

A silver microscope is positioned in the center of the top section, set against a background of a light blue network of interconnected nodes and lines. To the right of the microscope, there are several blue icons representing bacteria and viruses. The text 'Alternatives to Antibiotics' is written in a bold, sans-serif font, with the letter 'A' being significantly larger than the rest of the words. The 'i' in 'Antibiotics' has a blue pill icon as its dot.

Alternatives to Antibiotics

2nd International Symposium
on Alternatives to Antibiotics (ATA)
Challenges and Solutions in Animal Production


OIE Headquarters, Paris, France
12-15 December 2016

Programme

A N D

Book of Abstracts

The bottom section of the cover features a light blue network of interconnected nodes and lines, similar to the top section. Interspersed within this network are various blue icons, including chemical structures, bacteria, and viruses.



**PROGRAMME
& BOOK
OF ABSTRACTS**

Alternatives to 
Antibitics

**2nd International Symposium
on Alternatives to Antibiotics (ATA)
Challenges and Solutions in Animal Production**

OIE Headquarters, Paris, France
12-15 December 2016

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WELCOME TO

2nd International Symposium on Alternatives to Antibiotics (ATA) Challenges and Solutions in Animal Production

OIE Headquarters, Paris, France
12-15 December 2016

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 the perspective from research funders

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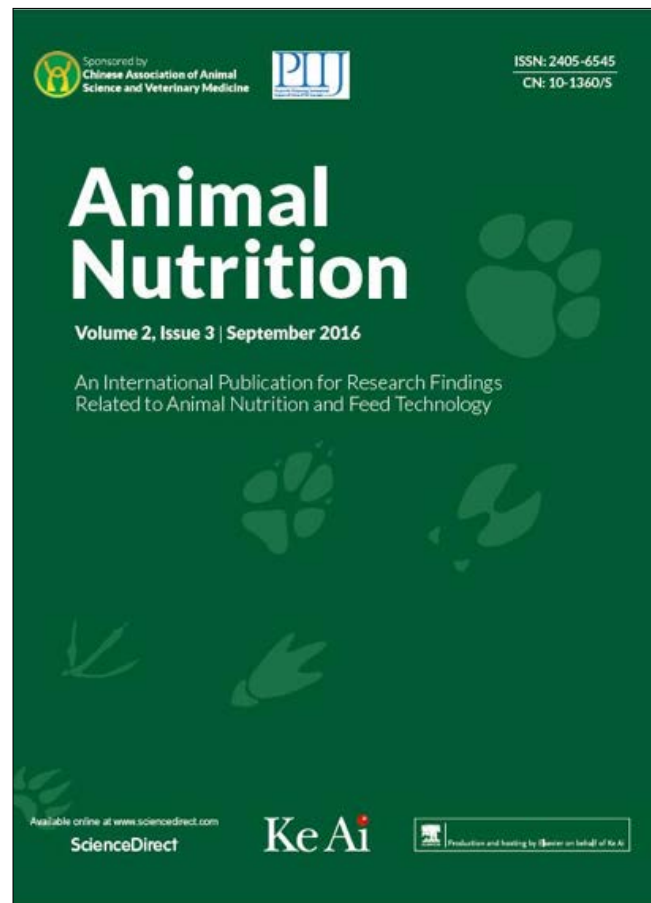
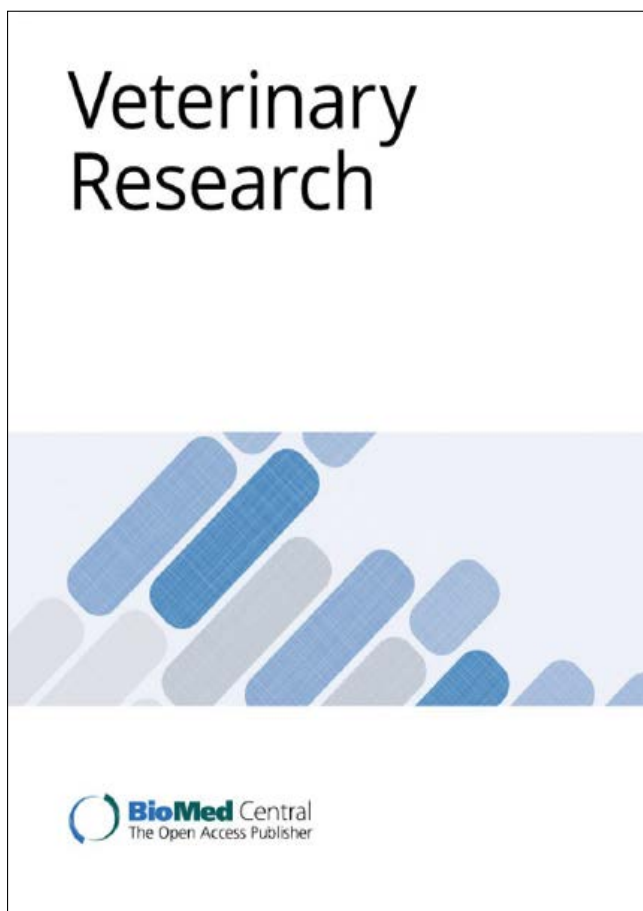
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OIE Headquarters, Paris, France
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SUPPORTING JOURNALS

The following journals have agreed to publish scientific outcomes from the symposium, which will be published in the form of review articles from the six symposium sessions. Review articles will provide in depth scientific information on promising new drugs and biologics that meet the definition of alternatives to antibiotics that could reduce the use of medically important antibiotics. Importantly, where feasible, the review articles will capture from the expert panel discussions challenges, opportunities, and solutions that will enable the research and development of alternatives to antibiotics and enable their registration and commercialization.



SUPPORTING ORGANIZATIONS



United States Department of Agriculture



2nd International Symposium on Alternatives to Antibiotics (ATA) Challenges and Solutions in Animal Production

OIE Headquarters, Paris, France
12-15 December 2016

PROGRAMME

DAY 1: MONDAY 12 DECEMBER 2016

Opening Ceremony – Keynote Presentation

18:00–18:15	Welcome Address	Monique Éloit DIRECTOR GENERAL, WORLD ORGANISATION FOR ANIMAL HEALTH (OIE)
18:15–18:30	Program Review – Objectives and Expected Outcomes	Cyril G. Gay NATIONAL PROGRAM LEADER, ANIMAL PRODUCTION AND PROTECTION, AGRICULTURAL RESEARCH SERVICE, USDA, BELTSVILLE, MD, USA
18:30–19:00	Keynote Presentation: New approaches to address antimicrobial resistance in animals	Harald Brüssow SENIOR RESEARCH SCIENTIST AND EXPERT, NESTLE RESEARCH CENTRE, NUTRITION AND HEALTH RESEARCH, HOST-MICROBE INTERACTION, SWITZERLAND
19:00–20:00	RECEPTION	

DAY 2: TUESDAY 13 DECEMBER 2016

Session 1 – Vaccines

CHAIRS: FILIP VAN IMMERSEEL, FACULTY OF VETERINARY MEDICINE, GHEENT UNIVERSITY, BELGIUM

KARIN HOELZER, OFFICER, HEALTH PROGRAMS, THE PEW CHARITABLE TRUSTS, USA

09:00–09:30	Prioritization of diseases for which vaccines could reduce antimicrobial use in animals	Elisabeth Erlacher-Vindel HEAD OF THE SCIENCE AND NEW TECHNOLOGIES DEPARTMENT, WORLD ORGANISATION FOR ANIMAL HEALTH (OIE)
09:30–10:00	Challenges and solutions for improved intestinal vaccinations	Eric Cox LABORATORY OF IMMUNOLOGY, FACULTY OF VETERINARY MEDICINE, GHEENT UNIVERSITY, BELGIUM
10:00–10:15	Heat stable <i>Bacillus Spores</i> as Vaccines	Simon L. Cutting SPOREGEN LTD, ROYAL HOLLOWAY UNIVERSITY OF LONDON, EGHAM, SURREY, UNITED KINGDOM
10:15–10:30	Benefits of a Herpesvirus of Turkey vector vaccine for Infectious Bursal Disease (HVT-IBD) in control of immuno-depression in broiler chickens and decrease of use of antibiotic medication	Stephane Lemiere MERIAL S.A.S., AVIAN GLOBAL TECHNICAL SERVICES, LYON, FRANCE
10:30–11:15	COFFEE BREAK: POSTER SESSION	

Session 1 – Vaccines (cont.)

11:15–11:30	Recombinant orally effective vaccine platforms expressing putative conserved antigens for reduced antimicrobial usage in poultry	Lisa R. Bielke THE OHIO STATE UNIVERSITY, DEPARTMENT OF ANIMAL SCIENCES, COLUMBUS, OHIO, USA
11:30–11:45	Opportunities and problems in vaccination against opportunistic pathogens: The example of <i>Clostridium perfringens</i> in calves and chickens	Evy Goossens DEPARTMENT OF PATHOLOGY, BACTERIOLOGY AND POULTRY DISEASES, FACULTY OF VETERINARY MEDICINE, GHENT UNIVERSITY, MERELBEKE, BELGIUM
11:45–12:00	Pasteurellaceae oral vaccine vector for economically important diseases of cattle	Robert Briggs NATIONAL ANIMAL DISEASE CENTER, AGRICULTURAL RESEARCH SERVICE, USDA, AMES, IOWA, USA
12:00–12:15	Reduction of antibiotic treatments in broilers by the use of a live Coccidiosis vaccine in rotation with anticoccidial feed	Jesus Rubio POULTRY BUSINESS UNIT, HIPRA, SPAIN
12:15–13:00	Session 1 Expert Panel	Elisabeth Erlacher-Vindel Eric Cox Simon L. Cutting Kemal Karaca Alicia Umiza OIE, FRANCE GHENT UNIVERSITY, BELGIUM SPOREGEN LTD, UNITED KINGDOM ELANCO, USA ZOETIS, SPAIN
13:00–14:00	LUNCH: POSTER SESSION	

Session 2 – Microbial-derived products

CHAIRS: BRUCE S. SEAL, OREGON STATE UNIVERSITY CASCADE, BEND, OREGON, USA

DJAMEL DRIDER, CHARLES VIOLETTE INSTITUTE, UNIVERSITÉ DE LILLE, INRA, LILLE, FRANCE

14:00–14:30	Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials	David Bikard INSTITUT PASTEUR, PARIS, FRANCE
14:30–15:00	Probiotic product development with fecal microbiome transplants as alternatives to antibiotics in broiler chickens	Brian Oakley COLLEGE OF VETERINARY MEDICINE, WESTERN UNIVERSITY OF HEALTH SCIENCES, USA
15:00–15:30	Novel antimicrobial compounds from microbial sources	Joseph O. Rich US DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, NATIONAL CENTER FOR AGRICULTURAL UTILIZATION RESEARCH, RENEWABLE PRODUCT TECHNOLOGY, PEORIA, IL, USA
15:30–16:15	COFFEE BREAK: POSTER SESSION	
16:15–16:45	Bacteriocins as alternatives to antibiotics: the case of enterocins DD14 and DD28	Djamel Drider INSTITUT CHARLES VIOLETTE, UNIVERSITÉ DE LILLE, FRANCE
16:45–17:15	Artilynsins – Novel antibacterial tools for industrial animal protein production	Stefan Miller LYSANDO AG, TRIESENBERG, LIECHTENSTEIN
17:15–17:45	Microbial-ecology guided discovery of antibiofilm compounds from marine sponges	Jason Kwan DIVISION OF PHARMACEUTICAL SCIENCES, SCHOOL OF PHARMACY, UNIVERSITY OF WISCONSIN—MADISON, MADISON, WISCONSIN, USA
17:45–18:30	Session 2 Expert Panel	David Bikard Brian Oakley Joseph Rich Stefan Miller Estelle Devillard Gérard Bertin INSTITUT PASTEUR, FRANCE WESTERN UNIVERSITY OF HEALTH SCIENCES, USA AGRICULTURAL RESEARCH SERVICE, USDA, USA LYSANDO AG, LIECHTENSTEIN ADISSEO, FRANCE EUROPEAN PROBIOTIC ASSOCIATION, FRANCE

DAY 3: WEDNESDAY 14 DECEMBER 2016**Session 3 – Phytochemicals**CHAIRS: **HYUN LILLEHOJ**, AGRICULTURAL RESEARCH SERVICE, USDA, BELTSVILLE, MARYLAND, USA**YANHONG LIU**, ASSISTANT PROFESSOR, UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA, USA

09:00–09:30	Dietary phytonutrients as alternatives-to-antibiotics in agricultural animals: Mode of action in modulating cross-talks among immunity, disease resistance and gut microbiota	Hyun Lillehoj ANIMAL BIOSCIENCES AND BIOTECHNOLOGY LABORATORY, AGRICULTURAL RESEARCH SERVICE, USDA, BELTSVILLE, MARYLAND, USA
09:30–10:00	Dietary phytonutrients enhance disease resistance in swine	Yanhong Liu UNIVERSITY OF CALIFORNIA, DAVIS, USA
10:00–10:30	Phytonutrients as alternative feeding strategy to improve performance of cattle without using an antibiotics	Sergio Calsamiglia ANIMAL NUTRITION AND WELFARE SERVICE, UNIVERSITAT AUTÒNOMA DE BARCELONA, SPAIN
10:30–11:00	COFFEE BREAK: POSTER SESSION	
11:00–11:30	Impact of dietary tannins on rumen microbiota in bovine	Mariano E. Fernández Miyakawa INSTITUTO DE PATOBIOLOGÍA, CENTRO NACIONAL DE INVESTIGACIONES AGROPECUARIAS, INSTITUTO NACIONAL DE TECNOLOGÍA AGROPECUARIA, BUENOS AIRES, ARGENTINA
11:30–12:00	Meta-analysis of broiler research shows that Varium™ results in feed efficiency equal to antibiotics	Fang Chi AMLAN INTERNATIONAL, CHICAGO, USA
12:00–12:45	Session 3 Expert Panel	Hyun Lillehoj Yanhong Liu Sergio Calsamiglia Ron Cravens Mariano E. Fernández Miyakawa Jamie G. Nickerson AGRICULTURAL RESEARCH SERVICE, USDA, USA UNIVERSITY OF CALIFORNIA, USA UNIVERSITAT AUTÒNOMA DE BARCELONA, SPAIN AMLAN INTERNATIONAL, USA INSTITUTO NACIONAL DE TECNOLOGÍA, ARGENTINA AVIAGEN INC., USA
12:45–14:00	LUNCH: POSTER SESSION	
Session 4 – Immune-related products		
CHAIRS: HENK P. HAAGSMAN , PROFESSOR OF MOLECULAR HOST DEFENSE, CHAIR OF THE DEPARTMENT OF INFECTIOUS DISEASES AND IMMUNOLOGY, UTRECHT UNIVERSITY, THE NETHERLANDS		
PETER M. HEEGAARD , PROFESSOR OF INNATE IMMUNOLOGY, DENMARK TECHNICAL UNIVERSITY (DTU), NATIONAL VETERINARY INSTITUTE, DENMARK		
14:00–14:30	Innate defense mechanisms and passive immunity	Peter M. Heegaard PROFESSOR OF INNATE IMMUNOLOGY, DENMARK TECHNICAL UNIVERSITY (DTU), NATIONAL VETERINARY INSTITUTE, DENMARK
14:30–15:00	Immunomodulators derived from host defense peptides	Albert van Dijk DEPARTMENT OF INFECTIOUS DISEASES AND IMMUNOLOGY, UTRECHT UNIVERSITY, THE NETHERLANDS
15:00–15:30	Egg shell membrane improves immunity of post hatch poultry: a paradigm for nutritional immunomodulation	Narayan C. Rath POULTRY PRODUCTION AND PRODUCTS SAFETY RESEARCH, FAYETTEVILLE, ARKANSAS, USA
15:30–16:15	COFFEE BREAK: POSTER SESSION	
16:15–16:35	Green algal sulfated polysaccharides: a natural alternative to antibiotics via modulation of the intestinal immune response	Mustapha Berri ISP, INRA, UNIVERSITÉ FRANÇOIS RABELAIS DE TOURS, NOUZILLY, FRANCE
16:35–16:55	Maintenance of vascular integrity via ARF6-GTP inhibition protects mice from MDR <i>Acinetobacter</i> infection	Ashraf S. Ibrahim LA BIOMED. RESEARCH INSTITUTE, HARBOR-UCLA MEDICAL CENTER, TORRANCE, CALIFORNIA, USA
16:55–17:15	Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhea	Chris Juul Hedegaard NATIONAL VETERINARY INSTITUTE, TECHNICAL UNIVERSITY OF DENMARK, FREDERIKSBERG, DENMARK

Session 4 – Immune-related products (cont.)

17:15–18:00	Session 4 Expert Panel	Peter Heegaard Henk Haagsman Narayan Rath Mustapha Berri Raksha Tiwari Stuart Reeves Robert Zolynas	DENMARK TECHNICAL UNIVERSITY, DENMARK UTRECHT UNIVERSITY, THE NETHERLANDS AGRICULTURAL RESEARCH SERVICE, USDA, USA UNIVERSITÉ FRANÇOIS RABELAIS DE TOURS, FRANCE ZOETIS, USA DIAMOND V, USA BAYER ANIMAL HEALTH GMBH, GERMANY
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DAY 4: THURSDAY 15 DECEMBER 2016

Session 5 – Innovative Drugs, Chemicals, and Enzymes

CHAIRS: CHENGBO YANG, ASSISTANT PROFESSOR, NUTRITION AND NUTRITIONAL BIOCHEMISTRY, DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF MANITOBA, CANADA

JIAN PENG, PROFESSOR, HUAZHONG AGRICULTURAL UNIVERSITY, PEOPLE'S REPUBLIC OF CHINA

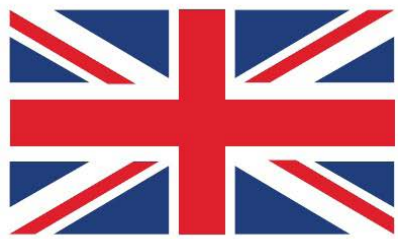
09:00–09:30	Non-resistance-inducing antibacterial polymers and nanomedicines		Song Liu ASSOCIATE PROFESSOR, DEPARTMENT OF BIOSYSTEMS ENGINEERING, UNIVERSITY OF MANITOBA, CANADA
09:30–10:00	Bacterial anti-virulence strategy as an alternative to antibiotics		Damien Maura DEPARTMENT OF SURGERY, DEPARTMENT OF MICROBIOLOGY AND IMMUNOBIOLOGY, HARVARD MEDICAL SCHOOL AND MASSACHUSETTS GENERAL HOSPITAL, SHRINERS HOSPITALS FOR CHILDREN BOSTON, BOSTON, MASSACHUSETTS, USA
10:00–10:30	COFFEE BREAK: POSTER SESSION		
10:30–10:45	Cationic amphiphilic non-hemolytic synthetic polymers as potential agents to combat bacteria with antibiotics resistance		Nanloh Yang CENTER FOR ENGINEERED POLYMERIC MATERIALS AND CHEMISTRY, CITY UNIVERSITY OF NEW YORK, USA
10:45–11:00	Reducing antimicrobial dependence through feed additives that bolster immune readiness, disease resistance, and production performance in farmed fish		Benjamin Beck UNITED STATES DEPARTMENT OF AGRICULTURE – AGRICULTURAL RESEARCH SERVICE, AQUATIC ANIMAL HEALTH RESEARCH UNIT, AUBURN, ALABAMA USA
11:00–11:15	Development of technique to circularly produce enzyme, probiotics and oligosaccharide, and solutions to replace antibiotics in a low-cost way		Nicole Zhou WUHAN SUNHY BIOLOGY CO, PEOPLE'S REPUBLIC OF CHINA
11:15–12:00	Session 5 Expert Panel	Chengbo Yang Jian Peng Song Liu Xianfeng Peng Joris Michiels Benjamin Beck Sam Barringer Laurence Rahme	UNIVERSITY OF MANITOBA, CANADA HUAZHONG AGRICULTURAL UNIVERSITY, PEOPLE'S REPUBLIC OF CHINA UNIVERSITY OF MANITOBA, CANADA GUANGZHOU INSIGHTER BIOTECHNOLOGY CO., LTD., PEOPLE'S REPUBLIC OF CHINA GHENT UNIVERSITY, BELGIUM AGRICULTURAL RESEARCH SERVICE, USDA, USA DIAMOND V, USA HARVARD MEDICAL SCHOOL, USA
12:00–13:00	LUNCH: POSTER SESSION		

Session 6 – Regulatory Pathways to Enable the Licensing of Alternatives to Antibiotics and Issues and Opportunities from Funders’ Perspective

CHAIRS: FAYE IOANNOU, EUROPEAN MEDICINE AGENCY, LONDON, UNITED KINGDOM

CINDY BURNSTEEL, OFFICE OF NEW ANIMAL DRUG EVALUATION, CENTER FOR VETERINARY MEDICINE, FDA, SILVER SPRING, MD, USA

13:00-13:30	EU approach to authorization of novel technologies with particular emphasis on alternatives to antibiotics	Faye Ioannou EUROPEAN MEDICINE AGENCY, LONDON, UNITED KINGDOM
13:30-14:00	FDA’s innovation initiative to evaluate novel emerging technologies and international cooperation in the area of innovation	Cindy Burnsteel OFFICE OF NEW ANIMAL DRUG EVALUATION, CENTER FOR VETERINARY MEDICINE, FDA, SILVER SPRING, MD, USA
14:00-14:30	Regulatory perspective from Asia	TBD
14:30-15:00	Do innovative solutions require novel regulatory paradigms?	Patricia Logie ADVISOR, GLOBAL FOOD ANIMAL INNOVATION, ELANCO, UNITED KINGDOM
15:00-15:30	The sequence of success: antibiotic alternative development from the startup perspective	Lucia Mokres CHIEF MEDICAL OFFICER EPIBIOIME, USA
15:30–16:00	COFFEE BREAK: POSTER SESSION	
16:00-16:45	Session 6 Expert Panel: Regulatory Pathways	David Mackay Jean-Pierre Orand Cindy Burnsteel Carel du Marchie Sarvaas Su-San Chang EUROPEAN MEDICINES AGENCY, UNITED KINGDOM FRENCH AGENCY FOR VETERINARY MEDICINAL PRODUCTS, FRANCE CENTER FOR VETERINARY MEDICINE, FDA, USA HEALTHFORANIMALS, BELGIUM PINGTUNG AGRICULTURAL BIOTECHNOLOGY PARK, COUNCIL OF AGRICULTURE, TAIWAN
16:45-17:30	Session 6 Expert Panel: Issues from Funders’ Perspective	Jean-Charles Cavitte Gary Sherman Dennis Dixon Luke Dalton Karin Hoelzer Stacy Sneeringer EUROPEAN COMMISSION, BELGIUM NATIONAL INSTITUTE OF FOOD AND AGRICULTURE (NIFA), USA NATIONAL INSTITUTES OF HEALTH (NIH), USA STAR-IDAZ, UNITED KINGDOM THE PEW CHARITABLE TRUSTS, USA USDA-ERS, USA
17:30-18:00	Conclusions and next steps	Cyril G. Gay NATIONAL PROGRAM LEADER, ANIMAL PRODUCTION AND PROTECTION, AGRICULTURAL RESEARCH SERVICE, USDA, BELTSVILLE, MD, USA



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The logo consists of an orange swoosh that underlines the text 'sunHY' in a teal, italicized sans-serif font.
sunHY 新华扬



**The Reference
in Prevention
for Animal Health**



**ORAL
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Antibiotics

**2nd International Symposium
on Alternatives to Antibiotics (ATA)
Challenges and Solutions in Animal Production**

OIE Headquarters, Paris, France
12-15 December 2016

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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 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 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 enhancement of 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 alternatives to antibiotics 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 and agriculture. 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 resistance 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**GUT MICROBIOME MODULATIONS - AN ALTERNATIVE TO ANTIBIOTICS?****Harald Brüssow**

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The gut microbiome is currently intensively studied for its association with many physiological and health aspects of the host (digestion, obesity, immunity development, cardiovascular disease, infectious diseases). Infectious diseases are frequently treated with antibiotics, but due to resistance development alternatives are urgently needed. Interventions that modify the gut microbiota composition can potentially positively impact on gut infections. Currently, there are four main ways to achieve that goal. First, by changing the nutrient intake the growth of specific beneficial microbes can be stimulated or inhibited. This essentially refers to gross diet change, fibres, and prebiotics. Yet, by using chemically defined human milk oligosaccharides researchers hope to achieve fine tuning of microbiome composition. Second, beneficial microbes can be directly (re-)introduced into the gut. This was tried with either the transplantation of an entire microbiota from a donor to a dysbiotic recipient (fecal microbiota transplantation for *Clostridium difficile* patients; vaginal microbiota transplantation to infants born by Caesarian section) or more defined with specific probiotics that might serve as keystone species to catalyze a different microbiota constellation. A third approach is to eliminate the undesired members of the gut microbiota with antimicrobials that target pathogens more specifically than antibiotics thereby avoiding collateral damage to beneficial bacteria. For this very specific anti-pathogen approach antibodies were used, sometimes produced from hyper-immunized milking cows. Currently also bacterial viruses (bacteriophages) that mostly show a host range restricted to a single bacterial species are explored as an alternative for antibiotic-resistant pathogenic bacteria. Finally, to a more limited extent the gut environment can be modified by physico-chemical interventions like pH modifying drugs. Pertinent features from clinical trials using these different approaches will be illustrated in the keynote lecture.



