
Alternatives to Antibiotics
International Symposium
Alternatives to Antibiotics (ATA)
Challenges and Solutions in Animal Production
The World Organisation for Animal Health (OIE)
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Abstracts

Program Overview

OBJECTIVES AND EXPECTED OUTCOMES

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Antibiotics are essential tools for animal and human health and concerns over antibiotic resistance and their prudent use in animals have garnered global interest. Yet insufficient attention has been given to the scientific breakthroughs and novel technologies that provide alternatives to antibiotics. The objectives of this symposium is to highlight promising research results and novel technologies that provide alternatives to antibiotics, assess challenges associated with their commercialization and use, and provide actionable strategies to support their development. The symposium will focus on the latest scientific breakthroughs and technologies that provide new options and alternative strategies for preventing and treating diseases of animals. Some of these new technologies provide the means for a One Health approach and have direct applications as medical interventions for human health, but the focus of the symposium is animal production, animal health, and food safety. The following five areas will be explored in detail through scientific presentations and expert panel discussions:

1. Alternatives to antibiotics: lessons from nature
2. Immune modulation approaches to enhance disease resistance and treat animal infections
3. The gut microbiome and immune development, health and diseases
4. Alternatives to antibiotics for use as growth promotants
5. Regulatory pathways to enable the licensure of alternatives to antibiotics

The major issue to be addressed is novel biocontrol approaches for reducing bacterial pathogens (and where applicable viral and parasitic pathogens) in food animal production that employ strategies specifically geared to reduce or eliminate drug resistance development.

The expected outcomes from the conference are four-fold. First will be the selection of key presentations from the conference that will be published in a special issue of the journal of Veterinary Microbiology. Presentations that reflect significant advances in the discovery and development of novel antimicrobials that present alternatives to conventional antibiotics will be selected. Second will be the publication of manuscripts from the symposium panel discussions that identify problems, solutions and recommendations for advancing the research and development of alternatives to antibiotics. Third will be the development of the conference website www.ars.usda.gov/alternativestoantibiotics with the posting of oral and poster presentations, relevant news items, scientific publications, and future events. Lastly, will be reporting the present research on new molecules and scientific findings on the alternatives that could be used in animal production at the March 2013 OIE Global Conference on the Prudent Use of Antimicrobial Agents.

Keynote Presentation

TOWARDS A NEW AGE OF ANTIMICROBIAL DISCOVERY

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The discoveries of penicillin and streptomycin in the 1940s signaled an era of discovery and application of a series of new therapeutic agents for the successful treatment of many microbial diseases. This was of enormous benefit to humankind and there was talk of "closing the book on infectious diseases". However, misuse and overuse, and the relentless appearance of resistant pathogens have dashed these hopes, leading instead to talk of a possible return to the pre-antibiotic era. Most major pharmaceutical companies have admitted failure to find new bioactive compounds and accordingly have discontinued their antimicrobial discovery programmes. Regrettably, during this time of discovery and development, little effort was directed towards understanding the roles of microbial natural products in nature. However, we are now witnessing an unprecedented change in the science of microbiology: the genomic revolution, driven by modern high throughput sequencing techniques is revealing astonishing new information about microbial biochemistry, chemical ecology, and the hidden potential for the production of millions of bioactive small molecules. These discoveries have enhanced the concepts of microbial community structures and their associated signaling pathways. It is obvious that this lexicon of bioactives will provide an inexhaustible source of novel therapeutic agents for modern medicine. How might these compounds be identified and produced in sufficient quantities for human and animal use? Fortunately, methods for the identification of the corresponding biosynthetic pathway gene clusters are being developed rapidly, and their isolation and expression in heterologous, designer hosts is improving. As alternatives, genetic or chemical triggering of the expression of so-called cryptic biosynthetic pathways are being investigated. Finally, dramatic technical advances in mass spectrometry and X-ray crystallography will provide high resolution procedures to identify natural product structures *in situ* together with identification of their cognate targets.

Session 1
Alternatives to Antibiotics: Lessons from Nature

Oral Presentations

1.1 ANTIMICROBIALS IN ANIMAL HEALTH: LESSONS FROM NATURE

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The discovery and development of antibiotics has led to dramatic improvements in the ability to treat infectious diseases and significant increases in food-animal production. Unquestionably, they represent one of the major scientific and medical advances of the 20th century. However, the emergence of microorganisms that are resistant to antibiotics has become a growing public health concern. To address this important issue, scientists, health specialists, and food-animal producers are searching for alternatives to conventional antibiotics.

