-feed antibiotics in swine production and the green development of husbandry industry. We hope this review will be also helpful for the full-chain antibiotics reduction including antibiotics in feed and for therapeutic purpose, which ultimately achieves the goal of no antibiotic residues in meat products.

Keywords: Antibiotics, Alternatives, Dietary manipulation, Management, China

Non-antibiotic treatments for honey bee diseases in the era of omics

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Honey bees provide pollination service to 35% of the world's food crops. The microsporidia parasite, *Nosema ceranae*, affects honey bee health in many ways and has been implicated in the steep global population decline of honey bees. Fumagillin-B is the only antibiotic approved for control of *Nosema* disease in honey bees and has been extensively used in North America for more than 50 years. However, Fumagillin-B has been linked to the problems of resistance development and off-target effects. Further, the product is, in fact, coming off the market due to supply issues. This formidable challenge has spurred scientists to look for alternative treatment options for *Nosema* disease in honey bees. Using genomic, comparative genomics, and transcriptomic approaches, we identified virulent factors that are essential for invasion and replication by *N. ceranae* in honey bees and genes and pathways implicated in host-*Nosema* interactions. We also identified the factors and processes involved in the battle between *Nosema* and the honey bee host, providing us with a list of potential targets for innovative therapeutics to break down the life cycle of the parasite. RNA interference (RNAi) is a remarkable process in which RNA molecules suppress gene expression by neutralizing specific targeted mRNA molecules and has provided unique opportunities in combating diseases caused by pathogens in a wide range of organisms. We explored the potential of RNAi as a therapy for controlling *Nosema* disease in honey bees through two different angles. First, we explored the possibility of silencing the expression of a *N. ceranae* virulence gene encoding polar tube protein 3 (PTP3) that is involved in the host cell invasion as a therapeutic strategy for controlling *Nosema* disease. Our studies showed that the oral ingestion of a dsRNA corresponding to the sequences of PTP3 could effectively suppress the expression of the PTP3 gene in *N. ceranae* infected bees and reduce the *Nosema* load. Secondly, we employed the RNAi strategy to reduce the expression of a honey bee gene, namely naked cuticle (*nkd*), which is a negative regulator of host immune function. Our studies found that *nkd* mRNA levels in adult bees were upregulated by *N. ceranae* infection and that RNAi-mediated knockdown of *nkd* transcripts could efficiently silence the *nkd* expression in *Nosema*-infected bees. We also found that the oral ingestion of dsRNA specific to *nkd* could lead to the upregulation of expression of several genes encoding Antimicrobial peptides (AMPs) such as *Abaecin, Apidaecin*, and *Defensin-1* that are regulated by the Toll pathway. Further, the oral ingestion of a dsRNA specific to *nkd* led to the significant reduction of *Nosema* spore loads and the extension of honey bee lifespan, clearly demonstrating that silencing the host *nkd* gene can activate honey bee immune responses, suppress the reproduction of *N. ceranae*, and improve the overall health of honey bees. The results of our studies strongly suggest that RNAi-based therapeutics hold great promise for the effective treatment of honey bee diseases and will have positive implications for bee disease management practices in the future.

Keywords: Honey bee, pollinator, Nosema, RNA interference (RNAi), therapy

In vitro and *in vivo* characterization of a Gly-substituted DLP4 cationic peptide against *Staphylococcus aureus* CVCC 546

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Staphylococcus aureus is notorious for its ability to become resistant to antibiotics, generating interest in novel antimicrobial strategies. The clinical development of antimicrobial peptides (AMPs) is currently under evaluation. However, most of these AMPs have modest direct antibiotic activity and toxicity. Thus novel peptide D13 designed and optimized from DLP4 with enhanced antibacterial properties was develped. *In vitro* and *in vivo* efficacies were evaluated.

Homologous sequence alignment, circular dichroism analysis, DNA gel retardation, flow cytometry, scanning electron micsroscopy (SEM), transmission electron microscopy (TEM), K^+ leakage and *In vivo* efficacy was tested against *Staphylococcus aureus* CVCC 546 in a mouse thigh infection model.

Base on homologous sequence alignment, a variant D13 from DLP4 with enhanced antibacterial properties was designed. The defensin D13 exerted potent antimicrobial activity against *S. aureus in vitro*, with the minimum inhibitory concentrations (MICs) in the range of 2 to 8 μg/mL. D13 exhibited lower hemolysis toward murine erythrocytes and cytotoxicity against RAW 264.7 cells than DLP4 (0.37% VS 0.42% of hemolysis and 80.9% VS 59.7% of survial, respectively) at the concentration of 128 μg/mL. D13 could distroy the cell membrane of *S. aureus* CVCC 546, resulting in an increase in the K^+ leakage. Gel retardation and CD spectra analyses demonstrated that D13 bound specifically to DNA and disrupted the DNA conformation. After treatment with 4×MIC D13 for 2 h, wrinkled and collapsed *S. aureus* cells were observed in SEM and TEM. Additonally, D13 was highly efficacious in a thigh model of infection with *S. aureus* CVCC 546, causing a 1.8 log10 reduction of CFU in thighs, and a downregulation of TNF-α, IL-6 and IL-10 levels.

The multiple modes of action of D13 against *S. aureus* may minimize resistance development of the target microorganisms. The result suggests that D13 could be a novel promising antimicrobial candidate to treat infectious deseases caused by *S. aureus* in livestock.

Keywords: D13, *Staphylococcus aureus*, antimicrobial peptides, mechanism

Afterlife of bacterial cell debris: Peptidoglycan in the gastrointestinal tract

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An ever-growing body of evidence suggests, that the microbiota in the gastrointestinal tract plays a key role in regulating host metabolism and health. While most attention is directed towards the living bacteria in the gastrointestinal tract, there is little focus on dead and decomposing bacteria. This is surprising, as biomass from dead and decomposing bacteria in the gastrointestinal tract can surpass that of living bacteria. A more holistic view of the microbiota is thus required, particularly including dead and decomposing bacteria.

A major part of the bacterial biomass is peptidoglycan, a complex and sturdy polymer forming the bacterial cell wall. It is built from long glycan strands held together by short peptide bridges. The glycan strand is composed of the *β*-(1,4)-linked sugars *N*-acetylglucosamine and *N*-acetylmuramic acid, the latter of which is uniquely found in peptidoglycans. The peptide bridge is composed both of common L-amino acids and extremely rare D-amino acids. A further structural peculiarity of peptidoglycan is, that the peptide bonds between amino acids in the peptide bridge are not only formed via their backbone but also their side chains, a feature not found in proteins.

Peptidoglycan fragments are continuously released into the gastrointestinal tract as bacterial cells divide, die and decompose. Together with the live microbiota, they could potentially affect the gastrointestinal functionality of the host. To help expand our knowledge about the interplay between diet, microbes and the host, innovative tools to study bacterial waste biomarkers as well as bacterial viability are required.

We have adapted a method used previously to estimate bacterial biomass in soil samples to study bacterial waste biomarkers by quantifying the peptidoglycan building block muramic acid in soluble and insoluble fractions of intestinal content. A novel microbial muramidase has recently been described in literature, which is capable to selectively hydrolyze peptidoglycan from bacterial waste while leaving the live microbiota untouched, thereby improving animal performance. Using our novel method to study bacterial waste biomarkers in digesta samples from animals fed with this novel microbial muramidase, we could show that the enzyme leads to an increase in the amount of soluble peptidoglycan debris by hydrolyzing large, insoluble bacterial waste. We believe that these large and insoluble bacterial waste fragments impair nutrient uptake and when selectively degraded leading to an improved gastrointestinal functionality.

Keywords: Gastrointestinal functionality, bacterial cell debris, peptidoglycan, muramidase, biomarker

Fighting Antimicrobial resistance by optimizing gastrointestinal functionality: A holistic approach

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The occurrence of antimicrobial resistance (AMR) is an issue of global concern and it is catalyzing consumer-driven and regulatory pressure to eliminate antibiotic use in animal production. By 2050 AMR is expected to be a leading cause of death in humans.

Modern animal production has consistently been intensified in order to improve productivity. Increasing stocking density increases the risk of infectious diseases outbreaks. In the past, such diseases were largely controlled by antibiotic growth promoters (AGPs). Even in the absence of clinical diseases, these compounds exerted a beneficial effect on farm animals, since they were capable to control dysbacteriosis and enteropathogens. Since the ban of AGPs in Europe in 2006, the incidence of intestinal problems and dysbiosis has steadily increased. Until recently, 'health solutions' such as AGPs, have provided the 'silver bullet' for gastrointestinal functionality in animal nutrition and health, however, the current industry approach conceals fundamental gaps in the understanding of gastrointestinal functionality and its interactions with husbandry practices including nutrition.

Antibiotic Free (ABF) production systems are a reality in several regions of the world and they are often combined with changes in housing condition, feeding management and health programs. These changes, in combination with strategic use of feed additives, are necessary to optimize the management of the myriad of factors that may influence gastrointestinal functionality.

In farm animals, effective gastrointestinal functionality is vital in determining their health, welfare and productive performance. Several definitions of gut health can be found in the literature; however, they often lack of a precise and unifying meaning or etiology. A new definition of gastrointestinal functionality has been recently offered: "gastrointestinal functionality is a steady state with the gastrointestinal tract and its microbiome are in a healthy equilibrium to perform normal physiological functions which play a critical role in overall health and well-being". This definition pivots around six key components, namely: the diet, effective intestinal barrier, host-microbiome interaction, effective digestion and absorption, effective immune status, and neuroendocrine function of the gut. Each of these components are intimately intertwined by multifaceted physiological mechanisms and pathways. Optimization of gastrointestinal functionality is crucial to increase nutrient digestibility and thus maximizing value from feed, to sustain host physiological functions such as innate and adaptive immunity and thus increasing resilience to environmental challenges, and finally, to maintain eubiotic conditions.

While it is clear that antibiotics must be used responsibly to ensure the health and welfare of animals, we advocate for the replacement of AGPs and reduction in the prophylactic use of antibiotics with alternative nutritional solutions and innovations combined with farm and health management. Future ABF production and its sustainability depends on the development a larger understanding and practical application of concepts related to gastrointestinal functionality that imply complete holistic management of the production system. Maintaining effective gastrointestinal functionality by integrating key husbandry and management practices in a holistic approach, is the most promising concept to promote farm animals' productivity, health and welfare, and to reduce globally the use of antibiotics in animal farming and decrease AMR.

Keywords: gastrointestinal functionality, antimicrobial resistance, nutrition, feed additives, holistic

Organic acids as antibiotic alternatives in monogastric animals

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Young animals have a high susceptibility to various stressors, including bacterial pathogens, oxidative stress and inflammation, leading to reduced growth performance, high mortality and morbidity rates and compromised animal welfare. The empirical benefits of using antibiotics to address animal health issues in animal agriculture (using therapeutic doses) and increasing the overall productivity of animals (using sub-therapeutic doses) are well established. This practice may lead to the spread of antimicrobial-resistant bacterial pathogens in both animals and humans, posing a significant public health threat, which is furthering the need to reduce the use of antibiotics within the animal production chain. Therefore, it is critical to develop cost-effective antibiotic alternatives for ensuring the long-term sustainability of animal production. Organic acids have been known for their ability to prevent food spoilage and extend the shelf-life of many perishable commodities. This capability brought attention to their possible usage in combating bacterial diseases and fighting infections. It has been demonstrated that organic acids have good potential as antibiotic alternatives in feeds for monogastric animal production. The potency of these acids is dependent on the physiological status of the targeted microorganisms and the physicochemical characteristics of the surrounding environment. The combination of different organic acids and other compounds (synergistic effect) such as essential oils seem to be a promising approach to improve the efficacy of organic acids in applications. High-throughput systems technologies have been developed recently, which will allow us to dissect the mechanisms underlying the functions of organic acids and facilitate the use of organic acids in monogastric animal production. This presentation summarizes the efficacy, feasibility and potential mechanisms of the application of organic acids as antibiotic alternatives in monogastric animal production.

Keywords: Organic acids, Medium chain fatty acids, Infection, Antibiotic alternatives, Monogastric animals

25-OH-D3: An indispensable tool to managing antibiotic free feeding programs for commercial broilers

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Antibiotic free (ABF) or raised without antibiotics (RWA) programs are accompanied by a dramatic increase in the incidence of enteritis in commercial broiler flocks. Enteritis is commonly a result of clinical/subclinical coccidiosis or clostridiosis intercurrent with coccidiosis to further cause necrotic enteritis. Researches suggested that maintaining a healthy immune system is one of the keys for success in an RWA program.

25-OH-D_3 (a metabolite of vitamin D_3) has been implicated to support anti-inflammatory reaction. Locally-produced 1a, 25-$(OH)_2$-D_3 (destined for autocrine/paracrine functions of vitamin D) from 25-OH-D_3 by 1a-hydroxylase in immune cells including monocytes, and macrophages inhibits pro-inflammatory cytokine expression and promotes anti-inflammatory cytokine production. This is mediated by inhibition of the nuclear factor *kappa*-B (NFkB), a transcription factor with a critical role in inflammatory response. As such, 25-OH-D_3 may become indispensable in managing the inflammatory nature of enteritis.

Coccidiosis and necrotic enteritis models were applied to 0-to-21-day-old broilers. The birds were challenged with either *Eimeria maxima* or *Eimeria maxima* plus *Clostridium perfringens*. Birds were fed 25-OH-D_3 dosed at either 34.5 (LD) or 69 (HD) mcg per kg diet.

HD birds exhibited higher serum 25-OH-D_3 level than LD birds regardless if they were challenged with *E. maxima* only or *E. maxima* plus *C. perfringens*. At day 21, seven days post *E. maxima* challenge, HD birds had 13 points mortality-adjusted feed conversion ratio (adj. FCRm) advantage over LD birds. In addition, HD birds had lower oocyst shedding (105,211/g) compared to LD birds (150,725/g). At day 21, seven days post *E. maxima* challenge and a day post three consecutive days of *C. perfringens* challenge, HD birds had 4 points advantage of adj. FCRm over LD birds. They also had higher IL-10 (an anti-inflammatory cytokine) gene expression than LD birds. Both HD and LD birds produced similar necrotic enteritis lesions and *E. maxima* lesions.

In conclusion, 25-OH-D_3 could be a convenient tool in managing RWA programs for broilers under the experimental model of disease-challenged conditions reported herein by ameliorating the inflammatory response associated with enteritis.

Keywords: 25-OH-D3, Antibiotic Free, Coccidiosis, Clostridiosis, Enteritis

Alternatives to Veterinary Antimicrobials (AVANT): A new EU project focused on diarrhoea in pigs

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AVANT is a 5-year EU Innovation Action project that will start in January 2020. The aim is to develop and test innovative alternatives to antimicrobials for management of post-weaning diarrhoea in pigs. This is one of the disease conditions for which most antimicrobials are used in livestock worldwide, and effective alternatives to colistin and zinc oxide are missing for treatment of infections caused by enterotoxigenic *Escherichia coli* (ETEC). The AVANT portfolio includes 7 interventions for which pre-clinical studies will be performed to test safety/efficacy and optimize product formulation and administration on an industrial scale. These interventions include a symbiotic product, in-farm faecal transplantation, anti-EHEC phage- and polymer-based products, immunostimulants and various feeding strategies targeting sows at farrowing or piglets at weaning. The most promising interventions will be selected for farm trials to assess their clinical efficacy. Moreover, the effects of these interventions on reduction of antimicrobial use will be determined at the study farms and the potential reduction in antimicrobial use attributable to the adoption of these alternatives at the EU level will be predicted by modelling. The inclusion of a variety of interventions with different modes of action offers the advantage of comparing the efficacy of different intervention measures under controlled conditions and provides the opportunity of integrating multiple interventions with synergistic effects. Moreover, this approach contributes to minimize the risks of the project if a single intervention will perform poorly in the pre-clinical phase.

The strategic objective of AVANT is to significantly reduce antimicrobial consumption by demonstrating field interventions that target the animal species and disease condition accounting for most antimicrobial use in the EU. The introduction of innovative interventions that reduce antimicrobial consumption, disease incidence and production losses is expected to have a great commercial and societal impact by revolutionizing the concept of disease control in the animal health industry and by minimizing the risks of AMR transmission, with positive consequences on animal welfare, public health and economy. In order to accomplish this ambitious goal, AVANT has assembled a highly inter-sectorial consortium with a balanced participation by the public (n=6) and private sector (n=8) that covers complementary types of knowledge (scientific and practical) and meets the high degree of cross-disciplinarily ('dry' vs 'wet' sciences) that is required to demonstrate innovative and sustainable alternatives to antimicrobials. The AVANT consortium comprises 4 leading industries in the animal health sector, 4 highly specialized SMEs, 5 prestigious universities and the Federation of Veterinarians of Europe. Collaboration between these sectors will ensure access to the knowledge, tools and infrastructures necessary to advance our

interventions rapidly and promote project activities and results to the relevant audiences effectively. Our 'multi-actor' consortium ensures that the innovative solutions developed by AVANT cover real needs and are applicable on real farms.

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Keywords: post-weaning diarrhoea, pigs, EU project, inter-sectorial consortium

SESSION 3
Innovative drugs, chemicals and enzymes

POSTER PRESENTATIONS

IN1
Evaluation on the effects of β-mannanase on intestinal health in broilers, based on 31 trials

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β-Mannans, a type of polysaccharide fibers found in most vegetable feed ingredients, are capable of provoking a wasteful innate immune response, which causes intestinal inflammation[1], and affects numerous metabolic functions[2] and performance[3]. Degrading the β-mannans with an enzyme can reverse the adverse effects[1-3].

The evaluation includes 31 trials conducted under field (28) and field-like (3) conditions, during the last six years to evaluate the effects of a β-1,4-mannanase on broiler performance and intestinal health. Data integrity and relevance of the trials was ensured by screening against a set of pre-established inclusion criteria. All lesions were scored according to a robust scoring system, developed by Elanco[4]. 3580 apparently healthy birds were euthanized and necropsied during the trials for collection of lesion scores related to bird health and welfare, and the scores of 23 conditions related to intestinal health were combined in an intestinal integrity index (I2). Statistically significant improvements were, among others, demonstrated on the I2 index (Control (C)=92.6 and β-mannanase (T)=93.6; P<0.0001), Cellular sloughing (Incidence: C=18.4% and T=14.3%; P<0.01), Excessive intestinal fluid (Incidence: C=17.6% and T=14.8%; P<0.05), Gross *E. acervulina* lesions (incidence: C=30.2% and T=24.7%; P<0.01), Litter Eater (Incidence: C=17.6% and T=14.1%; P<0.01), Feed passage (Incidence: C=12.3% and T=10.1%; P<0.05), and pododermatitis (Incidence of severe lesions: C=18.5% and T=13.8%; P<0.001).

The evaluation demonstrated that the use of a β-1,4-mannanase feed enzyme to degrade β-mannans may improve intestinal health and reduce the incidence and severity of pododermatitis.

Keywords: β-Mannananase, intestinal health, broilers

IN2
The Efficacy of Sodium Humate to Control Diarrhoea and Support Performance of Fattening pigs

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Sodium Humate (HNa) is the sodium salt of humic acid derived from the decomposition of organic matter from plants and animals. HNa can inhibit bacterial and fungal growth and decrease levels of mycotoxin contamination as well as food poisoning. This study was conducted in 10 WOA fattening pigs in a commercial farm in Chonburi province of Thailand.

According to the study, 42 pigs at average weight 29.46 kg were divided into 3 equal groups (14 pigs/group): C-control with common feed, TA-Treatment A with HNa 1 kg/ton feed, and TB-Treatment B with HNa 2 kg/ton feed. Pigs were fed for 21 days. The daily clinical observation was done once a day. Individual weighting was conducted after 21 days feeding. Statistical analysis was by a t-test and significant difference at $P<0.05$.