SESSION 1

Vaccines

ORAL PRESENTATIONS

1.1 PRIORITISATION OF DISEASES FOR WHICH VACCINES COULD REDUCE ANTIMICROBIAL USE IN ANIMALS

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Antimicrobial resistance 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. To combat antimicrobial resistance, the World Organisation for Animal health (OIE) develops science-based intergovernmental standards and guidelines covering terrestrial animals and aquaculture.

The OIE also contributed to the development of the WHO Global Action Plan on Antimicrobial Resistance, supported by the OIE's 180 Member Countries through a Resolution unanimously adopted in May 2015.

As a contribution to the global actions to address antimicrobial resistance, and in consideration of the use of vaccines to prevent diseases as one of the possible options to reduce the use of antimicrobial agents at the global level, the OIE convened an *ad hoc* Group on Prioritisation of Diseases for which Vaccines Could Reduce Antimicrobial Use in Animals in April 2015.

Animal diseases of global importance for which availability and use of vaccines could reduce the use of antimicrobial agents in animals were identified and recommendations were made to better target research programmes for new or improved vaccines. The Group focused on pigs, poultry and fish as a first step and considered priority animal diseases and associated drivers for antibiotic use.

The outcome of this work was the development of rankings of priority animal diseases by species for which new or improved vaccines could potentially reduce reliance on antimicrobials, with the aim of providing direction for priority investment to policy makers, research communities, and the private sector.

Towards this goal of preserving the efficacy of antimicrobials and reducing reliance on them, ensuring availability of safe and effective vaccines is essential. Targeting priority diseases for development of vaccines should help focus efforts towards new technologies, innovative formulations, and alternate routes of administration to more efficiently elicit protective immune responses.

Reference

World Organisation for Animal Health (2015). – Report of the *ad hoc* Group on Prioritisation of Diseases for which Vaccines Could Reduce Antimicrobial Use in Animals. Annex 5, 35-50. Available at: www.oie.int/fileadmin/Home/eng/International_Standard_Setting/docs/pdf/SCAD/A_SCAD_Sept2015.pdf

1.2 CHALLENGES AND SOLUTIONS FOR IMPROVED INTESTINAL VACCINATIONS

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Most pathogens cause disease by either colonizing mucosae or by using mucosae as a port of entry. Mucosae have developed ingenious mechanisms to preventing pathogens from infecting the host consisting out of barriers, innate defense mechanisms and adaptive immunity. However, mucosae also continuously encounter environmental antigens and are colonized by commensals. This is especially true for the intestinal mucosa, which comes in contact with many harmless food antigens and the normal intestinal gut flora. To cope with these harmless antigens several tolerance mechanisms exist. So to induce antigen-specific immunity, a vaccine has to pass protective barriers and to overcome innate defense and tolerance mechanisms before it can activate the gut-associated lymphoid tissue (GALT). The GALT consists of organized lymphoid tissue, the Peyer's patches and isolated lymphoid follicles, localized underneath a follicle-associated epithelium containing M cells which can transfer antigen to the GALT.

To induce antigen-specific immunity, a vaccine has to: 1. reach in an immunogenic form the epithelium close to the GALT, 2. pass the epithelial barrier to reach cells which can present the antigen to the lymphocytes of the GALT; 3. induce an antigen-specific immune response which can prevent the pathogen to cause disease. Oral vaccines can be subdivided in live attenuated pathogens, vector-based vaccines or dead vaccines. The latter are inactivated pathogens, particles which target, carry, contain and/or protect the antigens or soluble subunits, which can be targeted to the immunisation sites in the gut thereby enhancing their uptake. Innovative adjuvants which target pattern recognition receptors, or other activation receptors on epithelial cells, antigen presenting cells and/or lymphocytes might be necessary to sufficiently stimulate the mucosal immune system. Often the desired outcome is a pathogen-specific IgA response which prevent colonization, invasion and/or neutralize toxins.

1.3 HEAT STABLE *BACILLUS* SPORES AS VACCINES

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Bacterial spores are robust dormant entities able to withstand exposure to high temperatures, UV irradiation and desiccation. A number of species of these aerobic bacteria are in current use as probiotics both for humans and for inclusion in animal feeds. Recently, a number of food products have entered the market where *Bacillus* spores are included and where spores can survive short-term exposure to temperatures as high as 235°C.

Bacillus spores have also been shown to be suitable for mucosal delivery of heterologous antigens. As vaccines mucosal delivery is attractive for well-understood reasons but the heat stability of spores is particularly useful for veterinary vaccines where the vaccine could be considered for inclusion in feed. Currently, a spore vaccine to White Spot Syndrome virus, an important disease of farmed shrimps, is being field tested in Vietnam. For veterinary vaccines a spore vaccine to *C. perfringens* infection in poultry has been developed in collaboration with the Univ. Ghent and funded by the EU Horizon 2020 program (SAPHIR). Finally, an oral spore vaccine to *Clostridium difficile* infection in humans is currently under phase 1 evaluation in Germany.

This presentation will summarise the utility of *Bacillus* spores for oral vaccination with a focus on veterinary vaccines.

1.4

BENEFITS OF THE HERPESVIRUS OF TURKEY VECTOR VACCINE OF INFECTIOUS BURSAL DISEASE (HVT-IBD) IN CONTROL OF IMMUNO-DEPRESSION IN BROILER CHICKENS AND DECREASE OF USE OF ANTIBIOTIC MEDICATION

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HVT-IBD vector vaccine allows early and life-long control of Marek's Disease and IBD in all types of poultry by hatchery vaccination, either *in ovo* or at day-old. Parameters studied in large-scale follow-up study included the mortality rate, daily weight gain (g), feed conversion index, final weight (kg), condemnation rate, age at slaughter (days), efficiency index and medication costs (US cents/kg). The vaccination programme was switched from classical vaccination in the field combining different IBD vaccines to the HVT-IBD vector vaccine. Production performance was calculated using the European Performance Index (EPI), in high IBD field challenge farms with a total of 35,655,852 chickens from 2,377 flocks in the former year and 38,306,065 chickens from 2,553 flocks the year after. Percentages of reported clinical IBD cases and described cases of very virulent IBD were compared between years. Mortality rate was found to decrease from 6.76% to 6.23%; daily weight gain increased from 55 g to 57 g; the feed conversion index decreased from 1.88 to 1.83; the final weight increased from 2.340 kg to 2.374 kg; the condemnation rate decreased from 0.58% to 0.39%; age at slaughter decreased from 43 days to 42 days; the EPI increased from 268 to 287 and medication costs decreased from 1.36 US cents/kg to 0.91 US cents/kg. Between years the percentage of reported clinical cases of IBD dropped from 4.33% to 0.19%. Control of immuno-depression induced by IBD was demonstrated by decrease of antibiotic medication cost when using the HVT-IBD vector vaccine, as compared to classical IBD vaccines. Such results have been then reported for example in Egypt or in China in the context of strong challenges of IBD virus infections associated to other diseases, as avian influenza in both areas, or more particularly in Egypt at the time of the study, infectious bronchitis involving variant strains.

1.5 RECOMBINANT ORALLY EFFECTIVE VACCINE PLATFORMS EXPRESSING PUTATIVE CONSERVED ANTIGENS FOR REDUCED ANTIMICROBIAL USAGE IN POULTRY

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Subunit vaccines which possess highly conserved antigens offer potential to provide cross-protection among multiple species of pathogen, and ability to express immune-enhancing molecules to promote immune response. A *Salmonella*-vectored subunit vaccine was developed in attenuated *S. Enteritidis* (SE) with high mobility group box 1 immune-enhancing sequence (H), peptidoglycan associated lipoprotein (P), and Omp18 protein Cj0113 (C) in different arrangements for evaluation against *S. Heidelberg* (SH) challenge. In experiment 1, chicks were orally vaccinated with SE-CPH, SE-HCP, SE-CHP on d1 and d14. On d17 all birds were challenged with SH, and SH detected on d23 and d28. Only SE-CPH reduced ($P<0.05$) cecal SH recovery, $0.34\pm 0.23 \log_{10}$ cfu when compared to unvaccinated control at $1.19\pm 0.26 \log_{10}$ cfu on d23. On d28, SE-CPH reduced recovery at 0.40 ± 0.40 . For experiment 2, chicks were orally vaccinated with SE-CPH, SE-HCP, or SE-CHP on d1. All chicks were orally challenged with SH on d7 and ceca collected on d28 and d35. Compared to unvaccinated control, only SE-CPH reduced ($P<0.05$) SH recovery on d28. Experiment 3 tested a *Bacillus subtilis*-vectored (BS) vaccine containing H, thrombospondin-related adhesive protein (T), and/or rhomboid-like protease 5 (M) for protection against *Eimeria*. Birds were orally vaccinated with BS-TMH, BS-TH, or BS-MH, on d4 and d14, followed by *Eimeria maxima* challenge on d21. At 28d of age, body weight (BW) and body weight gain (BWG) were recorded. Mortality was documented to determine vaccine efficacy. Eight days post-challenge BW was higher in chicks vaccinated with BS-TMH and BS-MH when compared with non-vaccinated chicks. BWG was significantly higher for all vaccinated groups when compared to controls. Mortality was 0% in the BS-TMH and BS-MH vaccinated groups compared to 17% unvaccinated group. These studies show that subunit vaccines are a viable option for development of vaccines against diseases in poultry to reduce the use of antimicrobials.

1.6 OPPORTUNITIES AND PROBLEMS IN VACCINATION AGAINST OPPORTUNISTIC PATHOGENS: THE EXAMPLE OF *CLOSTRIDIUM PERFRINGENS* IN CALVES AND CHICKENS

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Clostridium perfringens ranks amongst the most widespread bacteria, with a ubiquitous environmental distribution in soil, sewage, food, faeces and as a member of the normal intestinal microbiota. It is an opportunistic pathogen that causes intestinal necrosis when host defence is altered, for example, by predisposing mucosal damage. *C. perfringens* type A causes economically important diseases mainly in animals in intensive rearing conditions. In broiler chickens, *C. perfringens* is responsible for necrotic enteritis, and the NetB toxin is essential to cause disease. However, vaccination with NetB provides only partial protection against experimental disease and multiple other *C. perfringens* antigens have been shown to provide some part of protection. In calves, *C. perfringens* causes necro-haemorrhagic enteritis. Current clostridial vaccines do not seem sufficient to protect cattle against disease. Recently, alpha toxin was shown to be essential for disease, which creates new opportunities for vaccine development. However, as for necrotic enteritis in broilers, also in cattle vaccination with the causative toxin alone does not seem sufficient to provide full protection.

This presentation will summarize the current problems in vaccination against *C. perfringens* and difficulties in vaccine development, and propose solutions to overcome these difficulties.

1.7 PASTEURELLACEAE ORAL VACCINE VECTOR FOR ECONOMICALLY IMPORTANT DISEASES OF CATTLE

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Oral delivery of modified-live *Mannheimia* and *Pasteurella* vaccines in feedstuffs or milk replacer elicits rapid mucosal and systemic immune response without necessitating individual handling of animals. Vaccine can therefore be delivered without specialized equipment or personnel at the point of first assembly of calves prior to transport to feedlot or stocker operations with demonstrated efficacy. These oral mass-delivered vaccines are safe and highly effective, reducing the use of treatment antibiotics and increasing weight gain in field trials. Adoption of this or similar technology is likely to reduce the antibiotic metaphylaxis often administered to high-risk calves. Further, these bacteria preferentially colonize the palatine and pharyngeal tonsils of cattle, desirable sites for antigen presentation. Because many important veterinary pathogens invade via respiratory or gut mucosa, adaptive mucosal immunity may be critical for control of infection and disease. The USDA is therefore investigating delivery of heterologous viral, bacterial, mycoplasma, and parasite antigens using modified-live bacterial vectors. Selected antigens of bovine viral diarrhea, *Mycoplasma bovis*, *Mycoplasma ovipneumoniae*, *Moraxella* spp. (pinkeye), and *Rhipicephalus* spp. (cattle fever tick) have been expressed by modified-live *Mannheimia* and/or *Pasteurella* vaccine strains. Preliminary animal trials have been conducted which demonstrate humoral and CMI response to the heterologous antigens.

1.8 REDUCTION OF ANTIBIOTIC TREATMENTS IN BROILERS BY THE USE OF A LIVE COCCIDIOSIS VACCINE IN ROTATION WITH ANTICOCIDIAL FEED

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Introduction

Antimicrobial resistance has become a global public health problem in humans and livestock. As a result, the European Union decided to establish a sustainable policy for the use of antibiotics in the veterinary sector. Furthermore, coccidiosis is still a major disease in broiler production and, economically speaking, it is the most important.

It has been already seen that the implementation of a coccidiosis vaccine in a rotation program yields a significant improvement in zootechnical results (Ronsmans *et al.* 2014). The objective of the study was to determine whether the vaccination with this vaccine affected the use of antibiotics in broilers. The hypothesis was that if intestinal health was enhanced, the overall health of broilers farms would improve.

Results

The study was performed in 21 farms in Belgium (56 houses in total). This corresponds to a total of 5,404,000 vaccinated broilers.

All results were categorized into three groups:

- 1/ results of cycle before vaccination,
- 2/ during vaccination,
- 3/ after vaccination.

All treatments were taken into account and were categorized into coccidiosis treatments, gastrointestinal treatments and non-digestive treatments

For coccidiosis treatments, there was a decrease of 76,47% of the use of different active molecules

For gastrointestinal treatments, there was a reduction of the amount of active molecule by 17,39%.

Regarding non digestive treatments, during the vaccination the amount of antibiotics per kg of chicken remain the same.

Discussions and conclusions

The data show that the implementation of a rotational program including coccidiosis vaccines could be one of the solutions to respond to the political expectations concerning antimicrobial resistance.



SESSION 1
Vaccines

POSTER PRESENTATIONS

RECOMBINANT EXPRESSION OF OMPA, OMPC AND BAMA PROTEIN AS UNIVERSAL VACCINE AGAINST *ESCHERICHIA COLI* IN MICE

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Pathogenic *Escherichia coli* (*E. coli*) are one of the primary pathogens in humans and domestic animals, and the infection is commonly caused by multiple serotypes, thus leading to an urgent need for universal vaccines. In this study, the outer membrane proteins (OmpA, OmpC and Surf_Ag_VNR domain (at position 448-810 aa) of BamA) were analyzed in silico for sequence homology. The result showed that all these proteins from *E. coli* CVCC 1515 share a high homology with other *Escherichia*, *Shigella* and *Salmonella* strains. Then the proteins were expressed in BL21 (DE3) using the auto-induction method. After purification, the recombinant proteins were estimated to be approximately 40 kDa by SDS-PAGE with the purity of 93.5% (rOmpA), 96% (rOmpC) and 93.5% (rBamA), respectively. Immunological analysis indicated that the titers of antiserum reached to 1:642,000 (anti-rOmpA), 1:240,000 (anti-rOmpC) and 1:736,000 (anti-rBamA) against the recombinant proteins and 1:140,000 (anti-rOmpA), 1:27,000 (anti-rOmpC) and 1:152,000 (anti-rBamA) against to the whole *E. coli* cells. Moreover, it also generated cross-reaction against *Shigella* and *Salmonella* stains. Opsonophagocytosis assay revealed that the antiserum induced the phagocytic activity of neutrophils against *E. coli*. Survival rate of mice vaccinated with rOmpA, rOmpC, rBamA and PBS was 50%, 50%, 80% and 20%, respectively. These data indicated that the rOmpA, rOmpC, rBamA proteins could serve as promising universal vaccine candidates for the development of protective subunit vaccine against bacterial infection. Additionally, the above protocol would provide the more feasible technical clues and choices for available control of key pathogenic *Escherichia*, *Salmonella* and *Shigella* in epidemic prevention of animal husbandry.

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AUTOGENOUS VACCINES IN GERMAN AND POLISH POULTRY PRODUCTION

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In Germany and Poland the use of autogenous vaccines against bacterial infections is an important component in poultry production. Both countries belong to the top five producers of poultry meat in the EU (Poland 1,783 million tons and Germany 1,512 million tons in 2014). During the last decade the use of autogenous vaccines as an alternative to antibiotic treatment increased in both countries.

The high flexibility of these custom-made vaccines under consideration of different sero-/toxotypes, new bacterial diseases and complex polybacterial infections is an important advantage against other strategies for controlling bacterial infections. Here we give a general overview about the immune response of poultry flocks against different kinds of autogenous vaccines regarding different adjuvants and pathogen combinations. In addition, we show how new pathogens like *Enterococcus cecorum* in ducks, *Bordetella hinzii* and variants of *Ornithobacterium rhinotracheale* in turkey and chicken can be reduced by autogenous vaccination. Finally, we compare the antibiotics consumption and other performance parameters in vaccinated and not vaccinated flocks. These data lead to the conclusion that autogenous vaccination as an alternative approach can successfully control numerous bacterial infections and reduce antibiotic treatment in poultry production.

IDENTIFICATION OF COMMON BACTERIAL ANTIGENIC MARKERS FROM BOVINE DIGITAL DERMATITIS LESIONS USING META-TRANSCRIPTOMICS IN COMBINATION WITH HIGH-DENSITY PEPTIDE-MICROARRAYS

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Bovine digital dermatitis (DD) is the most important infectious cause of lameness in dairy cattle, and a major contributing factor to welfare problems and economic losses in the dairy cattle industry worldwide. DD is a disease that involves chronic dermal inflammatory processes and destruction of collagenous and connective tissues. Multiple *Treponema* species, many of which are not-yet-cultivable, are strongly implicated in disease progression. Despite the economic and welfare importance of this disease, no effective vaccine is available; and there is presently very little knowledge concerning efficacious immunoprophylactic antigens against DD.

It is highly likely that DD-associated treponemes possess considerable antigenic variation, as cows exhibit a variable humoral response against different isolates of *Treponema*. Hence, combinations of antigens from multiple *Treponema* species should be used for the development of disease prevention measures. As treponemes from DD lesions are extremely difficult to culture, identification of these antigens is challenging. To circumvent this problem, we studied the *in situ* gene expression patterns of the microbiome in DD-affected skin lesions and the host antibody response directed at the site of infection. By metatranscriptomics we measured the *in situ* genome-wide transcriptome of the bacterial population in DD-affected skin lesions from 21 dairy cows. From the transcriptome data, we identified a panel of *Treponema* genes that were highly expressed in multiple animals, and we monitored the host immune response to these target genes using high-density peptide microarrays. By this approach, we identified a small group of antigenic proteins, which were expressed in the majority of the samples, and demonstrated antigenicity when screened against sera from infected animal. Future studies will show if these proteins represent candidates for the development of novel biomarkers or vaccines.

WHAT IS THE BEST WAY TO DELIVER *CLOSTRIDIUM PERFRINGENS* ALPHA TOXIN TO CHICKENS TO YIELD PROTECTION AGAINST AVIAN NECROTIC ENTERITIS?

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Necrotic enteritis in broiler chickens is caused by *Clostridium perfringens*, and there is currently no effective vaccine for necrotic enteritis. NetB was identified as the essential toxin to cause disease, however, the pathogenesis of necrotic enteritis is still not completely understood. Although not essential to cause disease, alpha toxin might also be important during disease and vaccination with either NetB or alpha toxin results in similar levels of protection against necrotic enteritis. Protection against experimental necrotic enteritis by subcutaneous vaccination of broiler chickens with both alpha toxin and NetB has extensively been investigated. Also the use of live attenuated *Salmonella* strains as vector for *C. perfringens* antigens has been explored.

In this study the immune development in broiler chickens after oral vaccination with live recombinant *Bacillus* spores expressing alpha toxoid on the spore coat was compared to the immune response after subcutaneous vaccination with the alpha toxoid. Oral vaccination of the birds resulted in both mucosal and systemic antibodies. Future *C. perfringens* challenge studies are planned to elucidate whether this immunization protocol can provide superior protection against experimental disease.

INVESTIGATING THE POULTRY ENTERIC VIROME: SELECTION OF CANDIDATE AGENTS AND GENES FOR TARGETED INTERVENTIONS

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Viral infections of the avian gastrointestinal tract negatively impact poultry health and performance. A better understanding of the complex viral community present in the poultry gut would allow the design and application of early interventions to reduce the viral load in order to improve gut health and reduce the incidence of enteric syndromes and secondary infections that will make the full transition to antibiotic-free poultry production more problematic. Our metagenomic investigations of the enteric viral community in turkeys and chickens have revealed numerous novel viruses that may play roles in the performance problems and enteric syndromes observed in the field. Some of these newly described viruses remain largely uncharacterized and may be present in some United States geographic regions and not others, and there may be unique and varied genotypes, pathotypes, and geographical isolates circulating in poultry flocks as well. With the exception of certain autogenous vaccines, no specific therapeutic agents currently exist to aid in the control and prevention of poultry enteric viral infections. Part of our current strategy to combat early viral infection in poultry is the development an enterotropic vaccine platform that will lead to highly efficacious vaccines to control enteric diseases of poultry. To this end, we have constructed infectious clones of an enterotropic Newcastle disease virus (NDV) vaccine strain expressing the major antigenic spike glycoproteins of a turkey enteric coronavirus (TCoV) field strain initially detected in our laboratory using diagnostic high-throughput sequencing. The recombinant viruses, rV4/TCoV-S1 and rV4/TCoV-S2, were rescued using reverse genetics technology and the expression of the TCoV S1 and S2 spike glycoprotein subunits was confirmed *in vitro*, and their safety and stability was assessed *in vivo*. This serves as a proof-of-concept for the use of viral metagenomic data to inform the design of recombinant vaccines targeting specific enteric viruses associated with enteric disease in poultry.



SESSION 2
**Microbial-derived
Products**

ORAL PRESENTATIONS

2.1 CRISPR TOOLS TO STUDY AND FIGHT PATHOGENIC BACTERIA

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CRISPR-Cas systems have emerged as a powerful biotechnological tool. The Cas9 protein is a RNA-guided nuclease that can be easily reprogrammed to target any sequence of interest. Our work focuses on the development of CRISPR-Cas9 tools to edit bacterial genomes and control gene expression. Recently we have also shown how CRISPR systems can be used as a sequence-specific antimicrobial. The Cas9 protein can kill bacteria when directed to cut in their chromosome. CRISPR systems can be programmed to target antibiotic resistance or virulence genes specifically, and delivered to bacterial populations using phage capsids. Altogether CRISPR are greatly expanding the toolbox of synthetic biology leading to exciting developments.

2.2

PROBIOTIC PRODUCT DEVELOPMENT WITH FECAL MICROBIOME TRANSPLANTS AS ALTERNATIVES TO ANTIBIOTICS IN BROILER CHICKENS

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There now exists a large body of evidence that gastrointestinal (GI) microbes make significant contributions to host nutrition and pathogen resistance. In the poultry industry, the cost of feed represents a majority of production costs, and therefore any improvement to feed conversion ratios that can be achieved by optimizing the microbiota could have significant value to the industry and serve as an alternative to growth-promoting antibiotics. We have recently completed work on several specific aims with a general objective of generating defined and transferable microbiota that can improve poultry performance and reduce pathogen colonization.

First, the GI microbiome of chicks from low- and high-efficiency genetic lines were characterized and compared using high-throughput DNA sequencing. Second, groups of chicks from each genetic line received microbiome transplants from their own and the contrasting donor line to compare growth, feed efficiency, and effects on the microbiome. In a separate experiment, serially passaged GI contents were transplanted to chicks and tested for efficacy in resisting colonization of *Salmonella* and *Campylobacter*.

Feed efficiency status of the donor birds made little difference to the performance of the recipient. Importantly, there were significant differences between inoculated versus uninoculated chicks as measured by body weight gain and feed efficiency. These differences appeared to be mediated by the microbiota: by several different metrics inoculated versus uninoculated birds had significantly different microbial communities at the end of the experiment. Microbiome transplants also significantly improved pathogen resistance.

These results strongly support the strategy of modulating the GI microbiota to improve broiler performance and food safety. Optimizing the microbiota of commercial poultry has great potential to provide value to the industry by reducing feed costs, improving food safety, reducing the carbon footprint of the industry, limiting regulatory burdens, and providing new probiotic products as alternatives to antibiotics. Further development focuses on characterizing and propagating effective inocula and improving our mechanistic understanding of the effects observed here.

2.3 NOVEL ANTIMICROBIAL COMPOUNDS FROM MICROBIAL SOURCES

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The use of antibiotics for agricultural and industrial applications is a controversial practice. In food animal production, antibiotics are widely administered through medicated feed to treat subclinical infections, as well as to prevent the spread of disease throughout the herd or flock. Antibiotics are broadly applied in apple and pear orchards to protect trees from bacterial infections. Moreover, they have even been described to control bacterial contamination in industrial fermentations. Resistance to antibiotics is an increasing public health problem, believed to originate, at least in part, to the practice of using antibiotics in non-clinical applications, especially the use of drugs important in human clinical medicine. While reducing overall antibiotic usage is part of a strategy to combat the spread of antibiotic resistance, it also limits the availability of acceptable interventions to prevent and control infections and contamination on the farm, which will directly impact global food security and safety as well as animal and human health.