Natural gene-encoded antimicrobial peptides constitute a ubiquitous and broadly effective defense mechanism that is being evaluated as an alternative to conventional antibiotics. Antimicrobial peptides have been isolated from most life forms and include bacteriocins, fungal peptide antibiotics, plant thionins and defensins, insect defensins and cecropins, amphibian magainins and temporins, as well as defensins and cathelicidins from higher vertebrates. Most antimicrobial peptides share common features, such as small size (12-100 amino acid residues), a net positive charge, and an amphipathic structure that facilitates interaction with negatively charged microbial membranes or other cellular targets. Compared with conventional antibiotics, which are generally active against bacteria or fungi, antimicrobial peptides often exert activity against a broad spectrum of microorganisms including bacteria, fungi, parasites, and enveloped viruses. In addition, unlike conventional antibiotics, which generally target a metabolic enzyme and may selectively induce resistance in microorganisms, antimicrobial peptides kill microbes mainly by membrane-targeting mechanisms, a mechanism that is inherently more difficult for microbes to circumvent by developing resistance. Here we discuss the antibiotic properties of antimicrobial peptides and their potential as alternatives to conventional antibiotics.

1.2 AVIAN CATHELICIDINS: PARADIGMS FOR THE DEVELOPMENT OF ANTI-INFECTIVES

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The broad-spectrum defense system based on host defense peptides (HDPs) is evolutionary very old and many invertebrates rely on this system to protect them from bacterial infections. However, in vertebrates the system remained important in spite of the superposition of a very sophisticated adaptive immune system. The cathelicidins comprise a major group of HDPs in mammals. About 7 years ago it was first described that cathelicidins are also present in birds. These recently discovered avian cathelicidins may serve as a paradigm to develop novel anti-infectives. Like the mammalian cathelicidins, avian cathelicidins exert direct antimicrobial activities but can also selectively boost host immune responses by regulation of cytokine production and recruitment of immune cells. In addition, it was found that chicken cathelicidins bind endotoxins and dampen the endotoxin-mediated inflammatory response. Different structural elements involved in bacterial killing and in immunomodulation were identified by molecular dissection, which enables the design of small HDP-based antibiotics with specific functions, i.e. having primarily immunomodulatory or antimicrobial activities. Since the immunomodulatory effects may, to a certain degree, be species-specific, we hypothesize that poultry-specific antibiotics can be developed based on avian cathelicidins.

1.3 ANIMAL-DERIVED ANTIMICROBIAL PEPTIDES AND SWINE HEALTH

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Antimicrobial peptides (AMPs) have attracted considerable attention for their broad-spectrum antimicrobial activity and less possibility to cause bacterial resistance. Our research focuses on animal AMPs in the following aspects. Firstly, we investigated the developmental expression pattern of porcine AMPs, PR-39, protegrin-1 (PG-1), prophenin-2, PMAP-23, and the effects of nutritional elements on major porcine AMPs gene expression. Results showed the gene expressions of porcine AMPs were steadily increasing from neonatal to 60kg, but decreasing significantly after 60 kg body weight. Further studies have shown PR-39 and PG-1 were significantly down-regulated 7-day post-weaning when weaning at 21, 28 and 35 days, respectively. Feeding trial results showed ZnO3000 (3000mg, zinc/kg diet) significantly increased PR-39 whereas ZnO100 (100mg zinc/kg diet) and ZnSO4 (100mg zinc/kg diet) obtained no significant results. Secondly, antibacterial activity, mechanisms of action and cytotoxicity of animal AMPs were investigated. Results showed PG-1 from swine and C-BF from snake were the most active peptides and their bactericidal activities were almost equal to aureomycin, towards not only the standard strains, but also isolated strains from fecal samples of weaning piglets with diarrhea. Furthermore, most tested peptides showed no adverse effect on *Bifidobacterium suis* and *Lactobacillus acidophilus*, while aureomycin and neomycin showed a marked inhibitory effect. In addition, all the tested AMPs were found to permeabilize bacterial membranes, while intracellular targets maybe exist for PG-1 and C-BF. Cytotoxicity tests showed that LfcinB, LFP-20, C-BF, PMAP-23, cecropin P1 and cecropin A exhibited the lower cytotoxic effects, while LL-37, PG-1, indolicidin and OG1 displayed higher cytotoxic activity among 128-256 μg/mL. Since LFP-20 was safe but had low antimicrobial activity while OG1 had considerable antimicrobial activity but high cytotoxicity as described above, modified peptides LF-6 with higher antimicrobial activity and OG2 with lower cytotoxicity were obtained from LFP-20 and OG1, respectively. Both LF-6 and OG2 were expressed using thioredoxin or intein as a fusion partner in *Escherichia coli*, with the yield of peptide at 5.6 mg/L and 6 mg/L, respectively, and the expressed peptides showed antimicrobial activities. Our research implied that piglet immunity could be improved by upregulation of secretory porcine AMPs expression, and some AMPs have potential for development as antimicrobial agents for substitution of feed antibiotics in pig diets.