During the study, no pig showed serious diarrhea to be detected by the observer. Final weight (kg/pig) after 21 days of feeding was 42.00±2.35, 42.50±3.91, and 43.96±3.96 for C, TA, and TB, respectively, $P>0.05$. Feed intake (kg/pig/day) was 1.22^{a}, 1.22^{a}, and 1.24^{b} for C, TA, and TB, respectively, $P<0.05$. ADG (g/day) was 597.14±112.06, 620.95±186.08, 690.68±188.51 for C, TA, and TB, respectively, $P>0.05$.

From this study, HNa at 1 and 2 kg/ton feed for 21 days of feeding showed the positive efficacy to the performance of fattening pigs. Pigs fed with HNa 2 kg/ton feed showed a significantly higher feed intake. In conclusion, HNa can be a possible alternative to antibiotics. Further study in the higher number of pigs will be conducted for reliable statistical analysis.

Keywords: Sodium Humate, efficacy, diarrhoea, performance, fattening

IN3
Inhibition of *Staphylococcus aureus* Biofilm Formation and Its Persisters by novel fungal defensin P2

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There is an urgent need to discover new active drugs to combat methicillin-resistant *Staphylococcus aureus*, which is a serious threat to humans and animals and incompletely eliminated by antibiotics due to its intracellular accumulation in host cells, aggregation of biofilms and persisters. The novel antimicrobial peptide P2 from fungus *Pyronema omphalodes* with multiple antimicrobial mechanisms can be used as candidates.

Confocal laser scanning microscopy (CLSM), crystal violet staining methods, qRT-PCR, intracellular antibacterial activity, cytokines regulation in vivo were used in this study.

S. aureus ATCC43300 and *S. aureus* E48 had the ability to produce biofilm, which were identified by the Congo red and crystal violet staining. The genes related to biofilm formation of the two strains were analyzed by PCR. P2 is a new fungal defensin which is screened from recently sequenced fungal proteome.The results showed that P2 inhibited the initial formation of biofilms, and P2 at 8×MIC achieved 99% inhibition rate and it also eliminated mature biofilms (elimination rate: 64.7%-81.4%). The above effect on mature biofilms was further confirmed by laser confocal microscopy. At the same time, the persister bacteria (approximately 99%) in biofilm were efficiently killed by P2 of 16×MIC within 24 h, which was superior to plectasin. P2 may inhibit/disrupt biofilm formation by regulating *SarA* and *icaD* genes expression. P2 exhibited the potent activity against intracellular MDR *S. aureus* (bacterial reduction in 80–97%) in RAW264.7 macrophages, which was better than vancomycin. P2 regulated the cytokine in mice challenged with *S. aureus* E48. Finally, we observed in vivo that 5 mg/kg of P2 inhibited the bacterial translocation and alleviated multiple-organ injuries (liver, spleen, kidney and lung), and improved the survival of *S. aureus*-infected mice (100%), superior to vancomycin (30 mg/kg). Moreover, P2 also had a good inhibitory effect on the biofilm in mice.

The novel fungal defensin antibacterial peptides P2 has a potent efficacy to *S. aureus* biofilms and persisters, which is superior to vancomycin and plectasin. P2 has some protective effect on mice model with *S. aureus* E48-induced peritonitis and biofilm. These data suggested that P2 may be a candidate for novel antimicrobial agents against MDR staphylococcal infections.

Key words: P2, *Staphylococcus aureus*, intracellular activity, anti-biofilm ability

IN4
Clearing the lipopolysaccharide after killing multiple-drug resistant *Escherichia coli* by chimeric peptides-A6 and G6

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Antibiotics rapidly kill pathogenic Gram-negative bacteria, but simultaneously accelerate lipopolysaccharide (LPS) release from the cell envelopes, which leading to downstream cascade of inflammatory and sepsis. It is known that some antimicrobial peptides (AMPs) have potent high antibacterial activity against Gram-negative bacteria. However, antibiotics and most AMPs cannot adequately clean LPS due to weak LPS-neutralizing capacity. Here, to clear "battlefield" - LPS after killing bacteria, the LPS-targeted "smart" chimeric peptides (SCPs)-A6 and G6 were generated by connecting LBP14 (targeting LPS) and N6 (killing pathogen) via rigid and flexible linkers, respectively, and their properties, functions and mechanisms were firstly determined *in vitro* and *in vivo*. Results showed that both linkers retained the independent original biological activities from each parent component. Both A6 and G6 exerted low toxicity and no bacterial resistance, and more rapidly killed multiple-drug resistant *Escherichia coli* and more effectively neutralized LPS toxicity than treatment from N6 alone. At a dose of 0.125 μmol/kg, SCPs-A6 and G6 enhanced the mouse survival (100%), superior to N6 (60%) and polymyxin B (40%, 5 μmol/kg), alleviated lung injuries by blocking mitogen-activated protein kinase and nuclear factor kappa - β p65 activation. It uniquely showed that SCPs-A6 and G6 may be promising dual-function candidates as novel antibacterial and anti-endotoxin agents to treat bacteria and sepsis.

Keywords: lipopolysaccharide, *Escherichia coli*, chimeric antimicrobial peptides

IN5
Effects of supplemental dietary gamma-aminobutyric acid on growth performance and stress indicators in broiler chickens raised at different stocking densities

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Stocking density has critical implications for the welfare, health, and productivity of commercial broilers. Gamma-aminobutyric acid (GABA) is a primary inhibitory neurotransmitter and widely used in the animal industry as a safe feed additive to improve growth performance and to alleviate physiological stress. However, the direct role of dietary GABA in alleviating the stress of high stocking density and interaction between two of them have never been investigated in broiler chickens. Therefore, this study was conducted to test whether dietary GABA could improve growth performance and alleviate physiological stress in broiler chickens raised at high stocking density.

A total of 900 one-day-old male broiler chicks (Ross 308) were assigned to 4 treatments with 10 replicates of 15 or 30 birds in a completely randomized design. A 2 × 2 factorial treatment arrangement was used with stocking density and dietary GABA as the main factors. Experimental diets were formulated to mix the basal diet with or without 100 mg/kg of GABA and fed to birds kept at 7.5 birds/m^2 (low stocking density; LSD) or 15 birds/m^2 (high stocking density; HSD).

Body weight gain was decreased in chickens raised at HSD compared with LSD-raised chickens during all phases (P<0.05). Feed intake was decreased in HSD-raised chickens compared with LSD-raised chickens during the starter and whole phases (P<0.01). Feed conversion ratio was decreased during the starter phase, however, it increased during the finisher phase in HSD-raised chickens. Relative liver weight was increased in chickens fed the GABA-supplemented diet compared with chickens fed the basal diet on day 21 (P=0.021) and was increased in chickens raised at HSD compared with chickens raised at LSD on day 35 (P=0.013). Blood heterophil to lymphocyte ratio was decreased in chickens fed the GABA-supplemented diet compared with chickens fed the basal diet (P=0.037). Also, the concentration of corticosterone in serum samples tended to decrease in chickens fed the GABA-supplemented diet compared with chickens fed the basal diet (P=0.088).

The present results demonstrate that increasing stocking density impaired growth performance. Dietary GABA did not affect growth performance, but it lowered the heterophil to lymphocyte ratio in blood and the concentration of corticosterone in serum regardless of stocking densities. Our finding suggests that dietary GABA is effective in mitigating stress responses, but the effect is independent to stocking density.

Keywords: gamma-aminobutyric acid, stocking density, growth performance, stress indicators, broiler chickens

IN6
High-performance plasma biomarker for Penicillin-G resistance in a model of *Staphylococcus aureus* bacteremia

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Rapid determination of antimicrobial susceptibility/resistance is the key factor in selecting an appropriate antimicrobial treatment and eradicating infections promptly. Conventional antimicrobial susceptibility tests (ASTs) are not precise enough and are very time consuming. In addition, resistant bacteria generally release an enzyme to convert the active antimicrobial agent to an inactive metabolite, and this motivated us to develop a liquid chromatography-mass spectrometry (LC-MS/MS) method in our previous study for the rapid determination of the resistance level as well as the selection of the correct antimicrobial treatment. In this study, we extended our previous exploration to determine the resistance of *Staphylococcus aureus* to penicillin-G in an animal-infection model by means of the LC-MS/MS rapid method. The method was successfully applied to the rapid determination of resistance in a *S. aureus* bacteraemia model. This newly developed method is able to determine the extent of antimicrobial resistance qualitatively and quantitatively within 1 h, and can be used to replace conventional AST methods which take 3 days to determine resistance.

Keywords: Mass spectrometry, antibacterial susceptibility test, chicken infection model, antibacterial resistance, Rapid diagnosis

IN7
In vitro Synergistic Potentials of Novel Antibacterial Combination Therapies against Pathogenic Bacteria

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Bacteria have remarkable abilities to acquire resistance against antibiotics by several mechanisms. New strategies are needed to block the development of resistance and to prolong the life of traditional antibiotics. This study aimed to increase the efficacy of existing antibiotics by combining them with the opportunistic phenolic compound gallic acid (GA) and its derivatives. Fractional inhibitory concentration (FIC) indexes of phenolic compound-antibiotic combinations against *Salmonella enterica* serovar Typhimurium, *Escherichia coli* and *Staphylococcus aureus* were determined. Based on the FIC indexes and clinical importance, 3 combinations were selected to evaluate their effects on the virulence factors of these bacteria. The *in vitro* cytotoxicity of GA and hamamelitannin in the *Rattus norvegicus* (IEC-6) cell line were evaluated. Phenolic compounds were demonstrated to yield considerable antibacterial effects as the MICs of epigallocatechin, GA and hamamelitannin found against different strains were (32–1024), (128–1024) and (512–≥2048) µg/mL, respectively. The FIC indexes of the combined antibacterials against these strains were 0.281–1.016. The ultrastructural morphology and time-kill assays showed that the GA-ceftiofur combination, and hamamelitannin-erythromycin and GA-ampicillin combinations more efficiently inhibited the growth of *S.* Typhimurium and *E. coli*, respectively, compared to the individual antibiotics. Biofilm viability and the swimming and swarming motilities of *S.* Typhimurium in the presence of GA-ceftiofur and *E. coli* in the presence of the hamamelitannin-erythromycin and GA-ampicillin combinations were more competently inhibited than individual antimicrobials. The 50% inhibitory concentrations of GA and hamamelitannin in IEC-6 cells were 564.55 µM and 988.54 µM, respectively. The phenolic compounds increase the efficacy of existing antibiotics might be by disrupting virulence factors. We can conclude that these antibacterial combinations are safe and can be potential medications to treat *S.* Typhimurium, *E. coli* and *S. aureus* infections in animals and humans. Further study to confirm this effect in *in vivo* system and to determine the precise mechanism of action should be undertaken to establish these combinations as medications.

Keywords: Antimicrobials, Antimicrobial resistance, Phenolic compound, Biofilm, Fractional inhibitory concentration indexes

IN8
Eimeria challenged study with natural coccidiosis prophylaxis on alternatives to anticoccidals

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One of the biggest challenges faced by the global poultry industry is coccidiosis. However widespread use of anticoccidials resulted in the reduction of sensitivity or even resistance worldwide. In view of this situation, more and more phytogenic molecules are introduced to anticoccidials market trying to offer alternative solutions.

The active ingredient of the product in this study is 3,4,5-Trihydroxybenzoicacid (THB) which had proven effect against *Eimeria* spp. both in vitro and in vivo. The mechanism is to reduce the activity of sporozoites as well as to inhibit invasion and proliferation in host cells.

The aim of this study is to evaluate the effect of natural base product, COZANTE™ on the performance of broiler challenged with *Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. maxima* comparing with ionophores (Salinomycin) and chemicals (Diclazuril and Narasin-Nicarbazin).

The result of the study was COZANTE™ 120 g/MT and 150 g/MT worked as well as ionophores and chemicals on decreasing lesion scores caused by *Eimeria tenella*, *E. necatrix*, *E. acervulina* and *E. maxima*. COZANTE™ 120 g/MT and 150 g/MT worked as well as Diclazuril and Narasin-Nicarbazin on decreasing OPG (oocysts per gram of feces) number and significantly better than Salinomycin. We also found lower mortality with all COZANTE™ groups than ionophores and chemicals. There was no significant difference on body weight ($p>0.05$) between all groups. There was no significant difference on FCR ($p>0.05$); but the significant difference between COZANTE™ 120 g/MT and positive control (1.7: 1.9).

Keywords: coccidiosis, *Eimeria*, Cozante, Thb, OPG

IN9
Effects of tylan removal and increasing dietary roughage concentration on liver abscess disease

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Liver abscess formation is a multifaceted disease of great importance to feedlot cattle health management with the potential to cause substantial economic losses to U.S. beef industry stakeholders. In 2011, reduction in industry profitability due to unrealized liver value was estimated to be $15.8 million. Tylosin is used to reduce liver abscess rates in US feedlots, but limited data are available to suggest whether increased roughage concentration in high energy diets is a viable strategy for managing this disease. Therefore, a study was conducted to determine the impact of typical finishing rations with and without tylosin and the titration of roughage concentration in finishing diets without tylosin on liver abscess prevalence and severity (score) at harvest.

A randomized complete block design with 4 treatments and 12 pen replications per treatment was used with 65-70 steers/pen (3,340 steers total; 796 lb. average arrival weight). Treatments included 7.1% corn stalks (DM basis) and 9.6 g/ton tylosin (7.1 TYL), 7.1% stalks without tylosin (7.1 NT), 13.1% stalks without tylosin (13.1 NT), and 19.1% stalks without tylosin (19.1 NT). Rumensin (monensin [fed entire study]) and Optaflexx (ractopamine [fed last 29 days of study]) were included in all treatments. Mean feeding period was 161 days.

Total liver abscess disease was reduced ($P = 0.006$) in the 7.1 TYL group (13.03%), compared with 7.1 NT (19.18%). The A+ (1.99%) and A+/ adhered (0.87%) were reduced ($P < 0.04$) in the 7.1 TYL group, compared with 7.1 NT (3.75% and 3.27%, respectively). When comparing 7.1 TYL to 13.1 NT and 19.1 NT, no difference in total disease or A-, A, or total A+ scores was observed ($P \geq 0.13$). The 7.1 TYL treatment had fewer ($P = 0.008$) A+/ adhered livers, compared with 13.1 NT (2.93%) and 19.1 NT (2.21%). In 7.1 NT, 13.1 NT, and 19.1 NT treatments, increased roughage content decreased ($P \leq 0.03$) total disease (19.8%, 11.88%, and 14.4%) and A+ scores (8.83%, 5.57%, and 6.01%). Increasing roughage to 13.1 NT and 19.1 NT treatments resulted in linear increased feed consumption (0.9 and 1.3 lbs/day, respectively), less total live weight gained (3 and 14 lbs, respectively), reduction in carcass weight (6 or 21 lb., respectively), and had increased feed: gain (0.31 and 0.59, respectively), compared with 7.1 TYL.

In conclusion, tylosin was effective for the reduction of total liver abscess occurrence and severity. While increased corn stalks were able to compensate for the effects of tylosin removal on liver abscess occurrence, dietary roughage was not effective for controlling the severe liver abscesses that negatively affect packing plants. In addition, cattle fed increased corn stalks were unable to compensate for the lower dietary energy intake, which reduced the total amount and efficiency of beef produced.

Keywords: liver, abscess, tylosin, roughage, cattle

IN10
Effects of tylan defined feeding duration and dietary roughage type on liver abscess disease

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The pursuit of a reduction in liver abscess prevalence in feedlot cattle is of great importance to health management and beef industry stakeholder profitability. Disease effects are multifaceted, but primarily materialize in liver condemnation, lost feedlot performance, and reduced carcass weight and quality grade. The impact is further magnified by the increased prevalence of severe disease (A-plus liver score) at harvest that is associated with carcass trim and condemnation. Tylan Premix (tylosin) is labelled for reduction of incidence of liver abscesses associated with *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*. To further understand liver abscess disease, a study was conducted to determine the impact of a defined tylosin duration feeding period and roughage type on liver abscess disease prevalence and severity (score) at harvest.

Five treatments with 8 replicates were used in a 2 x 2 + 1 incomplete factorial design utilizing 9,396 steers. Treatments included corn silage without tylosin fed (SILNOTY), corn silage with tylosin fed continuously (SILALLTY), corn silage with tylosin fed continuously until the last 30 days prior to harvest (SILBAATY), corn stalks with tylosin fed continuously (STKALLTY), and corn stalks with tylosin fed continuously until the last 30 days prior to harvest (STKBAATY). Roughage was formulated at an equal forage neutral detergent fiber (NDF) level in each diet. With the exception of 234 heads/pen in Block 6, 235 heads/pen was enrolled in the remaining blocks. Tylosin treatment (10 g/ton [100% dry matter basis]) was initiated after finishing ration adaptation. Mean feeding period was 152 days.

Tylosin and roughage source both impacted liver abscess formation. Total liver abscess prevalence and A-plus score were significantly reduced ($P<0.001$) by 47.6% and 62.4%, respectively, by feeding tylosin continuously (SILALLTY), compared with not feeding tylosin (SILNOTY). Both total liver abscess prevalence and A-plus score significantly increased ($P < 0.05$) for cattle fed stalks as a roughage source, compared with SILALLTY and SILBAATY. A trend ($P = 0.06$) was observed for increased total liver abscess prevalence in SILBAA (19.7%), compared to SILALLTY (17.2%). No significant difference in total liver abscess prevalence was detected for STKBAA (21.0%), compared with STKALLTY (21.9%).

In conclusion, these results demonstrate the importance of Tylan Premix as a therapeutic feed additive for reducing the incidence of liver abscesses. Removal of tylosin in the silage and stalks diets during the defined feeding duration period did not have a substantial impact on liver abscess prevalence or severity. Steers fed stalks had an increase in total liver abscess disease and A-plus score, compared with steers fed silage and tylosin. These results do not support the use of corn stalks as a roughage source at a similar forage NDF level as silage for the reduction of liver abscess disease prevalence or severity.

Keywords: liver, abscess, tylosin, roughage, cattle

IN11
Inhibitory effect of SCFA and MCFA on contaminats of liquid pig feed and intestinal bacteria

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Microbial contaminants present in liquid pig feed seem to be susceptible to short chain fatty acids (SCFA) and medium chain fatty acids (MCFA). Adding these acids triggers growth inhibition and this would be valuable to optimise production, storage and hygiene of liquid pig feed. Similarly, intestinal microbes found in pigs seem to be susceptible to SCFA and MCFA but may depend on bacterial strain and other conditions. The objective of this *invitro* study was to determine the growth inhibitory effect of several SCFA and SCFA+MCFA combinations on 6 bacterial and 2 yeast strains. The SCFA and/or MCFA products (1 l/t) were added to a liquid bacterial growth media after which the media was inoculated with 9% v/v overnight cultured microbial inocula. Growth media pH and temperature were adjusted for tested strains as follows: *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*: TSGY, pH 5, 37°C; *Clostridium perfringens*: TSGY, pH 6.2, 37°C, anaerobic; *Streptococcus suis*: TSB with 2% serum, pH 7, 37°C microaerophilic; *Campylobacter jejuni*: BHI, pH 6.5, 37°C, microaerophilic; *Candida humilis* and *Saccharomyces cerevisiae*: YM, pH 5, 25°C. Growth inhibition was determined measuring culture optical density at 600nm at multiple time events over a 24h period, except for *Campylobacter jejuni* where another method was used at 24h. Growth inhibition (%) per product was statistically analysed relative to the negative control using a two-tailed Student t-test. All products inhibited the growth of tested bacterial strains dependent on time after inoculation, except for the growth of *Campylobacter jejuni*. The SCFA+MCFA combination showed superior growth inhibition, whereas lactic acid had the lowest efficacy. Only the SCFA+MCFA combination was able to inhibit the growth of yeast cells effectively. *E. coli* showed a time-dependent susceptibility to SCFA and MCFA. In conclusion, SCFA and SCFA+MCFA inhibited growth of several bacterial strains. The SCFA+MCFA also inhibited yeast cell growth.