Ongoing research in our laboratory on developing new biobased products has identified several promising antibacterial candidates (table below). Specifically, we have identified a variety of bacterial, fungal, and viral products, both large- and small-molecules, that have a range of antimicrobial activities. Amongst these compounds are compounds with exquisite specificity towards the target pathogens. Identification, production and characterization of these novel antimicrobials and their potential applications will be discussed.

Antimicrobial Compound	Source	Target	Specificity	Agricultural/ Industrial Production Problem
Endolysins	Various bacteriophage	Gram positive bacteria	Narrow	Infections of industrial fermentations
Liamocin	<i>Aureobasidium pullulans</i>	Streptococcus species	Narrow	Mastitis, septicemia, neonatal mortality
Laparaxin	<i>Lactobacillus paracasei</i>	Gram positive bacteria	Broad	Food borne pathogens, drug resistant pathogens
Unknown	<i>Bacillus</i> sp.	<i>Lactobacillus</i> species	Narrow	Infections of industrial fermentations

2.4

BACTERIOCINS AS ALTERNATIVES TO ANTIBIOTICS: THE CASE OF ENTEROCINS DD14 AND DD28

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Bacteriocins are proteinaceous antimicrobial peptides produced by Gram-negative and Gram-positive bacteria [Drider and Rebuffat, 2011]. They are used as food preservatives and anticipated to be used in human and veterinary medicines to help overcoming the worrisome issue caused by the increasing bacterial resistance to antibiotics. Recently, we have isolated six antagonistic strains from meconium. These strains were identified by molecular methods including MALDI-TOF mass spectrometry and 16S rDNA sequencing as *Enterococcus faecalis* 14, *E. faecalis* 28, *E. faecalis* 90, *E. faecalis* 93, *E. faecalis* 97, and *E. faecalis* 101 [Al Atya *et al.*, 2015]. According to this study, the aforementioned antagonism was attributed to production of lactic acid and bacteriocins, named enterocins DD14, DD28, DD90, DD93, DD97 and DD101. Further analyses were undertaken to unveil the antibacterial capabilities of these bacteriocins alone or in combination with erythromycin and kanamycin towards inhibiting methicillin-resistant *Staphylococcus aureus* (SARM) grown under planktonic and biofilm cultures [Al Atya *et al.*, 2016a]. According to this study, these combinations led to synergistic effects as performed by assessment of the minimal inhibitory concentration (MIC), followed by a checkerboard and time-kill kinetics experiments. Moreover, we have established that combinations of enterocin DD14 with nisin and colistin provide meaningful data towards *Escherichia coli* from swine origin, even strains carrying the *mcr-1* gene, which is responsible for resistance to colistin [Al Atya *et al.*, 2016b]. These examples added to those reported in the literature delineate the potential of bacteriocins to be used as agents with antibiotic augmentation capability.

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2.5

ARTILYSINS – NOVEL ANTIBACTERIAL TOOLS FOR INDUSTRIAL ANIMAL PROTEIN PRODUCTION

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A growing world population is demanding an increased production of animal protein. However, industrial animal production still involves massive use of antibiotics, whereas antibiotic resistance has become a serious problem worldwide. Thus, there is a clear need for novel antibacterial solutions for industrial animal food production, with a significantly reduced risk of resistance formation.

Artilysin[®]s constitute a novel class of antibacterial agents with a new, targeted mode of action. Artilysin[®]s are recombinant fusion proteins consisting of an endolysin combined with an amphipatic or cationic targeting peptide. Artilysin[®]s destabilises the bacterial cell envelope and kills the bacteria, including multidrug-resistant strains, due to an internal pressure inside the bacterial cell within a minute. As Artilysin[®]s do not require an active bacterial metabolism for its activity, they show a superior bactericidal effect against bacterial persisters, which are very difficult to address by other means.

Artilysin[®]s have been designed that are effectively killing a broad set of *Campylobacter* spp. strains *in vitro* but are also active reducing the *Campylobacter* load in food production animals, or eliminating *Vibrio parahaemolyticus* a major problem in industrial aquaculture of e.g. shrimp.

Besides industrial animal production, private animal husbandry is involved in transmission of antibiotic resistant bacteria. Dogs or cats with diagnosed Otitis media have been effectively treated in several case studies with Artilysin[®]s developed to kill either *P. aeruginosa* or *S. aureus*.

With this new mode of action, Artilysin[®]s kill the target bacteria, independent whether these are antibiotic resistant, forming biofilms or in a 'persister mode'. Importantly, resistance development against all Artilysin[®]s tested was not observed on all strains investigated. Furthermore Artilysin[®]s are readily biodegradable using a CO₂-evolution test according to OECD 301 B. In summary, Artilysin[®]s are novel antibacterial proteins well suited to support industrial animal protein production.

2.6

MICROBIAL-ECOLOGY GUIDED DISCOVERY OF ANTIBIOFILM COMPOUNDS FROM MARINE SPONGES

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Bacteria have a breathtaking ability to biosynthesise potent antibiotic compounds, which have been adopted by humans for human and veterinary medicine. The widespread use of these natural molecules by humans, however, has greatly increased the prevalence of resistant strains since the 1940s. It is thought that antibiotics mediate antagonistic interactions amongst bacteria in nature, but little is known about how bacteria avoid exerting selective pressure for resistance. Learning how small molecules are used in the environment, therefore, could offer strategies to modify human use of antibiotics to reduce resistance selection. To that end, we have been investigating the behaviour of complex microbiomes associated with marine sponges. The largely unculturable bacteria associated with these simple filter-feeding invertebrates have been implicated in the production of many compounds that are cytotoxic and perhaps protect the sponge from predation. Many compounds that interfere with biofilm formation have also been reported from marine sponges, which we hypothesised might be produced in order to prevent the settlement of nonsymbiotic bacteria on internal channels of the sponge. We have found that amongst sponge individuals of the same species, antibiofilm activity is correlated with increases in bacteria not normally found in that microbiome, suggesting that active compounds are being transiently upregulated. We are using comparative shotgun metagenomics and metatranscriptomics to identify the biosynthetic pathways that are upregulated in these 'perturbed' microbiomes, in combination with chemical isolation to determine how the sponge microbiome protects itself from nonsymbiotic bacteria. A custom bioinformatics pipeline allows us to assemble and separate the genomes of hundreds of bacteria from one metagenome, quickly identify which species are responding to infection, and which pathways are upregulated. This information can be used to guide efforts to culture producing bacteria or to clone and express pathways of interest for compound production in the laboratory.



SESSION 2
**Microbial-derived
Products**

POSTER PRESENTATIONS

MEDIUM CHAIN FATTY ACIDS TO REDUCE ANTIBIOTIC RESISTANCE

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Antibiotics are an integral part of industrialized livestock production. The indiscriminate use of antibiotics in animal agriculture has been subjected to a critical scrutiny by governments and consumers. Retailers worldwide increasingly want antibiotic-free food, preserving for as long as possible the ever-diminishing arsenal of antimicrobials effective in humans.

To make sure farming remains profitable there will be more need for healthy alternatives. Thanks to a well-defined mode of action and proven results in practice it's known that the use of Medium Chain Fatty Acids (MCFA) in animal feeds is the preferred solutions all over the world to reduce the use of AGP's.

In the low pH environment of the stomach, un-dissociated MCFA molecules are capable of penetrating the phospholipid bilayer of the bacterial cell membrane, thereby destabilizing it which causes leakage of the bacterial cell content. Inside the bacterial cell, MCFA's encounter a near-neutral environment resulting in accumulation of dissociated MCFA molecules and protons(H+) in the bacterial cytoplasm. Intracellular acidification will eventually lead to killing of the bacterium. Even at non-bactericidal concentrations, MCFA can have a dramatic effect on pathogen persistence. By reducing the virulence of bacterial pathogens, the outcome of disease is altered and intestinal and systemic colonization is reduced, as shown in scientific trials. The combination of these antibacterial actions will result in a beneficial microbial ecosystem and thus a higher villus/crypt ratio favoring the digestive and absorptive capacities of the intestines. Next to promoting gut health, MCFA have a positive influence on the immunity of the animal. White blood cells (neutrophils) remain more active making the animal also more resistant against non-digestive disorders.

MCFA's have proven worldwide to be a reliable partner in taking the hurdle towards producing animals on a cost effective way without using any preventive antibiotics.

REDUCING THE NEED FOR THE USE OF ANTIMICROBIALS IN LIVESTOCK PRODUCTION THROUGH SPECIALTY FEED INGREDIENTS

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Antimicrobial resistance (AMR) is a present danger and future threat for human as well as animal health. Prophylactic use of antibiotics in human health care and animal production is a key driver for the rapid development of AMR. It has been demonstrated that reduction in antibiotic use may reduce prevalence of AMR pathogens in human and animal populations. Therefore, antimicrobial growth promoters (AGP) and prophylactic use of antibiotics are under pressure in certain countries. The European commission decided to ban all AGP in 2006. The Netherlands, for example, implemented very strict policies for the application of antibiotics. The total sales of antibiotics decreased from 2009 to 2015 by 58.4%. The reduced use of antibiotics in the Netherlands reported initial indications of lowered prevalence of bacterial resistance. Vaccination, stricter biosecurity programs, a more targeted administration of antibiotics and nutrition can be successful strategies in reducing antibiotic use whilst maintaining animal performance. Targeted feed ingredients can be applied to improve microbial quality of drinking water and feed, enforce the mucosal barrier of the host and stabilize the intestinal microbiota. Various classes of feed additives have been developed including organic acids, short- and medium chain fatty acids, prebiotic sugars and fibers, probiotics, botanicals and microbial derived additives from yeasts and fungi. Similar health and productivity responses in swine and poultry may be obtained compared with AGP by combining specific feed additives with optimal feed, farm and health management. In addition, specialty feed ingredients can be applied for pathogen specific approaches for example to reduce the risk of intestinal colonization and transmission of *Salmonella* spp. The scientific data and practical experiences to date highlight the significant potential functional feed ingredients have in strategies to reduce use antibiotics.

BIOSECURITY INSIDE THE CHICKEN – A NEW VIEW OF MAINTAINING HEALTH IN CHICKEN FLOCKS WITH LITTLE USE OF ANTIBIOTICS

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The classic tools to maintain or restore the health of livestock were biosecurity, vaccines, anti-coccidials and antibiotics, both as AGPs and prophylaxis. If antibiotic reduction has to be achieved, it is logical to look at the defences already available to the bird as well, as no commercial farm can ever reach specific pathogen free status. The birds first defence is still on the biological outside, the beneficial intestinal microbiome in the intestine.

A previous metagenomic study clearly showed that *Bacillus subtilis* PB6 can increase the share of beneficial intestinal bacteria. The present study aimed to further investigate the change to known beneficial parts of the microbiome, and the resulting effects on the intestinal pH. The study used 80 old white laying hens (64 weeks), randomly divided into three groups fed none, 0.5kg or 1 kg of a *Bacillus subtilis* additive, respectively. Performance parameters (egg production, weight, mass, eggshell weight and thickness) as well as bone mineralisation improved significantly after 6 for the lower and even faster for the higher dosage. The *Bacillus subtilis* addition improved gut microflora balance by leading to a significant ($p < 0.05$) increase in *Lactobacillus* and *Bifidobacterium* while significantly decreasing *Clostridium* and *Coliforms*. Parallel to the increase of *Bifidobacteria* and *Lactobacilli*, a decrease in pH could be observed in intestinal content (-0.2), caecal content (-0.3) and excreta (-0.1).

The results show that *Bacillus subtilis* PB6 has potential to reinforce the intestinal microbial balance by reducing potentially pathogenic organisms, such as *Clostridium* and *Coliforms*. At the same time, it can promote desired groups such as *Lactobacilli* and *Bifidobacteria*. *Bacillus subtilis* PB6 can therefore play an important role in biosecurity concepts, aimed at using the chickens' internal defence mechanisms against potential pathogens. Further studies of enhancing the effect of a combination of *Bacillus subtilis* PB6 and a prebiotic are currently ongoing.

ENVIRONMENTAL IMPACT ON POULTRY GROWTH AND MORTALITY

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Food-producing animals account for 80% of antibiotic use in the United States of America, according to the US Food and Drug Administration. Not all of these are shared class, but good on-farm practices may reduce the need for prophylactic, nontherapeutic antibiotics. In this study, water chlorination, litter quality and antibiotic use were examined in relation to poultry growth and mortality. The study was comprised of 8 treatment groups, 8 replicates per treatment, with 20 chicks per replicate group for a total of 1,280 chicks. Results indicate water chlorination treatments had little effect on animal growth and mortality independent of antibiotic treatments or litter condition. Antibiotics had a negative effect (-2%) on chicken final live weight raised on new, clean litter. While on previously used litter, antibiotic feed had a positive effect on animal weight (+4%). The highest live weights at the end of the study were observed in birds raised on antibiotic treatment and on used litter (2.87 kg/bird) closely followed by birds raised without antibiotic treatment and on new litter (2.86 kg/bird). However, after taking into account the higher mortality of the first group, the latter yielded a greater net weight of poultry (+6.5%). While this 6.5% greater yield of product in itself will not offset the cost of changing litter, a more difficult element to quantify, consumer preference and retailer demand for an 'antibiotic free' product will likely impact the decision to change the grow-out environment.

PROPHAGE INDUCTION FROM *STAPHYLOCOCCUS AUREUS* BOVINE MASTITIS ISOLATES

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Mastitis is a major concern to the dairy industry as it is the main cause of economic losses to producers due to reduced milk production, low milk quality, and costs of animal treatment. The use of antibiotics to treat bovine mastitis has raised concerns over the development of antibiotic resistant bacteria, antibiotic residues in milk and milk quality. Furthermore, *Staphylococcus aureus* is the most common cause of mastitis and is of particular concern due to low cure rates following treatment. Research into non-antibiotic treatments for *S. aureus* mastitis is a critical need. Bacteriophage and endolysins have the ability to meet this need. Therefore, the process of phage induction was investigated as a means to isolate and identify novel bacteriophage specific to *S. aureus*. Twenty-two *S. aureus* bovine mastitis field isolates were obtained from bulk tank milk samples originating from dairy farms in Central California. Pulsed-Field Gel Electrophoresis was performed on these isolates and it was determined there were 15 genetically distinct isolates. Prophage induction was accomplished by exposure to Ultra Violet light. Optical Density and colony-forming units (CFU) were tracked for 5 time points following exposure to UV light. Lysates from each time point were spot tested onto the control strain *S. aureus* ATCC 19685 as well as the 15 field isolates. Nine bacteriophage were isolated from this process and two *S. aureus* field isolates were found to be more susceptible to bacteriophage attack than *S. aureus* 19685. Prophage induction offers the potential to quickly screen, identify and characterize bacteriophage and their endolysins for use in non-antibiotic therapies of mastitis.

IN VITRO ASSESSMENT OF IMMUNOMODULATORY PROPERTIES OF BACILLUS-BASED PROBIOTICS

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For decades, it has been thought that antibiotic growth promoters (AGPs) efficacy was mainly due to their antibiotic activity. However, there is more and more evidence that AGPs have a strong role in inhibiting intestinal inflammation (Niewold, 2007). Thus, non-antibiotic compounds showing immunomodulating properties, such as probiotics, could be good candidates to replace AGP. Importantly, such properties seem to be strain specific. The objective of this study was to investigate the immunomodulating properties of the new *Bacillus subtilis* probiotic strain 29784 in comparison with two other commercially available *B. subtilis* strains (Bs A and Bs B).

We conducted *in vitro* inflammatory assays using Caco-2 cells in stimulated and non-stimulated conditions. *B. subtilis* strains, or a positive control, epigallocatechin gallate (EGCG) known for its anti-inflammatory properties, were applied to differentiated Caco-2 cells monolayer, exposed, or not, to the inflammatory mediator IL-1beta. Trans Epithelial Resistance (TER) and IL-8 production were then monitored as indicators of intestinal permeability and inflammation, respectively.

As expected, TER was increased by EGCG. The 3 *B. subtilis* strains had different impact on TER: Bs A decreased it, Bs B had no effect and *B. subtilis* 29784 increased it. IL-1beta induced inflammation as shown by an increase in IL-8 production. All strains tested were able to significantly reduce IL-8 level. In these conditions, however, *B. subtilis* 29784 was the only strain able to reduce the inflammatory response to the same level of what obtained with EGCG.

Our results clearly show that 1) different *B. subtilis* strains can have different levels of efficacy in modulation of inflammatory response and intestinal permeability and 2) *B. subtilis* 29784, could be a good alternative to AGP since it shows the potential to reduce intestinal inflammatory status and to enhance intestinal barrier, and this could at least partially explain the improved animal performances shown in previous studies.

ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS: SCREENING FOR SAFE PROBIOTICS

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Introduction

Gut health is a major factor in the optimum performance of production animals. It is becoming increasingly clear that intestinal microbiota mediates key physiological processes thereby influencing the host. Probiotics can positively impact these processes and are thus seen as a promising tool in settings where antibiotic growth promoters are not used in animal production. However, no two probiotic strains are the same and the importance of a thorough screening process to select strains, which are both efficient and safe in use is crucial. Here, we report the study of different strains of potential probiotics in regard to safety and efficacy.

Methods

Several hundreds of strains of *Bacillus* have been screened with regards to robustness, performance and safety. Here we focus on findings related to safety of a potential product. EFSA recommended methods were first used to study absence of toxicity and antimicrobial resistance. Additional methods to support the safety profile of a probiotic were also applied to complete the analysis. These methods rely on whole genome sequencing and in silico analyses of the genetic potential of the strains.

Results

The screening of a large library of strains has resulted in a deeper understanding of the complexity of probiotics. Even strains closely related can possess very different characteristics in term of safety and efficacy. We present results on related strains of *B. subtilis* and discuss differences in hemolysis, antimicrobial resistance profile, genomic potential related to antimicrobial resistance and strain performance.

Discussion

Probiotics possess a large potential to enhance animal performance in settings where antibiotic growth promoters are not used. However, no two strains are the same and some may possess unwanted characteristics such as resistance to antibiotics and harboring of antimicrobial resistance genes. Therefore thorough screening and understanding of potential product candidates is essential to develop new, safe probiotic products.

VIRULENCE FACTORS AND PROBIOTIC INHIBITION OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM COMMERCIAL BROILER PRODUCTION IN BRAZIL

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, a syndromic infection in poultry, as well as subclinical disease. This study aimed to investigate the prevalence of virulence associated genes of *E. coli* isolates from commercial Brazilian broiler production and to determine the efficacy of the cell free supernatants (CFS) of 12 *Bacillus* strains to inhibit the growth of potential APEC *in vitro*. Whole intestinal tracts were excised from commercial broilers across 4 farms and sections were collected from the duodenum, jejunum and ileum; all sections were used to isolate and quantify *E. coli* based on selective CHROMagar media. A previously defined pentaplex PCR assay was used to screen all *E. coli* for five virulence-associated genes (*cvaC*, *iss*, *iucC*, *tsh*, *irp2*); isolates harbouring ≥ 2 genes were identified as APEC. CFS were obtained by centrifuging 18 hour Trypticase Soya broth cultures at 10000 rpm (10 minutes at 4°C) and filtering through 0.2 μm . Inhibition assays were performed in 96-well, by monitoring OD at 595 nm of *E. coli* cultures with or without CFS every 15 minutes for 14 hours using a FlexStation Multi-Mode Microplate Reader. Percentage inhibition was calculated by comparing the OD of the treated well to the 0.4 OD reached by the control well. 45.83% of all *E. coli* isolates contained ≥ 2 virulence genes; only 0.67% contained 5 genes. *Irp2*, the gene encoding yersiniabactin, was the least prevalent, identified in 11.2% of all isolates, while the colicin *cvaC* gene was the most prevalent (49.33%). *E. coli* growth inhibition by the 12 *Bacillus* was variable, with significant differences in average inhibition between strains. Understanding APEC virulence profiles in a flock allows the use of targeted prevention and treatment measures, as these populations provide an underlying risk to poultry health and welfare.

MULTI-STRAINS BACILLUS DFM-MEDIATED COMPETITION AND EXCLUSION IN ATTACHMENT OF PATHOGENS TO BROILER INTESTINAL MUCUS *IN VITRO*

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Competition with pathogens for adhesion and colonization of the mucosal surfaces in monogastric animals are possible protective mechanisms of probiotics, also known as direct-fed microbials (DFMs). Therefore, the aim of this study was to investigate the competitive exclusion potential of a multi-strains *Bacillus* commercial DFM containing 2.5×10^9 cfu/g *Bacillus* spores (Enviva Pro, Danisco Animal Nutrition), against *Escherichia coli* K88, *Clostridium perfringens* ATCC 3626 (type B), *C. perfringens* ATCC 13124 (type A) and *Salmonella* Typhimurium ATCC 23564. The DFM was mixed with a buffer, and tested at 1×10^8 , 1×10^7 , 1×10^6 and 1×10^5 cfu/ml. Solutions of mucus (0.1 mg protein/ml 0.1 M HEPES-1x Hanks, pH 7.4) isolated from 21-day old broiler jejunum, ileum and caecum were immobilized on polyethylene terephthalate wells, to which the DFM and a [³H]thymidine-labelled bacterial suspension of adjusted density ($A_{600}=0.25$) were added. After 1-hour incubation, unbound bacterial cells were removed and the amount of adhered cells determined by scintillation counter. The effect of DFM on pathogen adherence was calculated as percentage of the adherence in the control wells. Each combination (mucus, sample dilution, pathogen) was tested as three replicate wells/run, and a total of three replicate independent runs/combination were performed. Statistical differences were analyzed in the Fit Model platform of JMP 11; significance was determined at $P < 0.05$. The DFM significantly inhibited adherence of all pathogens at 1×10^8 cfu/ml; 1×10^7 also significantly reduced adhesion, although overall level remained high, around 70-95%. The most consistent anti-adhesive effect was seen against *C. perfringens* ATCC13124. The origin of the mucus (jejunum, ileum or caecum) had no significant effect on the inhibitory effect of the DFM. These results suggest that despite being an inert form, the *Bacillus* spores, are capable of reducing the adhesion of pathogenic bacteria to the gut wall and can compete for adhesion, potentially reducing colonization and disease incidence.

COMPREHENSIVE COUNTER MEASURES AGAINST MYCOBACTERIAL INFECTIONS USING PROBIOTICS AND NOVEL VACCINES

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The rise of anti-microbial resistance (AMR) against antibiotics is a significant problem in animal health, especially among animals infected with members of the genus mycobacterium. Infection of dairy cows with *Mycobacterium avium* ss *paratuberculosis* (*M. ap*) results in the development of Johne's disease (a.k.a paratuberculosis) with significant economic losses that can reach \$500/cow. The current inactivated vaccine is not effective in controlling the infection and does not prevent shedding of *M. ap* from infected animals, further spreading infections to milk and meat. We developed an oral murine model of paratuberculosis that showed a beneficial effect of administration of *Lactobacillus casei* AATCC 334 on the outcome of infection with *M. ap*. Interestingly, *in vitro* analysis of the probiotic extract of *L. casei* or commercial Nisin (Lantibiotic class I) showed a significant inhibition of *M. ap* survival especially among clinical isolates of *M. ap*. On the other hand, Pediocin (non-Lantibiotic class II) has no effect on the survival of *M. ap* suggesting the potential use of only Nisin to reduce *M. ap* presence in food. The effect of Nisin on the integrity of *M. ap* cell wall was further confirmed by preliminary analysis of treated *M. ap* samples with scanning electron microscopy. Milk samples spiked with *M. ap* also showed significant decline of *M. ap* survival when milk was treated with Nisin or protein extracts of *L. casei*. Finally, we developed novel genetically modified organisms (GMO) based on live *M. ap* that elicit strong immune responses following immunization. In both murine and caprine models of paratuberculosis, the GMO were shown to provide superior protection against *M. ap*. Overall, we believe we have excellent probiotic products that can eliminate mycobacterial infections from food sources and effective vaccine candidates that could help in controlling infections in dairy herds without the need of using antibiotics.

ENGINEERED BACTERIOPHAGE THERAPEUTICS AGAINST MULTIDRUG-RESISTANT PATHOGENS

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Since the introduction of penicillin over 70 years ago, the number of patient deaths caused by infectious diseases has dramatically decreased and antibiotics have become among the most commonly prescribed drugs. However, the selective pressure imposed by antibiotic overuse has led to the emergence of bacterial strains resistant to virtually all antimicrobials and has renewed interest in the development of lytic bacteriophage (or phage) therapeutic and prophylactic agents. The advantages of using phage to combat infectious diseases include minimal disruption of resident flora, lack of cross-resistance with antibiotics, low toxicity and self-limiting dosing. Despite these attractive features, translational development of natural phage has been hindered mainly by the difficulty of accessing bacterial hosts within biofilms, the rapid emergence of resistant bacteria to a single phage, and above all, by the narrow host specificity of phage compared to antibiotics. The need for customized and complex combinations of natural phage to achieve adequate host range activity has made their development as licensed therapeutics very difficult. Genetic engineering of phage genomes can overcome these hurdles; however, broadly applicable methods for efficient construction of defined mutations in virulent phage genomes are still in their infancy. Thus, we developed a completely cell-free phage engineering method that allows rapid and iterative editing of viral genomic DNA. In parallel, we sequenced and annotated the genomes of ~300 MDR *P. aeruginosa* clinical isolates, and subsequently determined the susceptibility of each isolate to specific phages. Through bioinformatics analysis in combination with our engineering platform, we have successfully collapsed the host range of a family into a representative phage. We further engineered wide host range phage to express secondary payloads, such as biofilm degrading enzymes and antimicrobial moieties, and demonstrated that these phages have improved activity compared to natural phage in *P. aeruginosa* models of infection.