1.4 PREBIOTICS AND PROBIOTICS IN ANIMAL PRODUCTION: PRESENT STATUS AND FUTURE PERSPECTIVES

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For many years, small amounts of antibiotics have been used as feed additives for farm animals in order to improve their growth rate and feed conversion efficiency. This practice has been banned in the EU since January 1, 2006 (EC Regulation No 1831/2003). Simply omitting the antimicrobial growth promoters (AGP) from the feed leads to approximately 10% less favorable feed conversion efficiency. Moreover, in the last years the cost of animal feed has dramatically increased due to large portions of arable land being used for growing biofuel crops. All this has led to enormous research and development efforts being made for the development of alternatives to the AGP. Currently, feed additives available as alternatives to the AGP can be subdivided into a limited number of major classes, which include the organic acids, etheric oils and herbs, enzymes, and finally the prebiotics and probiotics. Prebiotics are non-digestible feed ingredients that selectively favor the multiplication or metabolic activity of a specific fraction of the intestinal microbiota. Most currently available prebiotic feed additives contain oligomers of specific mono- or disaccharides, obtained either by controlled degradation of the naturally occurring polysaccharides, or by synthetic polymerization of disaccharides. For several prebiotics, including fructo-oligosaccharides and arabinoxylan-oligosaccharides of a specific degree of oligomerization, beneficial effects on feed conversion efficiency have been documented. Additionally, for mannan-oligosaccharides and for arabinoxylan-oligosaccharides, protective effects against *Salmonella* colonization have been documented. Probiotics are single or mixed cultures of living microorganisms, which beneficially affect the host by improving the properties of the indigenous microbiota. Currently available probiotics most commonly contain one or more strains of *Lactobacillus* spp., *Enterococcus* spp., *Bacillus* spp. or *Saccharomyces* spp. Effects on feed conversion efficiency and daily weight gain largely depend on the successful delivery of viable microorganisms to the lower intestinal tract of the animals. Hurdles to be overcome include the incorporation in the feed and survival of the feed pelleting process, as well as survival during gastric transit. Spore forming strains in this context definitely have an advantage. Future developments and progress in this field may come from metabolome analyses leading to a better understanding of the cross-feeding phenomena taking place in the intestinal tract and from the availability of *in vitro* cultures of new genera and taxa of beneficial indigenous intestinal microorganisms.

1.5 HEAVY METALS AS ALTERNATIVES TO ANTIBIOTICS: PANACEA OR PANDORA'S BOX?

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Metals are used in trace amounts in food animal production to maintain the normal physiology and healthy status of animals. In Europe and the United States, copper (Cu) and zinc (Zn) at levels much higher than their physiological requirements have been touted as alternatives to the use of antibiotics for growth promotion purposes. Gut microbiology is also impacted by supplementing high levels in feed, and commensal and pathogenic enteric bacteria both must adapt in order to survive under such conditions. We examine copper as one example of the potential conundrum of using metals as alternatives to antibiotics. *Enterococcus* spp., well-known gut commensal organisms that can cause nosocomial human infections, may acquire resistance to copper via a transferable copper resistance (tcrB) gene that is carried on a plasmid. In Europe, the plasmid also carries genes for macrolides [erm(B)] and glycopeptide (vanA) resistance while in North America tet(M) is also commonly present, though vanA is notably absent in U.S. agriculture. Owing to the full saturation of tet(M) and erm(B) among U.S. nursery swine enterococci, Cu supplementation at 125 ppm does not select further for tetracycline and erythromycin resistance, respectively, despite the highly determined linkages among the three genes: tet(M), erm(B) and tcrB. However, in cattle where tet(M) and erm(B) resistance among enterococci is not complete, Cu at 100 ppm appears to have a sparing effect on tylosin resistance ($P < 0.001$) but none on tetracycline resistance ($P=0.218$). The Gram-negative commensal bacteria such as *Escherichia coli* tell a different story altogether with the pco gene cluster conferring transferable resistance to Cu via plasmid-borne pcoD gene. A major public health issue concerning these bacteria at the moment is resistance to the 3rd and 4th generation cephalosporins such as ceftiofur which have been labeled as critically important by the WHO. In the United States, known linkages of the ceftiofur resistance gene blacmy-2 with tetracycline resistance determinants (particularly tetA) have complicated efforts to control resistance through prudent use of antibiotics. Localization of the CMY-2 gene to an Inc group of plasmids in *E. coli* and *Salmonella* in the United States has afforded some opportunity to attempt to control resistance by selecting for strains that harbor competing plasmids. Our work suggests that copper supplemented at 125 ppm in swine favors the pco gene, and the presence of the pco gene selects against the CMY-2 gene (odds ratio = 0.29; $P < 0.0001$). Thus, while copper supplementation can co-select for copper and some antibiotic resistance factors, it may also be shown to disfavor other more critical resistance factors.