Keywords: SCFA, MCFA, bacteria growth inhibition, yeast growth inhibition, SCFA and MCFA combination

IN12
Effects of dietary fiber in weaning pig diets on growth performances, nutrient digestibility and intestinal health

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This study aimed to determine the effect of dietary fiber level for weaning pig on growth performances, nutrient digestibility and intestinal health from intestinal morphology and bacterial count parameters. Total of 30 weaning pigs at 21±3 days of age were kept in the individual cage and fed randomly with 3 experimental diets consisting of three different levels of total dietary fiber (DF) at 130, 140 and 150 g/ kg with the same ratio of insoluble fiber per soluble fiber at 4.0. All diets were formulated with nutritional balance using the source of insoluble and soluble dietary fiber from grounded rice hull and pectin. Pigs were fed *ad libitum* with experimental diets for 28 days after weaning. Growth performances were determined for the weekly and overall period. Fecal characteristic was determined daily for calculated of diarrhea incidence. At d29 after fed with experimental diet, four pigs per treatment were randomized and euthanized for the collection of tissue samples in different segments of the small intestine (duodenum, jejunum and ileum). The sample of gut digesta from ileum, caecum and colon were collected for the microbial count. The intestinal morphology was determined by a light microscope at 40X magnificent. For digestibility study, twelve weaned pigs were housed in an individual cage and fed 7 days with an experimental diet for fecal collection. The nutrient digestibility of experimental diet using indirect method and 0.5% chromic oxide was mixed in the diet as an indicator. The FCR of weaning pig at the first week was improved (P=0.023), however, there was not significantly different (P>0.05) on growth performances of weaning pig evaluated overall period (28 days). The diarrhea incidence numerically decreased in a high level of dietary fiber. The nutrient digestibility coefficient of energy and protein in weaning pig were not different among treatment diet while digestibility coefficient of crude fat and fiber tended to increase (P =0.082 and P= 0.074) when the increasing of DF level. The dietary fiber was not affected to villus height, crypt depth and villus height per crypt depth ratio (VH: CD) of duodenum and jejunum. The increasing of DF level in diet tended to increase the villus height and enhance VH: CD especially at DF level 140 g/kg diet. The bacterial count in gut content was not affected by dietary fiber level. In conclusion, a dietary level at 140 g/kg in weaning pig diet by adding grounded rice hull could improve feed efficiency and decreases diarrhea incidence of the pigs at the first week after weaning without any effect on nutrient digestibility, intestinal morphology and some bacterial count in gut content.

Keywords: weaning pig, dietary fiber, digestibility, intestinal health

IN13
The use of the dry-off facilitator velactis (cabergoline) in selective dry cow therapy

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The dry period is a crucial resting period for the dairy cow that is close to calving and in transition to a new lactation. One important objective for drying off dairy cows is to minimize the risk of intramammary infections (IMI) and ensure a healthy production in the next lactation.

The level of milk production before drying off and the incidence of milk leakage during the week after the last milking are known to be risk factors for new IMI during the early dry period. The reason why it is recommended to reduce the level of milk production at dry-off is that, the higher the milk production at the moment of dry-off, the higher the risk of new IMI. As mentioned above, for every 5 kg increase in milk production at dry-off above 12.5 kg, the odds of a cow having an IMI at calving increases by 77% (Rajala-Schultz *et al*., 2005). The reduction of milk leakage resulting from decreased milk production could also decrease the incidence of new IMIs.

Velactis (cabergoline) is a dry-off facilitator that offers a novel way to reduce milk production at the time of drying off. It facilitates the drying off procedure when used together with the abrupt cessation of milking. Velactis contains cabergoline, a prolactin release inhibitor which acts on the hypothalamus and suppresses prolactin production in the pituitary gland. Prolactin is stimulates milk production by the alveolar cells of the mammary gland, thus causing milk to accumulate in the udder. By reducing prolactin, Velactis acts to decrease milk yield in dairy cows at dry-off. As a result, there is also a reduction in the risk of milk leakage, new IMI and discomfort at dry-off and during the dry period. Studies show that one intramuscular injection of Velactis at dry-off significantly decreases milk leakage the first day after dry-off by 81% and consequently decreased new IMI across the dry period and immediately post calving by 21% (Hop et al, 2019). In a recent study in commercial dairy herds in the UK (Bradley *et al*., 2019), Velactis use was associated with a lower incidence of clinical mastitis in the following lactation. Uninfected quarters from cows receiving both Velactis and an internal teat sealant (ITS) at drying off were at a significantly lower risk of developing clinical mastitis in the first 100 days-in-milk in the subsequent lactation compared with quarters in cows receiving ITS alone or antibiotic alone. When used in combination with an ITS, Velactis offered a clear alternative to antibiotic DCT in uninfected cows. Moreover, the decrease in the number of cases of clinical mastitis will result in reduction in the use of antibiotics for treatment of cows with mastitis.

In summary, dry-off facilitators like Velactis are a useful aid for drying off modern dairy cows in the current era of minimising antibiotic dry cow therapy whilst having a positive effect on udder health and animal welfare.

Keywords: cabergoline, Velactis, mastitis, dry-off, antibiotic

IN14
A paper-based microfluidic device (DON-Chip) for rapid and low-cost deoxynivalenol quantification in food, feed and feed ingredients

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Mycotoxin contamination causes over 5 billion dollars of economic loss per year in the North American food and feed industry. A rapid, low-cost, portable and reliable method for on-site detection of deoxynivalenol (DON), a representative mycotoxin predominantly occurring in grains, would be helpful to control mycotoxin contamination. In this study, a paper-based microfluidic chip capable of measuring DON (DON-Chip) in food, feed and feed ingredients was developed. The DON-Chip incorporated a colorimetric competitive immunoassay into a paper microfluidic device and used gold nanoparticles as a signal indicator. Furthermore, a novel ratiometric analysis method was used to improve detection resolvability. Detection of DON in aqueous extracts from solid food, feed or feed ingredients was successfully validated with a detection range of 0.01-20 ppm (using dilution factors from 10-10^4). Compared with conventional methods, the DON-Chip greatly reduces the cost and time of mycotoxin detection in the food and feed industry.

Keywords: paper-based microfluidic device, deoxynivalenol quantification, competitive immunoassay, low-cost, on-site detection

SESSION 4
Phytochemicals

ORAL PRESENTATIONS

Sensing and reacting: micronutrients and phytochemicals in gut health

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The gastrointestinal tract is confronted with a cornucopia of diverse chemicals, pathogens and physicochemical states that it must analyze and react to appropriately to optimize nutrition and to defend against harm. It presents the largest and most vulnerable body surface that faces the outside world. Integrated responses to these challenges require the gut to sense its environment. This it does through a range of detection systems for specific chemical entities, pathogenic organisms and their products, and physico-chemical properties of its contents. Receptors for nutrients and micronutrients include taste receptors, free fatty acid receptors, peptide, micronutrient and phytochemical receptors, many of which are located on enteroendocrine cells. Hormones released by enteroendocrine cells act locally, on other organs such as the pancreas, and via the nervous system to optimise digestion. Pathogen detection is both through antigen presentation to T cells and through pattern recognition receptors (PRRs). Activation of PRRs triggers local tissue defence, for example, by causing release of antimicrobials from Paneth cells. Toxic chemicals, including pharmaceuticals, are sensed and then avoided, expelled or metabolized. Bacterial products are also detected. Sensory information is communicated to four major effector systems: the enteroendocrine hormonal signalling system; the innervation of the gut, both intrinsic and extrinsic; the gut immune system; and the local tissue defence system.

Phytochemicals are a special component of foods. They occur in low amounts, and add little directly to nutrition. Phytonutrients can contribute to improved nutrient conversion, reduced food spoilage, antimicrobial actions, improved palatability, enhanced gut health, including immune defense and mucosal growth promotion. They provide signals to the intestine that can have beneficial downstream effects.

They are thus valuable food additives that can promote gut health and improve animal productivity. Many of the phytochemicals act on specific receptors, and commonly these receptors belong to the TRP (transient receptor potential) class of receptors. For example, capsaicin from peppers acts on TRPV1 receptors and cinnamaldehyde acts on TRPA1 receptors. However, our knowledge of the receptors for phytochemicals is incomplete.

Major challenges include determination of the mechanisms of action of phytochemicals, quantitative evaluation of their effects, determining how they interact with the microbiota, and investigation of their benefits at specific life stages, for example in growers, in pregnancy, in early life, and under environmental threat.

Mechanisms of Baitouweng Decoction in the Treatment of Diarrhea Caused by *Escherichia coli*

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Baitouweng decoction (Pulsatilla decoction, PD), from Treatise on Febrile Diseases in the Eastern Han Dynasty, is a classical prescription in traditional Chinese medicine that has therapeutic effects on wetness-heat-induced diarrhea, Which is composed of four herbs Baitouweng (Pulsatillae Radix), Huang Lian (Coptis Rhizome), Huang Bai (Cortex Phellodendri), and Qin Pi (Cortex Fraxini). Although PD has a good therapeutic effect on diarrhea caused by Escherichia coli or its toxins, the experimental study of its antibacterial activity in vitro is not very good. So how does PD kill bacteria?

Escherichia coli (*E. coli*) is an intestinal infectious disease that is harmful to animal health and even causes death. Lipopolysaccharide (LPS) is a biologically active substance and exists in the outer membrane of pathogenic *E. coli*. In the infectious foci, LPS is released from dead *E. coli* to local microenvironments and transported to tissues and organs. LPS displays a potent ability to induce inflammatory responses. Microvascular endothelial cells (MVECs) are important sites for the exchange of substances inside and outside the blood vessels, forming a major defense barrier in the body. MVECs play an important role in maintaining normal physiological and immune functions of the body and maintaining homeostasis. The current study found that the target area for pathological changes of various diseases is MVECs. LPS induces MVECs injury, which leads to disorder of function and inflammation of the intestine.

Neutrophils, which are polymorphonuclear leukocytes, form into the blood circulation after being matured in the bone marrow. They are the innate immune cell of the body, participate in the body's immune response and enhance the defense of the body. Neutrophils are powerful effector cells for clearing bacterial infection and controlling inflammation. Neutrophils function as the first line of defense against bacterial infection. Once they recognize the signals of infection, neutrophils rapidly transmigrate to the infected tissue through vascular endothelial cells and exert its role in resisting pathogens and controlling inflammation. Many studies have shown that impaired MVECs are key factors in inducing many diseases and their complications in the body. The bactericidal function of PMNs is closely related to the state of MVECs. MVECs play an important role in fighting with bacterial infections in PMNs through multiple pathways. Our previous studies found that the bacterial toxin (LPS) released by *Escherichia coli* can damage the microvascular endothelial cells, thus weakening the function of trans endothelial neutrophils to kill bacteria. PD and its active ingredients have the function of inactivating toxins and protecting microvascular endothelial cells from bacterial toxins. Therefore, neutrophils can release granulase after crossing microvascular endothelial cells to kill bacteria.

Keywords: Pulsatilla decoction, *Escherichia coli*, microvascular endothelial cells, neutrophils, kill bacteria

An anthocyanin-rich purple potato extracts reduce high fat diet and lipopolysaccharide (LPS) induced obesity and low-grade gut inflammation

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The gut epithelium acts as a physical and chemical barrier against pathogenic invasion and toxic metabolites. However, this barrier function can be compromised by high-fat diet and gut inflammation. This study aims to determine health benefits of anthocyanin-rich purple potato (PP) in low-grade inflammation obese mouse model. A dose-dependent inhibitory effect of PP supplementation was found to prevent the elevation of pro-inflammatory mediators (e.g. TNF-α, IL-1β, IL-6 and MCP-1) and changes of plasma lipid profile (e.g. total cholestrol, HDL and LDL) caused by HFD and low-grade inflammation. In addition, PP supplementation ameliorated the inflammation-induced loss of tight junction proteins such as ZO-1 and Clds and pro-inflammatory cytokine expression, while restored the expression of colonic anti-inflammatory cytokines IL-10 and microbial recognition receptors. As such, PP supplementation contributes to maintaining the intestinal epithelial barrier function and restore normal host defense function. Lastly, the PP supplementation at high dose was shown to shape gut microbiome by promoting probiotic growth that further leads to modification of the fecal metabolic profile in mice experiencing low-grade inflammation. These findings suggest that the purple potato derived phenolics is a promising anti-inflammatory agent; and thus, increasing consumption of deep color root vegetable contributes to chronic disease prevention through promoting gut health, due to gut playing a crucial role in maintenance of overall health.

Keywords: anthocyanin, gut health, inflammation, gut barrier, high-fat

Dietary resistant potato starch alters immunological status and microbial populations in swine to limit *Salmonella*

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Recent limitations to antibiotic use in livestock species in the U.S. have heightened research efforts to identify alternatives to maintain swine health and production. Prebiotics as feed additives, such as resistant starches, have been shown to support the growth and functions of beneficial members of the intestinal microbiota to increase microbial butyrate production, which is known to play an influential role in maintaining colonic homeostasis and moderating host immune responses. To investigate the potential benefit of resistant starches in swine production, pigs were fed either a standard diet or the same diet supplemented with 5 percent raw potato starch (RPS) for 21 days post-weaning. Pigs fed RPS had increased levels of butyrate in the cecum, a short-chain fatty acid known to affect host immune status. Mucosa-associated bacterial communities were significantly different between the two groups; for example, proteobacteria, commonly associated with intestinal inflammation, was reduced in the RPS-fed pigs. Changes in host responses indicative of enhanced mucosal defenses were observed; a network analysis of host and microbial changes in the cecum revealed that regulatory T-cells correlated positively with butyrate concentration, luminal IgA concentrations, expression of IL-6 and DEF1b, and beneficial mucosa-associated anaerobic bacteria. These positive effects on intestinal health prompted a follow-up study investigating the potential protective benefits against challenge with *Salmonella enterica* serovar I 4,[5],12:i:-, a serovar of increasing prevalence. Following 4 weeks of the RPS amended diet, pigs were challenged with the *Salmonella* strain and fecal shedding was monitored for 21 days, at which point *Salmonella* colonization of various tissues was assessed. Pigs fed RPS shed less *Salmonella* in their feces, and tended to have lower quantities of *Salmonella* in their intestinal tissues and cecal contents. Furthermore, a correlation was observed between butyrate concentration in the cecal contents and cumulative *Salmonella* fecal shedding over the 3 week period. Collectively, these data suggest a beneficial effect of RPS on the intestinal microbiota, host immune response, and colonic homeostasis that reduced colonization and shedding of an important human foodborne pathogen.

Keywords: *Salmonella*, resistant starch, immunomodulatory, intestine, dietary

Phytonutrients: The Next Generation

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Is food (and feed) medicine, as Hippocrates said? Perhaps not, but humans have recently (re) discovered that food not only provides nutrients for maintenance, it also contributes to their health. The animal production community has come to the same realization. Feed – critical to production performance – also affects animal health. In parallel, phytonutrients have developed as a relatively new category of feed ingredients capable of improving farm animal performance. This review will discuss some of the challenges of using phytonutrients in animal feed as growth promoting agents, in light of the recent progress of biological sciences.

The expectation of simple, silver-bullet solutions – widespread at all levels of the animal production industry – limits progress. Improving farm animal health and production is a complex problem that calls for complex solutions. When the mechanism triggered by an ingredient is understood, it opens the possibility of developing multiple-ingredient solutions that can robustly improve production when applied in the field. Today, the consensus is that phytonutrients are valuable feed ingredients *because* they kill pathogens in the gut of the animal. This is based on early observations that phytonutrients kill most pathogens *in vitro*, later substantiated by improved animal performance when used *in vivo*. This promoted the use of only the phytonutrients able to show antimicrobial effects *in vitro*, neglecting all others. In the last 10 years, research questioned this consensus and proposed a new one: a host-mediated response. This was enabled by the progressive appreciation that in a farm animal, production is a trade-off with other physiological processes. For example, farm animals are prone to high levels of inflammatory response which consumes nutrients that would otherwise be allocated to growth. This realization opened a new avenue for thinking in which, instead of killing pathogens, an ingredient could mitigate the response of the animal to its environment. Additionally, the appreciation of the gut as an intelligent sensory organ, and not only a tube for digestion, also paved the way for an alternative phytonutrient paradigm. Specific sensory receptors expressed by enteroendocrine cells detect low concentrations of dietary phytonutrients; once the signal is received and the information analyzed, the gut responds locally or conveys a signal to a distant organ. The response – for example improvement of digestion or lowered inflammatory response – materializes in improved performance or health status. Acknowledgement of such alternative mechanisms of phytonutrients will allow for the consideration of other phytonutrients aside from those that kill pathogens *in vitro*.

Regulatory positioning of key ingredients is undoubtedly a challenge of the next decade. Phytonutrients (as well as other ingredients such as probiotics) improve performance of farm animals because they help mitigate the response to the environment, especially via their immune effect. How can one develop such tools without being able to properly position them in the market because of regulatory limitations? The progress of science also needs to be integrated at the regulatory level to enable continued sparking of innovation.

Keywords: gut physiology, gut sensing, health, phytonutrients

Science-based use of plant extracts to improve animal health in post-antibiotic era: where are we?

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In recent years there are frequent pathogenic challenges all over the world that has caused immense economic loss in the animal production industry. Antimicrobial resistance can be attributed to some extent for these frequent challenges. Increasing concerns and reports about antimicrobial resistance in recent years have led to explore alternatives to antibiotics as growth promoter in animal production. Among different alternatives plant extracts have shown promising results in terms of zootechnical parameters, immune modulation and bringing down the negative effects of different pathogenic challenges. Recent scientific findings reveal that plant extracts in small concentrations can exerts their effect directly in the animals, leading to a host mediated response. This effect is beyond antimicrobial effect of plant extracts that was originally thought. With different molecular biology and nutrigenomics tools, now we have better in depth understanding of the mode of actions of different plant extracts at the level of genes, receptors and cell signaling pathways. This will help in developing new solutions to improve animal health and performance in near post antibiotic era.

In-vitro antibacterial activity of phytobiotic against *Eschericia coli* and *Mycoplasma gallisepticum*

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Avian Pathogenic *Eschericia coli* (APEC) and *Mycoplasma gallisepticum* are bacteria that commonly cause respiratory and digestive tract infections in poultry. Antibiotics such as enrofloxacin, tylosin, colistin have been quite extensively used in Indonesia to treat such diseases. However, since they are critically important to human medication, alternatives are needed. This study aimed to determine in-vitro antibacterial activity of 10 Indonesian plants known to have antimicrobial activities that were extracted with ethanol against *Eschericia coli* and *Mycoplasma gallisepticum*.

Sappan (*Caesalpinia sappan*) wood, guava (*Psidium guajava*) leaves, red ginger (*Zingiber officinale var. Rubrum*) rhizome, elephant ginger (*Zingiber officinale var. Rosc*) rhizome, nutmeg (*Myristica fragrans*) fruit, roselle (*Hibiscus sabdariffa*) calyx, and Indonesian bay (*Syzigium polyanthum*) leaf extracts were found to be effective against *E. coli* strain O78.K80.H12 (ATCC 43896). Sappan wood and guava leaves extracts showed the lowest minimum inhibitory concentration (MIC) of 256 µg/mL. Sappan wood and guava leaves extracts also showed additive effect against *E. coli*.

Only sappan wood and red ginger rhizome extracts showed to be effective against *Mycoplasma gallisepticum* strain S6 (ATCC 15392), with the lowest MIC of 400 and 800 µg/mL, respectively. Combination of sappan wood and red ginger rhizome inhibited the growth of *Mycoplasma gallisepticum* at concentrations of 200 : 400 µg/mL and 400 : 800 µg/mL.

This study showed that ethanol extracts of sappan wood, guava leaves, and red ginger rhizome were potential alternatives to antibiotics against *Eschericia coli* and *Mycoplasma gallisepticum*.