PROTECTIVE EFFECT OF BACILLUS SPP. PROBIOTICS AGAINST PATHOGENIC *E. COLI* IN AN *IN VITRO* PORCINE CELL MODEL

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E. coli, especially enterotoxigenic *E. coli* (ETEC F4, F5, F18, F41) infections cause major morbidity and mortality losses in swine production. Antimicrobial activities of probiotics *in vitro* represent novel tools to effectively reduce *E. coli* infections in swine. The present study aims to evaluate the antimicrobial activities of Chr. Hansen *Bacillus* spp. probiotics (*B. subtilis* HLB, *B. licheniformis* CH200, *B. subtilis* CH201 and the newly selected probiotic *B. subtilis* EB15 strains) in preventing *E. coli* colonization of the porcine jejunal IPEC-J2 cell line *in vitro*. Pathogenic *E. coli* strains used as *in vitro* challenge models were ETEC O149:K91:F4ac, EHEC O147:K89:F4, *E. coli* O101:K:F5 and *E. coli* O149:K88a. There was a significant reduction in ETEC O149:K91:F4ac, *E. coli* O149:K88a and *E. coli* O101:K:F5 colonization in the presence of the new EB15 probiotic and *B. subtilis* CH201 strain ($P < 0.005$). Although an F4 strain as well, EHEC O147:K89:F4 colonization was not affected by the presence of any of the *Bacillus* spp. strains. Overall, our data suggest that probiotic bacilli vary greatly in terms of their antimicrobial activities, their protective effect is most likely to be pathogen specific, but as for studies with ETEC, the EB15 strain has a considerably broader spectrum of antimicrobial activities than any other Chr. Hansen probiotic bacilli commercially available. The *in vivo* challenge trial conducted with ETEC O149:K91:F4ac confirms the *in vitro* findings for the new EB15 probiotic strain.

ISOLATION OF NOVEL PROBIOTIC CLOSTRIDIALES FROM THE CHICKEN GI TRACT THAT HAVE ANTI-PATHOGEN ACTIVITY

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The removal of antibiotics during food-animal production will require development of new strategies to maintain animal health, optimize nutrition and control pathogens. Utilizing naturally occurring bacteria as probiotics has several advantages including viability, ability to compete against ecologically similar taxa and a natural rationale for regulatory approval. Indigenous species of non-toxin producing, anaerobic bacteria of the Clostridiales promote anti-inflammatory immune responses in the mammalian gut by activating T-regulatory cells and these bacteria make up a large proportion of the monogastric animal intestinal microflora. Our hypothesis is that selecting spore-forming, non-toxin producing bacterial taxa closely related to known pathogens offers potential for competitive exclusion of pathogenic bacteria. Consequently, three percent chloroform treatment of chicken gastrointestinal contents was completed for one hour to remove vegetative bacterial cells followed by anaerobic culture of surviving spores. Approximately 40 axenic isolates were obtained that were distinct from one another morphologically and phenotypically based on Gram staining and sulfide production. Moreover, by genomic analyses some of the strains represent novel species based on the standard of less than 97 percent similarity comparing the full-length 16S rRNA gene sequences to their known closest cultured representative species, primarily Clostridia. Growth reduction utilizing lawns of *Clostridium perfringens*, *C. septicum* and *C. difficile* was demonstrated by zones of inhibition produced by eight cecal and five mid-gut newly identified isolates when placed on the respective pathogen lawns. At this time the antimicrobial mechanism is unknown, although several interesting genes such as potential prophage holins and endolysins were identified by sequencing genomes of the newly obtained isolates. Results from these investigations further demonstrate that newly identified, potential probiotic bacterial cultures can be isolated and identified for future use to improve animal and human health.

ISOLATION OF POTENTIAL NOVEL PROBIOTIC BACTERIA FROM CANADA GOOSE FECES

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Removal of antibiotics during food-animal production, e.g. poultry, will require developing innovative strategies to maintain animal health and optimize nutrition. Utilizing naturally occurring bacteria as probiotics has several advantages including viability, ability to compete against ecologically similar taxa and a natural rationale for regulatory approval. Indigenous species of spore-forming, fiber-fermenting bacteria promote anti-inflammatory responses in the mammalian gut by activating immune cells and these bacteria make up a large proportion of the monogastric animal gastrointestinal microflora. Enriching avian feces for chloroform-resistant bacteria will select for bacterial spores that represent potentially non-toxin producing bacteria that could be utilized as probiotics for poultry or another avian species. Therefore, our hypothesis is that selecting spore-forming bacterial taxa closely related to known pathogens offers potential for competitive exclusion of pathogenic bacteria. Also, isolation of potential probiotic bacteria from a variety of avian species could be of value for commercial poultry production and the minimal result will be discovery of previously undiscovered bacteria. Three percent chloroform treatment of Canada goose (*Branta canadensis*) feces was completed for one hour to remove vegetative bacterial cells, followed by anaerobic and aerobic culture of surviving spores. Twenty-six axenic, endospore containing isolates were obtained that were distinct from one another morphologically and phenotypically based on Gram staining. Twelve anaerobic Gram positive (3) and negative (9) isolates were obtained by culturing on Brucella blood with vitamin K and hemin or reinforced clostridial hveg hydrolysate with L-cysteine, Na acetate and starch agars. Fourteen aerobic Gram positive (4) and negative (10) isolates were cultured using the two aforementioned agars and nine (9) aerobes could subsequently be propagated on lysogeny broth (LB). Results from these investigations further demonstrate that newly identified, potential probiotic bacterial cultures can be isolated from free-ranging species and identified for future use to improve animal health.

DEVELOPING ALTERNATIVES TO ANTIBIOTICS USE: REDUCTION OF *CAMPYLOBACTER* COUNTS ON CHICKEN WINGETTES BY A CHITOSAN BASED COATING OR USE OF PROBIOTIC (*LACTOBACILLUS* SPP.) ISOLATES

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The presence of *Campylobacter* on poultry products remains one of the leading causes for foodborne illness. The reduction in the use of antibiotics in animal agriculture has increased the need for alternative forms of improving food safety. The use of lactic acid bacteria (LAB) as a bio-preservative/protective culture in food commodities is an ancient technology that is safe and natural. In this study, 13 *Lactobacillus* spp. isolates were screened by a chicken skin dipping model to evaluate the potential to reduce *C. jejuni* counts. From this screening assay, 4 isolates (isolates 1-4) which produced >1 log reduction in *Campylobacter* counts were chosen for further evaluation in a chicken wingette model. In replicate trials, chicken wingettes were inoculated with *C. jejuni* (~7 Log CFU/mL) and treated with either a *Lactobacillus* broth culture or a BPD control (n=5 samples/treatment). *Campylobacter* counts were determined at 0, 1, 3, 5 or 7 days post treatment. *Campylobacter* counts were log₁₀ transformed and data were analyzed using ANOVA with the PROC MIXED procedure of SAS. Isolates 2 or 4 were the most effective and consistently reduced *Campylobacter* counts from day 1 through day 7 (P<0.05). In follow-up studies, isolates 2 and 4 were subjected to additional testing aimed at assessing potential synergistic activity between the *Lactobacillus* isolates and their combination with a 2% chitosan (CH) solution. Each isolate by themselves, CH or their combination significantly reduced *Campylobacter* counts (~1-2.5 log reduction) from day 1 through 7. The combination of isolates+CH reduced *Campylobacter* counts on wingettes, but this treatment did not demonstrate any additional reduction compared to each individual treatment alone. Our studies demonstrate the potential use of CH or *Lactobacillus* isolates as a protective culture to reduce *Campylobacter* counts on raw poultry.

Keywords

Campylobacter jejuni – Food safety – Poultry – Protective culture.

A NEW *BACILLUS SUBTILIS* SHOWS SIMILAR EFFICACY THAN BACITRACIN IN GROWING BROILERS

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Bacillus-based direct-fed microbials are of particular interest to improve gut health and performance of broilers. Indeed, they have the ability to first modify gut microbiota, and second to remain viable after pelleting. Thus, they could be considered as alternative to the use of antibiotic growth promoters. The objective of the present experiment was to investigate the effect of a new *Bacillus subtilis* on the performance of broilers compared to bacitracin methylene disalicylate (BMD) treatment.

A total of 1,800 day-old male broiler chickens, Cobb 500, were randomly allocated according to a factorial design with three treatments (12 replicates of 50 birds) and reared until 42 days in floor pens. The experimental treatments were: T1, negative control (basal diet, corn-based); T2, T1 + BMD at 55 ppm; T3, T1 + *Bacillus subtilis* strain 29784 at 1.108 CFU/kg of feed. Feed intake (FI) and body weight gain (BWG) were measured and feed conversion ratio (FCR) were calculated at 35 and 42 days.

At d35, FCR was improved by both treatments T2 and T3 (respectively -3.3% and -2.9%; $P < 0.0001$). In addition, no difference was observed between T2 and T3 for FCR at this age. At d42, BWG did not differ between T2 and T3, and the *Bacillus* strain allows a numerical improvement of BWG when compared to T1 (+1.5%; NS). Concerning FCR, both treatments T2 and T3 significantly improved this parameter by comparison with T1 (respectively -4.8% and -3.5%; $P < 0.0001$).

These results showed that a new *Bacillus subtilis* strain improves broiler performance, and the level of improvement is similar to that obtained with BMD, suggesting that this new *Bacillus subtilis* might be considered as an alternative to AGP.

CONTROLLING DYSBACTERIOSIS THROUGH NATURALLY OCCURRING BACTERIOPHAGE IN POULTRY INTESTINES

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The intestines of chickens and turkeys are known to be home to a wide diversity of bacteria, the microbiome, colonising shortly after hatch. What is less well known is that there is also a large community of viruses, the virome, found in neonates and some of these viruses are vertically transmitted from the parents into the egg e.g. chicken astrovirus. Early viral infections, including embryonic infections, can result in gut tissue damage that may predispose birds to later bacterial imbalances (dysbacteriosis) thus affecting performance and possibly resulting in specific diseases. We and others have recently discovered by next generation sequencing and metagenomics studies of poultry gut contents, from growth retarded birds, that in addition to these microbiomes and viromes, there is a high abundance of bacteriophage present. Bacteriophage are naturally occurring viruses whose host organisms are bacteria and they have been used as alternatives to antibiotics in human phage therapy in Georgia, Russia and Poland for a number of years.

The presence of high levels of bacteriophage in commercial poultry suggests a natural control mechanism for bacteria. Bacteriophage of the order *Caudovirales* were detected from the families *Siphoviridae* and *Myoviridae*, with fewer representatives of the *Podoviridae*, and less still of the *Leviviridae* and unassigned *Microviridae*. The majority of the bacteriophages in our study were identified as targeting *E.coli*, *Enterococcus* and *Bacteroides* species. Identification and culture of specific strains of bacteriophages that have an obligatory lytic stage to ensure lysis of target bacteria of economically relevant importance could facilitate the production of a cocktail of naturally occurring bacteriophages to be administered early in the broiler rearing period before dysbacteriosis occurs, often at 2-3 weeks of age, but after normal enteric bacterial colonisation has been established by day 3 (intestines) – day 14 (caeca) post hatch.

INTRAMAMMARY INFUSION OF A LIVE CULTURE OF *LACTOCOCCUS LACTIS* IN LACTATING MICE: PRELIMINARY DATA

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Bacterial mastitis is one of most important disease on dairy farms worldwide, generally treated using antibiotic therapy. Antibiotics present some disadvantages including transmission of antibiotic resistance and appearance of residues in milk and dairy products. Development of alternatives is therefore encouraged in an age of mounting antibiotic resistance. Lactic acid bacteria are considered a good alternative to this therapy. Aim of the study was to evaluate the effects of an intramammary infusion of a live culture of *Lactococcus lactis* into the mammary glands of healthy mice. The animal study was conducted in accordance with both institutional guidelines and international laws and policies. L5 and R5 udders of 27 healthy female mice were considered in the study: 12 animals were infused with 100µl of a live culture of *L. lactis* (8×10^8 CFU/ml in Trypticase Soy Broth) (Group 1), 8 received an infusion of PBS (100µl) (Group 2) and 7 were left untreated and used as control (Group 3). Mice were sacrificed 24 h after treatments and L5 and R5 mammary glands were explanted. Histological investigations were performed using a semiquantitative scoring system (0-3) to assess the lesions. The number of *L. lactis* retrieved per gram from treated glands was determined on agar plates. Moderate to diffuse alveolar and interstitial neutrophils and interstitial *lymphoplasmacytic infiltration* were observed in 8/12 glands of the Group 1; only two udders of the Group 2 showed slight to moderate inflammation. The remaining glands showed no histological changes. *L. lactis* was isolated from treated glands at a mean value of 8.6×10^6 CFU/g. These preliminary data suggest an immune response in *L. lactis*-treated udders. Further investigations are needed to evaluate the potential use of this treatment for the mastitis therapy.

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NISIN: A PROMISING ALTERNATIVE TO ANTIBIOTIC FOR INHIBITION OF *CLOSTRIDIUM DIFFICILE* IN A HUMAN COLONIC CONDITIONS

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Clostridium difficile is the most frequently identified enteric pathogen in patients with nosocomially acquired, antibiotic-associated diarrhoea and pseudomembranous colitis. Although metronidazole and vancomycin are effective, an increasing number of treatment failures and recurrence of *C. difficile* infection are being reported. Use of probiotics, particularly metabolically active lactic acid bacteria, was recently proposed as an alternative for the medical community. The aim of this study was to assess a probiotic candidate, nisin Z producer *Lactococcus lactis* UL719, competitiveness and nisin (Nisaplin®) capacity to inhibit *C. difficile* in a model of human colon. Bacterial populations were enumerated by qPCR coupled with propidium mono azide treatment. *L. lactis* UL719 was able to survive and proliferate under simulated human colon, did not alter microbiota composition, but failed to inhibit *C. difficile*. While a single dose of 19 µmol/L (5× the MIC) was not sufficient to inhibit *C. difficile*, nisin at 76 µmol/L (20× the MIC) was effective at killing the pathogen. In this condition, *C. difficile* was inhibited by 2.3 log₁₀ after 1 h that lasted for 8h. Nisin (at 76 µmol/L) caused some temporary changes in the microbiota. Ruminococaceae group was reduced by 3.7 log₁₀ after 24 h while Lachnospiraceae, Lactobacillaceae and Bifidobacteria were reduced by 1.5, 1.3 and 1 log₁₀, respectively but returned to their initial population after 24 h. Regarding the short chain fatty acid profile, a decrease of all acid concentrations was noted, especially acetate and butyrate, except with propionate. These results highlight the capacity of *L. lactis* UL719 to survive under simulated human colon and the efficacy of nisin as an alternative in the treatment of *C. difficile* infections.

MICROCIN J25 AND REUTERIN. TWO NATURAL ANTIMICROBIAL COMPOUNDS WITH A HIGH POTENTIAL FOR INHIBITION OF SALMONELLA

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Microcin J25 (MccJ25) is a particular lasso peptide produced by *Escherichia coli* and composed of 21 amino acid residues. Previous studies have shown that MccJ25 exert a potent inhibitory activity against *Enterobacteriaceae*, including several pathogenic *E. coli*, *Salmonella* and *Shigella* strains. In the present study, we first evaluated the antibacterial activity of MccJ25 against *Salmonella* Newport ATCC 6962 and compared this inhibition activity to reuterin, an antimicrobial compound produced by some *Lactobacillus reuteri* and to rifampicin. The results have shown that the tested strain of *S. Newport* was significantly more sensitive to MccJ25 than reuterin and rifampicin. In order to confirm these results in a higher volume, the antibacterial activity of MccJ25, reuterin and rifampicin was evaluated using the CLSI antimicrobial susceptibility assay. Interestingly, MccJ25 was shown to exhibit a bacteriostatic effect only when tested in 24-well microplate. Indeed a moderate growth of *S. Newport* was observed after 8 hours of incubation. This bacteriostatic activity was not observed with reuterin and rifampicin. Further analysis has showed that this bacteriostatic activity could be associated with the development of adapted or resistant variant of *S. Newport* to MccJ25. Further assays are underway to confirm the present results in swine colonic fermentation conditions.

PHAGE ENDOLYSINS AS PUTATIVE *CLOSTRIDIUM PERFRINGENS* THERAPEUTIC

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Clostridium perfringens is a major necrotic enteritis causing bacterial pathogen in poultry, and a source of food poisoning and gas gangrene in people. *C. perfringens* can also cause mild to severe enteritis in pigs. In the EU, the occurrence of *C. perfringens* associated necrotic enteritis in poultry has increased as antibiotic use has decreased. As the US moves away from use of antibiotics in animal feed, we can expect an increase in necrotic enteritis with subsequent losses from morbidity and, in subclinical cases, losses from decreased chicken weights. Alternatives to antibiotics in animal feed will be needed in the near future. The genomes of 43 unique *C. perfringens* isolates from chicken were sequenced, examined for peptidoglycan hydrolase enzymes by homology to known enzymes. There were more than 120 putative peptidoglycan hydrolases (primarily phage endolysins) identified that clustered into 15 groups according to homology [less than 50% amino acid identity between groups and more than 90% amino acid identity within group]. Of 15 representative lysins (one from each group) four lysins were shown to have high lytic activity against all 43 of the initial isolates as well as 9 porcine (all tested) in plate lysis assays but not other Gram positive or Gram negative species tested. Activity was also demonstrated in both zymogram, and turbidity reduction assays. The domain architecture and relative activity of the four lysins has been determined. Production of nanoparticles composed of peptidoglycan hydrolases as alternative antimicrobials will be described.



SESSION 3
Phytochemicals

ORAL PRESENTATIONS

3.1

DIETARY PHYTONUTRIENTS AS ALTERNATIVES-TO-ANTIBIOTICS IN AGRICULTURAL ANIMALS: MODE OF ACTION IN MODULATING CROSS-TALKS AMONG IMMUNITY, DISEASE RESISTANCE AND GUT MICROBIOTA

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New antibiotic regulatory policies affecting agricultural animal production now challenge animal scientists to think outside of the box to develop alternative strategies for sustainable animal agriculture. For those animal infectious diseases for which effective vaccines are lacking, there is a critical need to develop novel feed additives that will serve as antibiotic alternatives. The gut represents a continuously evolving ecosystem where a dynamic interaction between host immune, neuroendocrine and entero-endocrine cells and the gut microbiota influences normal physiological development, response and homeostasis. This presentation will discuss novel ways to use dietary phytochemicals to enhance animal growth and modulate host physiological responses. Phytochemicals are non-nutritive, plant-derived chemicals, many with disease-preventing properties. A growing body of scientific evidence has demonstrated that many of the health-promoting activities of phytochemicals are mediated through their ability to improve host defence against microbial infections and tumors. During the last 10 years, our research has provided science-based evidence for the beneficial effects of certain phytochemicals in the poultry growth and immune system. Many of these phytochemicals are now commercially used to increase the growth and reduce disease-associated losses in poultry and livestock. However, it is of primary importance to understand the mechanisms supporting the effect of a product in order to improve positioning and efficacy. New studies suggest that certain phytochemicals reduce the negative consequences of enteric diseases, in part, through the alteration of the gut microbiome. This presentation will discuss new strategies to enhance animal growth and modulate innate immunity against enteric pathogens using various dietary phytochemicals.

3.2

DIETARY PHYTONUTRIENTS ENHANCE DISEASE RESISTANCE IN SWINE

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It is imperative that various antibiotic-free feed technologies are developed to prevent and control infectious diseases due to the restriction of using in-feed antibiotics. These potential feed technologies include but not limited to the dietary feed additives which represent potential 'alternatives to antibiotics', either by altering microbial populations in the gastrointestinal tract or by influencing the host immune system. The immune system of pigs is vital as its proper functioning protects the pig from diseases and maintain health. It also causes inflammation which contributes to the animal's ability to fight off infection but also inhibits growth performance by reducing feed intake and diverting amino acids and nutrients away from growth. Therefore, it is now clear that reducing inflammation would benefit pig health. Our work has shown that several plant extracts benefit swine health. *In vitro* studies reported that several plant extracts (anethol, capsicum oleoresin, carvacrol, cinnamaldehyde, eugenol, garlicon, and turmeric oleoresin) suppressed ($P < 0.05$) pro-inflammatory cytokine secretion from lipopolysaccharide-stimulated porcine alveolar macrophages. Results from an *in vivo* *Escherichia coli* (*E. coli*) challenge study showed that dietary capsicum oleoresin, garlicon, or turmeric oleoresin reduced ($P < 0.05$) diarrhea of *E. coli*-challenged pigs. Feeding these three plant extracts also decreased inflammatory responses of *E. coli*-challenged pigs, as indicated by reduced ($P < 0.05$) white blood cell numbers, serum pro-inflammatory cytokines, and acute phase proteins compared to the control pigs fed standard diet. A potential mechanism of action is that plant extracts may enhance gut mucosa health and attenuate the overstimulation of the immune system. The microarray data from the same *in vivo* study indicated that these plant extracts counteracted ($P < 0.05$) the effects of *E. coli* by reducing the expression of genes involved in antigen presentation or other biological processes of immune responses. Another *in vivo* study with porcine reproductive and respiratory syndrome virus (PRRSV) challenge showed that feeding these three plant extracts to nursery pigs enhanced the pigs' immune responses to a PRRSV challenge and alleviated negative impacts of infection as indicated by reduced ($P < 0.05$) viral load, decreased serum inflammatory mediators, and shortened ($P < 0.05$) the time of fever in PRRSV-infected pigs. In conclusion, dietary phytonutrient feed additives improve pig health and disease resistance by modulating inflammation.

3.3 PHYTONUTRIENTS AS AN ALTERNATIVE FEEDING STRATEGY TO IMPROVE PERFORMANCE OF CATTLE WITHOUT USING ANTIBIOTICS

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Antibiotics play or have played a relevant role in animal production as growth promoters (AGP) and therapeutic tools. However, increasing public health and consumer concerns on antibiotic resistance are leading current policies toward the reduction and/or abolition of its use. The EU banned the use of AGP in 2006 (Directive 1831/2003/CEE). The focus of our research is to search for alternative feeding additives that will enhance production performance without using AGP in ruminants, by either modifying rumen fermentation or enhancing intestinal function. Feeding a combination of cinnamaldehyde, eugenol and capsicum oleoresin (CAPO) was able to shift the microbial populations in the rumen resulting in an increase in propionate (+10,5%), and a reduction in acetate (-5%) and ammonia-N (-13%), improving energy and protein retention. These phytonutrients also resulted in higher milk production (+3%), body weight gain (+2.8%) and feed efficiency (+2,6%). These results are similar to those obtained with AGP. Furthermore, CAPO changed feeding behavior resulting in more time spent eating (+54%), higher dry matter (+9%) and water (+18%) intake, and more stable rumen pH. More recent studies have shown that Sucram, a sweetener, upregulated the expression of glucose transporters in enterocytes. Glucose is the main energy source for enterocytes, and increasing glucose absorption contributes to a better intestinal function. Furthermore, CAPO increased indicators of acute immune response (increased neutrophil:lymphocyte ratio) in dairy cows. By strengthening the defense mechanisms, nutrients are diverted to production purposes from immune responses. The drive to reduce the use of antibiotics in animal production has opened new opportunities to explore different strategies to achieve high performance without using antibiotics. Phytonutrients are enhancing cattle production at different levels including modulation of rumen fermentation, behavior and immune capacity of animals.

3.4

IMPACT OF DIETARY TANNINS ON RUMEN MICROBIOTA IN BOVINE

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Tannins added in the diet are being used to improve nutrition and to control enteric diseases in farm animals and appear to be a promising alternative to antibiotic growth promoters. In previous studies we found that tannins have antibacterial activity against *Clostridium perfringens* and are effective for long periods of time and prevent these anaerobic bacteria to develop resistance. Many reports indicate that inclusion of tannins in feed can improve performance and health in ruminants. It is speculated that tannins modify the digestive process not only by binding dietary protein but also through modulation of gastrointestinal microbiota. Our aim was to evaluate *in vivo* the impact of SilvaFeed RBM, a blend of tannins specifically designed with the aim of optimizing the ruminal microbiota. The rumen microbiota composition and its interplay with rumen physiological parameters were studied at different times in fistulated steers. High-throughput sequencing of 16S rRNA gene (Illumina MiSeq platform) was used to analyze the effects of SilvaFeed RBM in rumen microbiota of six Holando-Argentino steers. A total of 9.871.395 sequences were obtained at five time points (0, 3, 5, 9 and 12 days after tannins supplementation). Bacterial diversity estimators tended to homogenize through tannins treatment and dysbiotic microbiota profiles were normalized. Firmicutes to Bacteroidetes ratio, a parameter linked to energy harvesting function in mammals, was gradually increased by tannins over time, mainly by favoring members of family Ruminococcaceae in detriment of genus Prevotella. Other fibrolytic, amylolytic and ureolytic bacteria were also modulated by tannins and methanogenic archaea were inhibited. Concomitantly, ruminal pH was significantly increased and remained stable until the end of the experiment. Our results suggest a link between microbiota normalization and ruminal physiology stabilization. More studies are required in order to evaluate tannins potential to efficiently and reproducibly improve ruminant nutrition and health.