1.6 BACTERIOPHAGES: THE ALTERNATIVES TO ANTIBIOTICS FOR ANIMAL FEEDS

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The emergence of strains that are resistant to antimicrobials as a result of antimicrobial usage in animal feeds is a public issue of great concern. Since July 2011, all the antimicrobials as AGP-replacers have been banned for use in animal feed in Korea. We have been investigating and commercializing bacteriophages to control pathogenic bacteria in livestock and farming environments by supplying with feeds. BIOTECTOR S1 is the first product consisting of bacteriophage specifically infecting *Salmonella gallinarum* (SG) and *Salmonella pullorum* (SP), which are responsible for fowl typhoid and pullorum disease, respectively. To estimate the preventive effect of BIOTECTOR S1 in livestock against *Salmonella gallinarum* (SG), the strain was orally challenged (5x106~5x108 PFU/ml/head) into *Salmonella*-free chickens (e.g. broilers, broiler breeders, and layers). One hundred thirty six male Ross broilers at 5 weeks old, 60 Ross broiler breeders at 67 weeks old, 60 Hy-line brown layers at 6 weeks old, and 60 Lohmann Brown layers at 6 week of age were assigned to three groups in each experiment (negative control, positive control and test group). All the phage treated groups represented significantly decreased level of mortality comparing with that of positive control groups: 73% lowered in Ross broilers, 53% decreased in Ross broiler breeders, 31% reduced in Hy-line brown layers, and 86% decreased in Lohmann Brown layers. Also, layer performance in Hy-line Brown was improved in the phage treated group: 3% increased in egg production, 2.4% increased in egg mass (g/day/bird) etc. In monitoring of SG recovery from cecums, phage treated group involved in 3~4 log10 PFU/g reduction comparing with SG level in control group (SG treated without phage dietary). However, any negative clinical symptom in internal organs (liver, spleen and pancreas) has not been observed in phage-treated groups. In addition, SG control efficacy in layers on usage of various feed additives such as antibiotics, organic acid and BIOTECTOR S1 was tested. The mortality in BIOTECTOR S1 treated group showed 20% while antibiotics treated-, organic acid treated-, or control group resulted in 55%, 65%, and 75% mortality, respectively. The improvement of lowering mortality by BIOTECTOR S1 mixed in poultry feed was confirmed in a field test resulting in reduction of mortality by 86% comparing with control. In conclusion, we suggest that bacteriophage itself could be a good replacement for antimicrobials and, also, it could be concurrently used with chemical antibiotics to enhance bactericidal effect in terms of controlling anti-drug resistant bacteria.