Keywords: *Mycoplasma gallisepticum, Eschericia coli, Caesalpinia sappan, Psidium guajava, Zingiber officinale*

In vitro and *in vivo* evaluation of therapeutic effects of neutrapath™ against *Salmonella* Typhimurium

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Antibiotic resistance in foodborne pathogens such as *Salmonella* is a major concern for public health safety. The poultry industry is compelled to seek alternative solutions to antibiotics in reducing the incidence of *Salmonella* colonization in broiler chickens at the farm level. NeutraPath is a formulated feed additive that features a proprietary blend of essential oils, medium-chain fatty acids and an activated toxin-adsorbing mineral. This formula has been shown to neutralize a variety of key virulence factors of pathogenic bacteria in addition to exerting direct bacteriostatic/bacteriocidal effects. This study was aimed to evaluate *in vitro* and *in vivo* effects of NeutraPath on *Salmonella enterica* sv. Typhimurium (ST) infection in broiler chickens.

An *in vitro* digestion model was used to simulate three gastrointestinal compartments with physiologically relevant pH and enzymatic conditions correspondent to that of crop, proventriculus and intestinal section respectively. In the *in vivo* trial, one-day old male broiler chicks were randomly allocated to one of three groups (n=30 chickens), i.e., Challenged control with non-treated feed and NeutraPath supplemented at 0.25% and 0.5% in feed. Chickens were orally gavaged with 10^6 CFU of live ST per chicken at 9-d old. Twenty-four hours post challenge, ceca-cecal tonsils were removed to evaluate *Salmonella* recovery and serum was collected for FITC-d determination. Differences between treatments were analyzed using one-way ANOVA.

In the *in vitro* trial, 0.25% NeutraPath significantly reduced total CFU of ST recovered in the proventriculus and intestinal compartments compared with control ($P<0.05$). NeutraPath treatment at 0.25% and 0.5% resulted in a 41.7% and 33.3% reduction in the prevalence of ST in ceca respectively compared to the challenged control ($P<0.05$ for both comparisons). Both dose levels also significantly reduced total ST CFU recovered in the ceca by 1.84 and 1.79 Log_{10} CFU/g compared to the challenged control ($P<0.05$ for both comparisons). Further, NeutraPath at both doses significantly reduced serum FITC-dextran levels ($P<0.05$).

Based on these *in vitro* and *in vivo* data, the NeutraPath treatment had the therapeutic potential to reduce ST colonization in broiler chickens and preserve the functional integrity of the intestinal barrier of chickens during ST challenge.

SESSION 4
Phytochemicals

POSTER PRESENTATIONS

PH1
Supplementation with encapsulated phytonutrients improves carcass characteristics in broilers

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Essential oils are aromatic volatile components of medicinal plants. Diverse bioactivity of these compounds makes them ideal candidates for use in animal health and nutrition. Indeed, essential oils are widely used as growth promoters to improve performance in poultry. In this study, a multiple-trial analysis was conducted to evaluate the efficacy and consistency of an essential oil blend (EOB; Phytomax, AVT Natural, India) in improving the carcass characteristics and performance of broilers.

Five independent research trials were conducted in a private poultry research farm in India. In each trial, performance and carcass characteristic of birds supplemented with EOB (250g/ton of feed) was compared with that of unsupplemented control (CT) group. A positive control (PC; Bacitracin, 500g/ton of feed) was included as a reference and compared to CT. Treatments were imposed during the entire growth phase from d 1-42 of life, with 6 to 10 replicates per treatment (20-25 birds/replicate; COBB430 and ROSS308 lines were used). Performance and mortality data were measured throughout the treatment period and summarized on d42. Carcass characteristics were evaluated in all experiments and blood samples were collected on d42 in experiment 5. Data were subjected to two-way ANOVA to test for the fixed effects of treatment, experiment and their interaction using GraphPad Prism 6 software. Dunnett's test was used to compare each of the treatment groups to the control group. Final body weight was increased with EOB (2,475 g; $P<0.09$) and PC (2,484 g; $P<0.02$) compared to CT (2,445 g), but there was no effect of treatment on FI (4,104, 4,112, & 4,125 g for CT, EOB, and PC respectively; $P>0.7$). Consequently, FCR was decreased with both EOB (1.67) and PC (1.67) compared to CT (1.70; $P<0.001$); however, a treatment by experiment interaction ($P<0.001$) revealed that improved efficiency was observed in only 3 of the 5 experiments. Mortality was decreased with both EOB (1.3%; $P\leq0.01$) and PC (1.6%; $P<0.05$) compared to CT (2.8%). Liver weight was increased with EOB (24.3 g/kg BW; $P<0.05$), but there was no difference between PC and CT (23.9 vs. 23.3 g/kg BW; $P>0.3$). Dressing weight and breast weight increased with EOB (783 & 281 g/kg BW; $P<0.005$) and PC (782 & 278 g/kg BW; $P<0.01$) compared to CT (767 & 269 g/kg BW). Serum cholesterol and abdominal fat decreased with EOB (136.8 mg/dL; $P<0.005$ & 13.3 g/kg BW; $P<0.1$) compared to CT (192.4 mg/dL & 14.3 g/kg BW), whereas PC had no effect on serum cholesterol (191.8 mg/dL; $P>0.5$) but decreased abdominal fat (12.9%; $P<0.02$) compared to CT. In conclusion, EOB positively influenced lipid metabolism and carcass quality with an improvement in zoo-technical parameters. Therefore, EOB can be used not only as a growth promoter but as a tool to improve meat quality in poultry.

Keywords: Phytogenics, Broilers, Caracass Quality, Meta-analysis, growth promote

PH2
IDENA, A long experience with new generation additives

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Since its creation in 1995, when the first cases of mad cows appeared in Europe, IDENA has focused all its R&D efforts on active ingredients from the botanical world to create a range of additives that substitute chemical molecules, growth factors, antibiotics and anticoccidians in animal feed.

A painstaking work in the sourcing of assets and scientific validation directly in farms to lead to targeted associations according to the different health and sanitary issues encountered in the livestock operations. This work also included the definition of the modes of action of these new molecules against coccidiosis parasites and pathogenic bacteria's but also, their activity on the beneficial bacteria in the digestive tract. For example, IDENA has developed applications to minimize the negative effects of coccidiosis in poultry, pigs and small ruminants.

This concept, which was first developed in the 2000s, provides the same or even better answers to conventional products. It is designed as a powder to be incorporated into feed and/or as a liquid to be introduced into drinking water or directly into the mouths of animals (piglets, calves and lambs).
In pig production, neonatal and weaning diarrhea is also a major concern at IDENA and receives highly effective solutions to accompany antibiotic reductions on farms.

Thus, applications have been studied and approved to manage and reduce the frequency of neonatal diarrhea in piglets. Other solutions are more focused on diarrhea around weaning.

All the alternative concepts designed by IDENA are combinations of several active ingredients working in synergy for a direct global effect on the pathogen, flora control and immunity. They benefit from a specific technology, ECHV, developed by IDENA, to protect and stabilize the assets.

IDENA is convinced that the replacement of antibiotics cannot be achieved by substituting one product for another. There are situations, sometimes very complicated, where even antibiotics no longer work. Improving livestock conditions and the sanitary environment is a priority to optimize the effectiveness of the programs implemented. Each farm is a special case that requires an adapted program.

Keywords: Alternative, botanic, coccidiosis, neonatal diarrhea, antibiotic reduction

PH3
Can a beneficial role of chitosan oligosaccharide supplementation make an alternative to antibiotic substitution in weaned pig?

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There is continued need for novel agents to improve intestinal function in weaned pigs. Some evidence suggests that chitosan oligosaccharide (COS) supplements, as a functional prebiotic, may enhance pigs' intestinal function for health benefit after weaning. Post-weaning stressors: nutritional, social and environmental stresses can affect feed intake, growth performance, and predisposition to diseases. Antibiotics are added to pig starter diets as growth promoters and to prevent infections. However, antibiotic addition to animal feed has been prohibited or limited in many countries due to antibiotic-resistance which threatens human health. The search for safe and environmentally friendly alternatives to antibiotic to promote health has become necessary in swine production. The objective of the present study was to test COS supplementation at 150 mg/kg with the molecular weight about 8 kDa and deacetylation degree (DD) about 90% as an effective alternative to antibiotic addition in weaned pigs' health and production. For the experiment, weanling pigs were divided into 3 groups (9 animals per group) and received either a basal diet, a supplemented diet with 150 mg/kg COS, or a supplemented diet with 110 mg/kg lincomycin for 56 days. Growth, feed conversion ratio, nutrient's ileal digestibility, small intestinal morphology and crypt cell proliferation were measured at 56 days of the experiment. The statistical significance (P<0.05) of the differences among the groups was determined by ANOVA in conjunction with Tukey's test. Compared with the control group, pigs supplemented diet with COS or with lincomycin significantly (P<0.05) showed: (i) consistently more digestible ileal contents (e.g. crude protein, crude fat, and calcium), (ii) increased absorption capacity (e.g. increased villus height/crypt depth ratio at the jejunum) and (iii) more active cell division (as indicated by Ki-67 marker of duodenal and jejunal crypt cells). In conclusion and implication, weaned pigs fed dietary supplementation at 150 mg/kg COS with 8 kDa and about 90% DD showed improvements in major nutrient digestibility and small intestinal morphology through cell proliferation similar to those of in-feed lincomycin. Therefore, supplementation of this COS characteristic may be an effective substitute for in-feed antibiotics during the post-weaning period.

Keywords: antibiotic, Chitosan oligosaccharide, dietary supplement, small intestine, weaned pig

PH4
A multi-hurdle approach using phytochemicals as natural alternatives to antibiotics for controlling *Campylobacter* in poultry

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Campylobacter is one of the leading causes of foodborne illness, resulting in an estimated 96 million cases of gastroenteritis and 21,000 deaths per year, globally. *Campylobacter* is prevalent in many food animals; however, the presence of *Campylobacter* on poultry products remains one of the leading causes of bacterial foodborne illness worldwide. *Campylobacter jejuni* naturally colonizes the ceca of chickens in high numbers, thereby leading to contamination of the carcass during slaughter. Therefore, a multi hurdle approach combining strategies to reduce *C. jejuni* cecal colonization in poultry gut, reducing survival in poultry products and the environmental persistence of this pathogen during poultry processing would potentially reduce the risk of human infections.

One of the missions of our laboratory has been to provide the poultry industry with efficient antibiotic alternatives for the control of *Campylobacter* in conventional and the organic poultry sectors. The use of phytochemicals as antimicrobial feed additives, food bio-preservatives and natural disinfectants is one such technology that is safe, effective and environmentally friendly. We have tested numerous phytochemicals for their anti-*Campylobacter* efficacy targeting three main areas (poultry production, post-harvest contamination, and limiting survival of pathogens in the processing environment) in a multi-hurdle approach from farm to fork. Our results indicate that plant-based, generally recognized as safe status (GRAS), compounds such as *trans*-cinnamaldehyde (obtained from cinnamon bark), eugenol (from clove oil), and carvacrol (from oil of thyme) are very effective in reducing *C. jejuni* in the poultry gut, on carcasses as well as inhibiting *C. jejuni* biofilms on common food processing surfaces. Using our work with eugenol as an example, in-water supplementation of 0.125% eugenol nanoemulsion consistently reduced *C. jejuni* colonization by at least 1.5 log CFU/g of cecal contents in 14-day old broiler chickens ($P<0.05$; 10 birds/treatment/trial). Eugenol was also effective in reducing the survival of *C. jejuni* on chicken skin and wings when applied as an antimicrobial wash or coating treatments. Washing the chicken skin with 0.5-2% eugenol for 1 min and antimicrobial coating of chicken wingettes reduced *C. jejuni* by 1-2 log CFU/sample ($P<0.05$). In addition, 0.25-1% eugenol was highly effective in inhibiting biofilm formation as well as inactivating a mature *C. jejuni* biofilm on common poultry processing surfaces. Follow up mechanistic studies (using real-time quantitative PCR and proteomics analysis) revealed that eugenol modulates key *C. jejuni* genes and proteins essential for intestinal colonization, persistence in the environment, and survival of the pathogen in meat products. (Funded by USDA-NIFA-OREI-2017-51300-26815).

Keywords: Campylobacter, Phytochemicals, Pre-harvest poultry, Post-harvest, Biofilm

PH5
Functional fermented proteins to replace medicinal zinc and reduce antibiotic treatments in pig production

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EP100i is a Lacto-fermented rapeseed protein designed to replace medicinal doses of zinc oxide and reduce antibiotic treatments in weaned pig production. In addition to a high digestibility of protein and fibre, the product modulates the bacterial composition of the gut flora and promotes beneficial gut bacteria. With this, improvements in the gut barrier and associated immune functions occur. As a result, high doses of zinc oxide, followed by antibiotic treatments, become unnecessary. This was tested in a large trial with weaned pigs fed individually with 1) a commercial control diet (negative control), 2) a control diet with 2500 ppm zinc oxide (positive control) and 3) with 10% EP100i included as protein in the feed formulation. Pigs were not given prescription antibiotics before and during the experiment unless fallen ill. Ill animals were treated separately and excluded from the experiment. The trial was carried out for 53 days starting at weaning. Each diet group had 6 replicas per diet group, with an average of 46 pigs per pen. Average daily gain, feed intake and animals excluded from the experiment, were recorded. Hindgut tissue and content, along with blood, was sampled from 10 slaughtered pigs from each diet group after 4 weeks (28 days) of diet supplementation. Microbiome, inflammation biomarkers and gut histopathology were analyzed to test the hypothesis. Overall, pigs from the control diet performed poorer in comparison with the groups with zinc oxide and EP100i. The EP100i and zinc oxide group both displayed similar gut modulation, growth performance and a higher number of animals completing the study. However, at the end of the experimental period, pigs from group EP100i were 1.6 kg heavier than those of the zinc oxide group. The hindgut of pigs from the control group was dominated by one bacterial group (*Prevotella* spp.). Pigs from the zinc oxide and EP100i group showed a significant increase in L*actobacillus* spp., Ruminococcaceae spp., and the Clostridiales group IV ($p<0.05$). Histomorphometry jejunum and colon was higher in the zinc oxide and EP100i groups than in the control group. However, histopathology and inflammation biomarkers were statistically significantly lower in pigs supplemented with EP100i than in the other groups ($p<0.05$). This suggested that feeding EP100i to piglets will result in a well-developed gut barrier function with a vast array of beneficial gut bacteria. Hence, pigs focus the energy into growing and becoming resilient to infections. We conclude that EP100i is an excellent alternative to the use of high doses of zinc oxide and antibiotic treatments in pig production

Keywords: Fermented, proteins, microbiome, inflammation, gut-development

PH6
Phytogenic feed additives as alternative to antibiotics in food animal production

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Phytogenic feed additives as alternative to antibiotics in food animal production

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Driven by regulatory actions and/or tied to customer demands for antibiotic-free meat, we have witnessed the ban on antibiotic growth promoters in key feed markets and observed a decline of the usage of antibiotics in animal feed. The ban on prolonged growth promoting and shotgun style prophylactic treatments is a major achievement in the battle against antibiotic resistance, a global threat to humanity.

Based on the available evidence, it is clear that phytogenic compounds represent a potential alternative to in-feed antibiotics. However, it is also known that there is no silver bullet and it is very challenging for phytogenic feed additives to replicate the action of antibiotics. A combination of additives and an integrative prevention program have proven to be a valid strategy to avoid bacterial resistance improving safety, efficacy and animal welfare.

Among the plethora of phytogenics, hops (*Humulus lupulus L.)* have caught our attention for their interesting properties. For several thousand years, hops have been utilized in folk medicine for their health-promoting effects. Hops are also widely applied in beer manufacturing due to their antimicrobial and preservative properties together with their bitterness. The phytochemicals in selected hop components have a chemical structure similar to ionophores. That is why they possess inhibitory activity against Gram+ bacteria and can be potentially used as a natural alternative to in-feed antibiotics.
Based on this knowledge, a new phytogenic feed additive, Anta®Phyt, containing selected hops phytochemicals and other plant ingredients was developed. Herewith, we report the results of the *in vitro* and *in vivo* characterization of its efficacy in broilers.

In vitro: Chicken ileal digesta were mixed with different concentrations of Anta®Phyt and monensin, as positive control. After incubation, lactic acid and volatile fatty acids were quantified. The application of Anta®Phyt significantly increased the production of acetic and propionic acid, while decreasing the production of lactic acid.

In vivo: Broilers were allocated to two groups. The treatment group received 400 g/t Anta®Phyt. Performance parameters were recorded. Dry matter content of faeces was determined. Faecal samples were analysed for specific rDNA sequences to monitor the intestinal microflora. The application of Anta®Phyt improved feed conversion by 2.1% and live weight gain by 3.6%, as compared to control. Moreover, it also decreased moisture content in the litter significantly by 7.21% and slightly reduced the microbial load of all analysed bacterial groups.

A shift from lactic acid production to propionic and acetic acid fermentation was observed. These short chain fatty acids contribute to the energy supply of the animal and stimulate gut epithelial proliferation. The comparable effects of monensin and Anta®Phyt, on the fermentation pattern observed *in vitro* and the slight reduction of microbial flora in the faeces *in vivo*, could be an indication for the antimicrobial activity of Anta®Phyt. Modulation of the intestinal flora may also explain the favourable effect on the litter quality. By preventing dysbiosis this plant based product is a potential natural alternative to antibiotics to improve broiler production.

Keywords: Phytogenic, feed additive, antibiotic alternative

PH7
Alternative to antibiotics effects of Quebracho tannin as an animal feed supplementation

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In pork production the early weaning is very efficiency way to increase sows reproductivity. And it is followed by the successful rearing of early weaned piglets. However, weaned piglets must rapidly adapt to dramatic changes in the social and physical environments, separation from maternal littermates, mixing with unfamiliar piglets, abrupt changes in diet from suckling the dam to ingesting solid feed from a feeder, and establishing a social hierarchy. Consequently, early weaning is very stressful for piglets. Diarrhea is the nemesis of the early weaned piglet. Enteropathogens infect the small intestine, which results in secretory or malabsorptive diarrhea. High death losses from diarrhea have dampened the enthusiasm for early weaning of artificially reared piglets. Usually, the nursing piglet is protected from enteropathogens by antibodies bathing the gut from the dam's colostrum and milk. Artificially reared early weaned piglets are protected from enteropathogens by feeding them diets containing additives, such as antibiotics. In addition, adding an antibiotic to the feed results in significant growth performance and improves food conversion rate.

The present study to assess the possibility of replacing antibiotics with one of phytochemicals QT, the treatment groups fed with dietaries addition of QT instead of antibiotics. This study used 21-day-old weaned piglets (Duroc × Landrace × Yorkshire; 6.51±0.17kg; 21±1d) divided to 3 groups. To assess the possibility is through analyzing the bodyweight changes, daily gain, hematology and biochemical index, blood amino acids level, diarrhea incidence rate, organs weight, and intestinal morphometric changes. The result showed big possibility of QT somehow could be replacing antibiotics.