3.5

META-ANALYSIS OF BROILER RESEARCH SHOWS THAT VARIUM™ RESULTS IN FEED EFFICIENCY EQUAL TO ANTIBIOTICS

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Several broiler experiments were conducted to develop a new non-medicated growth promoter for poultry. To better understand this research meta-analysis was utilized to combine data examining the effects of Varium on poultry production. The clinical studies were conducted over a three-year period at four facilities using four disease models. A slope ratio based on feed: gain was used for the main comparison. The control treatments, which had no Varium, Antibiotics, or Necrotic Enteritis (NE) challenge, were set at 100. The slope ratio values for the different treatments can be compared to the Control or to each other. Slope ratio values above 100 indicate that those birds had poorer performance than the Control birds. When birds were challenged to induce necrotic enteritis and not given Varium or an antibiotic the slope ratio was 123.4, much higher than the Control treatments at 100. Thus, as expected, challenging birds to induce necrotic enteritis (NE) decreased the efficiency of their feed utilization. Challenged birds that did not receive Varium or an antibiotic had the highest slope ratio value in the analysis. Feeding unchallenged birds either an antibiotic or Varium resulted in slope ratios that were below 100; these birds had better feed efficiency than the controls. There was not a difference between feeding the unchallenged birds an antibiotic or Varium with values of 96.8 and 97.3, respectively. Feeding NE-challenged birds with Varium or an antibiotic resulted in similar slope ratios. Because birds were challenged slopes were above 100, but were much lower than the 123.4 of the challenged control birds. The slope ratios were the same for challenged birds fed either Varium or an antibiotic; 107.7. Slope ratio when feeding both to challenged birds was 103.2. In conclusion, feeding broilers Varium resulted in feed: gain improvement equal to an antibiotic in unchallenged or challenged broilers.



SESSION 3
Phytochemicals

POSTER PRESENTATIONS

THE *IN VITRO* ACTIVITY OF HEXAHYDRO- β -LUPULONE LAURIC ACID ESTER (6HLE) AND THE EFFECTS OF DIETARY 6HLE ON BROILER PRODUCTION

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The active constituents of hops are mainly β -acids containing lupulone and other active ingredients. β -acids are bitter and highly unstable but they show anti-microbial activity against gram-positive bacteria. During the last eight years, Insighter® has developed hexahydro- β -acid (δ HL) through hydrogenation of β -acid, which further increased the antimicrobial activity against *Clostridium* spp. and significantly improved product stability. However, the stability has still not reached the stability criteria of feed additives. Further efforts to increase stability and anti-microbial activity led to the hexahydro- β -lupulone lauric acid ester (δ HLE) which exerts antimicrobial activity through the release of hexahydro- β -acid (δ HL) in the hindguts. *Clostridium perfringens* is the main cause of necrotic enteritis (NE) in broilers. The minimum inhibitory concentration (MIC) of the antimicrobial activity of hexahydro- β -lupulone against *Clostridium perfringens* is 0.01-0.1 μ g/mL, indicating that δ HL exhibits strong *in vitro* activity against Gram-positive bacteria. In our *in vivo* feeding trial, 800 Chinese yellow feathered broilers of 21 days old were randomly assigned to 4 groups of 200, which were individually caged and fed basal diet (negative group), basal diet + AGPs (virginiamycin, 20 ppm)(positive group), commercialized diet + 1.5 ppm (trial group 1) and 3 ppm (trial group 2) of experimental diet incorporated with (δ HLE). After reaching 30 days old, the enteric microorganisms and their production performance were analyzed. The results showed that supplementation of δ HLE dose-dependently decreased ($P<0.05$) the population of *Clostridium perfringens* in the gut compared with positive group. Therefore, we concluded that dietary δ HLE reduced the number of *Clostridium perfringens* in broiler chickens and decreased the incidence of NE.

A STUDY ON THE PRODUCTION PERFORMANCE OF PIGLETS ADMINISTERED WITH TANNALBIN REPLACING AGPS AND ZNO IN SWINE FEED

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Tannic acid is a general term for a large class of polyphenol substances originating from plants. Tannic acid has an extensive range of biological activities including antimicrobial, antiviral, and antioxidant properties, intestinal astringent action, and neutralization of enterotoxins. But the defects – the reduced palatability due to its astringent taste, the irritation it brings to the operators and its discoloration (turning black) when in touch with water – restrict their direct application in animal feed. Tanpro™ (containing 50% tannic acid) is a product of Tannalbin created through special processes of chemical reactions between soy protein and tannic acid (screened and optimized to have strong astringent activity). The current experiment determined the antidiarrheal effects of Tanpro on post-weaning piglets and the effects of Tanpro on animal production performance to compare the effects of Tanpro, Colistin Sulphate and ZnO on diarrhea and performance of weaned pigs. A total of 210 healthy 24-days-old post-weaning Duroc*Landrace*Yorkshire hybridized piglets of similar weight were randomly assigned to 7 groups and fed basal diet (group 1), basal diet + Zn (from ZnO) 2250 ppm (group 2), basal diet + Colistin Sulphate 20 ppm (group 3), basal diet + Tanpro 1000 ppm (group 4), 2000 ppm (group 5), 3000 ppm (group 6) and 4000 ppm (group 7) respectively. The trial was performed by self-help feeding in sextuplicates and lasted two weeks during which diarrhea rate, ADG and FCR of each group were compared. Tanpro dose dependently reduced ($P<0.05$) diarrhea rate and improved ($P<0.05$) growth performance of weaned pigs compared with group 1. Pigs fed the diets supplemented with 4000 ppm Tanpro had lower ($P<0.05$) diarrhea rate and greater ($P<0.05$) growth performance than diets supplemented with 2250 ppm of Zinc or 20 ppm of Colistin Sulphate. In conclusion, these results indicate that supplementation of 4000 ppm Tanpro may be an efficacious alternative to ZnO and Colistin Sulphate.

IN SITU PRODUCTION OF PREBIOTICS

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In animal husbandry, infectious disease is commonplace and antibiotics are routinely used in large amounts. The aim of this project was to develop a feed supplement as an alternative to antibiotics in the form of a prebiotic feed which could increase bacterial diversity and beneficial bacteria as they combat pathogens in the GI-tract. Because of the current cost-effectiveness of antibiotics, novel products should be cost effective, but current prebiotics do not fulfil this requirement. In this endeavor, the production of prebiotics was moved from the industrial setting to instead take place within the gastrointestinal tract of the animal, which is what we refer to as ***in situ* production of prebiotics**. In this report, we used a waste product from the potato industry, namely potato pulp along with pectinolytic enzymes to produce prebiotics. In an *in vitro* digestion, 24.6% of the potato pulp could be water solubilized by enzymes and this solubilized galactose-rich fraction can then be fermented by bacteria present in the piglet terminal ileum. The fermentations resulted in high levels of organic acids as determined by HPLC, lactate in particular, and an increase in the Genera *Lactobacillus* and *Veillonella* as determined by deep sequencing of the 16S rRNA gene which suggest some prebiotic potential of potato pulp. Enzymes in combination with potato pulp were then fed to weaning piglets. We found up to 40% of the theoretically maximum amount of solubilized fiber in the gastrointestinal content, which was released within 20 minutes, suggesting that *in situ* production of fiber is feasible. A pilot study testing an experimental infection with pathogenic field strains of *Escherichia coli* F4+ was performed by feeding the pulp and enzyme supplement to weaning piglets challenged with *E. coli* F4+. Water-solubilized fibers were observed in the intestines of the animals, but the experimental challenge did not result in a clinical infection. Overall, *in situ* production of fibers is possible in the weaning piglet, although it remains to be confirmed *in vivo* if these fibers are indeed prebiotic and/or inhibitory against post-weaning diarrhea.

Ox-C-BETA™ LIVESTOCK, A NOVEL ANTIBIOTIC ALTERNATIVE FOR ENHANCING PRODUCTIVITY AND DISEASE RESISTANCE IN MULTIPLE FOOD ANIMAL SPECIES

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Ox-C-beta™ Livestock is the first product based on a novel class of biologically active carotenoid oxidation products. Its active ingredient, OxBC, is derived from the complete autoxidation of beta-carotene and is composed predominantly of β -carotene-oxygen copolymers. Laboratory-based research established that OxBC possesses dual immunological activities including 1) priming of innate immune defenses to more readily detect and respond to pathogens and 2) limiting the extent and promoting the resolution of inflammatory reactions.

Field trials with broiler poultry show that supplementation with OxBC improves final body weights by 3 to 4.5% compared to un-supplemented birds. *Clostridium perfringens* challenge trials modeling subclinical necrotic enteritis (NE) observed that OxBC reduced mortality of birds. In NE challenge trials, OxBC supplementation improved body weights by 7 to 11.5% compared to un-supplemented birds. Significant reductions in intestinal *C. perfringens* levels and lesion severity were also observed in birds receiving OxBC. OxBC-supplementation also benefits the growth performance and clinical health of hogs. Swine trials indicated that the benefits of OxBC supplementation were obvious in the post-weaning stage, where improvements in daily weight gain reached as high as 23% and incidence rates of post-wean diarrhea were reduced by over half compared with negative controls. The product also outperformed positive antibiotic controls. Significant enhancements of growth performance were also observed by inclusion of OxBC to pre-wean creep feed, as well as during the grower and finisher phases of production. Across multiple trials in broilers and hogs, OxBC provides consistent and economically meaningful enhancements to critical measures of productivity and disease resistance. In general, the benefits of OxBC are equivalent to, and in some cases superior to, those obtainable with in-feed antibiotic regimens in widespread commercial use.

EFFECTS OF ZINC, COPPER, OREGANO OIL AND RACTOPAMINE AS ANTIBIOTIC ALTERNATIVES ON BACTERIAL RESISTANCE AND GROWTH PERFORMANCE IN SWINE

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Supplemented antibiotics have been used in animal agriculture for growth promotion and routine prophylaxis. However, the worldwide increase in antibiotic resistance has prompted the exploration of alternatives in the form of heavy metals, essential oils and β -adrenergic agonists. We investigated the effects of zinc, copper, oregano oil and ractopamine in swine as alternatives to growth promotion and prophylactic in-feed antibiotics in two trials. The first trial on piglets was arranged in a 2x2x2 complete factorial design, factors being copper, zinc, and oregano oil, and included the reference diets of chlortetracycline (CTC, 22mg/kg prophylactic dose and 4mg/kg sub-therapeutic dose). The second trial on finisher pigs was arranged in a 2x2x2 complete factorial design, factors being copper, zinc, and ractopamine. Faecal samples were collected every seven days during each study period. Faecal suspensions were plated onto selective and differential agar for *Escherichia coli* and *Enterococcus* spp. (as plain and supplemented with antibiotics at CLSI breakpoint concentrations). Two isolates were chosen from each agar plate, streaked to blood agar and evaluated for phenotypic resistance via the TREK Sensititre[®] system. The results of the first study did not show a significant association between antibiotic alternatives (copper, zinc and oregano oil) and drug resistance, with the exception of copper supplementation which showed a strong negative association with multi-drug resistant phenotypes (including to 3rd generation cephalosporins). CTC and copper added separately to the piglets' diet each showed a paradoxical reduction in ceftriaxone resistance possibly tied to associations of chromosomal *pcoB* and *tet(B)* genes versus plasmid-borne *tet(A)* and *bla_{CMY-2}* genes. The results of the second study demonstrated that supplementation of copper or zinc in combination with ractopamine did not improve average daily gain in pigs; however, inclusion of ractopamine alone improved carcass leanness as well as feed efficiency while not measurably affecting antimicrobial resistance among *E. coli*.

HEAVY METALS, ESSENTIAL OILS, AND B-ADRENERGIC AGONISTS AS ALTERNATIVES TO ANTIBIOTICS AND THEIR IMPACTS ON BACTERIAL RESISTANCE IN BEEF CATTLE

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Sub-therapeutic antibiotics have been used in animal agriculture to increase feed efficiency, promote growth, and prevent disease. Public health risks relating to the global rise of antimicrobial resistance have led to research on alternative feed supplements, including heavy metals, essential oils, and β -adrenergic agonists. We aimed to measure the impact of antibiotic alternatives by investigating the effects of zinc, menthol, and ractopamine on antimicrobial resistance among commensal enteric bacteria of cattle in two trials. The first trial on feeder cattle was arranged in a 2x2 factorial design with zinc and menthol. The second trial on finisher cattle was arranged in a 2x2 factorial design with zinc and ractopamine. Faecal samples were collected every seven days during each study period. Faecal suspensions were plated onto selective and differential agar for *Escherichia coli* and *Enterococcus* spp. (both as plain and also supplemented with antibiotics at CLSI breakpoint concentrations). Two isolates were selected from each agar plate, subcultured to blood agar and analysed for phenotypic resistance via the TREK Sensititre[®] system. In the first study, there were limited direct effects of the treatment factors on *E. coli* resistance phenotype; however, menthol (alone and in combination with zinc) exhibited a suppressive effect on both the total log₁₀ CFU of *E. coli*, and the relative concentration of *E. coli* that grew on MacConkey + 16 µg/ml tetracycline media. In the second study, ractopamine exhibited no associations with either endpoint as main effect or modifier, while elevated levels of zinc were associated with increased relative and absolute levels of tetracycline resistance on day 21 (mid-trial) as measured both via log₁₀ CFU and resistance prevalence among isolates. It seems likely that co-selection favoured such observations and further studies are underway to better understand this observation.

ASSESSMENT OF *NIGELLA SATIVA* EXTRACT AS A POTENTIAL ANTIBIOTIC ALTERNATIVE FEED SUPPLEMENT FOR WEANED SWINE

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New technologies are needed to help livestock producers to maintain health and wellbeing of their animals while minimizing risks of disseminating antimicrobial resistant bacteria to humans or animals. *Nigella sativa* (NS) is a plant containing bioactive constituents such as thymoquinone. Extracts of NS enhance immune function, improve performance and reduce enteropathogen colonization in poultry and small ruminants but studies in swine are lacking. Presently, we assessed the effect of NS extracts on intestinal carriage of wildtype *E. coli* and *Campylobacter* and a challenge strain of *Salmonella* Typhimurium orally inoculated (10^9 colony forming units, CFU) into newly weaned piglets 6 to 18 h before initiation of treatment. In study one, treatments were administered via gavage of an aqueous NS extract at doses equivalent to 0, 0.15 or 0.45% diet dry matter ($n = 6$ pigs/treatment). In study two, equivalent doses were administered via supplementation of the pigs' daily ration ($n = 6$ pens/treatment). Analysis of bacterial populations in gut samples collected 28 h or 9 days after initiation of treatment in studies one and two revealed tendencies of linear effects ($P < 0.09$) of treatment on *E. coli*, with populations recovered from NS extract-treated pigs being 0.7 to 1.8 log units lower than those recovered from jejunal, cecal and rectal contents of controls (which ranged from 6.05 to 8.10 \log_{10} CFU/g). Gut populations of *Campylobacter* and Typhimurium were unaffected ($P > 0.10$) by NS treatment in both studies. Feed intake and weight gain over the nine days of study two were unaffected by treatment ($P > 0.10$), averaging 2.85 ± 0.67 and 1.28 ± 0.70 kg, respectively, but feed efficiency was improved linearly ($P < 0.05$), achieving 0.28 ± 0.21 , 0.46 ± 0.11 and 0.54 ± 0.16 kg body weight gain/kg dry matter intake in 0, 0.15 and 0.45% NS-treated pens, respectively.

SELECTED POLYPHENOLS AS GROWTH PROMOTER

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Plant tissues are rich in a wide variety of secondary metabolites which have been found to have *in vitro* antimicrobial properties. Tannins, a type of plant polyphenol, are widely distributed among plant species in which they play a protective role against pathogen attack. Tannins in different plant species have specific physical and chemical properties which enclose very different biological activities. A series of *in vitro* and *in vivo* studies from our laboratory have been performed to characterize properties of different natural polyphenols, resulting in the selection of some of them to design a suitable blend to replace and improve the use of antibiotics as growth promoters. These selected tannins showed bacteriostatic and bactericidal activities against different pathogens, including *Clostridium perfringens* and antitoxin properties without evident induction of bacterial resistance. Also, this combination of selected natural compounds was able to regulate the intestinal microbiota, the physiologic host function, the morphology of the gastrointestinal tract, and the resistance to different infectious diseases of broilers including necrotic enteritis (NE). The weight gain was improved when compared to non-growth promoter control under all the different conditions tested: experimental farm (1.8%), experimental pathogen challenge (5-22%) and commercial farm (4.8%). A two years evaluation in large commercial settings of different countries with a specifically designed product based on previous lab trials (SilvaFeed HG) corroborated our previous findings. In all of the analyzed farms, comparing to controls using antibiotic growth promoters, an improvement of intestinal health, lesions, weight gain and reduction of mortality were measured, showing an improvement of productive parameters with cost reduction. The available information supports the use of specific mixture of tannins as alternative to antibiotic growth promoters.

VARIUM™ REDUCES MORTALITY AND IMPROVES FEED CONVERSION OF BROILERS ON A COMMERCIAL FARM

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Varium is a natural growth promoter for poultry, shown to reduce mortality and enhance feed efficiency in broiler chickens. Because of these results, a study was conducted using Varium on a commercial broiler facility in Peru. The objective of this study was to determine how Varium affected the performance of commercial broilers. Cobb-500 broilers (n = 49,540 Control; 53,025 Varium) of both sexes raised in a total of 14 houses were used in a study conducted at a farm located in Chancay, Lima, Peru. The diets used for the research were the commercial diets typically used on the farm. Feeds for the Control treatment were the standard diets typically used in commercial farms, while the feeds for the Varium treatment were the Control feeds supplemented with 1 kg Varium /MT feed (0.1%). Both treatments included an in-feed antibiotic. Feeding Varium decreased mortality by 40% compared to birds not fed Varium from hatch to 35 days of age. Weekly decreases in mortality were 54%, 19%, 27%, 50% and 53% for weeks 1 to 5, respectively, when Varium was fed. Birds fed Varium had an overall FCR of 1.49 compared to 1.64 for the birds on the Control feed. Females had a larger feed conversion response than males. Weight gain for broilers fed Varium was 2,129 g and the Control group was 2,110 g from hatch to 35 days of age. Reduced standard deviation of the live weight at d35 indicated improved homogeneity in the Varium group. Improvements in mortality and feed conversion resulted in increased European Efficiency Index from 350 to 400. Adjusted to 1,000,000 birds the added revenue from feeding Varium was over \$220,000 (US) with a return-on-investment for Varium of over 10:1. In conclusion, feeding Varium to commercial broilers in the study decreased mortality and improved FCR, while maintaining weight gain. Improved income on Varium fed birds showed a significant return-on-investment for Varium.

THE EFFECTS OF PLANT EXTRACTS ON GROWTH PERFORMANCE, SERUM BIOCHEMICAL PARAMETERS, DIGESTIVE ENZYME, APPARENT DIGESTIBILITY AND DEVELOPMENT OF IMMUNE ORGANS OF BROILERS

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The objective of this study was to investigate the effects of a plant extract product (Impim: dandelion extracts and glycyrrhiza extracts) applied in feeding broilers. Nine hundred day-old Cobb commercial broilers were randomly allocated into three treatments with three replicates in each treatment, with 100 broilers in each replicate. The three dietary treatments were 1) Control group (CON), basal diet; 2) Impim group, basal diet + 0.02% Impim; 3) Antibiotics group, basal diet + 0.002% Colistin sulfate premix + 0.004% Aureomycin premix. The trial period was 53 d.

The results showed that, ADG was increased by 2.37% ($P > 0.05$), average daily feed intake was increased by 0.32% ($P > 0.05$), and death and culling rate was decreased by 32.09% ($P < 0.05$) in broilers of Impim group than antibiotics group. Compared with antibiotics group, digestibility of Ca, P, CP and ether extract (EE) of Impim group were significantly improved 34.10%, 24.17%, 18.82% and 18.98% respectively ($P < 0.05$). Compared with antibiotics group, amylase activity and lipase activity of broilers fed with Impim had been significantly improved by 18.25% and 17.27%, respectively ($P < 0.05$). Serum total protein was increased by 9.91% ($P < 0.05$) in broilers of Impim group than antibiotics group. Glutamic oxalacetic transaminase (AST) was increased significantly in antibiotic group compared with control group, while AST in Impim group was the same statistically with control group. Spleen index of Impim group was increased significantly ($P < 0.05$) than antibiotics group at the 21 day and 42 day. These results show that plant extracts could promote the secretion of digestive enzyme and improve digestibility of nutrients, therefore improve growth performance. In addition, our results indicate that plant extracts improve fatty acids metabolism, alleviate liver damage and improve immune organ development although further studies are needed.

ALTERNATIVES TO ANTIBIOTICS: APPLICATIONS OF EMERGING THERAPIES

STUDY OF THE GROWTH INHIBITION CAPACITY AND THE RECOVERY CAPACITY OF A COMMERCIAL ESSENTIAL OILS BLEND AGAINST E.COLI, IN COMPARISON WITH COLISTIN ANTIBIOTIC

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Antibiotics resistance is a global concern and phyto-genic additives are new antibiotic alternatives identified to reduce the use of drugs in feed. The aim of this study was to investigate the growth inhibition capacity and the recovery capacity of an essential oils mixture against *E. coli* at inhibitory and sub-inhibitory concentrations (MIC and Sub-MIC). Bacteria growths were compared with a positive antibiotic control: colistin. First, an *E. coli* growth monitoring (\log_{10}) was performed from 0 to 6 hours in the presence of both antibacterial substances (essential oils vs. colistin) at different concentrations (MIC, MIC/2 and MIC/4). Then, a second *E. coli* growth monitoring was performed after elimination of antimicrobial agents by centrifugation previously in contact with the bacteria during 90 minutes. The recovery capacity is the difference of *E. coli* population (\log_{10}) between initial time corresponding to the elimination and 24 hours. Both antibacterial substances demonstrated a concentration-dependent growth inhibition capacity at sub-MIC concentrations. Unlike the essential oils mixture, colistin had a bactericidal trend; the *E. coli* recovery capacity was non-existing after exposure to a MIC colistin concentration and equal to the negative control when using essential oils blend MIC. However, an MIC/2 exposure to colistin results in a 3 times higher population instead of MIC/2 essential oils blend and negative control. This study showed the bacteriostatic activity of the essential oils blend at sub-MIC concentrations. Sub-MIC colistin exposure increases significantly the *E. coli* recovery capacity. A hypothesis was the modification of the bacteria metabolism due to an adaptation. Using a sub-MIC dose of essential oils blend did not change the *E. coli* recovery capacity. Further studies are needed to investigate the mode of action including resistance patterns.

OREGANO ESSENTIAL OIL INDUCES SOD1 AND GSH EXPRESSION THROUGH NRF2 ACTIVATION AND ALLEVIATES HYDROGEN PEROXIDE-INDUCED OXIDATIVE DAMAGE IN IPEC-J2 CELLS

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Oregano essential oil (OEO) has long been used to improve the health of animals, particularly their intestinal health. The health benefits of OEO are generally attributed to antioxidative actions, but the mechanisms remain unclear. Here, we investigated the antioxidative effects of OEO and their underlying molecular mechanisms with porcine small intestinal epithelial (IPEC-J2) cells. We found that OEO treatment prior to hydrogen peroxide (H_2O_2) exposure increased the cell viability and prevented lactate dehydrogenase (LDH) release into the medium. H_2O_2 -induced reactive oxygen species (ROS) and Malondialdehyde (MDA) were remarkably suppressed by OEO. OEO dose-dependently increased mRNA and protein levels of the nuclear factor-erythroid 2-related factor-2 (Nrf2) target genes, Cu/Zn-superoxide dismutase (SOD1), and g-glutamylcysteine ligase (GCLC, GLCM), as well as intracellular concentrations of SOD1 and glutathione. OEO also increased intranuclear expression of Nrf2 and the activity of an antioxidant response element reporter plasmid in IPEC-J2 cells. The OEO-induced expression of Nrf2-regulated genes and increased SOD1 and glutathione concentrations in IPEC-J2 cells were reduced by Nrf2 small interfering (si) RNAs, counteracting the protective effects of OEO against oxidative stress in IPEC-J2 cells. Our results suggest that OEO protects against H_2O_2 -induced IPEC-J2 cell damage by inducing Nrf2 and related antioxidant enzymes.