Session 1

Poster Presentations

1.7 *IN VITRO* ANTIMICROBIAL ACTIVITY OF MATERIAL FROM 450 EUROPEAN PLANT SPECIES TOWARDS *CLOSTRIDIUM PERFRINGENS*, ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) AND *CAMPYLOBACTER JEJUNI*

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To identify potential alternatives to in-feed antibiotics, the EU FP6 project REPLACE screened 450 European plant species (representing 100 families), collected around five geographical locations in Europe, for antimicrobial properties against *Clostridium perfringens*, enterotoxigenic *Escherichia coli* (ETEC) and *Campylobacter jejuni* (only tested with 100 of the species). The plant samples were dried, grinded and stored at room temperature in the dark. The antimicrobial activity towards *C. perfrin*gens was tested in chicken ileum extract medium (pH). The ETEC *strain E. coli* O149:K88 was investigated in two *in vitro* assays; survival of the ETEC strain in pig stomach content (pH 4.2-4.5) and growth of the ETEC strain in pig small intestinal content (pH 6.5-7.0). The inhibitory levels were arbitrarily defined as high, medium, low, or insignificant. For *E. coli* tested in small intestinal content, the inhibitory levels were defined as bactericidal, bacteriostatic, growth inhibitory, growth retarding, or insignificant. When added directly as 5% (w/w) dry powder to the assays, approximately 250 of the plant samples showed no significant inhibitory effect whatsoever against either of the tested bacteria. The remaining approximately 200 plant samples showed antibacterial activity in a dose-dependent manner. *Clostridium perfringens* was in general most prone to inhibition, particularly by several members of the families Cistaceae, Ericaceae, Fagaceae and Rosaceae. The ETEC strain was particularly inhibited by members of the families Juglandaceae, when tested in stomach content, and members of Brassicaceae, Juglandaceae, Liliaceae and Ranunculaceae in small intestinal content. For *C. jejuni* the antimicrobial effect was highest with members of Fagaceae, Juglandaceae and Liliaceae.

1.8 ANTIVIRAL ACTIVITY AND MECHANISM OF ACTION OF FUCOIDAN FROM *CLADOSIPHON OKAMURANUS* AGAINST NEWCASTLE DISEASE VIRUS

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Fucoidan is a sulfated polysaccharide found in the cell wall matrix of brown algae. Recent studies have demonstrated that sulfated polysaccharides have antiviral properties. These compounds are much less cytotoxic than conventional antiviral drugs, and are therefore excellent candidates for novel antiviral drug development. Newcastle Disease Virus (NDV) causes fatal infections of poultry, resulting in major economic losses to the poultry industry worldwide. Numerous NDV outbreaks occur despite vaccination programs, which underline the need for alternative prevention and control strategies. This study aims to determine the mechanism of action for the *Cladosiphon okamuranus* fucoidan antiviral activity against NDV. Further, we determined *in vitro* the 50% cytotoxic concentration (CC50) and the 50% effective concentration (EC50) to determine the fucoidan therapeutic index. In Vero cells, fucoidan showed potent antiviral activity against La Sota NDV strain and a therapeutic index (CC50/EC50) of 2000. In contrast, ribavirin had weaker antiviral activity, required higher concentrations to inhibit NDV, and exhibited substantial cell cytotoxicity (therapeutic index 1.7). In time-of-addition studies with fucoidan, we demonstrated a viral inhibition at early stages of infection (minute 0 to 60 post-infection). In the fusion inhibition assay, fucoidan significantly inhibited syncytia formation when it was before the cleavage of fusion protein, indicating a specific interaction between fucoidan and the fusion protein. Using a mesogenic NDV strain, we further showed that addition of fucoidan during the first hour post infection significantly inhibited NDV penetration and reduced HN protein expression by 98%. Finally, ribavirin-resistant NDV isolates remained susceptible to fucoidan's antiviral activity and there was no difference in the fucoidan EC50 between wild-type and ribavirin-resistant NDV. Fucoidan-resistant NDV developed slowly, requiring many passages. Using an embryonated chicken egg *in vivo* system, we found that fucoidan potently inhibited La Sota NDV *in vivo* at a concentration of 0.25 µg/mL. RT-PCR analysis data showed that 16 µg fucoidan per embryo suppressed viral RNA synthesis by 99.8%. These data show that *C. okamuranus* fucoidan is a potent antiviral compound that may substantially benefit the poultry industry and also provides a better understanding the mode of antiviral action of sulfated polysaccharides.