Keywords: Tannin, Weaned piglet, Diarrhea, Blood hematology, blood biochemical index

PH8
Effect of oregano essential oil on SOD and GSH-Px activities and mRNA expression in the kidney and liver tissues of broilers

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In-feed antimicrobials have led to an antibiotic resistance fast escalating and dissemination of antibiotic resistance throughout the food chain. This experiment was conducted to evaluate the impact of oregano essential oil (OEO, contains 10% of essential oil from *Origanum vulgare ssp Hirtum* and 90% of inert carriers, named as Phytogen from Meritech) on activities and expression of mRNA and GSH-Px in kidney and liver tissues in the broilers. A total of 1000 one-day-old birds were randomly allotted to 5 diet treatments, with each treatment contained 4 replicates with 50 broilers per replicate: C (control with basal ration); A (AGP, C + Chlortetracycline 20 g/t +Virginiamycin 10 g/t feed); O1,O2 and O3 (Control plus 100, 150 and 200 g OEO/t diet, respectively). The results indicated that the supplementation of OEO (100,150 and 200g/t) into diets significantly improved SOD activity and up-regulated SOD mRNA expression level in the kidneys; OEO (150 g/t) of O2 group significantly increased renal GSH-P_X activity and OEO (100 and 150g/t) in O1 and O2 groups up-regulated GSH-P_X mRNA expression level in the kidneys of birds when comparing with C and A groups during the entire experiment. In addition, the hepatic levels of SOD of broilers in O1 and O2 groups were significantly increased comparing with the control and A groups. In conclusion, our results indicated that OEO contributed a better positive effect on anti-oxidative capacity in the kidney and the liver of broilers and this provided the useful insight to the development of potential alternative to antibiotic growth promoters for broiler feed.

Keywords: Broilers, Oregano essential oil, Glutathione peroxidase, Superoxide dismutase, mRNA expression

PH9
Oregano essential oil improved growth performance, meat quality and intestinal health of broilers

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The aim of this study was to evaluate the effects of oregano essential oil (OEO, contains 10% of essential oil from *Origanum vulgare ssp Hirtum* and 90% of inert carriers, named as Phytogen from Meritech) on growth performance, meat quality, intestinal microflora and morphology in broilers. A total of 400 Ross-308 one-day-old chicks were randomly divided into 4 diet treatments and the experimental period was 35 days. The treatment diets were: C (control with basal ration); A (C + Chlortetracycline 20 g/t +Virginiamycin10 g/t diet); O1 (C+ OEO 100g/t diet); O2 (C+ OEO 150g/t diet). The results showed that the supplementation of OEO or antibiotic in the diets significantly increased the average daily gain compared to the control group. Comparing to the control and antibiotic groups, OEO (150 g/t feed) in O2 group significantly improved feed conversion ratio of broilers at 35 days of age, and also improved meat quality through reducing shear force at 24 h after slaughter. The supplementation of OEO (150 g/t feed) significantly increased jejunum villus height, villus height-crypt depth ratio and muscle thickness compared with the antibiotic group. The rectal contents of *Lactobacillus* and *Bifidobacterium* counts of broilers fed diets containing OEO were significantly higher, and *Escherichia coli counts* was lower than those fed with antibiotic or control diet. In addition, OEO in O2 group significantly up-regulated the anti-apoptotic protein (along with decreased expression) of Bcl-2 in the jejunum compared with the antibiotic group. The results suggested that the dietary OEO supplementation improved broiler performance and meat quality through modulating intestinal bacteria and morphology. Therefore OEO could be used as an alternative for antibiotic growth promoter in the broiler diets.

Keywords: Broilers, Oregano essential oil, Growth performance, meat quality, intestinal health

PH10
Dietary oregano essential oil improved the growth performance and intestinal health in the weaned piglet

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Antibiotics play the important roles in the animal growth promotion and disease prevention but antibiotic growth promoters (AGPs) will be banned in China due to the public concern in 2020. The aim of the present study was to investigate the effects of supplementation of oregano essential oil (OEO, contains 10% of essential oil from *Origanum vulgare ssp Hirtum* and 90% of inert carriers, named as Phytogen from Meritech) on growth performance, intestinal morphology, rectal microflora, and serum immune function of piglets fed diets with or without AGPs. Sixty weaned piglets (initial body weight (BW) 7.12 ± 0.26 kg) were randomly allotted to 3 treatments, with each treatment contained 4 replicates with 5 piglets per replicate: C (control with basal ration); A (C with Chlortetracycline 50 g/t + Virginiamycin 20 g/t feed); O (C with OEO 250g/t feed). The results showed that supplementation of both OEO and antibiotics in the diets significantly increased the average daily gain of piglets comparing with the control group. Increased gain: feed was also detected in the OEO-fed piglets. Piglets fed OEO demonstrated the significantly higher jejunum villus height, villus height-crypt depth ratio, and muscle thickness compared with antibiotic and control groups. The rectal content *Lactobacillus* and *Bifidobacterium* counts of piglets fed diets containing OEO were significantly higher than those fed antibiotic or control diet. OEO supplementation predominantly decreased the the *Escherichia coli* counts of rectal and fecal content. Serum IgA and IL-10 concentration were enhanced in piglets fed the diet with supplementation OEO compared with the control and antibiotic groups. Furthermore, in-feed antibiotics increased expression of genes involved the immune functions in the jejunum. We could conclude that oregano essential oil can be used as a feed supplement to improve the gut health and immunity of piglets and may be a potential alternative to antibiotics in the weaned piglets.

Keywords: Weaned piglets, Oregano essential oil, Growth performance, Intestinal microflora, Immune level

PH11
Oxidized Derivatives of β-Carotene Support Immune Function and Help Optimize Growth Performance in Food Producing Animals

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β-carotene confers health benefits that occur independent of its provitamin-A activity. A nutritive antioxidant activity has been the proposed mechanism underlying these effects. However, the significance of the antioxidant role has not been clearly established, and efficacy trials with β-carotene have produced inconsistent results.

Our research shows that β-carotene very readily undergoes spontaneous oxidation in air and is transformed into a complex mixture consisting predominantly of copolymers with oxygen. The copolymer mixture, which we term OxBC, is active in supporting innate immune function. Immunological actions include priming of innate immune defenses to recognize and respond to early stage infections as well as a reduction in overzealous inflammatory activity.

In feeding trials with broiler poultry and pigs, dietary supplementation with low levels of OxBC (synthetically produced) led to reductions in *Clostridium perfringens* in broiler poultry and *E. coli* in piglets. Furthermore, supplementation with OxBC leads to lower incidence of disease and improved growth performance in piglets and chickens. Additional findings of increased immunoglobulin levels in colostrum and milk of sows receiving OxBC highlights the potential for further improvements in piglet health by enhancing passive immune transfer. The positive outcome of these trials has led to the successful commercial application of OxBC as an alternative to antibiotics for growth promotion and disease prevention in the Philippines and elsewhere in South East Asia.

The phytogenic origins of OxBC are confirmed by work showing the presence of β-carotene derived copolymers in a variety of plant-based feed stuffs. Other carotenoids, such as lycopene, lutein and canthaxanthin, also undergo spontaneous oxidation to form similarly active copolymer mixtures. The demonstrated activities of OxBC combined with the apparent ubiquitous presence of carotenoid derived copolymers in nature leads us to propose that these compounds are the actual source of carotenoid activities (apart from the well know provitamin A role of certain carotenoids). The copolymers represent a newly discovered class of phytogenic compounds with potential application as antibiotic alternatives in the feed industry. OxBC can be readily produced in highly pure form by synthetic manufacturing procedures and is easily handled, characteristics that further facilitate its commercial application.

Keywords: Oxidation, Carotenoids, Immune-Function, Phytogenic

PH12
Effect of a characterized citrus extract on poultry performances

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Nowadays, plants extract are more and more used in animal feed, as alternatives to antibiotic growth promoters. Some of them such as citrus extract had already shown beneficial effect on zootechnical performances and health. However, all citrus extracts can vary a lot in terms of concentration of active compounds. These variations may provoke efficacy differences. The study realised aimed to characterise a standardized natural citrus extract in order to identify the main components responsible for the effect. Then, the effect of this Standardized Natural Citrus Extract (SNCE) was evaluated through a meta-analysis in poultry.

After freeze drying the citrus extract at 50 mg/mL (Nor-Spice® AB, Nor-Feed SAS, France), three HPLC analysis methods were performed using HPLC-UV-DAD-DEDL (Shimadzu) and (HPLC UV-MSMS, Esquire 3000 Plus, Ion trap, Bruker) chromatographic systems. Analysis were performed using Hypersil RP-C18 column (4.6 mm, 250 mm, 5 µm) and data were analysed using DataAnalysis software. The main components of the product were identified using dereplication. In parallel, a meta-analysis was performed based on the results of seventeen trials realized between 1995 and 2018 with this Standardized Natural Citrus Extract. Each trial had 2 poultry groups, a control group (CTL group) fed with standard diet without supplementation and a SNCE group (SNCE group) supplemented with SNCE at dose between 250 and 400 ppm. Feed Conversion Ratio (FCR) and Average Daily Gain (ADG) were monitored in every trials. Trials were conducted worldwide. Statistical analysis were performed using Student test (T-test) and GraphPad Prism 7 (GraphPad software, USA).

Spectral data from HPLC analysis allowed to identify approximately 30 secondary metabolites among carboxylic acids, phenolic acids, coumarin, flavones, flavanones and flavanols.

Concerning the17 trials, 16 have shown a significant effect on FCR or ADG of broilers chickens ($p<0.05$, T-test). On average, SNCE supplementation on broilers significantly reduces the FCR by 2.3% and increases the ADG by 4.4% ($p<0.05$ Fisher Test)

According to these data, Standardized Natural Citrus Extract supplementation seems to be an effective solution in order to improve zootechnical performances of broiler chickens and to offer a good alternative to antibacterial growth promoters.

Keywords: citrus extract, standardization, microbiota, poultry, plant extract

PH13
In-feed resin acids improve small-intestinal mucosal characteristics of broiler chickens during dysbiosis challenge

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In-feed coniferous resin acids had previously shown to reduce both duodenal inflammatory T cell infiltration and small intestinal matrix metalloproteinase (MMP) activity towards collagen type I and type IV in the ileum of non-challenged broiler chickens, indicating a protective effect of resin acids on intestinal barrier integrity by preservation of the basal membrane and the extracellular matrix. Here we fed coniferous resin acid composition (40% natural resin acids in wheat flour) to study whether resin acids affect mucosal histology and collagen-degrading activity in a diet-induced challenge model, in which increased MMP expression and collagen breakdown were previously shown. Male Ross 308 hatchlings were randomly divided into three dietary treatments: 1) non-challenged control (NCC), 2) challenged control (CC), and 3) CC+ RAC at 250 g/ton (RAC); 10 replicate pens/treatment and 28 chicks/pen. The CC diet was produced by the inclusion of 7.5% rye (replacing an equal amount of wheat) for all dietary phases (starter d1-13, grower d14-26, finisher d27-39). One bird/pen was sampled for duodenal, jejunal and ileal tissue on d26. Duodenal samples were processed by routine histology into haematoxylin-stained slides and measured by light microscopy for the length of villi and the depth of crypts. Homogenized tissue samples from all intestinal areas were measured for the relative activities of collagen type I and type IV degradation by EnzChek™ kit (Thermo-Fisher Diagnostics, USA).

Birds performance, as measured by daily weight gain and feed conversion, was significantly lower for CC than NCC ($p<0.05$). Feed conversion was better for RAC than CC for the starter period ($p<0.05$), but otherwise performance was similar for CC and RAC treatments. Mortality was unaffected by the treatments. The relative ileal activity of collagen type I and type IV degradation was higher for CC than NCC (by +38% and +86%, respectively; $p<0.05$ for both). In RAC, the activity of collagen degradation was similar to NCC for both collagen types. In duodenum and jejunum, the collagen-degrading activity was unaffected by the treatments. The challenge significantly shortened duodenal villi and reduced the depth of crypts, thus thinning the entire mucosal surface (NCC vs. CC $p<0.05$ for both variables). In RAC, the length of villi and depth of crypts were similar to NCC and differed significantly from CC (RAC vs. DCC $p<0.05$ for both variables). In conclusion, the diet-induced challenge increased relative collagen-degrading activity in the ileum and caused alterations in histological parameters. In-feed resin acids prevented these diet-induced changes and maintained mucosal characteristics similar to those in birds without a dietary challenge. Thus, the dietary amendment by coniferous resin acids may partly protect intestinal mucosa of broiler chickens during intestinal disturbances.

Keywords: Resin acids, Feed additives, Intestinal Health, Gut Microbiota, Matrix metalloproteinases

PH14
The effects of mesobiliverdin containing algae on gut microbiota in broilers

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Gut inflammatory bowel diseases (IBDs) are costly and serious diseases in the livestock industry. New animal feeds that promote livestock gut health and control IBDs without antibiotics are needed. It exploits recent discoveries on mesobiliverdin (MBV), an analog of the animal metabolite biliverdin known to protect against IBDs and new synthetic strategies for producing it from kilogram (kg) amounts of microalgae. Two hundred and eighty-eight Arbor Acres broilers were reared from day 1 to day 30 and randomly allotted to 6 dietary treatments (four pens/treatment and 20 birds/pen). The settings of each group are as follows: a. control, b. antibiotic growth promoter (AGP, 0.1 % amoxicillin), c. 0.5% algae (AL), d. 1.0% algae (AH), e. 0.5% MBV enriched algae (MBVL), f. 1.0% MBV enriched algae (MBVH). The chicken feed of control group was no antibiotic addition and group of AGP containing 0.1 % amoxicillin. Group c and d were adding 0.5% and 1% spirulina algae powder respectively. Group e and f were adding 0.5% and 1% MBV enriched algae powder respectively.

During the whole experimental period, the chickens showed the good survival rate (99.7%). The live weight, average daily gain and feed efficiency did not differ for the different dietary treatments. For investigate the microbiota population, we showed that the effect of algae and MBV enriched algae on firmicutes/bacteroidetes (F/B) ratio in broilers. Firmicutes and Bacteroidetes are most common phylum in chicken ceca, with Proteobacteria and Cyanobacteria accounting for the remainder. Interestingly, we further analyzed the data of families and genera and found that algae powder can effectively increase the ratio of *Lactobacillaceae* and *Lactobacillus* respectively, and the most obvious is MBV enriched algal treatment group. For histological examination, current data indicated that antibiotic treatment decrease the villi length of duodenum and ileum in broilers and there have similar effect in jejunum. Moreover, we found that MBV containing algae can improve the intestinal health by elevated the villi length in duodenum, jejunum and ileum, especially the 1.0% MBV enriched algae treatment. In conclusion, we suggest that MBV containing algae can improve gut health in broiler chickens.

Keywords: algae, mesobiliverdin, biliverdin, gut microbiota, chicken

PH15
Potency of *Andrographis paniculata* and *Origanum vulgare* extracts in poultry

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The aim of this study was to determine the effects of phytobiotics combination of *Origanum vulgare* and *Andrographis paniculata* water extracts (FOA) given in feed on the performance, intestinal bacteria, and Eimeria spp. oocysts in feces, of broiler and layer chickens as alternative to Antibiotics Growth Promoter (AGP).

One-day old broiler chicks (Cobb) were divided into three groups of FOA, Zinc Bacitracin (ZB, as an AGP group), and Control. At day 28, body weight of FOA and ZB groups were significantly higher than the control group ($p < 0.05$). FCR was shown best in ZB group followed by FOA and Control. In the intestines, total number of *Lactobacillus* spp. and *Bacillus* spp. was higher in FOA group compared to ZB and control groups, meanwhile *Escherichia coli* and *Salmonella* spp. were lower.

In 14-days-old layer chickens that were infected with live coccidia vaccine (peroral, 5 doses), higher oocyst per gram (OPG) reduction after 7 days of treatment was observed in FOA and Amprolium (as an anticoccidial) group (82.53% and 92.02%, respectively) compared to the control group.

In conclusion, combination of *Origanum vulgare* and *Andrographis paniculata* extracts can be used as AGP replacements in feed.

Keywords: *Origanum vulgare, Andrographis paniculata*

PH16
The effects of *Thunbergia* on sulfatrimethoprim excretion in Nile tilapia (*Orechromis niloticus*)

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In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. The antibiotics are commonly administered by water immersion and medicated feed, which could obtain a uniform dose and avoid any potentials for high localized concentration. During a long cultivated period, animals are continuingly fed with antibiotics for disease prevention, so it might increase risk of antimicrobial residue in animal products. Unfortunately, very little information on the pharmacokinetics of various antibiotics was evident in each fish species. Among approved antibiotics in food animals, sulfonamide was presented a low rate of bacterial resistance by disc diffusion method. Since fish are poikilotherms, the drug excretion could probably be different from other species. Therefore, it might be interesting and worthwhile to understand the pharmacokinetic of sulfonamide in the fish. The study outcome should contribute to minimizing fish consumers' risk from antibiotic residues.

Thunbergia laurifolia Lindl. belongs to the family Acanthaceae, which is commonly known as Rang jeud in Thailand. This plant is traditionally used in Thailand for centuries as an antidote for several poisons and drug overdose. This experiment was performed in 4-month-old Nile tilapias (*Orechromis niloticus*), 10 fish for control and 10 fish for treated group. They were fed with commercial diet for 14 days before experiment started. At day 0, treated fish was supplemented with thunbergia at 500 mg/kg BW for 12 hours before single intramuscular injection with Sulfatrimethoprim at 50 mg/kg BW. Blood samples from all fish of both groups were collected at 0 (before sulfatrimethoprim injection), and at 0.5, 1, 2, 3, 6, 12, 24 hours and 7, 14 days and further examined for liver, kidney, muscle and also pancreatic functions. Biochemical tests; albumin, AST, BUN, cholesterol, creatinine, glucose, total protein and triglyceride were measured by Liquid Stable Reagent Trinder's, colorimetric, and kinetic methods. Results demonstrated that plasma concentration of sulfonamide was significantly low at the first thirty minute and unable to detect at the120th hour in thunbergia treated fish. All blood biochemical results of kidney, liver and pancreas were not different among control and thunbergia treated fish, which indicated that all fish did not have any adverse effects on their vital organ functions.

Therefore, it might be concluded that *T. laurifolia* extract had a detoxification effect by the significant reduction of sulfadiazine in fish plasma. Furthermore, *T. laurifolia* could be an alternative medicine as anti-inflammatory, antioxidant, anti-microbial, hepatoprotective effects.

Keywords: Sulfatrimethoprim, Thunbergia laurifolia Lindl, pharmacokinetic, Tilapia, alternative medicine

PH17
Raising pigs without antibiotics thanks to algae-based solutions

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Antibiotic resistance is one of the top five public health concern according to WHO. Consequently, Olmix has developed a complete and adapted program aiming at accompanying producers to decrease the use of antibiotics and thus limit the development of antibiotic resistance thanks to the use of natural algae-based solutions.

The program was implemented in a farrow-to-finish pig operation of 840 sows in France. The farm was submitted to a detailed audit in December 2015, from which an action plan was prepared and agreed with all collaborators. The action plan aimed at reducing antibiotic use from 100% of the pigs being systematically treated with one or several antibiotics, to a maximum of 10% of the pigs being treated with only one antibiotic.

Several recommendations constituted the action plan. An improved cleaning and disinfection protocol was implemented. Adjustments were made in the farrowing room in order to improve the comfort of newborn piglets. Biosecurity management was reinforced by a stricter quarantine program, more control of inputs to the farm and the action against rodents. The use of 5 innovative marine algae-based products, used in environment, feed or drinking water, completed these different measures. The action plan was implemented step-by-step from February 2016 to April 2017.

The results of this case study show that the use of antibiotic was strongly reduced to reach 94.5% of piglets raised without antibiotics. Moreover, global performance in maternity was improved. These results demonstrate that it is highly important to adapt the strategy to each situation and confirm the need of a global approach to reduce the use of antibiotics in farms.

Keywords: algae-based

PH18
Raising broilers without antibiotics thanks to algae-based solutions

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A major challenge in the animal farming industry will be to reduce the use of pharmaceuticals while maintaining growth performances in order to support the growing demand for meat. Interest for exploring natural bioactive products, able to improve animal health or act against infections, has increased. This study will present a case of using seaweed as an additive in an industrial poultry farm with the aim to reduce the use of antibiotics.