OREGANO ESSENTIAL OIL DECREASED SUSCEPTIBILITY TO OXIDATIVE STRESS-INDUCED DYSFUNCTION OF INTESTINAL EPITHELIAL BARRIER IN RATS

DYSFUNCTION OF INTESTINAL EPITHELIAL BARRIER IN RATS

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The protective effects of oregano essential oils (OEO) against oxidative stress in intestine of rats were investigated. A total of thirty Wistar rats were randomly allocated to five groups with six replicates per groups and treated for two weeks as follows: the control group (CT); the negative control group (NC); the negative control treated orally with 20 mg vitamin E/kg body weight (VE); the groups treated orally with 5 mg OEO/kg body weight (LO) and 10 mg OEO/kg body weight (HO). On day 15, all treatment groups, except CT, received 0.1 mmol/kg bw of diquat i.p. dissolved in normal saline. CT group received an equal dose of normal saline i.p. Rats offered OEO diet had an increased villus height and the ratio of villus height to crypt depth compared with NC. Consistently, we found that the expression of Occludin and ZO-1, the two major tight junction proteins in epithelia, was significantly increased by OEO treatment compared with NC. HO significantly decreased the concentrations of ROS levels in jejunal mucosa and digesta. Both LO and HO enhanced the activities of plasma SOD and GSH-Px and decreased the MDA concentration in rats under oxidative stress. Rats offered OEO supplemented diet had higher *Lactobacillus* population and lower *E.coli* population under oxidative stress. Rats offered OEO supplemented diets had a lower *TNF- α* and *IL-6* mRNA expression in the jejunum compared with NC. These results revealed that OEO exerted a protective effect against diquat-induced oxidative injury in intestine of rats.

ALTERNATIVE APPROACHES TO TREATMENT OF URINARY TRACT INFECTIONS IN DOGS: A PILOT STUDY

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Bacteria represent the most common cause of urinary tract infection (UTI). Bacterial UTIs occur as a consequence of ascending migration of pathogens through the genital tract and urethra to the bladder, ureters, and one or both kidneys. Antimicrobial drugs are the cornerstone of treatment of UTIs. Because of the chronic nature of UTIs and the potential for antibiotic resistance, a natural approach to prevention and treatment is desirable. Clinical research suggests the best natural options for long-term prevention include consuming cranberry, mannose, and probiotics. The purpose of this study was to evaluate the efficacy of a polyphenolic extract from cranberry juice concentrate (35%) in canine cystitis. Enrollment was considered for 10 dogs with clinical signs consistent with bacterial cystitis confirmed by positive urine culture. The product was daily orally administered for 60 days. Complete blood count, serum biochemistry profile, right lateral abdominal radiograph and urinalysis were performed to exclude the presence of predisposing conditions for cystitis and to monitor the effects of the treatment. The evaluation were made at day 0 (D0), 15 (D15), 30 (D30) and 60 (D60) after the start of the treatment. The Wilcoxon-Mann-Whitney-Test was used to evaluate differences between the times; $p < 0.05$ as significance level was chosen. A significant decrease in protein, epithelial cells, leukocytes and bacterial counts were observed in urine at D60 compared to D0. All dogs at D60 showed a negative urine culture. Haematological analysis showed a significant decrease of leukocytes and neutrophils. Moreover ultrasonography revealed a significant improvement of the urinary bladder mucosal irregularity and thickening. This study, even if performed on a limited canine population, suggests a potential efficacy of the product on canine bacterial cystitis. A larger study need to be performed to confirm these data.

EFFICACY OF FORMULATED CARVACROL ON *CAMPYLOBACTER JEJUNI* IN VITRO AND ELECTRONIC MICROSCOPY APPROACHES

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Bacteria from the *Campylobacter* genus are well known as the leading cause of foodborne diarrheal disease worldwide with *Campylobacter jejuni* and *Campylobacter coli* representing the most frequently involved species. The main source of infection is the meat from poultry origin mostly contaminated during evisceration. Thus, reducing *Campylobacter* concentrations in the intestinal tract and particularly in the caeca may help to decrease flesh colonization and human infections by the bacteria. Some natural substances have interesting antimicrobial properties. Studies have reported the antibacterial effect of carvacrol against bacteria from the *Campylobacter* genus. As essential oils compounds are often absorbed before they reach the last part of the intestinal tract, they do not get to the site of *Campylobacter* growth. A new galenic formulation (Phodé Sciences, France) has been created to resolve this issue. This product contains a liquid formulated core based on carvacrol, and a specific solid carrier. In the present study, we compared the efficacy of carvacrol and the formulated carvacrol, against *Campylobacter jejuni* ATCC 33291 using a broth microdilution method. The new formulation of carvacrol has the same efficacy as the carvacrol alone ($P > 0,05$). We also compared the mechanism of action of both products by Scanning and Transmission Electron Microscopy. The new galenic formulation still showed the same results as pure carvacrol. Treated cells showed wrinkles, clefts and blisters. We noticed too, large membrane blebs caused by separation of the plasma membrane from outer membrane, with leakage of the cytoplasmic content into the intermembrane space. In spite of the galenic, the new formulation of carvacrol has the same activity as non-formulated carvacrol *in vitro*. The next step will be to test the new formulation on chicken *in vivo*.

PHYTOCHEMICALS REDUCE *CAMPYLOBACTER JEJUNI* COLONIZATION FACTORS AND TRANSCRIPTION OF VIRULENCE GENES *IN VITRO*

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The foodborne pathogen *Campylobacter* is the leading cause of bacterial gastroenteritis in humans resulting in an estimated 96 million annual infections globally. In the United States, an estimated 1.3 million cases of Campylobacteriosis occur each year largely due to consumption of contaminated poultry products. Chicken act as the reservoir host for *Campylobacter*, wherein the pathogen colonizes the intestine, especially the ceca thereby leading to contamination of carcass during slaughter. Motility and attachment to intestinal epithelium are the two major factors responsible for *Campylobacter* colonization in chicken. Reducing the expression of aforementioned factors could potentially reduce *Campylobacter* survival in chickens and risk of subsequent human infections. This study investigated the efficacy of sub-inhibitory concentrations (SICs, concentration not inhibiting bacterial growth) of three, generally regarded as safe (GRAS)-status phytochemicals (*trans*-cinnamaldehyde 0.01%, carvacrol 0.002%, eugenol 0.01%) in reducing the major colonization factors (motility, epithelial adherence) critical for survival of *C. jejuni* in chickens. In addition, the effects of the phytochemicals on the expression of critical colonization genes were studied using real-time quantitative PCR. All experiments had duplicate samples and were replicated three times on three strains of *C. jejuni* (Wild type S8, NCTC 11168, 81-176). Data were analyzed using ANOVA with GraphPad ver. 6. Differences between the means were considered significantly different at $P < 0.05$. All phytochemicals reduced *C. jejuni* motility and adhesion to chicken primary enterocytes ($P < 0.05$). Real-time PCR revealed that all phytochemical treatments reduced the transcription of critical chicken colonization genes in *C. jejuni* ($P < 0.05$). Results suggest that *trans*-cinnamaldehyde, carvacrol, and eugenol could potentially be used to control *Campylobacter* colonization in chickens and reduce the incidence of human foodborne illnesses. Follow up studies investigating the efficacy of in-water supplementation of *trans*-cinnamaldehyde, carvacrol and eugenol in reducing *C. jejuni* colonization in the chicken gut and their effect on the chicken cecal microbiome are currently underway.

USE OF NATIVE PLANTS FROM ARGENTINA TO CONTROL *SALMONELLA* ENTERITIDIS IN POULTRY

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Salmonella infection is a major cause of bacterial enteric illness in both human and animals and poultry production is usually associated with the contamination of this food-borne pathogen. Among other measures, antimicrobials are traditionally used to control *Salmonella* which provides a selection pressure resulting in the preferential spread of resistant microorganisms, not only between *Salmonella* strains, but also among other species of *Enterobacteriaceae*. Therefore new alternatives to antimicrobials are needed to guarantee the food safety for the consumers. The aim of this work was to assess the efficacy of two additives based on native plants from Argentina to diminish the excretion of *Salmonella* *Enteritidis* in an experimental infection model in broilers. Groups of 15 chickens were fed from the first day of life with regular feed added with 0.1% of desiccated and ground native plants from Argentina, belonging to the family *Leguminosae* (plant 1) and family *Anacardiaceae* (plant 2). A control group of birds was also included. At day 6, chickens were individually challenged by oral gavage with 10^7 CFU of *S. Enteritidis* and individual samples were taken by cloacal swabbing at the 3rd, 7th and 12th day post-infection (dpi) to determine the excretion of the microorganism. Samples were enriched using tetrathionate broth and thereafter detection of the strain was done on XLD agar plates. At the 3rd dpi, 8/15, 0/15 and 2/15 birds respectively from control, plant 1 and plant 2 groups were positive for *Salmonella*. At the 7th dpi, *Salmonella* was isolated from 8/15, 3/15 and 5/15 birds of control, plant 1 and plant 2 group. Finally, at the 12th dpi, 7/15, 2/15 and 4/15 of the birds respectively from control, plant 1 and plant 2 groups excreted the pathogen. Both plants were effective to diminish the excretion of *Salmonella*, particularly during the first days after challenge; the effect of native plant 1 was more evident. In productive conditions, reduced excretion of *Salmonella* from infected animals at early stages is crucial to control contamination in the environment. Therefore, the use of these alternative natural products, combined with appropriate biosecurity management, may be a useful tool to diminish and avoid the use of antibiotics to control the pathogen in poultry and consequently to improve the food safety for the consumers.

EFFICACY OF A CHESTNUT-BASED ADDITIVE TO PROTECT AGAINST FOWL TYPHOID IN LAYING HENS

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Fowl typhoid is caused by *Salmonella Gallinarum* biotype *gallinarum* (*S. gallinarum*) and may lead to important economic losses in the global poultry industry, mainly due to mortality (from 10% to 100%) and drop of egg production in laying hens. The disease has been officially eradicated from commercial poultry in many developed countries, but still subsists as an endemic infection in most developing countries and non-commercial farms. Antimicrobial treatments are commonly used to reduce mortality but it is unlikely that treatment will completely eliminate fowl typhoid from an infected flock. Furthermore, in the last years augmented resistance of *S. gallinarum* strains for some antibiotics commonly administered has been observed. In this work, the use of chestnut extracts as an alternative to prevent or treat fowl typhoid was assessed. Sixteen-week-old laying hens were divided in three groups of 14 birds each. Hens from control group received commercial feed and hens from treated groups received normal feed plus 5 ppm of chestnut extract from 24 h before infection onwards (Group 1) or from 24 h after infection onwards (Group 2). All hens were orally challenged with 10^6 CFU of *S. gallinarum* and mortality was recorded for 10 days. Seventy-one percent (10/14) of the hens died from fowl typhoid in the control group. In the treated groups, mortality was of 36% (5/14) in Group 1 and of 50% (7/14) in Group 2. Reduction of the mortality was significant in the Group 1 ($P= 0.0465$) but not in Group 2 ($P= 0.1886$). Thus chestnut extracts may be useful to prevent fowl typhoid in the flocks, although once the animals are infected, the additive may not be completely efficient to reduce mortality. Combined strategies such as preventive feed additives, vaccination and biosecurity measures seems to be the most likely approach to improve the control of fowl typhoid and may help to eradicate the disease from countries where it is endemic.

MATERNAL DIETARY APPLICATION OF SEAWEED EXTRACT (LACTOSHIELD®) IMPROVES THE PERFORMANCE OF PIGS IN THE POST-ANTIBIOTIC ERA

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Supplementation of feed with seaweed extract has been shown to have a positive effects on performance, immune status and intestinal morphology and microflora in sows and post-weaned pigs. It was hypothesized that maternal seaweed extract (LactoShield®) supplementation would enhance the growth performance of offspring pigs. Pregnant sows were fed with either 1) a basal lactation diet or 2) a basal lactation diet supplemented with LactoShield® from day 107 of gestation until weaning (28 days of age). The LactoShield® supplement was supplied by BioAtlantis Ltd. (Kerry, Ireland). At weaning the piglets were moved to separate houses according to their dietary treatment (basal group, n=33 and LactoShield® group, n=34). The amount of feed given and the amount of waste feed removed was recorded daily. Piglets were weighed at birth and at 7, 11, 18, 26, 32, 40, 46, 53, 60, 67 and 78 days of age. Feed intake and body weights were used to calculate average daily gain (ADG) and gain to feed ratio (GFR). Pigs from LactoShield® supplemented sows showed improved performance. Pigs in the LactoShield® group had greater ADG on days 0-7, 25-32, 40-46, 53-60, 25-53 and 25-78 ($P < 0.05$) compared with pigs in the control group. A statistically significant higher average body weight was also observed in the LactoShield® treated group on days 7, 32, 46, 53, 60, 67 and 78 ($P < 0.05$). Assessment of performance parameters demonstrated that pigs weaned from LactoShield® supplemented sows had a significantly improved ADG and average body weight. ADG was improved in the critical early period after weaning and from weaning to termination of the experiment at 78 days of age. As such LactoShield® supplementation could be a useful management tool for pig producers in this post-antibiotic era and could help to improve margins in a difficult marketplace.

TANNINS ARE A USEFUL TOOL TO AVOID LIVER ABSCESES IN FEEDLOT CATTLE

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In ruminants that are subject to diets with high content of concentrates, such as feed-lots conditions, frequent episodes of ruminal acidosis are observed. Many of these episodes occur are subclinical, without apparent signs but producing a series of disorders that negatively impact the animal productivity. Liver abscess is one of the conditions caused by acidosis in the ruminal environment, originated by the injury that occurs in the ruminal epithelium due of the low-pH. That injury in the epithelium of the rumen makes it more permeable for the passage to the blood (System porta) of bacteria such as *Fusobacterium necrophorum* and *Corynebacterium pyogenes* that cause bacterial thrombus impacting the liver. Liver abscesses results in lower consumption of dry matter, lower daily weight gain, and lower conversion efficiency as well as liver confiscation at the time of slaughter. Antibiotics Growth Promoters (AGP) are commonly used in feedlot animals to reduce the incidence of hepatic abscesses. It has been observed that vegetable extracts (i.e. tannins) can prevent ruminal acidosis and have a strong antibacterial activity as well, so they could be an important tool for the prevention of hepatic abscesses. In order to test the efficacy of a mix of natural tannins in the prevention of hepatic abscesses emergence, 495 heifers divided into two groups, were fed during two months with the same basal diet, but added only with 0.25% (MS) of a blend of tannins (Silva feed Bypro), or with 40 mg /kg DM of monensin. The analysis of abscesses in the livers at the slaughter showed a 1.2% of prevalence in the tannin group vs 6.1% in the monensin group ($p < 0.05$), suggesting that the addition of selected blend of tannins can significantly reduce the prevalence of hepatic abscesses in animals in confinement.



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ORAL PRESENTATIONS

4.1

INNATE DEFENSE MECHANISMS AND PASSIVE IMMUNITY

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Innate defense mechanisms comprise generalized host reactions towards infection, aimed at quickly containing and removing the infectious microorganism. This is accomplished by a highly coordinated sequence of events profoundly changing the population of cellular and soluble factors in the affected tissue. In the aftermath of these events tissue homeostasis is restored, resolving the acute phase of the response and activating the adaptive immune system. Innate defense mechanisms include activation of local stromal and immune cells, the induction of cytokine and chemokine messengers and the resulting attraction and activation of neutrophils (also known as inflammation), the induction of antimicrobial peptides and acute phase proteins, and, finally, the activation of the complement system. The innate selection of antibodies found in the neonate, whether acquired during gestation through the placental blood supply or post-delivery by ingestion of colostrum and milk per definition also forms part of the innate defense system.

As alternatives to antibiotics, innate immune mechanisms are highly relevant as they offer general, rapid, evolutionary robust, non-resistance inducing ways to protect, at least partially, against infections, optimally increasing resistance against disease by allowing the adaptive immune system time to take over protection in time to stop disease from developing. For products based on innate defense mechanisms to become attractive leads as alternatives to antibiotics in the animal production they obviously need to match the efficiency and ease of use of traditional antibiotics, however they also need to be very cost-efficient, broadly applicable, and with low adverse effect levels and high safety, as well as being acceptable to consumers. As an example of a class of biological molecules displaying most of these characteristics, natural immunoglobulin pools, isolated from slaughterhouse blood, whey or other low value side streams and having a very wide range of specificities will be discussed in more detail.

4.2

IMMUNOMODULATORS DERIVED FROM HOST DEFENCE PEPTIDES

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While antibiotics have been indispensable for the treatment of bacterial infections, the increase in antibiotic resistance and lack of new antibiotic compounds, greatly limits the available treatment options. Therefore, development of novel anti-infective therapies is crucial for the treatment of bacterial infections in the future. In the search for new anti-infective templates, host defense peptides, such as cathelicidins, have gained a lot of interest. Of these cathelicidins, the chicken cathelicidin-2 (CATH-2) has been shown to be an interesting template, with broad-spectrum antimicrobial activities and potent immunomodulatory activities.

In order to investigate the efficacy of CATH-2 as an immunomodulatory agent *in vivo* the stable

D-amino acid analog of CATH-2 (D-CATH-2) was administered to chickens *in ovo* at three days before hatch. First, *in ovo* live imaging of zirconium-89 labeled CATH-2 peptide by positron emission tomography was carried out to study the uptake by chicken embryos, a technique to study peptide distribution *in vivo*. By *in ovo* injection of labelled CATH-2 peptides, uptake of the peptide by the embryo was seen from 4 hours after injection onwards followed by accumulation in the gastrointestinal and respiratory tract. D-CATH-2 administration *in ovo* partially protected chicks against a systemic *Salmonella* infection or a respiratory *E. coli* infection in the first week posthatch. In the salmonellosis model mortality was 50% reduced by administration of the peptide and the number of birds with clinical symptoms was even 69% less than in control infected chickens. In the colibacillosis model the reductions in mortality and morbidity were 30% and 52%, respectively.

It is concluded that prophylactic treatment with immunomodulatory cathelicidin-derived peptides may reduce the likelihood of bacterial infections of young animals. The elevated threshold above which infections occur may help to reduce the use of conventional antibiotics.

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4.3

EGG SHELL MEMBRANE IMPROVES IMMUNITY OF POST HATCH POULTRY: A PARADIGM FOR NUTRITIONAL IMMUNOMODULATION

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Improving natural immunity can reduce susceptibility to infection, improving animal health and eliminating prophylactic use of antibiotics. However, there are very few affective means to enhance natural immunity to a wide range of infections. Considering the natural diets of most birds and mammals, it is intuitive that the newborns often depend upon wild and biodiverse diets that can be pro-immunomodulatory because of their bioactive protein constituents. We hypothesized that the egg shell membranes (ESM) which constitute a significant part of egg shell refuse and abound with many bioactive proteins and peptides including enzymes, antimicrobials, and cell regulatory proteins, can potentially exert epigenetic immunomodulation similar to natural diets which contain many such factors. Besides, the presence of different microbial proteins, originating from bacterial contaminants of these eggshells, can help establish immunity against microbes. We conducted two *in vivo* experiments to evaluate the effect of fresh and hatchery ESM provided at a concentration of 0.5% in diet during the first two weeks of growth. We measured the effects on growth and different physiological parameters including serum antibodies at 3 and 5 weeks of age. Chickens receiving either types of ESM supplement showed moderate improvement of growth, decreased blood corticosterone and heterophil/ lymphocyte ratios, and increased IgG and IgM at three weeks of age. The chickens receiving 0.5% HESM showed resistance to lipopolysaccharide induced body weight loss and decreased expression of pro-inflammatory and increased expression of anti-inflammatory cytokine genes in the spleen. Thus, our results show that ESM supplement, during post hatch growth can improve immunity, impart resistance to endotoxin induced changes, and decrease stress variables. We believe the effects are due to the bioactive factors such as enzymes, growth factors, and cell regulatory proteins which most likely modulate the early development of immune system and build a wide range of resistance and tolerance to disease.

4.4

GREEN ALGAL SULFATED POLYSACCHARIDES: A NATURAL ALTERNATIVE TO ANTIBIOTICS VIA MODULATION OF THE INTESTINAL IMMUNE RESPONSE

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Alternative prophylactic strategies are needed to limit the use of antibiotics in animal husbandry. One potential solution is to limit the level of infections via stimulation of the innate immune response. Green algae have emerged as a rich source of bioactive natural compounds that can be used as a new generation of growth enhancers capable to potentiate the immune function and improve animal health. Recently, a crude extract of marine sulfated polysaccharides (MSP) prepared from the green macroalgae *Ulva armoricana* harvested from the shores of northern Brittany (France) was evaluated, *in vitro*, as an antibacterial compound against bacterial strains found in livestock animals as well as for its immunomodulatory activity on differentiated porcine intestinal epithelial cell line IPEC-1. We showed that this MSP extract was able to inhibit the bacterial growth of several pathogenic bacteria and stimulate mRNA expression of intestinal immune response mediators such as IL1 α , IL1 β , IL-6, IL-8, TNF α , TGF β , CCL20, PPAR γ , a ligand-activated transcription factor, and the TLR2 receptor. The MSP extract was further purified and the low molecular weight water-soluble sulfated polysaccharides fraction (ulvan) confirmed its capacity to stimulate the expression of cytokines such as CCL20, IL8, and TNF α , both at mRNA and protein levels. By using human embryonic kidney HEK 293 reporter cell lines for pattern recognition receptors, we found that this ulvan fraction act mainly via interaction with the TLR4 receptor. In addition, western blot analysis of ulvan-treated HEK 293-TLR4 cells showed an increase in the Akt signaling pathway and nuclear factor- κ B (NF κ B) phosphorylation, able to induce cytokine gene expression. Used as a feed additive, the MSP may constitute a new prophylactic strategy to increase intestinal health of livestock animals, and thereby reducing the subsequent use of antibiotic treatment. Furthermore, the ulvan fraction could be used, in vaccination strategies, as an immunostimulating adjuvant compound to effectively complex and deliver TLR ligands to relevant immune cells.

4.5 MAINTENANCE OF VASCULAR INTEGRITY VIA ARF6-GTP INHIBITION PROTECTS MICE FROM MDR ACINETOBACTER INFECTION

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Background

Multi-drug resistant (MDR) Gram negative bacteria (GNB) infections (e.g. caused by *Acinetobacter baumannii* [AB]), portend global health and well-being. Thus, novel methods to treat these infections are needed. Our solution relies on altering the immune response to GNB infections. Specifically, we propose to develop therapies that inhibit ARF6-GTP formation, a molecule essential for lipopolysaccharide (LPS)-mediated vascular leak often associated with septicemia. We hypothesize that by pharmacologically inhibiting ARF6-GTP we will preserve vascular permeability, reduce tissue edema and prevent organ failure while having no adverse effect on immunity-based clearance of the pathogen.

Methods

AB-mediated ARF6-GTP formation in human umbilical vein endothelial cells (HUVEC) and the effect of ARF6-GTP inhibitors was studied by immunoprecipitation (IP) and trans-well permeability assays. HUVEC VE-Cadherin expression was tracked by immunofluorescence. Contribution of ARF6-GTP to AB virulence *in vitro* and *in vivo* was studied by reduction of ARF6-GTP expression (siRNA) and by using ARF6^{-/-} mice, respectively. ARF6-GTP Inhibitors were evaluated for their protective effect in neutropenic mice with AB pneumonia.

Results

AB LPS activated HUVEC ARF6-GTP and increased trans-well permeability via TLR-4 signaling. ARF6-GTP inhibitors reduced AB-mediated ARF6-GTP activation and abrogated HUVEC trans-well permeability by ~70% and ~85%, respectively ($P < 0.05$). Further, AB disrupted HUVEC junctions via reduction of VE-Cadherin surface expression and the inhibitors reversed this effect. HUVEC with reduced ARF6-GTP expression and ARF6^{-/-} mice were resistant to AB-mediated trans-well permeability and infection, respectively. Finally, a water soluble ARF6 inhibitor (NAV-5093) significantly improved survival of mice ($n = 10$ per arm) when compared to placebo (21-day survival of 90% vs. 0% survival for placebo, $P < 0.004$) with surviving mice appearing healthy.

Conclusions

AB virulence is reliant on inducing vascular permeability via activation of ARF6-GTP formation. Inhibition of ARF6-GTP formation reduces vascular leak and enhances survival of AB infected mice. Development of ARF6-GTP inhibitors as a novel treatment of MDR-GNB is warranted.

4.6

SWINE PLASMA IMMUNOGLOBULINS FOR PREVENTION AND TREATMENT OF POST-WEANING DIARRHOEA

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Post-weaning diarrhoea (PWD) is a common condition in intensive swine production and is caused by enteric enterotoxigenic *Escherichia coli* (ETEC) infection. With the aim of developing a sustainable product for protection against PWD, natural antibodies (immunoglobulins) derived directly from inexpensive raw materials were investigated as an alternative to antibiotics and zinc. Natural porcine IgG was isolated directly from slaughterhouse pig plasma waste by expanded bed adsorption chromatography, and used in small-scale challenge models of PWD weaner piglets in which the purified porcine IgG (pplgG) was provided orally to the weaner piglets to observe the effect on disease symptoms.

In three ETEC challenge experiments, modelling PWD, oral pplgG led to faster clearance of the *E. coli* challenge strain compared to non-pplgG treated challenged groups. In two of the experiments the ETEC-challenge resulted in diarrhoea, and pplgG reduced disease symptoms faster than in the control groups, even compared to a group given dietary zinc oxide. Collectively these results indicate that pplgG could be an alternative to antibiotics and dietary zinc for treatment and prevention of PWD.