1.9 ACTION OF ORGANIC ACIDS IN DIETS CONTAMINATED BROILERS EXPERIMENTALLY WITH *SALMONELLA* SP.

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In Brazil, the study of organic acids is relevant as alternative to production, the health requirements for its poultry products. The need to replace antimicrobials in diets aimed to evaluate the effects of three organic acids. Three experiments were conducted separately with CobbX chicks from one day old. In each experiment, 200 chicks were distributed in five treatments and four replications of ten chicks each, in a controlled environment. The ration of corn and soybeans followed the nutritional requirements without the addition of animal products or preservatives are experimentally contaminated with *Salmonella* sp. and supplemented to one organic acid (acetic, formic, or propionic acid) in four different concentrations (0.5, 1.0, 1.5, and 2.0%). The feed was supplied in the period of eight to twenty-one days old. There was also a control group composed of contaminated feed without added acid. Three parameters were analyzed: the efficiency of acid to reduce or eliminate the *Salmonella* sp. from fed rations, performance for weight gain / feed intake / feed-to-gain ratio and the presence of the pathogen in cloacal swabs and organs pool (liver, heart, and gallbladder) of a bird of each plot at the end of 21 days. Data were analyzed by polynomial regression, the relative frequency of observation and estimation scores for isolation and recovery of the pathogen, respectively. For the assay with acetic acid, it was found that levels of 0.5, 1.0, 1.5, and 2.0% have not eliminated or reduced *Salmonella* sp. rations; favor weight gain and improved feed conversion linearly and the recovery of the pathogen was evident in 30% of cloacal swabs and 50% of the organs pool. To formic acid, it was found that levels of 1.5% and 2.0% reduced contamination by *Salmonella* sp. but no level change the performance of the broilers, the presence of bacteria was observed in 70% of cloacal swabs and 50% of organs pool. For propionic acid, it was observed that the level of 0.5% reduced the contamination of the feed and the other (1.0%, 1.5%, and 2.0%) eliminated the pathogen in the diet. However, the propionic acid treatment negatively altered food intake, showing quadratic effect on feed conversion in broilers. We observed relative frequency of isolation of the pathogen in cloacal swabs of 80% and 10% in organs pool. Propionic acid reduced and eliminated *Salmonella* sp. of contaminated feed and promoted less isolation of bacteria in the organs pool. However, it proved to be unsuitable for use in broilers in the period from eight to twenty-one days of age by lower palatability and adversely affected performance.

1.10 LIVER FUNCTION AND BACTERIOLOGY OF ORGANS IN BROILER INOCULATED WITH NALIDIXIC ACID-RESISTANT *SALMONELLA TYPHIMURIUM* AND TREATED WITH ORGANIC ACIDS

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Salmonella is configured between the emerging pathogens of major importance for poultry breeding stock, especially for issues related to food safety. An experiment was carried out with 630 one-day-old broilers to evaluate the effects of organic acids when birds were experimentally inoculated with *Salmonella Typhimurium*. Liver damage and the persistence of the bacterium in the organs were evaluated. Broilers were distributed in a completely randomized experimental design of a 3X3 factorial arrangement of six treatments with seven replicates of 15 birds each. Treatments consisted of experimental challenge (saline solution, nalidixic acid-resistant *Salmonella Typhimurium* via gavage or via feed) and of feeding an organic acid blend or not. Birds were inoculated with saline solution or the bacterium via gavage at one day of age, or were offered a feed with or without the organic acid blend for the period of seven to 14 days of age. A dose of 5.0x102 colony-forming units (CFU)/0.5mL of *Salmonella Typhimurium* was used for inoculation both via gavage and via feed. The following parameters were evaluated: relative liver weight, liver histopathology, liver and serum biochemistry, and bacteriological analyses of the ceca, crop, spleen, and liver and heart pool. At 21 and 28 days of age, the liver of the non-inoculated groups were significantly lighter as compared to the other treatments. Birds fed organic acids, independently from *Salmonella Typhimurium* inoculation route, presented lower bacterial isolation rates in all organs tested. Birds inoculated in the crop and treated with organic acids presented lower *Escherichia coli* CFU counts ($p < 0.05$). Birds inoculated with *Salmonella* presented significant changes ($p<0.05$) in liver enzymes as detected by serum biochemistry, as well as in liver histopathology. It was concluded that organic acids effectively controlled *Salmonella Typhimurium*, and did not cause any liver damage.