The experiment was undertaken on a total of 411,459 broilers receiving 4 seaweed- based products (11 batches) against a control population of 634,453 broilers (14 batches). Two different genetics of broilers were tested (JA957 and JA987) under high population density, 32 birds per square meter. Slaughtering was undertaken after 32 days. Results show that broilers which received seaweed-based products experienced a drastic decrease of antibiotic use (-92%) while maintaining and/or improving growth performances such as the weight at slaughter and the Food Conversion Ratio (FCR). Moreover, the condemnation rate at slaughter and economic mortality was reduced by 25 and 68% respectively for JA 987.

In conclusion, seaweed-based products introduced in the animal feed through an adapted program reduced the use of antibiotics without losing on animal growth performances. Such results indicate a global improvement of animal health despite intense farming practices which are inclined to progress worldwide within the coming years.

Keywords: Seaweed-based

PH19
Evaluation of efficacy of essential oil blend as alternative to anti-biotic growth promoters in broilers

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Essential oils are complex mixture of structurally diverse volatile compounds produced by aromatic and medicinal plants. Essential oils comprise structurally diverse volatile compounds, which are demonstrated to have unique bioactivity, especially when used in combination. Several studies confirmed the beneficial effects of essential oils in animal health and nutrition, especially as a performance enhancer for commercial poultry production. With the prohibition on the use of antibiotics as growth promoters (AGPs) in different countries and with increasing concerns about the microbial resistance, focus on the use of essential oils as an alternative for anti-biotic growth promoter in broiler diet is increasing exponentially. In this study, we evaluated the growth promoting properties of selected blend of essential oils (EOB), in comparison with different anti-biotics used as growth promoter in commercial boiler birds.
A total of 980 ROSS 308 birds were randomly divided in to 7 groups comprising, control, T1 (BMD) T2 (Tylosin), T3 (Virginiamycin), T4 (Oxy-tetracycline), T5 (EOB 250g/Ton of feed) and T6 (EOB 750g/Ton of feed). Anti-biotics – bacitracin, tylosin, virginiamycin and oxy-tetracycline were used as positive control. FCR, feed intake, body weight gain, mortality and histomorphological data were collected during the study period. FCR and body weight gain of EOB supplemented birds, from both dosage groups, resulted in highly significant ($p<0.001$) improvement when compared to control. Additionally, T6 (750g EOB supplementation) resulted in highly significant ($P<0.001$) difference in FCR and body weight again, when compared to BMD, tylosin, virginiamycin and oxy-tetracycline anti-biotic groups. Similarly, T5 (250g EOB supplementation) showed statistically significant ($P<0.05$) difference in FCR and body weight gain, when compared to oxy-tetracycline and virginiamycin supplemented groups. Among BMD, tylosin and T5 groups, there wasn't any significant difference in weight gain and FCR. Cumulative mortality was less in all groups including control, probably due to best in class farm practices, in spite of that, reduced mortality was observed in EOB supplemented groups, when compared to control. These results infer the dose dependent growth promoting activity upon EOB supplementation in broilers and its efficacy was better than anti-biotics virginiamycin and oxy-tetracycline and comparable to BMD and tylosin as growth promoter, even in the low dose (250g/ton of feed) EOB supplementation. Thus, even low dose of EOB can be effectively used to replace anti-biotics such as BMD, tylosin, virginiamycin and oxy-tetracycline in broiler diets, as an efficient alternate anti-biotic growth promoter.

Keywords: phytogenics, broilers, AGP, Zootechnical performance, alternatives to AGP

PH20
Combination program trial using two different plant extract additives improved immune and zootechnical parameters in broilers

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Phytogenics are secondary metabolites of plants with diverse bioactivity. During the last two decades, phytogenics have been widely used in animal health and nutrition because of their antimicrobial, antioxidant, immunomodulatory and growth promoting properties. Independent studies confirmed growth promoting and immune-modulatory effects of two different essential oil blends (EOB) in broilers. EOB1 comprises a blend of Oregano, Clove and Cinnamon essential oils and EOB2 contains blend of turmeric oleoresin, capsicum oleoresin and piperine. The aim of this study was to evaluate the efficacy of these two formulations in improving zoo-technical and immune-modulatory performance of broiler, in a combination program feeding trial.

A total of 1000 day-old ROSS 308 chicks were randomly divided in to 5 groups comprising negative control, positive control (BMD), T1 (EOB1 250g/t of feed), T2 (EOB2 250g/t of feed) and T3 (EOB2 and EOB1). In T3, birds were supplemented with EOB2 (250g/t of feed) for 14 days followed by EOB1 (250g/t of feed) from day 15 to 42 days of age. FCR, Feed intake, body weight gain, NDV and IBD anti-body titer levels were monitored. One-way ANOVA was used to compare different treatment groups using GraphPad Prism 6 software. Tukey's multiple comparison test was used, in order to draw treatment difference. Improvement (P<0.0001) in FCR and BW gain were observed in the positive control (1.69, 2367.7g), T1 (1.69, 2375.4g) and T3 (1.68, 2391.4g) groups, compared to that of control (1.72, 2329.6g) birds. FCR (P < 0.01) and BW gain (P<0.001) were found to be greater in T3 group birds, compared to BMD supplemented birds. Supplementation of BMD, EOBs alone and in combination did not affect the feed intake (P > 0.05) of birds compared to control. An increase (P<0.0001) in NDV antibody-titer levels was observed in the positive control (5073.4) and all three treatment (4767.1 to 8068.3) groups, compared to control (547.7) on 42d. T1 group NDV antibody titer value (8068.3) was greater (P<0.0001) than the positive control. Similarly, an increase (P<0.0001) in IBD antibody titer value was observed in positive control (872.9) and all treatment groups (803.5 to 892.7), compared to control (319.5). There was not any difference in IBD antibody titer values (P>0.1) among the treatment and positive control groups. These results show that supplementation of EOB2 in the starter phase, followed by EOB1 during the grower phase can improve both immune and zoo-technical parameters.

Keywords: plant extracts, broilers, zootechnical performance, AGP, immunity

PH21
Dietary resin acid supplementation improves the performance of sows and piglets

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Coniferous resin acids have anti-inflammatory and antimicrobial effects. We have previously shown that dietary resin acids improve the reproductive performance of sows and increased the production and immunoglobulin G -content of colostrum. Here we studied the effect of a tall oil -based resin acid composition (RAC) with 8.5% resin acids to the performance sows and their offspring.

Fifty-six Polish synthetic line 990 sows were allocated to two dietary treatments. The standard lactation feed was amended with 0 kg/tn (Control; C) and 1.0 kg/tn (Treated; T) of RAC from 2 wk before to 4 wk after farrowing. The parity number, backfat thickness at start and end of lactation, course and length of parturition, number of total born, born alive and stillbirth piglets, number and weight of piglets and weight of litter at weaning were recorded for the sow. The piglets were weaned at 4 wk of age. From both diet groups, 80 castrated male and 80 female weanlings were housed in groups of eight and allocated to C and T dietary treatments, to reach a total 320 piglets in the following four treatments: CC, CT, TC, TT. The piglets were weighed at 0, 2 and 6 wk after weaning, and recorded for daily feed intake, feed conversion ratio (FCR) and mortality. For statistical evaluation of the performance data, ANOVA was used.

Dietary RAC decreased the course of parturition, the number of stillborn piglets, and piglet mortality before weaning, increased piglet weight at birth, and resulted in an average of 0.96 more piglets per litter ($p<0.05$). For 2 wk post-weaning, piglet average daily gain (ADG) was lower for the CC than other dietary groups ($p<0.05$). ADG remained significantly lower in CC than CT group for 6 wk post-weaning. Average daily feed intake was higher in piglets of the CT group than in TC group ($p<0.05$). FCR was better in piglets of TT group than in piglets of the TC group. In conclusion, the performance of sows and piglets was improved by dietary RAC supplementation at lactation and nursery respectively.

Keywords: resin acid, sow, piglet, performance

PH22
Evaluation of the impact of garlic and cinnamaldehyde application on *Salmonella* recovery at end of broiler growout

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The final days prior to broiler transport is a critical time to reduce or eliminate foodborne pathogenic bacteria. Compounds such as allicin, deriving from garlic as well as cinnamaldehyde have shown to mitigate growth of gram-negative bacteria, including *Salmonella*. However, *in vivo* research implementing a combination of these compounds is limited. The objective of this study was to evaluate the effects of the Alliin Plus (AP) product, a combination of freeze-dried garlic and cinnamaldehyde, on reducing cecal, environmental and crop prevalence of *Salmonella* at the end of growout.

A total of 216 male broilers at 35 days of age were placed in one of the following treatments: 1) Control (CC), 2) AP in mash feed (AP-F), and 3) AP in drinking water (AP-W). Dosage of AP was 900 g per ton of feed or 1 g/L so that total expected daily intake per bird was equivalent for both treatments. Each treatment had 6 replicate pens with new litter (3 pens/room) and 12 birds/pen. A *Salmonella* Typhimurium (STM) challenge was performed 7 days post placement and all birds were gavaged with ~10^8 STM CFU of nalidixic-acid resistant strain. Impact of AP on STM challenge was measured in cecum. Four birds/pen were sampled on day 7 and 12 post-challenge (i.e. 14 and 19 days of treatment). The day before each sample collection (6 days and 11 days post-challenge), the litter surface of each pen was sampled using intermittently stepped-on drag swabs. Furthermore, crop samples were collected on day 12 post challenge (19 days of treatment). To test if *Salmonella* prevalence would change with feed-withdrawal practice, on the 12-day collection, two pens/treatment were either full-fed, 6-h off feed or 12h off-feed 6h off-water prior to sampling.

Although all litter was *Salmonella*-positive, AP-W at 14 days had the lowest prevalence (p=0.029) as there was no recovery of *Salmonella* with direct plating (>10^2 cells/mL sample). There was no difference on feed-withdrawal, therefore data was combined at the 12-day collection. This was also observed with cecal recovery of STM that was, although not significant, a trend of over a log reduction with the AP-W treatment (1.85 ± 0.51, p=0.470) relative to CC (3.07 log ± 0.48). Additionally, cecal recovery showed no statistical difference at 12 days post challenge. On day 12 post challenge crop also showed a trend of lower recovery of STM in AP-W (0.23 ± 0.15) relative to than in CC (1.39 ± 0.64; p=0.174). Results were similar in AP-F group where cecal recovery on day 7 trended of a log reduction relative to CC (1.49 log CFU ± 0.82; p=0.261) and crop recovery on day 12 was lower than CC (0.15 ± 0.15; p=0.133). These preliminary results suggest that garlic and cinnamaldehyde may help to reduce the prevalence of environmental and cecal colonized *Salmonella* prior to processing, therefore a possible water or future in-feed application at the end of grow out. Future studies at larger magnitude and dose titrations are warranted.

Keywords: *Salmonella*, poultry, allicin, cinnamaldehyde, water-application

PH23
Inclusion of lignocellulose in semi-purified diet on performance and duodenal morphology of broilers

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Antibiotics have been added in feed as growth promoters to reduce overall health problems and improve growth performance in past several decade. Nowadays, the use of antibiotic in modern broiler production has been prohibited from many countries throughout the world. However, one of the potential candidates as effective alternative to in-feed antibiotics is lignocellulose (LC). LC are the major structural component of plant cell wall and mainly composed of lignin and three polysaccharides: cellulose, hemicellulose, and pectin. Some reports exhibited that LC are profitable to gut health and structure as well as stimulating the hydrochloric acid secretion. Therefore, the current research was aimed to evaluate the effect of dietary LC in semi-purified diet on performance and duodenal morphological alterations of broilers. One hundred sixty 10-day-old Ross 308 chicks were divided into 4 groups, each with eight replicates of five birds. They were fed the semi-purified diet included with LC at 0 (control), 60, 80 and 100 g/kg until 45 days of age. Results showed that diets containing various levels of dietary LC clearly affected broiler performance and duodenal tissue. Compared to the control group, body weight and weight gain tended to be higher with increasing dietary LC levels and significantly increased ($p<0.05$) in the 80 and 100 g/kg LC groups. Whereas, feed intake significantly decreased ($p<0.05$) in both 80 and 100 g/kg LC groups, results in an improved feed conversion ratio in all the LC groups ($p<0.05$). With increasing dietary LC levels, crypt depth (CD) in duodenum (CD) was significantly shallow ($p<0.05$) in the 80 and 100 g/kg LC groups. Duodenal villus height (VH) and VH to CD ratio increased linearly ($p<0.01$) in all the LC groups than the control group. There were no significant ($p>0.05$) differences in duodenal villus area among the dietary treatments. In conclusion, dietary LC can be added in semi-purified diet up to 100 g/kg to enhance broiler performance as a result of stimulation of duodenal morphological maturation. These results suggest that LC have a high potential to use as alternative strategy to antibiotic growth promoters due to their ability to support gut development.

Keywords: Broilers, Duodenal morphology, Growth performance, Lignocellulose, Semi-purified diet

PH24
Sunflower meal inclusion rate and the effect of exogenous enzymes on broiler performance

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Worldwide, corn and soybean meal are the most used conventional feed ingredients for broiler diets. Nutritionists are constantly in search of alternative affordable and nutritious feed ingredients due to high feed costs. One promising ingredient is sunflower meal (SFM). It is rich in protein content, methionine, and does not have antinutritional factors. However, its use in broilers has been limited by low levels of lysine, high crude fibre and non-starch polysaccharides. This study was conducted to determine if inclusion levels of SFM can be increased, and if the efficacy of exogenous enzymes (EE) on broiler performance is altered by SFM inclusion rates.

All experimental procedures were approved by the Animal Ethics Committee at the University of Pretoria (Project No. EC042-18). A commercial 4-feeding phase of pre-starter (1-9d), grower (10-20d), finisher (21-28d) and post-finisher (28-35d) was followed. Two SFM inclusion rates were used; low sunflower meal (BSL) and high sunflower (BSH). SFM contained 36% crude protein. SFM inclusion in BLS was 3% throughout the phases, whereas in BSH, inclusion was 7.5, 10, 13 and 13.5%. Each SFM inclusion, had a Negative Control (NC); a Positive Control diet (PC) with additional 80kcal Apparent Metabolizable Energy (AMEn). Additionally, xylanase (X), xylanase plus beta-glucanase (XB), xylanase plus beta-glucanase plus protease (XBP), xylanase plus amylase plus protease (XAP) enzymes were added to the BSL and BSH NC diet formulation to make 12 treatments. One-day old male Ross 308 chicks (n=1,920) were placed on pens (2m*1m) and allocated to the treatments (n=8 replicate/treatment) from day 1 – 35. Pen bird weight (BW) and feed intake (FI) was determined on day 9, 20, 28 and 35d and feed conversion ratio (FCR) calculated. Data were statistically analysed (P<0.05) using a randomized two-way ANOVA model.There was no interaction (P>0.05) between SFM inclusion and Enzyme treatment for all parameters throughout the study period. Increasing SFM inclusion reduced FCR at 9d (1.15 vs 1.17), but affected performance parameters at any period thereafter. There was a significant main effect of enzyme treatment on BW gain to 35d. Birds fed the XAP enzyme gained more weight (2.69kg) than either the PC (2.61kg) or NC (2.62kg) while other enzyme combinations had no effect on 35d BW gain. A limitation of this study was that there were no significant differences between the NC and PC diet with 80kcal/kg more AMEn. This could be due to the energy density of the diets being above the requirement and limited the ability to separate enzyme effects on FCR. In conclusion, our data suggest that SFM can be increased to at least 7.5% in starter feed and 10% in grower and finisher diets without negatively affecting performance, and that the addition of XAP enzymes can improve BW gain of broilers grown to 35d of age.

Keywords: sunflower meal, non-starch polysaccharides, exogenous enzymes, efficacy, inclusion rate

PH25
Herb based complexes for improving the quality of the microbiome

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Herbal compounds possess antimicrobial properties and therefore can represent true alternatives to antibiotics. The greatest disadvantage of herb originated compounds is that the biological activities vary largely on the origin, the harvesting time or on the applied extraction procedure.

During our research an optimized extraction procedure was established for plant oils and extracts which enabled the minimizing of the biological variation and resulted in stable and reproducible antimicrobial properties for various herbal compounds towards multiple obligate and facultative pathogens of livestock. As a second step, during the screening procedure, a unique quality control system was introduced where instead of defining exact chemical compositions; plants, plant oils and extracts were categorized based on their biological activities observed in multiple *in vitro* studies towards isolates of the pathobiome, such as *E. coli*, *Clostridium perfringens, Brachyspira* species or *Lawsonia intracellularis.*

With using the optimized extraction procedure and the biological activity data of over hundred screened herb originated drugs, different feed additives were developed which prevents the pathobiome development and helps in maintaining the normal microbiome in livestock. The optimized compositions of phytochemicals were tested in target species such as poultry and pigs. *In vivo* trials were conducted on small scale laboratory farms and on large production farms, with either excellent or low hygienic circumstances.

Based on the results of several *in vivo* trials, it was concluded that the application of herbal extract containing feed additives can serve as alternatives to antibiotics, result in significantly less diseases and increased production parameters, meat quality and overall wellbeing, especially on farms with several hygienic issues and low farm management involvement. Stabilizing herbal compounds with optimized extraction procedures help in maintaining a stable and reproducible biological activity, which is a crucial parameter in terms of industrial scale production.

Keywords: pathobiome, selective inhibition, screening, minimal inhibition concentration

PH26
Study of cost effective feed additives to replace AGP in poultry chickens, improving productive parameters and reducing antimicrobial resistance spread

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Antibiotic growth promoters have been used for decades in animal production to maintain health and improve productive parameters. Global concern about the recurrent emergence and spreading of antimicrobial resistance is challenging the livestock producers to find cost effective alternatives. The use of phytogenic compounds, probiotics and organic acids appears as a feasible option due to their ability to emulate the bioactive properties of antibiotics. The present study was planned to compare (1) a standard AGP program against two antibiotic free programs: (2) a combination of probiotic- yeast moss - chestnut/quebracho extracts blend and (3) a chestnut/quebracho extracts blend. A total of 1,575 animals distributed in 3 treatments with 15 replicates each one was followed from 0 up to 42 days of life. Weight gain, feed conversion, live weight/feed conversion relation, mortality, and overall health were routinely measured and recorded. Intestinal health was evaluated at day 21 and 42. Cecal and litter samples were taken for microbiota analysis, moisture, bacteriological determinations and antimicrobial resistance environmental spread.

The weight gain and feed conversion were improved by both non-AGP programs when compared to AGP, 1.051 vs 1.077 and 1.072 for treatment 2 ($p>0.05$) and 3 ($p>0.05$) respectively at day 21. At day 41 no significant differences were observed among treatments, however birds in treatment 3 showed higher weight and improved feed conversion (2.978 vs 3.003 kg; 1.728 vs 1.715), while birds from treatment 2 only showed improved feed conversion (1.728 vs 1.714). Microbiota analysis show a differential effect of all treatments, particularly alpha diversity, number of species and specific groups of beneficial bacteria improved by non-AGP treatments. Food pad lesions and intestinal health was similar in AGP or non-AGP programs, with most in 0 lesion score. Both non-AGP treatments showed an important tendency to reduce antibiotic resistance in representative bacteria from intestine and litter. Bacteriological parameters, weight gain, feed conversion rate, reduction of mortality was improved by non-AGP, producing an improvement of productive parameters with costs a reduction. The available information supports the competitive use of these alternative programs and an important cost reduction by the use of specific mixture of polyphenols.

Keywords: phytochemicals, productivity, poultry, antimicrobial resistance, polyphenols

PH27
The dose of chestnut and quebracho polyphenols alters rumen microbiota profile and production of volatile fatty acids in bovines

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Previous studies showed that inclusion of polyphenol-rich plant extracts in feed improves performance in ruminants, but the effect may vary depending on the dosage used. It has been shown that polyphenols modify the digestive process through modulation of gastrointestinal microbiota and bypass of protein digestion. The rumen houses a highly complex microbiota which is able to convert indigestible plant mass into energy mainly through production of short-chain fatty acids (SCFA).