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POSTER PRESENTATIONS

THE IMPACT OF DIETARY SWINE PLASMA IMMUNOGLOBULINS ON THE INTESTINAL MICROBIOTA AND GENERAL HEALTH IN WEANER PIGLETS

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Porcine IgG was isolated directly from slaughterhouse pig plasma waste by large-scale expanded bed adsorption chromatography. The resulting preparation of purified porcine IgG (pplgG) was shown to harbour reactivity against enterotoxigenic *Escherichia coli* (ETEC) (Hedegaard *et al.* 2016) and thus has the potential to be an alternative to antibiotics for treating and preventing diarrhoea caused by ETEC. Before launching *in vivo* experiments addressing the effect of pplgG on ETEC induced diarrhoea we studied the effect of enterally administered pplgG on weaner piglet health and intestinal microbiota composition.

Weaner piglets receiving either 1 or 4 grams of dietary pplgG for up to 14 days after weaning showed no change in systemic IgG levels, and no disease signs including the absence of intestinal pathology as compared to an age matched control groups receiving no pplgG. In weaning piglet challenged with ETEC we studied the effect of pplgG on the intestinal microbiota, and observed that pplgG decreased shedding of haemolytic bacteria faster as compared to control groups without changing the amount of faecal non-haemolytic bacteria. Further studies on the effect of pplgG on weaner piglet microbiota will be presented. Thus pig slaughterhouse plasma is indicated as a safe and sustainable source of antibodies for oral administration to control of ETEC infections in pigs.

ENGINEERING CHIMERIC ANTIBODIES AIMED FOR PASSIVE MUCOSAL IMMUNIZATION AGAINST HRSV

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Human Respiratory Syncytial Virus (HRSV) is the leading cause of acute lower respiratory tract infection in infants and frequently causes severe disease in the elderly. There is no licensed HRSV vaccine. As an alternative, the prophylactic treatment with an HRSV fusion protein targeting monoclonal antibody (mAb) is often recommended for infants that are at risk for developing HRSV infection. However, the high cost that is associated with the current mammalian cell-based mAb manufacturing systems hampers the broad implementation of this therapy. Also, it is possible that IgA type antibodies against HRSV may contribute even better to protection. Therefore, we aim to compare the effectiveness of IgG with secretory IgA based passive immunization against HRSV. In this context, we choose for the transient expression platform in plants that permits rapid small scale production of IgG and IgA based antibody versions [1].

In this study, we have genetically fused three different single domain antibodies that are specific for the HRSV fusion protein F [2] to the fragment crystallisable part (Fc) of different murine and human monomeric IgA and IgG antibodies. In order to obtain all different permutations we took advantage of the GoldenBraid2.0 cloning system [3]. Since secretory IgA are the predominant antibodies in mucosal surfaces, they might be more effective than monomeric IgA and IgG in virus neutralization; therefore, IgA based antibodies will also be tested in their dimeric and secretory forms. A total of 15 different versions of these chimeric antibodies against HRSV have been engineered and produced in *Nicotiana benthamiana* via *Agrobacterium tumefaciens* – mediated transient expression. HRSV neutralization potency of the antibodies was confirmed *in vitro* by plaque reduction assay. In the future, we aim to further characterize these antibody fusions for neutralization effectiveness by challenge experiments in animal models.

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PREPARATION OF THE EGG WITH BROADLY PROTECTION AGAINST DIARRHEA CAUSED BY ENTEROTOXIGENIC *ESCHERICHIA COLI*

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The aim of this study was to produce and characterize egg yolk immunoglobulin (IgY) against the enterotoxigenic *Escherichia coli* (ETEC) as well as to evaluate the anti-adhesion activity of IgY *in vivo*. Specific IgY was obtained from the yolk of hens immunized with fusion protein EtpA-FliC and FliC-FliC and EtpA proteins. The IgY was obtained from egg yolks using the ammonium sulphate precipitation method and it was characterized by SDS-PAGE, Western-blot and ELISA, demonstrating that anti-adhesion IgY strongly reacted specifically against EtpA-FliC and FliC-FliC FliC and EtpA proteins. IgY was more resistant to Trypsin compared to pepsin. IgY was stable when the temperature was below 70 °C or pH>3 while complete loss of IgY activity was observed when the temperature exceeds 70 °C or pH 3. Finally, the anti-adhesion activity of three IgYs was evaluated using a BALB/c mouse model. Anti-EtpA-IgY effectively reduced adherence of ETEC strains H10407 to mouse intestines tract ($P < 0.05$). Anti-EtpA-FliC-IgY significantly inhibit ETEC strains E519 adhesion ($P = 0.01$).

Anti-FliC-FliC-IgY significantly reduced adherence of ETEC strains H10407 and E519 to mouse intestines tract ($P = 0.01$). Anti-EtpA-FliC-IgY and Anti-FliC-FliC-IgY only had the tendency of inhibiting colonization ($P = 0.09$) when infected mouse with ETEC strains 44815. These results demonstrate that anti-adhesion IgY affords broadly significant protection against ETEC colonization, suggesting that anti-adhesion IgY has further application in passive immunotherapy of ETEC diarrhea.

LACTOFERRICIN TOPICAL EMULSION FOR THE TREATMENT OF ATOPIC AND FOLDS CANINE DERMATITIS: PRELIMINARY DATA

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Atopic and folds dermatitis are associated with high numbers of yeasts and bacteria on the skin surface. Generally topical antiseptics and anti-inflammatory treatments are required. The present study evaluated the potential beneficial role of a topical emulsion containing lactoferricin 7.5%, verbascoside 0.1% and glycerophosphoinositol-lysine 2% in dogs affected by dermatitis. Ten dogs were included according to good general practice guidelines. The emulsion was daily applied using one pump spray at 10 cm of distance. Animals were clinically evaluated at day 0 and 14 using Canine Atopic Dermatitis Extent and Severity Index (CADESI) and visual scale (VAS) score. Cytological smears were also done. Samples were quantitatively judged for keratinocytes, cocci and *Malassezia* spp. presence using a semi-quantitative score method (0: <5/100x; 1: 5-10/100x; 2: 10-20/100x; 3: 20-40/100x; 4: >40/100x). Descriptive statistical analysis was performed. The Wilcoxon signed rank test for paired samples was used to determine the mean differences between evaluated parameters before and after treatment ($P < 0.05$). The following significant results were obtained: 6.90 and 2.10 for CADESI ($P = 0.007$); 4.30 and 3.10 for VAS ($P = 0.016$); 2.90 and 1.20 for keratinocytes ($P = 0.002$); 2.00 and 0.80 for cocci ($P = 0.008$). A negative trend of the mean of *Malassezia* spp. was observed (0.80 and 0.40) even if the differences were not statistically significant ($P = 0.063$). The present research, even if it was an uncontrolled study performed on a small number of dogs, suggests that daily applications of tested emulsion are effective in reducing bacterial overgrowth and clinical signs in skin folds and atopic dermatitis. Further studies on a large number of dogs are needed following this pilot study to confirm these results.

ANTIMICROBIAL DEFENSE BY INTESTINAL NEMATODES

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Increasing antibiotic resistance in pathogens resulting in less effective antibiotics poses a severe threat to human health, highlighting the urgency for the development of novel antimicrobial agents. Antimicrobial peptides (AMPs) are an ancient component of innate immunity, distributed across all multicellular organisms, providing protection against various pathogens. Furthermore, many AMPs have been shown to modulate immune responses. Parasitic nematodes are widespread in nature, infecting humans and animals alike. As nematodes induce long term chronic infections that comprise tissue migrating life stages without inciting overt inflammatory immune responses, we hypothesize that intestinal nematodes release factors which interfere with the host microbiota and thus exhibit antimicrobial activities. Therefore, excreted/secreted (ES) products are collected from *ex vivo* cultured nematodes (*A. suum*, *T. suis*, *H. polygyrus*), fractionated by adsorption chromatography and methanol gradient elution, and tested for antimicrobial activity. Radial diffusion assays with ES fractions demonstrate antimicrobial activity against gram+ and gram- bacteria. The fractions undergo peptide mapping by liquid chromatography-tandem mass spectrometry analysis. Peptide sequencing reveals the presence of lysozyme, chitinase, antimicrobial peptides including members of the cecropin and *Ascaris suum* antimicrobial factor families. Crystal violet assays to assess static biofilm development suggest decreased biofilm formation in the presence of nematode ES products. Hence, preliminary results indicate that nematode ES products possess promising antimicrobial and anti-biofilm activities. Ongoing efforts are aimed at characterizing specific factors responsible for observed antimicrobial activities and whether these factors interfere with invasion and replication of pathogenic bacteria. Future studies will explore the mechanisms of interference and the therapeutic potential of the nematode-derived antimicrobials in infectious disease models.

A COMPARISON OF CLINICAL AND ECONOMIC OUTCOMES WHEN METAPHYLACTICALLY ADMINISTERING EITHER A NOVEL DNA IMMUNOSTIMULANT OR TILMICOSIN TO BEEF CALVES AT MEDIUM – HIGH RISK OF DEVELOPING BOVINE RESPIRATORY DISEASE IN THE FEEDLOT

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Bovine respiratory disease (BRD) is a major source of morbidity and production loss in the beef and dairy industries. The objective of this study was to evaluate the efficacy of a novel DNA Immunostimulant (Zelnate[®]), labeled to aid in the treatment of BRD, versus the currently approved antibiotic Tilmicosin (Micotil[®]) when administered for the control of BRD in feedlot cattle. The inclusion criteria consisted of healthy cattle who were categorized as being of medium to high risk for the development of BRD by the Investigator and the participating feedlot. On Day 1, 2004 calves were enrolled into the study population (Tilmicosin, N=1002; DNA Immunostimulant, N=1002). All calves were followed for 56 days. On Day 57, calves remaining on the study were weighed and subsequently eligible to return to commerce.

Descriptively, across both treatment groups, BRD morbidity, BRD repulls, BRD chronicity, overall BRD mortality, and BRD case-fatality estimates were observed to be 10.7%, 18.3%, 29.4%, 0.6%, and 3.3%, respectively.

Overall, the raw BRD morbidity estimates for Tilmicosin (7.65%) and the DNA Immunostimulant (13.84%) were evaluated for non-inferiority based upon a 10% margin of difference (determined a priori) of having no clinical significance. The inferential statistical analysis performed on the difference (-6.19) observed a 95% confidence interval of -8.91% to -3.47%. Therefore, the BRD incidence of the DNA Immunostimulant was observed to be non-inferior to Tilmicosin (lower bound within 10% margin of difference). The time to first BRD diagnosis was significantly less for the DNA Immunostimulant (22.6 days) compared to Tilmicosin (28.1 days). No significant differences ($p>0.05$) between the treatment groups were observed in the remaining study outcome variables: BRD repulls (Tilmicosin = 17.9%, DNA Immunostimulant = 11.1%), BRD chronicity (Tilmicosin = 27.9%, DNA Immunostimulant = 29.1%), overall BRD mortality (Tilmicosin = 0.44%, DNA Immunostimulant = 0.50%), BRD case-fatality (Tilmicosin = 3.95%, DNA Immunostimulant = 2.99%), average daily gain (Tilmicosin = 2.96 lbs/day, DNA Immunostimulant = 2.91 lbs/day), dry-matter intake (Tilmicosin = 12.96 lbs/day, DNA Immunostimulant = 12.81 lbs/day), and feed:gain (Tilmicosin = 4.50, DNA Immunostimulant = 4.55). In this study, the DNA Immunostimulant was shown to be a viable non-antibiotic option for metaphylaxis in classes of cattle like that reflected in the current study population. No adverse events were observed in this study.



SESSION 5
**Innovative Drugs,
Chemicals,
and Enzymes**

ORAL PRESENTATIONS

5.1 NON-RESISTANCE-INDUCING ANTIBACTERIAL POLYMERS AND NANOMEDICINES

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The problem of rising antibiotic resistance has caught the world's attention. This talk will cover two major strategies polymer and material scientists have adopted to combat bacterial infections. The design and synthesis of non-resistance-inducing antimicrobial polymers (such as dendrimers) will be presented as the first strategy. Those antimicrobial polymers are designed in such a way to only act on bacterial superficial structures to decrease the possibility of inducing bacterial resistance and also show high selectivity for pathogens over host cells. The second strategy is to apply nanotechnology to achieve effective delivery of broad-spectrum potent biocides to pathogens with minimal toxicity to host cells. Three examples of this strategy will be given:

1. using nanotechnology to allow post-treatment recovery of biocides for minimal toxicity on topic wounds;
2. using nanotechnology to enable potent non-selective biocides to selectively target and destroy pathogens, i.e. be selective against pathogens over human cells; and
3. using nanotechnology to achieve responsive release of potent broad-spectrum biocides.

5.2

BACTERIAL ANTI-VIRULENCE STRATEGY AS AN ALTERNATIVE TO ANTIBIOTICS

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The overuse of antibiotics in both food industry and healthcare is contributing to the increase and spread of antibiotic resistant bacteria at an alarming rate. There is an urgent need for alternative strategies that may reduce the use of antibiotics in food producing animals, agriculture and aquaculture. Targeting bacterial virulence is a promising alternative strategy because as opposed to antibiotics:

- 1) does not impose strong selective pressure on bacteria that favors the evolution of additional mechanisms of resistance and persistence;
- 2) does not affect viability, and thus it should not disrupt beneficial microbiota;
- 3) it might have broad applications if a conserved virulence mechanisms is targeted; and
- 4) antibiotic resistant bacteria are predominantly sensitive to anti-virulence drugs.

As a proof of principal we targeted the MvfR Quorum Sensing (QS) virulence pathway of the bacterial human opportunistic pathogen *Pseudomonas aeruginosa* that also infects vertebrate and invertebrate animals, including insects and fish, as well as plants. Using a whole-cell high-throughput screen of ~300.000 compounds, we identified 40 MvfR QS inhibitors (QSIs). Subsequent structure-activity relationship studies yielded highly potent second generation QSIs with IC_{50} s around 200 nanomolar in live *P. aeruginosa* cells. These QSIs block *P. aeruginosa* MvfR-regulated functions, including biofilm formation and therefore have also a therapeutic potential in the context of chronic infections. Pharmacological inhibition of MvfR virulence *in vivo* using MDR *P. aeruginosa* demonstrated further the feasibility of our approach. In addition, the potential broad applicability of our technology was tested against *Escherichia coli* invasion of intestinal epithelial cells. As such, these and QSI-like molecules provide new hope for the development of next-generation of anti-bacterial agents that would provide an efficient alternative to antibiotics for the prevention, control and treatment of bacterial infections in in food producing animals, agriculture and/or aquaculture.

5.3

CATIONIC AMPHIPHILIC NON-HEMOLYTIC SYNTHETIC POLYMERS AS POTENTIAL AGENTS TO COMBAT BACTERIA WITH ANTIBIOTICS RESISTANCE

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The global health threat from infections involving antibiotic resistant bacteria (superbugs) has created a pressing need to develop new antibacterial agents. Antimicrobial resistance to antibiotics causes thousands of deaths and adds billions of dollars to health care budget in the United States alone. Synthetic amphiphilic polymers based on the design principles of natural host defense antimicrobial peptides (AMPs) have generated enormous research interest in the last decade due to their cost effective synthesis and structural versatility. The inability of bacteria to gain resistance towards synthetic amphiphilic polymers as opposed to conventional antibiotics has been demonstrated. One of the major challenges toward therapeutic applications of synthetic amphiphilic polymers is their toxicity to mammalian cells. Our ongoing investigations in the area of synthetic antibacterial polymers have been focusing on cationic amphiphilic non-hemolytic synthetic polymers systems. Toxicity of the synthetic amphiphilic polymers toward mammalian cells has been a concern, and amphiphilic polymers with potent antibacterial activity and concomitant low hemolytic activity toward mammalian cells are our goals. Our investigations on the antibacterial and hemolytic activities of synthetic amphiphilic polymers focus on the effects of topological placement of hydrophilic and lipophilic structure segments. Various structural parameters examined included: amphiphilic balance, spatial arrangement of cationic and hydrophobic groups, random copolymer and homopolymer architecture, and cationic charge density. Examples of some of the most promising systems reported will be expounded. One such system showed the control of spacer arm lengths to charged groups and copolymer composition can serve as one of the effective structural parameters in the synthesis of polymers with highly selective (bacteria over mammalian cells) antibacterial activity.

5.4

REDUCING ANTIMICROBIAL DEPENDENCE THROUGH FEED ADDITIVES THAT BOLSTER IMMUNE READINESS, DISEASE RESISTANCE, AND PRODUCTION PERFORMANCE IN FARMED FISH

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Aquaculture is the fastest growing sector of animal agriculture. However, sustainable expansion and intensification of aquaculture worldwide has been severely hampered by disease. In the US catfish industry, the largest segment of US aquaculture, disease-based mortality levels can reach nearly 60% over the course of a production cycle. Two Gram-negative bacterial pathogens, *Flavobacterium columnare* (columnaris disease) and *Aeromonas hydrophila* (motile *Aeromonas septicemia*), represent the largest sources of mortality in the industry. Despite their importance, there are currently few effective weapons available to combat either pathogen. Antibiotic usage is on the rise, closely followed by increasing numbers of antibiotic-resistant isolates. Catfish producers are eager to gain protection against disease in a more natural and cost-effective manner, i.e. through diets supplemented to provide for both mucosal health and performance. Here, we evaluated a *Saccharomyces cerevisiae* fermentation product called Diamond-V XPC. The trial featured four levels of inclusion which were added to a commercial 32% protein floating catfish ration. Following six weeks of feeding, we observed heightened resistance to columnaris disease and saw significant increases in the levels of immune effectors in the serum including lysozyme, complement, and immunoglobulin. Additionally, we also present findings on fortification of commercial diets with the enzyme phytase; which targets and destroys phytic acid, a potent anti-nutrient and mineral chelator that is abundant in plant-based catfish diets. Animals fed diets amended with phytase showed markedly improved mineral uptake, hematologic and immune parameters, growth, and feed conversion. Recent largescale adoption of these amendments in the US catfish industry has been associated with increased animal performance and reduced disease mortality. Our results stress the importance of understanding and prioritizing the protective benefits of dietary ingredients for aquaculture species alongside more typical consideration of a species' minimal nutritional requirements.

5.5 DEVELOPING OF TECHNIQUE TO CIRCULARLY PRODUCE ENZYME, PROBIOTICS AND OLIGOSACCHARIDE, AND SOLUTIONS TO REPLACE ANTIBIOTICS IN A COST-EFFECTIVE WAY

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Enzyme, probiotics and oligosaccharide are antibiotic alternatives in animal feed and each has its own advantages and disadvantages. When these three products are used in feed simultaneously, the combination can have better results but lead to very high cost. It will eventually reduce the profitability of feed mills. Thus, there is a need of better solution that can be practiced to reduce the inclusion cost. We developed a new technique to circularly produce enzyme, probiotics and oligosaccharide. The technique includes three production process lines:

- 1) To screen enzyme producing strains which are sensitive to diet, oligosaccharide hydrolase producing strains and probiotics which are sensitive to fermentation medium by a high throughput technique, and combine with submerged liquid fermentation at high density, to effectively lower down the cost of feed enzymes and oligosaccharide hydrolase;
- 2) To produce oligosaccharide efficiently by using the hydrolase;
- 3) To inoculate the probiotics to the remaining materials from oligosaccharide production process to manufacture probiotics products.

We developed accurate and efficient solutions to replace antibiotics for different diet formulations and in different stages of animals.



SESSION 5
**Innovative Drugs,
Chemicals,
and Enzymes**

POSTER PRESENTATIONS

A STUDY ON THE APPLICATION OF ENTERIC-RELEASING MICROENCAPSULATED BENZOIC ACID REPLACING AGPS IN SWINE FEED

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Developing effective and safe alternatives to AGPs has become the top priority in the feed industry because of increasing concerns with antibiotic resistance and food safety. Benzoic acid, with broad-spectrum antibacterial activities, can be a practical alternative but its large-scale application in feed industry is limited due to irritating odor, poor palatability, tendency to cake and sublime. These disadvantage could be overcome by Superstin-ER that is a 70% benzoic acid enteric-releasing micro-encapsulated product developed by Insighter® and can be passed stomach and released in intestine. Two animal trials were conducted on the effects of Superstin-ER on the diarrhea index of post-weaning piglets (Experiment 1) and growing-finishing pigs (Experiment 2), respectively. In the experiment 1, 120 healthy post-weaning hybridized piglets (Duroc x Landrace x Yorkshire, average body weight: 6.89 ± 0.61 Kg) at the age of 25 days were randomly assigned to 4 treatment groups.

Treatment 1 (Control group) was fed a corn soybean meal based diet (Basal diet).

Treatment 2 (AGPs group) was fed a basal diet supplemented with 20 ppm Colistin Sulphate and 75 ppm chlortetracycline.

Treatment 3 (Superstin-ER group 1) was fed a basal diet with 2000 ppm Superstin-ER.

Treatment 4 (Superstin-ER group 4) was fed a basal diet with 3000 ppm Superstin-ER.

In the experiment 2, 120 healthy growing pigs (Duroc x Landrace x Yorkshire, average body weight: 17.52 ± 1.35 Kg) at the age of 52 days were randomly assigned to 4 treatment groups. Treatment 1 (Control group) was fed a corn soybean meal based diet (Basal diet) supplemented with 3000 ppm benzoic acid. Treatment 2 (AGPs group) was fed a basal diet supplemented with 20 ppm Colistin Sulphate and 75 ppm chlortetracycline. Treatment 3 (Superstin-ER group 1) was fed a basal diet with 2000 ppm Superstin-ER. Treatment 4 (Superstin-ER group 4) was fed a basal diet with 3000 ppm Superstin-ER. Two animal trials were conducted in three times and lasted 2 weeks.

In the experiment 1, diarrhea rates were reduced significantly and ADG/FCR were improved significantly in the AGPs group and the Superstin-ER groups ($P < 0.05$) when compared with the control group. The Superstin-ER group 2 was significantly superior over the AGPs group and the Superstin-ER group 1.

In the experiment 2, the feed intake of the control group was significantly lower than the AGPs group and the Superstin-ER groups. ADG/FCR was significantly improved in Superstin-ER group 2 ($P < 0.05$) when compared with the control group. In conclusion, enteric-releasing microencapsulated benzoic acids can effectively reduce diarrhea rate in piglets, improve feed intake and growth performance.

EFFECTS OF DIFFERENT DOSAGES OF BUTA-ER IN FEED ON THE GROWTH PERFORMANCE, APPARENT DIGESTIBILITY OF NUTRIENTS, SLAUGHTER PERFORMANCE, INTESTINAL MORPHOLOGY AND MICROBIOTA IN BROILERS

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The use of sodium butyrate in animal feed has several disadvantages including odor emission, moisture absorption, deliquescence and volatilization. Buta-ER™ is a coated calcium butyrate and developed by Guangzhou Insigher Biotechnology Co., Ltd. It contains 50% calcium butyrate and can overcome the disadvantages that sodium butyrate has. This study was to investigate the effects of different dosages of Buta-ER™ in feed on the growth performance, apparent digestibility of nutrients, slaughter performance, intestinal morphology and microbiota in broilers. 2400 day old Chinese yellow feathered chicks were randomly assigned to 8 groups with 6 replicates each group that were fed basal diet (control group), basal diet + Colistin 20 ppm Sulphate + 20 ppm Stafac (virginiamycin) (AGPs group), basal diet + 0.15% sodium butyrate (sodium butyrate group), basal diet + 0.10%, 0.20%, 0.30%, 0.4%, 0.50% of Buta-ER™ (trial groups) respectively. The trial lasted 49 days. The results showed that Buta-ER™ significantly improved the growth performance of broilers when compared with control and AGPs groups ($P < 0.05$). 0.3% Buta-ER™ supplementation had the best effects. 0.3% Buta-ER™ supplementation increased the apparent digestibility of crude fat and crude fiber when compared with AGPs group ($P < 0.05$). The slaughter performance had a tendency to be higher ($P > 0.05$). The villus height in duodenum and jejunum was significantly increased ($P < 0.05$). The number of *Lactobacillus* in the cecum was significantly increased ($P < 0.05$), while the number of *Escherichia coli* was decreased significantly ($P < 0.05$). When compared with the sodium butyrate group, 0.3% Buta-ER supplementation had better growth performance ($P < 0.05$). In conclusion, Buta-ER™ improves gut health, increase the number of beneficial bacteria and promote growth performance of broilers, indicating Buta-ER™ is a promising antibiotic alternative in poultry production.