High-throughput sequencing of 16S rRNA gene was used to study the temporal changes in microbiota composition of eight rumen-fistulated steers supplemented with four doses of chestnut and quebracho plant extracts (0, 0.075, 0.15 and 0.30 kg per ton) in a 4x4 Latin-square design. In each sample, the pre- and post- prandial pH of the ruminal liquor was measured and the SCFA profile was determined by HPLC. Bioinformatics and statistical analysis of microbiota variability was performed using QIIME2 software.
6.724.119 reads of 16S rRNA gene V3-V4 region were obtained in total. A significant variation in rumen microbiota beta diversity was detected between animals and between samplings ($p<0.001$), but pre- and post-prandial samples showed very limited variation, with virtually identical patterns in both series of data. A smaller but significant effect was observed between polyphenol doses on alpha and beta diversity parameters. Steers with higher doses tend to have lower richness ($p=0.09$) and lower Shannon's diversity index ($p=0.03$), which indicates a more even and balanced microbial community in treated animals. When comparing with the not-supplemented control group, all three polyphenol doses reduced the relative abundance of phylum Euryarchaeota, which includes the methanogenic archaea responsible for greenhouse gas production. The two higher doses also increased the relationship between Firmicutes and Bacteroidetes (F/B). The dose of 0.15% was the one with the highest F/B ratio and the lowest relative abundance of methanogenic bacteria. Regarding SCFAs production and pH, a strong correlation was observed between these parameters and rumen microbiota diversity. Steers with lower number of bacterial species had a more acidic ruminal pH ($p=0.0004$), and higher ratio of C2/C3 in their SCFA profile ($p=0.0013$).

The results showed that the groups supplemented with chestnut and quebracho polyphenols have similar changes compared to the control group, but the modulatory effect on the diversity and composition of rumen microbiota and SCFA production profile varies depending on the dosage of extract used, reaching an optimal effect at intermediate doses. Therefore, the dose of polyphenols is a parameter that can be optimized since differentially alters ruminal physiology.

Keywords: microbiota, phytochemicals, rumen, bovines, polyphenols

PH28
Eugenol attenuates inflammatory responses and enhance barrier functions during lipopolysaccharide (LPS)-induced inflammation in porcine intestinal epithelial (IPEC-J2) cells

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Eugenol is an essential oil component which is known to possess anti-microbial, anti-inflammatory and anti-oxidative properties, however the effect of eugenol on porcine gut inflammation has not yet been investigated. In this study, an *in vitro* lipopolysaccharide (LPS)-induced inflammation model in porcine intestinal epithelial cells (IPEC-J2) was used.

The results showed that eugenol pre-treatment significantly suppressed the LPS-stimulated interleukin 8 (IL-8) level and the mRNA abundance of tumor necrosis factor α (TNF-α); enhanced the LPS-stimulated mRNA abundance of tight junction proteins *zonula occludens-1 (ZO-1), occludin (OCLN), claudin-1(CLDN-1), claudin-3 (CLDN-3)* and the mRNA abundance of the following nutrient transporters; *B^0-system neutral amino acid co-transporter (B^0AT1), system ASC sodium-dependent neutral amino acid exchanger 2 (ASCT2), apical sodium-dependent glucose transporter 1 (SGLT1), excitatory amino acid transporter 1 (EAAC1)* and *peptide transporter 1 (PepT1)*. In addition, eugenol improved the expression and even redistribution of ZO-1, tended to increase TEER value and maintained barrier integrity and tightness.

A low dose of eugenol showed to have a positive effect on attenuating inflammatory responses and enhancing selectively permeable barrier function during LPS-induced inflammation in the IPE-J2 cell line.

Keywords: eugenol, LPS-induced inflammation, inflammatory responses, barrier function, IPEC-J2 cells

PH29
Beneficial properties and mechanistic study of a phytogenic formulation, Rotam-CS, for avian coccidiosis

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In the interests of food safety and public health, edible plants and their compounds are now re-emerging as an alternative veterinary medicine for meat-producing animals. Here, we studied the impact of Rotam-CS, on coccidiosis, a protozoan disease, growth performance and drug resistance in chickens. First, we found that Rotam-CS was therapeutically effective against coccidiosis in chickens as evidenced by a survival rate, gut pathology, fecal oocyst excretion and anti-coccidial index. Next, we showed that Rotam-CS significantly increased body weight gain and decreased feed conversion ratio in chickens. Mechanistic study showed that Rotam-CS inhibited the life cycle of *Eimeria* species. Overall, this work suggests Rotam-CS as a potential remedy for avian coccidiosis via interference with protozoan life cycle.

SESSION 5
Immune-related products

ORAL PRESENTATIONS

Passive Immunity and IgG-like antibodies as an alternative to antibiotics

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With the surge of the world-wide antimicrobial resistance problem (see e.g. Review on Antimicrobial Resistance (2016), https://amr-review.org/) severe actions are needed to limit the use of antimicrobial drugs in the human as well as the animal sector. The animal production sector demands a huge consumption of antibiotics frequently used with little indication e.g. for animal growth performance enhancement and routine prevention rather than treatment of disease.

A prerequisite for alternatives to antibiotics to gain widespread acceptance in the animal production sector is that they are truly cost-efficient at the same level as antibiotics which as a class of drugs are very efficient, inexpensive and very broadly applicable. Non-vaccine immunization, also known as passive immunization offers an interesting alternative to antibiotics on one side and active immunization (vaccination) on the other side. Passive immunization offers a unique combination of sustainability, broad coverage, cost-effectiveness and absence of risk of anti-microbial resistance induction in production animals. In addition, passive immunization offers an often overlooked possibility of efficiently and safely treating or preventing infectious diseases with unknown and/or multi-factorial origin, making it particularly relevant for a range of major animal production diseases including perinatal as well as weaning associated intestinal disease.

The presentation will provide background on passive immunity approaches and give examples of applications within pig, cattle, poultry and fish production, showing recently published as well as unpublished research in the field. The performance of passive immunization approaches such as oral IgG and IgY, specific, polyspecific and non-specific immunoglobulin preparations, and modified immunoglobulins alone and in combination with other bioactive compounds will be compared with vaccination, maternal vaccination, breeding based strategies, and the use of probiotics and bacteriophages. Sources and production methods will be taken into account with a focus on sustainability, ease of use, low cost and efficiency, all of which are prerequisites for the widespread use of any alternative to antibiotics in the animal production sector.

Keywords: Passive immunization, animal production diseases, IgG, IgY, alternative

Host defence peptides with anti-microbial and immunomodulatory activities as antibiotic alternatives

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With the increasing emergence of antibiotic-resistant pathogens, antimicrobial peptides (AMPs) have been studied as alternatives to antibiotics based on their broad spectrum of bactericidal activity and selectivity. Cationic antimicrobial peptides are highly conserved in all organisms and are effective against many bacteria, including multidrug-resistant bacterial strains, by disrupting the bacterial membrane based on their cationic nature. However, the direct activity of cationic AMPs towards the microbial membrane is dependent on physiological conditions such as salt and serum. Increasing evidence indicates that direct microbial killing may not be the primary role of cationic AMPs in the body, and efforts to determine the role of cationic AMPs have focused on the immunomodulatory properties of cationic AMPs. The immunomodulatory activity of cationic AMPs is complex and includes anti-infective immune modulation such as the induction of chemokines and cytokines, pro/anti-inflammatory activity, direct chemotaxis, wound healing, angiogenesis, apoptotic activity and adjuvant activity. The immunomodulatory activity of cationic AMPs also varies depending on the cell type. Because of their ability to modulate the immune response, cationic AMPs are called host defense peptides (HDPs). Chicken NK-lysin (cNK-lysin) is a homologue of human granulysin which is found in the cytolytic granules located in human natural killer and cytotoxic T lymphocytes. It was previously demonstrated that cNK-lysin is hugely expressed in *Eimeria*-infected intestinal lymphocytes, suggesting a role in parasite infection. Subsequent studies have shown that cNK-lysin and cNK-2, the core α-helical region of cNK-lysin, can kill *Eimeria* sporozoites by disrupting the parasitic membrane. Interestingly, cNK-2 exhibits higher antimicrobial activity than the original peptide and even melittin indicating that the modification of the natural sequence can improve efficiency. In mammals, granulysin acts as an immunomodulatory peptide by serving as a chemoattractant for lymphocytes and modulating the expression of chemokines and cytokines. The present study demonstrates that cNK-2 has immunomodulatory properties as an HDP, including inducing chemokines/cytokines, an anti-inflammatory response, signaling pathway activation and internalization into chicken cells. By contrast, the antimicrobial effects of cNK-2 were reduced under physiological salt conditions. The responses of HD11 cells and primary monocytes to cNK-2 were also studied to understand the role of cNK-2 in innate immunity. The findings presented here provide advanced insight on how the chicken immune response is modulated by HDPs.

Keywords: antimicrobial peptide, host defense peptide, chicken, coccidiosis, alternatives to antibiotics.

Innovative enterobactin-specific egg yolk antibodies for controlling Gram-negative pathogens

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Passive immunization with specific egg yolk antibodies is emerging as a promising alternative to antibiotics for the treatment and prevention of various mucosal infectious diseases in food animals. Enterobactin (Ent)-mediated high affinity iron acquisition is a universal and critical contributor for Gram-negative mucosal pathogens to survive in the hosts. Given the bacteriostatic effect of lipocalin resulting from its potent Ent-binding ability, immune intervention directly targeting Ent is promising for iron-dependent pathogen control. In this study, we developed a novel Ent conjugate vaccine that has several significant advantages, such as ease of preparation, induction of high titer of Ent-specific antibodies in rabbits as well as chickens, and the ability of Ent-specific antibodies to bind various Ent derivatives including the salmochelins that help enteric pathogens evade sequestration of siderophores by host lipocalins. Notably, the *in vitro* growth assays provided compelling evidence that the Ent-specific antibodies function similarly as lipocalin to interfere with Ent-dependent growth of Gram-negative mucosal pathogens (e.g. *E. coli*, *Salmonella*, and *Campylobacter*) under iron-restricted conditions. Subsequently, we evaluated different vaccination regimens for production of hyperimmune Ent-specific egg yolk antibodies. Different layers (Barred Rock, Rhode Island Red), Ent conjugate vaccines (BSA-Ent, KLH-Ent, or CmeC-Ent), and immunization routes (intramuscular *vs* subcutaneous) were used in multiple immunization trials. The levels of specific antibodies (IgY) in serum and egg yolk were measured using immunoblotting and ELISA assays. The KLH-Ent triggered immune response in Barred Rock layers via intramuscular route, leading to significantly increased titer of Ent-specific IgY in serum (up to 4 fold) and yolk (up to 8 fold). Subcutaneous immunization of Rhode Island Red pullets with KLH-Ent or CmeC-Ent dramatically increased Ent-specific IgY in serum (up to 2,048 fold) and yolk (up to 1,024 fold). However, the BSA-Ent immunization did not induce anti-Ent IgY significantly. The lyophilized Ent-specific hyperimmune egg yolk IgY is being evaluated for its passive immunization efficacy in protecting chickens from the infections caused by *Campylobacter* and avian pathogenic *E. coli*. Together, this study reports an efficient method to prepare innovative Ent conjugate vaccines for inducing high level of Ent-specific antibodies. We also optimized vaccination regimen in layers and produced hyperimmune Ent-specific egg yolk IgY that has significant potential for prevention and control of Gram-negative infections in food animals.

Keywords: Egg yolk antibody, passive immunization, Enterobactin

High throughput screening for natural host defense peptide-inducing compounds as alternatives to antibiotics

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A rise in antimicrobial resistance demands novel alternatives to antimicrobials for disease control and prevention. As an important component of innate immunity, host defense peptides (HDPs) are capable of killing a broad spectrum of pathogens and modulating a range of host immune responses. Enhancing the synthesis of endogenous HDPs has emerged as a novel host-directed antimicrobial therapeutic strategy. To facilitate the identification of natural products with a strong capacity to induce HDP synthesis, a stable chicken HTC macrophage cell line expressing a luciferase reporter gene driven by an avian β-defensin 9 (*AvBD9*) gene promoter was constructed through lentiviral transduction and puromycin selection. A high throughput screening assay was subsequently developed using the stable reporter cell line to screen a library of 584 natural products. A total of 21 compounds with a minimum Z-score of 2.0 were identified. Secondary screening in chicken HTC macrophages and jejunal explants further validated most compounds with a potent HDP-inducing activity in a dose-dependent manner. A follow-up oral administration of a lead natural compound, wortmannin, confirmed its capacity to enhance the *AvBD9* gene expression in the duodenum of chickens. Besides *AvBD9*, most other chicken HDP genes were also induced by wortmannin. Additionally, butyrate was also found to synergize with wortmannin and several other newly-identified compounds in *AvBD9* induction in HTC cells. Furthermore, wortmannin acted synergistically with butyrate in augmenting the antibacterial activity of chicken monocytes. Therefore, these natural HDP-inducing compounds may have the potential to be developed individually or in combinations as novel antibiotic alternatives for disease control and prevention in poultry and possibly other livestock species.

Keywords: Host defense peptides, antimicrobial peptides, HDP-inducing compounds, high throughput screening, poultry

Making the transition from research trials to field application

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Since the mid 1990's, there has been a global shift away from the prophylactic or growth-promoting antibiotics (AGP) in poultry feed, due to legislation or consumer pressure. Therefore, there is a reasonably long history of the development of AGP alternatives. In spite of this history, the transition to AGP-free production is often not a smooth one for individual companies, or even individual farms within a company.

Ideally, a replacement product for AGP should be economically feasible, simple to apply consistently under field conditions, be accepted by consumers, not promote microbial resistance, and most importantly, be efficacious. The challenge faced by the industry is to replace a strategy that was broadly effective (i.e. AGP generally work under a wide variety of conditions, and the impact of AGP was typically most pronounced under poor production conditions). A transition to alternatives, however, will also require a higher standard of housing, husbandry, biosecurity, management, and nutrition. Controlled research trials are a necessary step towards commercial implementation, but caution must be used when evaluating research trial results.

Many proposed alternatives to antibiotics have been investigated in research conditions, with varying degrees of success. However, the translation to success under commercial conditions has often been problematic. There are several reasons for this. First, many controlled studies fail to include a proper negative control in which a challenge an be demonstrated. It is assumed that equivalent performance of a test product to antibiotics proves efficacy. However, in the absence of a demonstrated microbial challenge, it is impossible to determine whether the equivalent performance to AGP is due to an ability to reduce the effects of infection, or the lack of an infection. Second, controlled experiments usually utilize a specific disease challenge model. Efficacy against a single challenge organism may indicate usefulness, but the field challenges will vary from one region to the next, from one farm to the next, and even over time on the same farm. Third, even studies utilizing natural challenge models are limited by the specific environment in which the study is conducted. Finally, because of the variety of potential challenges that may be encountered in the field, a single alternative product with a specific mode of action is not likely to be effective against the variety of different pathogenic organisms that may be encountered.

A successful, commercially-viable AGP-free strategy will likely involve the utilization of multiple products, with complementary mechanisms of action, and will need to be tailored to each production unit. Although this may take time and effort, it is likely to be the only path to success.

Reprogramming the innate immune system as an alternative

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The aim of vaccination is to induce specific immunological memory that helps the adaptive immune response to mount an adequate response against a specific pathogen. Accruing evidence, however, suggests that some widely used vaccines protect in a-specific fashion against unrelated pathogens. This generic protection may be explained by innate immune memory or so-called "trained immunity". In recent years it was discovered that innate immune cells like macrophages and Natural Killer cells exhibit sustained memory that helps these cells to respond to re-infection. Various microbial products have been shown to "train" the innate immune system and their effects seem to be exerted by epigenetic changes.
Host defense peptides (HDPs) are small molecules present in all vertebrates and show both antimicrobial and immunomodulatory activities. Cathelicidins are cationic HDPs with an important function in the early vertebrate host response against invading pathogens. These peptides are secreted at mucosal surfaces by leukocytes and epithelial cells upon interaction with pathogenic microorganisms. If cathelicidins or cathelicidin-derived peptides are administered to experimental animals, it was observed that these animals were protected against subsequent infections. Here we report that the protective effects may be partly explained by "training" of the innate immune system.

It is concluded that reprogramming the innate immune system of farm animals by microbial products, HDP-derived products or combinations thereof may be a strategy to reduce antibiotic use.

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Efficacy of dried egg product administered to male broiler chickens during experimental necrotic enteritis

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Despite vaccination, medication, and management efforts, *Clostridium perfringens* (*Cp*) continues to cause necrotic enteritis (NE) worldwide resulting in devastating economic losses to the poultry industry. Coccidiosis is considered a trigger for NE outbreaks in flocks and thus efforts to control *Eimeria* spp. infection may ameliorate NE incidence and severity. Consumer apprehension about antibiotic usage in poultry is increasing and alternative solutions are needed. This study assessed the impact of a dried egg product (DEP) containing anti-interleukin 10 (IL-10) IgY neutralizing antibody on performance of broilers raised under antibiotic free (ABF) management practices during experimental NE challenge. A randomized complete block design was used to evaluate 6 doses (0, 143, 287, 358, 430, and 573 U/MT) of a DEP under experimental NE consisting of, Cocivac® B52 (Merck Animal Health, Kenilworth, NJ) spray vaccination on day of hatch according to label, and 10^8 cfu/bird/day of *Cp* in feed on d 18, 19 and 20. An additional *Cp* unchallenged control was included (NNC). Cobb 500 males were housed at 50 birds/pen in 70 floor pens (10 pens/treatment) for 42 d. Diets were administered in 3 feeding phases 0-14 (Starter), 14-28 (Grower), and 28-42 d (Finisher). A basal diet was mixed for each phase and DEP was added to each basal diet to make treatment diets, which were identical in ingredient and nutrient composition. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), unadjusted and adjusted for mortality (MA), and production efficiency index (PEI) were determined for each feeding phase. On d 20, 4 birds were randomly selected from each pen for NE lesion scoring. Statistical analyses were conducted using JMP (v. 14.1, SAS Institute, Cary, NC) using pen as the experimental unit with treatment as a fixed effect and block as a random effect. During Starter, growth performance was not different (P=0.25 to P=0.78) between 0 DEP treatment compared to NNC. Inclusion of DEP during Starter linearly improved (P<0.01) BW, ADG, MA_ADG, FCR, MA_FCR, and PEI. *Cp* challenge during Grower markedly reduced (P<0.0001) BW, ADG, MA_ADG, FCR, MA_FCR, survival, and PEI and MA_ADFI (P=0.06), and increased (P<0.0001) NE lesions scores in the 0 DEP treatment compared to NNC indicating a successful *Cp* challenge. NE lesion scores reduced (P<0.0003) linearly with increasing DEP supplementation. Addition of DEP linearly improved BW, ADG, MA_ADG, FCR, MA_FCR, survival, and PEI during (P<0.0005) Grower; BW and PEI (P<0.0001) and FCR and MA_FCR (P<0.088) during Finisher; and BW, ADG, MA_ADG, FCR, MA_FCR, survival, and PEI during the 42-d trial (P<0.0006). Including 573 U/MT of DEP improved FCR (P<0.005) by 10, 15, 12, and 13 and MA_FCR (P<0.004) by 10, 13, 13, and 12 points during Starter, Grower, Finisher, and overall, respectively, compared to 0 U/MT of DEP. In conclusion, DEP significantly improved bird performance during experimental NE compared with unsupplemented birds under same environmental challenge conditions and coccidiosis vaccination practice.

Keywords: coccidiosis, necrotic enteritis, broiler performance, dried egg product, IgY

Yeast cell wall immunomodulatory and intestinal integrity effects on broilers challenged with *Salmonella* Enteritidis

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The objective of this study was to evaluate the immune effects and the dynamics of intestinal integrity in broilers challenged with *Salmonella* Enteritidis (SE) and treated with yeast cell wall (YCW). One hundred birds were housed in isolators at 1 day of age and divided into 4 treatments: G1- Birds challenged with SE; G2- Birds not challenged and supplemented with YCW (*Saccharomyces cerevisiae*, ImmunoWall® from ICC Brazil, at 0.5 kg/MT); G3- Birds not challenged and not treated/medicated and; G4- Birds challenged with SE and supplemented with YCW (same inclusion). The challenge was administered orally at 2 d with 10^8 CFU per bird. SE was quantified in crop and ceca contents at 8, 14 and 21 d. Circulating lymphocyte and monocyte subsets, as well as phagocytic cells were evaluated at the same time ages. Samples of ileum, ceca, and liver were collected at 14 d (8 birds/treatment) for histopathology. Specific IgA for SE in feces was evaluated also at 14 d. Intestinal mucosa permeability was assessed in 8 birds/group at 4, 8, 14 and 21 d by passage of a marker (Dextran-FITC, 3-5 kD) from intestinal lumen to blood. The data were analyzed by ANOVA and the means compared by Tukey's test at 5% of significance. At 4 d, G1 presented the highest intestinal permeability (significantly different from the treated group [G4]). Circulating leukocytes counts were higher in the non-SE challenged groups (G2 and G3). Despite this, challenged groups consistently presented higher numbers of various cell subtypes, especially at 14 d (APCs, monocytes, suppressor monocytes, and the series of helper T lymphocytes and cytotoxic T lymphocytes). Treatment was effective in controlling leukopenia and in preventing some of the immune subset fluctuations provoked by the challenge, such as for APCs and cytotoxic cells. The number of phagocytic cells was increased by challenge at 8 d, while the YCW decreased this effect. G4 presented the highest number of reactive animals, as well as the highest level of anti-*Salmonella* IgA. The challenge induced marked inflammatory responses in the intestine and liver (assessed by lymphocyte counts, section area, goblet cell counts, tissue architecture). Treatment was effective in improving tissue inflammatory signs such as lymphocyte infiltration in cecum, but not liver. The challenge with SE induced changes in all evaluated systems; however, intestinal integrity and some immune parameters were improved by dietary YCW in challenged birds.

Keywords: *Saccharomyces cerevisiae*; Poultry; intestinal permeability; IgA

SESSION 5
Immune-related products

POSTER PRESENTATIONS

IM1
Characterization of *in-ovo* administered innate immune stimulants for prevention of early chick mortalities due to yolk sac infection

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Early chick mortality (ECM) due to yolk sac infection (YSI) and colibacillosis causes substantial economic loss to the broiler industry of Saskatchewan. Moreover, at the sub-clinical level these infections lead to retarded growth, poor carcass quality, weak response to vaccination, and increased susceptibility to other infections. Currently, culling of chicks with navel defects and *in ovo* or post hatch administration of antibiotics is used for prevention of ECM due to YSI. However, increased antibiotic resistance in the general public, carry over antibiotic residues in the food chain and contamination of poultry meat with antibiotic resistant foodborne pathogens are emerging as significant food safety and public health risks associated with the use of antibiotics in poultry. In addition, the use of antibiotics in the animal industry poses a serious environmental hazard. Thus, to eliminate or reduce these risks and to preserve effective treatment options for public health, rational development of immune prophylactic/immune therapeutic alternatives to antibiotics (on the basis of immune principles and pathogen sensitivity) appears to be an urgently needed realistic approach.

In this context, we have previously demonstrated that *in ovo* administration of 50µg, 20µg or 10µg CpG ODN/embryo or a combination of 10µg CpG ODN plus 15µg poly I:C/embryo resulted >81% survival following severe experimental YSI compared with non-treated or PBS treated groups which exhibited 43% and 60% survival respectively. In the present studies we investigated additional combinations of innate immune stimulants with respect to control of ECM due to experimental YSI. We noticed a significantly improved immune synergetic effect of formulations including: (i) CpG20 µg + avian beta defensin 10µg or (ii) CpG 20µg + poly I:C 15µg or (iii) CpG 20µg + Cyclic polyphosphazene (CPZ) 10µg or (iv) triple combination of CpG 20µg + poly I:C 10µg+ CPZ 10µg. Though, *in ovo* administration of these immune stimulants showed no impact on cecal colonization of *Salmonella*.

Studies to investigate the effect of *in ovo* co-administration of innate immune stimulants and Marek's disease (MD) vaccine showed non-significant differences on post (pathogenic *E. coli*) challenge (PC) survival rates or levels of MD vaccine virus replication in groups treated with (i) innate immune stimulants (ii) Marek's vaccine (iii) co-administration of immune stimulants and MD vaccine. However, relatively lower PC survival rates (compared with other experiments) were observed in these studies. We presume that this may be the result of immune suppression due to live MD vaccine. While, this immune suppressive effect was reduced with an increased amount of CpG ODN.

In conclusion, our data suggest that the above described formulations may serve as potential candidates for replacement of antibiotics for the prevention and control of ECM due to YSI. Furthermore, on basis of these data we may assume that involvement of diverse immune pathways with the use of multiple innate immune stimulants may offer more robust protection from multiple pathogens. However, in this regard further characterization of a repertoire of innate immune stimulants will be helpful in achieving our final goal of increased productivity without use of antibiotics in poultry production.

Keywords: Innate Immune stimulants, Alternatives to antibiotics, Early chick mortality, Yolk sac infection, Avian Patogenic *E. coli* (APEC)

IM2
Dietary β-glucan alters gut health parameters and reduces *Salmonella* shedding in pigs

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Salmonella, while not typically a pathogenic agent in pigs, can cause disease in humans through contaminated food products. Therefore, methods to limit *Salmonella* colonization and/or shedding in pigs are warranted to reduce the incidence of food borne infections. The generation and spread of antimicrobial resistance genes are a major public health concern and non-antibiotic methods are needed to improve animal health and prevent the spread of food borne illnesses. The impact of dietary β-glucan, an innate immunomodulating agent, on intestinal gene expression, circulating monocyte response to innate agonists, and *Salmonella* shedding in pigs was investigated. At weaning (3 wks of age), pigs were fed a control diet or diet modified with *Saccharomyces cerevisiae* β-glucan for the duration of the study. Monocytes were isolated from whole blood at 2 and 4 wks post diets, stimulated *ex vivo* with various innate agonists, and TNF and IL-1β production measured by ELISA. Ileal and cecal tissues were collect at 4 wks post diet from a subset of pigs for gene expression and RNA scope analyses. The remaining pigs were challenged with *Salmonella enterica* serovar I,4,[5],12:i:- and monitored for *Salmonella* shedding over the next 3 weeks. In the cecum, expression of tight junction and mucin stabilizing genes were upregulated in β-glucan fed pigs compared to controls. Increased expression of MUC2 (porcine mucin gene) in the cecum villi and crypts of β-glucan fed pigs was also detected. However, when stimulated with Pam3CSK4 (TLR2 agonist) monocytes from β-glucan pigs produced less IL-1β compared to cells from control pigs. Pigs fed dietary b-glucan had significantly reduced *Salmonella* shedding over the 3 wk period. The reduction of *Salmonella* shedding may be possible through induction of a tolerant phenotype, as circulating monocytes had reduced responses to TLR agonists. The altered gut health parameters may also indicate that epithelial barrier functions were enhanced in β-glucan fed pigs. Overall, β-glucan may serve as a non-antibiotic dietary additive to limit the shedding of the foodborne pathogen, *Salmonella,* through alterations of the local and peripheral immune responses.

Keywords: Beta-glucan, pig, immunomodulation, *Salmonella*, intestine

IM3
Novel hyperimmune egg yolk IgY antibodies developed against protective antigens of *Eimeria* and *Clostridium perfringens* protect against coccidiosis and necrotic enteritis

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Passive immunization with pathogen-specific egg yolk antibodies (IgY) is emerging as a potential alternative to antibiotics for the treatment and prevention of various human and animal diseases. The use of IgY offers several advantages in that laying hens are an excellent source of high-quality polyclonal antibodies, which can be collected noninvasively from egg yolks. With the increase in regulations on the use of antibiotic growth promoters and rise in consumer demand for poultry products from 'Antibiotic Free' or 'No Antibiotics Ever' flocks, the quest for alternative approaches intensified in the recent years. Successful strategies developed as antibiotic alternatives should be both safe for humans and animals, be easily administered, economically feasible and have significant beneficial impact on health and performance. In this report, we describe successful application of egg yolk IgY antibodies to prevent and treat coccidiosis and necrotic enteritis (NE), two most important enteric diseases of poultry which cost industry more than $ 10 million. A series of experiments were conducted to investigate whether passive immunization with oral supplementation of these egg yolk powders as source of *Eimeria*- and *Clostridium perfringens* (CP)-specific antibodies would have any protective effect upon coccidiosis and NE infection in broiler chickens. Four antigens selected from *Eimeria* and CP were proven to be the best therapeutic antibodies in newly hatched broiler chickens when using avian coccidiosis and NE disease challenge models developed at ARS. This is the first report that shows the effectiveness of *Eimeria*- and *Clostridium*-specific egg yolk IgY antibodies against the prevention and treatment of coccidiosis and NE.

Keywords: egg yolk antibodies, passive immunity, coccidia, *clostridium*, necrotic enteritis

IM4
Characterization of NK-lysin antimicrobial protein genes, and their activities, in rainbow trout (*Oncorhynchus mykiss*)

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The regulation, and number, of antimicrobial peptides (AMPs) is not well understood in commercially-important fish species. Based on the new rainbow trout genome assembly, we identified 6 new saposin-like AMP genes and their chromosomal locations. Protein sequence alignments and in silico modeling show that the proteins encoded by those genes belong to the Nk-lysin AMP sub-family (termed: Nkl 1, 2, 3, 4 and Nkl-like a & b).Transcriptomic data show that expression of nkl1-nkl4 mRNAs occurred in many tissues. By contrast, the nkl-like a & b mRNAs are mostly expressed in immune-related tissues. The effects of various aquaculture stressors, and a disease challenge (*F. psychrophilum*; Fp) in rainbow trout, were examined using RNA sequencing. Abundances of nkl1, nkl2, nkl4, and nkl-like a were downregulated by high-temperature and salinity stress, and nkl3 and nkl-like b were downregulated by high-temperature. In the Fp challenge study, abundances of nkl3, nkl4, nkl-like a and nkl-like b, were significantly affected by genetic line (resistant vs non-resistant) and treatment (PBS or Fp), which were further verified by qRT-PCR with spleen tissue sampled at 4 post-challenge time points (6 h, 24 h, 48 h and 144 h). This work represents an initial characterization of these AMPs in rainbow trout, with ongoing in vitro work to characterize how these AMPs affect flavobacterial pathogens, their biofilms, and survival and replication of novirhabdoviral pathogens. Understanding the distribution, regulation and bioactivity of these AMPs may enable rational design of approaches to reduce infectious disease in commercial aquaculture.

Keywords: Antimicrobial Peptides, Nk-lysin, Rainbow trout

SESSION 6
Regulatory pathways to enable the licensing of alternatives to antibiotics and incentives from stakeholders to support their development

ORAL PRESENTATIONS

US FDA's Regulatory Pathway for Alternatives to Veterinary Antimicrobials

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Increased awareness of antimicrobial resistance drives the need to identify and develop alternative products that can be introduced into the marketplace. In the United States, the Agency responsible for the evaluation of the products depends on the product, the primary mechanism of action of the product, and the specific marketing claim. FDA-CVM is responsible for the approval of food additives and new animal drugs. Alternatives to antimicrobials that do not act primarily through direct stimulation of the immune system and are intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function in man or other animals are evaluated as new animal drugs.

New animal drugs are approved for specific indications and conditions of use (e.g. dosage regimens, species, animal class, withdrawal times, prescription status, etc.) which are developed based on a demonstration of effectiveness while balancing any risks associated with target animal safety, human food safety, human user safety, and environmental impact. Congress established the statutory standards for the evaluations of these components.

These regulatory standards can be met through "non-standard" approaches, including the use of published studies, foreign studies, or validated model studies. FDA-CVM has developed processes to assist sponsors with innovative and novel technologies, including those to be used for antimicrobial alternatives, to discuss approval pathways early in the project development plan. FDA-CVM also collaborates with international regulatory agencies to reduce divergent studies for global registration and therefore facilitate drug approvals. FDA-CVM also incentivizes drug development through fee waivers that may be applicable to a sponsor interested in developing an alternative to antimicrobials.

Promoting the authorization of alternatives to veterinary medicinal antimicrobials in the European Union

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Antimicrobial resistance (AMR) to medicinal products for human use and veterinary medicinal products (VMPs) is recognised as a major threat to human and animal health in the European Union (EU) and worldwide. Encouraging and fostering the development of new, alternative medicines that could prevent or treat resistant infections and reduce the need for use of conventional antimicrobials is one of the pillars of fighting against the AMR threat and a high priority for the European Medicines Agency (EMA) and the European medicines regulatory network. The 2016-2020 strategy on antimicrobials of the Committee of the Veterinary Medicinal Products (CVMP) of EMA includes an action to explore measures that could be taken to promote the development and access to market of alternatives to antimicrobials. The new Regulation (EU) 2019/6 strengthens actions to tackle AMR and foster innovation.

In this context, the CVMP has drafted a Reflection Paper 'Promoting the authorisation of alternatives to antimicrobials in the EU' aimed at performing a gap analysis of the measures currently in place and additional measures that could be implemented to promote the development, authorisation and use of VMPs that may represent an alternative approach to the use of conventional antimicrobials, with special focus on alternatives to antibiotics, in animals. Existing gaps were identified through reflection on previous experience with such products at EMA, discussion with regulators from other regions, feedback from stakeholders, and review of the outcome of previous conferences on the subject. Some additional measures and activities to fill the key gaps identified are proposed in the document.

Gaps were identified in a) the existing EU regulatory framework (e.g. lack of consistent terminology, uncertainty on product classification and the existence of new regulatory paradigms for which specific guidance is not current available) which indicate the need for establishing appropriate, harmonised requirements in the legislation and specific guidance on the technical requirements for the authorisation of this type of products. Gaps were also identified in b) the support given to developers and applicants who would benefit from additional measures such as early access to scientific, regulatory and procedural advice, increased incentives to small, medium companies and from the creation of pull incentives to help bringing the most promising or relevant alternatives to the market. Finally, some gaps in c) the area of collaboration and communication with stakeholders were also recognised. The creation of a platform of communication and dialogue with stakeholders on development of alternatives to antimicrobials and the drafting of a roadmap to establishing priorities and setting targets to monitor success of measures implemented are amongst the potential measures proposed.

In conclusion, the results of the analysis identified a number of gaps for which additional measures are proposed and could be implemented to promote the development, authorisation and use of alternatives to antimicrobials in veterinary medicine in the EU. This will require a long-term approach and a set of coordinated actions with stakeholder engagement across the regulatory network and industry. The CVMP Reflection Paper was recently published for public consultation on the EMA webpage (https://www.ema.europa.eu/en/cvmp-reflection-paper-promoting-authorisation-alternatives-antimicrobials-eu) and is open for comments until 30 April 2020.

Legal framework for the approval/designation of alternatives to antibiotics

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Ministry of Agriculture, Forestry and Fisheries of Japan is promoting the developments of alternatives to antibiotics (ATAs) to combat antimicrobial resistance in veterinary fields. ATAs include, but are not limited to, vaccines, cytokines, enzymes, immunomodulators, immunostimulants, organic acids, probiotics, herbal medicines and bacteriophages. In Japan, these products are divided into two different categories based on their active ingredients and label claims (purposes for use), veterinary medicinal products (VMPs) and feed additives, regulated by different laws.

The Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145 of 1960) regulates products, including human medicinal products and VMPs, at each stage of development, manufacturing (importing), marketing, retailing and usage. The purpose of the Act is to improve health and hygiene by providing the control required for securing the quality, efficacy and safety of pharmaceuticals, quasi-pharmaceutical products, cosmetics, medical devices, regenerative medicine products (hereinafter referred to as "pharmaceuticals, etc.") and for preventing the occurrence or spread of health and hygiene-related hazards caused by the use of those pharmaceuticals, etc. by taking measures against designated substances, and by taking necessary measures for the promotion of research and development of pharmaceuticals, medical devices and regenerative medicine products which fulfill particularly high medical needs. In accordance with the Act, a person intending to market a VMP shall, for each product, obtain marketing approval of the Minister of Agriculture, Forestry and Fisheries with respect to its marketing. The approval shall not be granted when the VMP does not possess effects indicated in the dossier, has harmful action outweighing its effects or does not have appropriate quality.

The Act on Safety Assurance and Quality Improvement of Feeds (Act No. 35 of 1953) regulates feeds and feed additives. The purpose of the Act is to contribute to public safety and stable production of livestock products by regulating the production of feeds and feed additives, setting official specifications for feeds, conducting tests of feeds in conformity with the official specifications so as to provide the assured safety and improved quality of feeds. Feed additives in the Act refer to those used in feeds by methods such as addition, mixture and infiltration to prevent deterioration of quality of feeds, to supply nutrient ingredients and other effective ingredients of feed and to promote efficient use of feed nutrient ingredients, which are designated by the Minister of Agriculture, Forestry and Fisheries after consultation with the Agricultural Materials Council. The Agricultural Materials Council reviews efficacy, residue and safety of candidate feed additives by the dossier submitted by the company.

In this presentation, processes toward approval of VMPs and designation of feed additives in Japan are shown and discussed.

Industry perspective on the registration of alternatives to antibiotics

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There is a clear and undisputed need to fight antimicrobial resistance, in the interest of both animal and public health. Also given the decreased entry of new veterinary antibiotic treatment options in the last decades, it is imperative to find and register alternatives for the currently available antibiotics. Health for Animals is aware and committed to this fight. There are numerous types of "alternatives to antibiotics" (ATA). The definition of ATA is not evident, as most ATA would also respond to the usual functional definition of an antibiotic. In addition, the definition of what a veterinary medicinal product is can be different depending on the geography. There are both "classic veterinary medicinal products" (vaccines, phytochemicals, etc…) and additives (organic acids, herbal, botanical and mineral compounds, etc.) and there are solutions that are not easily classified in the previous categories (bacteriophages, in feed antibodies, a-specific immunostimulators, gene editing technology derived products…). The hurdles from a regulatory perspective are numerous, from an industry and from an authority perspective.

A first hurdle lies in the definition and classification as alluded to above. In particular, new technologies risk to be confronted with an unclear classification, which leads to the second hurdle. The second hurdle is within the type of regulatory pathway and the inherent lack of predictability associated it Current frameworks are based on precise and clear requirements for a specific product-claim combination and cannot easily deal with ambiguity and the specificities of new technologies. Regulators so far have proven hesitant to allow claims such as reducing the need/use of antibiotic treatment. The assessment of safety and efficacy of new technologies and approaches will require new assessment paradigms, while a benefit-risk assessment will learn to consider more the secondary benefits. A lack of regulatory convergence globally is a third hurdle. VICH should be the place where we proactively define technical requirements, also for new technologies. We continue to observe a *tendency* to start from a regional or local framework. The animal health industry and all stakeholders must support OIE initiatives to obtain modern and flexible regulatory systems, to control illegal and falsified medicines and to implement the OIE standards in general. A fourth hurdle that should be taken into account proactively is the public and consumer acceptance of new technologies, which has proved not easy and should not be taken for granted even when we know the science backs us up. The fifth and crucial hurdle is creating a regulatory environment that can foster and stimulate innovation even and especially in an environment characterized by risk and uncertainty. The main driver for establishing such regulatory environment is sufficient protection of the data created, allowing sufficient time for an appropriate return on investment. HealthforAnimals and the animal health industry in general have a long-standing commitment regarding antibiotics. Since 2017, we have defined five principles in our "Antibiotics Commitment". These guiding principles include the judicious and responsible use of antibiotics, the promotion of disease prevention and an increased access to products and expertise and the Investment in development of products for prevention and treatment, including of course, alternative to antibiotics.

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