EFFECT OF ZINC OXIDE SOURCES AND DOSAGES ON INTESTINAL ENTEROBACTERIACEAE AND GUT INTEGRITY OF WEANED PIGLETS

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Zinc oxide (ZnO) can be supplied at pharmacological dosage (2400 mg/kg of Zn) in diets of weaned piglets in order to improve performance through adjusting gut health. In this study, the effects of regular ZnO (110 and 2400 mg/kg of Zn) were compared to a potentiated ZnO source (HiZox[®]) at low dose (110 and 220 mg/kg of Zn). High iron level was used to induce gastro-intestinal disturbances: diets including regular ZnO contained 100 or 500 mg/kg of Fe from FeSO₄, vs. 500 mg/kg of Fe for diets with the potentiated ZnO. Each of the six treatments was replicated in four pens (2 piglets/pen, 20d of age at start) during 15 days. Animal performance, *Enterobacteriaceae* counts by qPCR and coliforms and *E. coli* counts in intestinal contents, and gut barrier and chloride secretion upon secretagogues in distal jejunum were assessed. Groups fed regular ZnO at 2400 mg/kg of Zn and potentiated ZnO at 220 mg/kg showed higher growth than other groups, irrespective of Fe content ($P < 0.05$). Piglets fed regular ZnO at 2400 mg/kg of Zn had significantly lower numbers of coliforms and *E. Coli* in distal small intestine than groups with 110 mg/kg of Zn from regular ZnO ($P < 0.05$). Also, supplementation with potentiated ZnO reduced coliform counts compared to 110 mg/kg of Zn from regular ZnO ($P < 0.05$), and numerically reduced *E. coli*. This was confirmed by results from qPCR analysis. Transepithelial electrical resistance (TEER) of jejunal mucosa was significantly ($P < 0.05$) higher for groups fed potentiated ZnO, compared with groups fed 110 mg/kg of regular ZnO, suggesting a better intestinal epithelial integrity. In conclusion, the potentiated ZnO at low dosage showed positive effects on the reduction of *Enterobacteriaceae* counts and improved gut epithelial barrier integrity, albeit similar to the effects of pharmacological dosage of regular ZnO.

EFFICACY OF A COMBINATION OF EUBIOTICS IN *ESCHERICHIA COLI* K88 CHALLENGED PIGLETS

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Weaning is critical for piglets as they are undergoing several stressful changes at a time their immune system is far from fully developed: separation from the mother, change from milk to solid feed, new housing environment ...Major consequences are decreased feed intake and impaired digestion. During this period, enterotoxigenic *E. coli* strains often cause colibacillosis. Treatments with antibiotic or high level of zinc oxide are usually prescribed to prevent or cure such diseases leading to the selection of resistant bacteria potentially affecting animal and public health. In this context, two studies were conducted to evaluate the efficacy of VevoStart[®], a combination of benzoic acid, essential oils compounds and a probiotic, in piglets challenged with *Escherichia coli* K88. For each study, 140 animals of 26 days of age were involved. The experimental treatments consisted of a basal control diet, and the same diet supplemented with either VevoStart[®], Colistin, or Zinc oxide. At day 4 of experiment, all animals were experimentally challenged with an oral dose of 5×10^8 CFU of *E. coli* K88. Quantification of *E. coli* by traditional plate culture methods and *E. coli* K88 by quantitative PCR were processed in fresh faeces. The first experiment showed VevoStart[®] significantly increased the daily weight gain and significantly improved the feed conversion ratio. *E. coli* fecal shedding measured with culture method was significantly reduced in piglets fed the colistin diet. In the second experiment an organic acid blend was added in the control, colistin and zinc oxide treatments. No significant differences in daily weight gain between the Control, VevoStart[®] and Colistin were observed. Medication with Zinc oxide significantly decreased feed intake and growth. The lowest fecal score and a reduced *E. coli* fecal shedding were noted in the colistin group although the Zinc oxide treatment significantly increased this parameter. In conclusion, nutritional solutions exist that can enable piglets to undergo the weaning period without using antimicrobial treatments while maintaining zootechnical performance.

ANTIMICROBIAL ACTIVITY OF SELECT ANTI-METHANOGENIC NITRO- AND THIO-CONTAINING CHEMICALS

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New technologies are needed to help livestock producers maintain optimal health and wellbeing in their animals while minimizing risks of propagating and disseminating antimicrobial resistant bacteria to human or other animal. Where possible, these interventions should contribute to the efficiency and profitability of animal production so as to avoid passing higher costs on to the consumer. Methane production within the rumen results in the loss of 2-12% of the gross energy consumed by the host, costing the U.S. cattle feeding industry as much as \$700,000/day or more. Rumen methanogenesis also contributes 20% of the U.S. emission of this greenhouse gas. Presently, we examined the antimicrobial activity of several potential anti-methanogenic chemicals to see if their applications may be combined to be more economically acceptable for producers. When tested against anaerobically-grown (in tryptic soy broth) pure cultures of *Salmonella* Typhimurium (ST) and *Escherichia coli* O157:H7 (EC), the potent methane inhibitor ethyl nitroacetate (9 mM) decreased ($P < 0.05$) mean specific growth rates by 26 and 36%, respectively, compared to that of controls (0.481 ± 0.05 and 0.357 ± 0.02 , respectively). Ethyl nitroacetate was bacteriostatic rather than bactericidal as evidenced by nearly equivalent ($P > 0.05$) maximum optical densities (0.40 ± 0.01 and 0.39 ± 0.03 at 600 nm, respectively) recorded after 24-h incubation (39°C). The methane inhibitors nitroethane and 3-nitro-1-propionic acid were ineffective in inhibiting ST or EC. When tested likewise, 9 mM 6,8-dithiolic acid (common name lipoic acid) decreased ($P < 0.05$) mean specific growth rates and maximal optical densities of ST by 98 and 65% and of EC by 93% and 59%, respectively. Mechanistically, evidence indicates that ethyl nitroacetate disrupts hydrogen transfer reactions whereas the antimicrobial effect of lipoic acid, a player in one carbon compound metabolism, may be mediated by pH.

ASSESSMENT AND ANALYSIS ON THE DIGESTIBILITY OF FEED INGREDIENTS BY USING REGRESSION EQUATIONS

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Animal production is a cost-driven industry and feed costs are the most important cost factor in animal production. Precision nutrition improves the probability of feed mills and producers. The quality of feed is depending on the quality of raw materials. The differences between each batch of raw materials will result in a quality fluctuation of final products. Thus, it is very important to measure the variations in the digestibility of raw materials in order to make accurate formulation for animals. It is not feasible for the feed industry to measure the digestibility of raw materials with a total fecal collection procedure. Therefore, we need to establish regression equations between non-nutrition indicators and feed utilization (e.g. digestibility). Then we built an industrial feasible model to evaluate the digestive utilization of raw material by analyzing non-nutrition indicators in the ingredients. Currently, this project has completed non starch polysaccharide (NSP) anti-nutritional factors detection in the corn, sorghum, rice bran, canola, cottonseed meal and flour from different sources in China and worldwide. The digestibility of dry matter and protein were evaluated by the Bionic Digestion Apparatus. With these regression equations that we established, we can provide more accurate formulation for animals although there are variations in raw materials and improve feed utilization efficiency.

CHEMICALLY SYNTHESIZED ANTIMICROBIAL PEPTIDES INHIBIT IN VITRO GROWTH OF *CAMPYLOBACTER* SPP

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Novel alternatives to traditional antibiotics are needed for food-animal production. One goal of our laboratories is to discover and evaluate antimicrobial peptides (AMP) to reduce foodborne bacterial pathogens during poultry production. AMPs permeabilize membranes and are found in most every class of living organism where they have evolved as a defense mechanism against invading microorganisms. Our working hypothesis is that AMPs can be identified that inhibit growth of *Campylobacter jejuni* then subsequently utilized to reduce gastrointestinal *Campylobacter* among commercially produced chickens. A set of eleven unique AMPs were chemically synthesized commercially and evaluated for ability to inhibit growth of two *C. jejuni* strains. Six of the AMPs assayed produced zones of inhibition on lawns of *C. jejuni*. These included: NRC-13, a variant of pleurocidin isolated from the American plaice-flounder; RL-37, a 37-residue AMP of the cathelicidin family which is expressed in bone marrow of the rhesus monkey; temporin, from the frog, *Rana temporaria*; a potent hybrid AMP (Cec-Mag) composed of residues 1-8 of cecropin A (from the *Cecropia* moth) fused to residues 1-12 of magainin 2 (from the African clawed frog, *Xenopus laevis*); dermaseptin from the skin of *Phyllomedusa* frogs; and the synthetic OAK, C12K-2b12. Three AMPs were chosen for further investigation on the basis of reported reduced cytotoxicity to mammalian cells: Cec-Mag, RL-37 and C12K-2b12. These AMPs produced zones of inhibition on lawn assays against 19 different bacteria, including *C. jejuni*, *C. coli* and *C. lari* as well as two strains of *Salmonella* and *Lactobacillus*. Modifications of the NCCLS M26A and Hancock assays were utilized to determine minimum inhibitory concentrations (MIC) in microtiter plates for these AMPs against three strains of *C. jejuni*. MICs were approximately 3.1 µg/ml for the AMP RL-37 and C12K-2b12, while the MIC for Cec-Mag was in the range of 12.5 to 50 µg/ml.

DEVELOPMENT OF ANTI-VIRULENCE DRUGS BY TARGETING THE SAERS TWO COMPONENT SYSTEM OF STAPHYLOCOCCUS AUREUS

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Staphylococcus aureus is a Gram-positive pathogen causing diseases in both humans and animals. In humans, the bacterium causes a wide range of diseases from skin and soft-tissue infections to various life-threatening diseases whereas, in animals such as cow, mastitis is a major staphylococcal disease. The success of *S. aureus* as a pathogen is due to the production of multiple virulence factors. The emergence of drug resistant strains including MRSA (methicillin resistant *S. aureus*) and VISA (vancomycin intermediate *S. aureus*) has made the treatment of the bacterial disease more difficult, calling for development of novel classes of drugs. One excellent target for novel drug development is the SaeRS two-component system, which controls the production of more than 20 staphylococcal virulence factors. The SaeRS TCS is composed of the sensor kinase SaeS and the response regulator SaeR. Animal experiments showed that the kinase activity of SaeS correlates with the bacterial virulence. To explore whether the SaeRS TCS is a viable target for anti-virulence drug development, we screened small molecule libraries for Sae-inhibitors by employing a promoter-GFP reporter system. By screening 10,000 compounds, we identified ~150 compounds that repress the Sae-regulated promoter. One of the compound (SKK1010) with low IC_{50} (4 μ M) was further characterized. At 8 μ M, the compound blocked the hemolysis of human erythrocytes by *S. aureus*, and protected HeLa cells from *S. aureus*-mediated killing. The IC_{50} -cytotoxicity of SKK1010 was 62 μ M, resulting in the therapeutic index of 15.5. In a microsomal stability assay, 80% of SKK1010 still remained intact at 30 min, showing an exceptional metabolic stability. Finally, in a murine infection model, SKK1010 showed a synergistic effect with vancomycin in protection of the mice from *S. aureus*-mediated killing, demonstrating the potential of the compound as an anti-virulence agent against *S. aureus* infection.

DEVELOPMENT OF NONTOXIC BUT SELECTIVE ANTIBACTERIAL NANOPARTICLES USING ANTIBODY CONJUGATED SOLID LIPID NANOCARRIERS AS AN ALTERNATIVE TO ANTIBIOTICS FOR WOUND TREATMENT

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Antibiotic resistance is one of the most challenging healthcare issues in the current century that threaten human life. To combat this problem, researchers and scientists have been working hard to search for alternatives to antibiotics. We intend to approach this problem from the perspective of material science by converting potent broad spectrum non-selective biocides into selective ones. The idea is to reduce the toxicity of potent broad-spectrum biocides by encapsulating them in biocompatible solid lipid nanoparticles (SLNs) and increase the selectivity of these SLNs by conjugating a bacterium-specific antibody onto the surface of the SLNs. A quaternary ammonium salt (QAS) newly developed in house was used as a model non-selective and toxic antibacterial agent (C17) to be encapsulated in the SLNs. Selectivity of these SLNs to Methicillin-resistant *Staphylococcus aureus* (MRSA) has been driven by a monoclonal antibody (mAb) conjugated on the SLNs surface. The Ab-conjugated antibacterial SLNs (C17-SLN-Ab) demonstrated superior antimicrobial activity over their antibody free counterparts. These biocide loaded SLNs showed significantly lower cytotoxicity to fibroblast cells in comparison to antimicrobial agent alone. We evaluated the selective binding activity by transmission electron microscopy and found that SLN-Ab bound to the MRSA membrane which did not happen in the case of *P. aeruginosa*. Also, SLNs did not show any significant attachment to MRSA. This work underlines the potential of antibody-bonded SLN to impart potent non-selective biocides with selective toxicity to pathogenic bacteria.

ANTIBACTERIAL ACTIVITY OF A RECOMBINANT ANTIMICROBIAL PEPTIDE MP1102 AGAINST *STAPHYLOCOCCUS AUREUS* AND *CLOSTRIDIUM PERFRINGENS* IN VITRO AND IN VIVO

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Antimicrobial peptide MP1102 (N9Q, L13V, and R14K), a new derivative of NZ2114, was designed and expressed in *Pichia pastoris* X-33. The yield of recombinant MP1102 (rMP1102) was 197.1 mg/l and its purity was about 96.4%. rMP1102 exhibited stronger potent *in vitro* activity against Gram-positive bacteria than native NZ2114, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium perfringens*. The minimum inhibitory concentrations (MICs) of rMP1102 against 20 clinical isolates of MRSA and *C. perfringens* were 0.004–0.23 μ M and 0.05–1.33 μ M, respectively, and their minimum bactericidal concentrations (MBCs) were 0.007–0.46 μ M and 0.91–7.28 μ M, respectively. In addition rMP1102 had high stability over a wide pH range of 2.0 and 10.0 and high thermal stability from 20 to 80 °C, and remarkable resistance to pepsin. It had also no hemolytic activity and cytotoxicity toward mammalian cells. The fractional inhibitory concentration index (FICI) indicated an additive or synergic effect between rMP1102 and bacitracin zinc, nisin, vancomycin, virginiamycin, aureomycin, and ampicillin against *C. perfringens* (FICI= 0.3125–1.0). Antibacterial activity of rMP1102 against *S. aureus* and *C. perfringens* was further assessed in a mouse thigh infection model. A dose of 5–20 mg/kg rMP1102 could kill over 90% of tested *S. aureus* ATCC43300 cells within 12 h, indicating that it had higher activity than vancomycin. Meanwhile, a decrease of 0.70 and 1.03 log₁₀ CFU/g *C. perfringens* CVCC 61 was observed after 24 h treatment with 10 and 20 mg/kg of rMP1102, respectively. Moreover, rMP1102 enhanced the survival rate of the mice infected *C. perfringens* CVCC 61, reduced the serum tumor necrosis factor (TNF)- α level (prevention or/and treatment). These results indicated that it is feasible for large-scale production of rMP1102 by the yeast expression system, and rMP1102 is a promising new antimicrobial agent for *S. aureus* and *C. perfringens* for further clinical applications.

Keywords

Antimicrobial peptide, *Clostridium perfringens*, Expression, rMP1102, *Staphylococcus aureus*.

Reference

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ESTABLISHMENT OF A CHICKEN ENTEROCYTE CULTURE SYSTEM TO SCREEN FACTORS THAT AFFECT INTESTINAL INTEGRITY

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Enteric health and integrity is important for overall animal health. Therefore, the understanding of mechanisms how enterocytes interact with different factors such as microbes, toxins, and other chemicals, is essential to evaluate substances that can protect against harmful agents and improve animal health and food safety. Enterocyte culture models afford fast and inexpensive screening method but there is no such model for avian enterocytes. The mammalian systems cannot adequately substitute for species specific screening. To address this need, we developed an enterocyte culture system using intestinal villi, harvested from broiler chickens. These cells are grown in DMEM containing a set of growth factors, polyamines, and serum which support their epithelial cell morphology and favor mucin production. To evaluate the effects of different chemicals, we plated the cells in 48 wells at a concentration of 5×10^3 cells per well, and on confluency treated them with selective chemicals which included vitamins (1, 25 dihydroxy vitamin D3, Trans retinoic acid, $1 \mu\text{M}$), fungicide (thiram $1 \mu\text{M}$), metabolic activators (dibutyryl cyclic AMP, a protein kinase A activator and phorbol myristate acetate (PMA), a protein kinase C activator, both at $1 \mu\text{M}$, and sodium butyrate 1mM), (*Salmonella* lipopolysaccharide (LPS) and *Staphylococcus* peptidoglycan (PGN), both at $1 \mu\text{g/ml}$), and screened for changes in cell morphology and viability at 24 h and 48 h of treatment. While none of the treatments affected cellular viability measured by Alamar blue, the retinoic acid and PMA showed significant morphological changes. The PMA treatment showed elongation of cells whereas the retinoic acid favored more flattened epithelial morphology. A preliminary study using label free quantitation proteomic analysis showed PMA upregulating the pathways of carbohydrate and cytoskeletal metabolism. These results show that the chicken enterocyte culture has potential as a screening tool for chemicals that affect enterocytes and study the mechanisms of their action.

ANTIOXIDANT EFFECTS OF GLUCOSE OXIDASE *IN VITRO*

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Certain enzymes are known to be active as antimicrobial agents. Glucose oxidase (GOD) is an oxidase capable of regenerating hydrogen peroxide. This experiment was conducted to investigate the antioxidant effects of GOD *in vitro*. Five treatments were assigned with 0, 0.01%, 0.02%, 0.03%, or 0.04% GOD, and 0.02% 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline(ETMDQI) in control treatment. Changes of POV values in soy oil and MDA content induced by liver homogenate during warm-bathing were measured. The results showed that:

- (1) Different dosages of GOD showed antioxidant effects on soy oil and 0.03% treatment was the best during the beginning period of test, but the antioxidant effects of all GOD treatments reduced after that;
- (2) The MDA content of liver homogenate in all treatments were inhibited but no significant effects ($P > 0.05$), but the best GOD dosage was also 0.03%.

It could be concluded that GOD showed better short-term antioxidant effects and the best dosage was 0.03% *in vitro*.

EFFECTS OF GLUCOSE OXIDASE ON THE NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF BROILERS

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Enzymes are known to be active as antimicrobial agents. Glucose oxidase (GOD) is an oxidase capable of regenerating hydrogen peroxide. This experiment was conducted in the commercial farm to study the effects of glucose oxidase (GOD) on nutrients digestibility and growth performance in broilers. One hundred and twenty-five AA broilers were randomly divided into five treatments with five replicates of five birds each. Positive control (PC) group fed basal diet, and treatment 1 to 4 fed basal diet added with 100g/ton, 200 g/ton, 300 g/ton, or 400 g/ton GOD, respectively. The experimental period lasted 40 d. The results showed: the treatment with 400 g/ton GOD significantly improved the digestibility of dry matter (DM) and crude protein (CP) digestibility ($P < 0.05$) in the broilers. Meanwhile, the average daily gain (ADG) and European broiler index (EBI) were significantly improved ($P < 0.05$) by adding 100-200g GOD per 10,000 broilers per day in broiler drinking water. In conclusion, the supplementation of GOD is an effective method in broiler feeds because it can improve the digestibility of DM and CP, ADG, feed conversion rate and EBI.

EFFECTS OF NON-STARCH POLYSACCHARIDE NSP ENZYMES ON THE GROWTH PERFORMANCE OF DUCK AND THE DIGESTIBILITY OF DRY MATTER (DM) AND CRUDE PROTEIN (CP) USING AN *IN VITRO* DUCK SIMULATIVE DIGESTION SYSTEM

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This experiment was conducted to investigate the effects of non-starch polysaccharide (NSP) enzymes on the growth performance of duck as well as the digestibility of dry matter (DM) and crude protein (CP) using an *in vitro* Duck-Simulative Digestion System. 720 0-day old cherry valley duck was randomly assigned into 4 treatments with 6 replicates of 30 birds each by one factor randomized block design, which was fed diet as followed: PC1 group fed normal duck basal diet, NC group fed diet with lower energy and CP than those in PC1 group (reduced 30kcal/kg, 0.25% respectively), PC2 group fed diet with higher energy and CP than those in PC1 group (increased 30kcal/kg, 0.25% respectively), NSP enzyme group fed NC diet with 0.2g/kg NSP enzyme, the whole feeding period was 37 days. And those four 17-25 days' duck diets were chosen to test DM and CP digestibility using an *in vitro* Simulative Digestion System by one factor completely randomized design. The result showed that:

- (1) In the 0-37 feeding periods, the average daily gain (ADG) in ducks of NSP group was significantly increased by 3.45% $P < 0.05$ compared with PC1 group and but there was no significant difference with PC2 group; also the EPI in NSP group was also improved during 0-37 days, which were significantly enhanced by 6.71% $P < 0.05$ and 6.38% ($P < 0.05$) compared to NC and PC1 group, respectively.
- (2) The digestibility of DM and CP in duck feed in 17-25 days duck diets were significantly higher than those in NC ($P < 0.05$ and PC1 ($P < 0.05$), higher than PC2 group but no significant difference ($P > 0.05$).

It could be concluded that NSP enzyme supplemented in the duck diet with lower energy and CP can improve duck growth performance and the matrix value of NSP enzymes was predicted to be 3000 kcal/kg and 25% CP/kg in duck feed.



SESSION 6
Regulatory
Pathways to Enable the
Licensing of Alternatives
to Antibiotics, and Issues
and Opportunities from
Funders' Perspective

ORAL PRESENTATIONS

6.1
EU APPROACH TO AUTHORIZATION OF NOVEL
TECHNOLOGIES WITH PARTICULAR EMPHASIS ON
ALTERNATIVES TO ANTIBIOTICS

Faye Ioannou

European Medicine Agency, London, United Kingdom

6.2

FDA'S INNOVATION INITIATIVE TO EVALUATE NOVEL EMERGING TECHNOLOGIES AND INTERNATIONAL COOPERATION IN THE AREA OF INNOVATION

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Alternatives to antimicrobials are an important area of development for products for food animals. In the United States, FDA is responsible for the approval of animal drugs. Congress established the statutory standard for approval of animal drugs; substantial evidence of effectiveness and reasonable certainty of no harm for human food safety. The animal drug approval process is an evidentiary process that answers the questions that provide the evidence to meet the statutory standard. Alternatives to antimicrobials may need a 'non-standard approach' to meet the regulatory requirements. CVM has developed processes to assist sponsors with innovative and novel technologies to develop appropriate evidence to meet the statutory standards. We encourage sponsors to meet with FDA/CVM early, to discuss their product and their proposed path to approval, and establish a development plan for their alternative product.

6.3 REGULATORY PERSPECTIVE FROM ASIA

Speaker to be decided

6.4

DO INNOVATIVE SOLUTIONS REQUIRE NOVEL REGULATORY PARADIGMS?

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Innovative alternatives to conventional antimicrobial therapies may arise in many different guises. Regulators, consumers and industry all agree though that they are of paramount importance and that we must work together to deliver them in order to preserve existing and new antimicrobials alike, for appropriate responsible use in the future. Regulation of veterinary medicines is an ever evolving area and we have seen new approaches to the assessment of Marketing Authorisation dossiers introduced on many occasions over the years. Perhaps most recently in terms of antimicrobial risk assessment, but previously also in the areas such as environmental risk assessment, pharmacovigilance, as well as refinements in safety and efficacy data provision, and statistical analysis – all underpinned by robust manufacturing quality data. As we bring novel molecules, be they large immune-modulating proteins such as Imrestor, enzymes, vaccines, antibodies, phage therapies, pre or probiotics, or other types of solutions, as yet unknown, we must ask if our current regulatory framework and assessment systems remain wholly appropriate.

For example are we in danger of excluding valuable molecules from our toolbox of potential agents to combat infections if we demand that all have the same level of efficacy as might be expected of a traditional antimicrobial? Are there more sophisticated models of clinical trials we could employ? Perhaps including more opportunity for confirmatory post approval work or some variety of provisional approval to permit gathering of large population based data sets might help to transform some of the early development candidates into the game changing products we are all striving to deliver as future alternatives to traditional antimicrobials?

6.5 THE SEQUENCE OF SUCCESS: ANTIBIOTIC ALTERNATIVE DEVELOPMENT FROM THE STARTUP PERSPECTIVE

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Early stage companies, known commonly as startups, play an essential role in the drug development ecosystem. As drivers of innovation, they are uniquely positioned to respond rapidly to unmet clinical needs, tackle drug discovery and development projects with high scientific and technical risk, pivot rapidly in response to drug candidate failures or to changing needs in the marketplace, and translate scientific discoveries into product candidates with extraordinary capital efficiency. These attributes are essential in order to address the growing threat of antimicrobial resistance in a timely manner. The steps required to move a novel compound from bench to bedside are fundamentally the same for startups and large pharmaceutical companies. However, startups face challenges that are not shared by their larger counterparts. They must achieve aggressive milestones with very limited investor funding and little to no revenue from existing products. The capital raised must pay for all overhead expenses associated with running a research program and a business, in addition to research and development costs. Investors expect high growth and rapid exit; if milestones are not met, the company will cease to exist. Furthermore, startups lack the internal resources and infrastructure of large companies, and often pay a premium for access to clinical research organizations, consultants, and other advisors. There are several opportunities for regulators to foster innovation by early stage companies, especially those developing antibiotic alternatives. These include supporting streamlined clinical trial designs that carefully consider the mechanism of action of the proposed drug candidate, timely review of regulatory submissions, waiving sponsor fees, and facilitating access to federal research facilities and other resources. A streamlined regulatory process not only reduces the cost of product development, but attracts investors by decreasing financial risk, which in turn encourages further investment in innovation.

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