1.11 SYNTHETIC ANTIBACTERIAL PROBIOTICS

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Foodborne bacterial gastrointestinal infections are significant causes of morbidity and mortality worldwide. Alarmingly, because of the extensive, non-therapeutic use of antibiotics in agriculture, foodborne pathogens are emerging that are resistant to our most potent drugs. We pursue an integrated research approach to reduce the use of therapeutic antibiotics in animal feed and treat gastrointestinal infections in animals and humans. We employ synthetic and systems biology technologies to engineer probiotic bacteria that reside in animal gastrointestinal tracts, and then express and release antimicrobial peptides (AMPs). Probiotic bacteria are part of the gastrointestinal microbiota and have known benefits for humans and animals. AMPs are proteins and can be readily produced by bacteria. What is unique in our approach is the use of synthetic biological switches to precisely control the overexpression and delivery of AMP molecules. AMPs are small molecules with remarkable bactericidal properties. Their use has been limited because they are quickly degraded by the host if administered orally or intravenously. We use probiotics as AMP-delivery vehicles. Probiotics are bile-resistant microorganisms that can be delivered safely in food or water. We engineer inducible AMP expression systems in probiotics. We examine the impact of controllable delivery of AMPs in swine and poultry challenged by infectious agents and explore AMP-carrying probiotics as alternatives to traditional antibiotics in agriculture. We also experiment with mice as models of human gastrointestinal tracts. We use established protein expression systems in probiotic bacteria to express bacteriocins, AMPs that are naturally produced by bacteria. We also study pathogen-specific AMPs. We experiment with probiotic species *Lactococus lactis*, *Lactobacillus acidophilus*, known to be safe for consumption by animals and humans. These species are also well-annotated with known genomes and have established microbiological and genetic engineering techniques. Synthetic molecular devices are engineered to be robust. With established metagenomics, proteomics, and bioinformatics techniques, we then analyze animal gut microbiomes and the relationships between probiotics, commensal microbes, and health. We quantify the presence of engineered probiotics and of expressed AMPs in intestines, and quantify the disease outcome for animals challenged by pathogens. A successful strategy to reduce this use of antibiotics would have significant public health, economic, and environmental impacts. Our approach is a radical shift from traditional drug discovery and delivery paradigms, and may constitute such a strategy. Modified probiotics may also have potential as countermeasures to bacterial infections.

1.12 TRIPLE-ACTING PEPTIDOGLYCAN HYDROLASE FUSION PROTEINS ERADICATE *STAPHYLOCOCCUS AUREUS* AND REDUCE RESISTANT STRAIN DEVELOPMENT

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There is a need for new antimicrobials since broad range antibiotics are believed to select for multi-drug resistant superbugs. Bacteriophage endolysins are peptidoglycan hydrolases (PGHs) that lyse the bacterial cell wall to allow nascent phage to escape and have desirable antimicrobial qualities. Phage and host have co-evolved, such that the endolysins target highly immutable bonds within a limited target species range. PGHs cause osmolysis by degrading extracellular PG, avoiding many of the classical resistance mechanisms (e.g. efflux pumps). PGHs are modular proteins amenable to genetic modification for the generation of novel fusions with multiple lytic activities. We have generated a fusion PGH combining the staphylolytic domains of the synergistic staphylococcal phage K endolysin LysK and the PGH bacteriocin Lysostaphin. The fusion retains the three unique catalytic activities of the parental molecules with an increased specific activity compared to both parental enzymes in turbidity reduction assays. Few bacteria can evade three simultaneous lytic activities. The recombinant protein disrupts *Staphylococcus aureus* SA113 biofilms more efficiently (at lower concentrations) than either parental molecules, and is less prone to resistance development both *in vitro* and *in vivo*. Cultures of *S. aureus* strain Newman develop ~2-fold increased resistance to the fusion during 10 rounds of liquid culture sublethal exposure (Minimum inhibitory concentration assay; MIC) compared to much higher parental enzyme MIC increases [LysK (~42-fold); Lysostaphin (~585-fold)]. Conventional antibiotics or the two parental enzymes in combination, tested in parallel were less effective than the triple fusion at reducing resistant strain development. In a rat model of nasal carriage, a triple acting fusion was able to reduce the *S. aureus* colonization to the same extent as mupirocin (~2 logs), whereas the parental molecules could not. Bacteria recovered from treated rats were found to retain the same sensitivity to the fusion molecule in both MIC and Plate Lysis Assays as the parental strain, prior to the experiment. The delivery of three unique PGH lytic activities in a single protein effectively treats *S. aureus* while reducing the risk of resistant strain development.

1.13 THE MULTIPLE SHP SIGNALING PEPTIDES FOUND IN *STREPTOCOCCI* GENOMES ALLOW CROSS-TALK BETWEEN DIFFERENT SPECIES

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Rgg-like proteins have been primarily identified and studied in streptococci, as transcriptional regulators controlling the expression of genes encoding various functions. Recently, we showed that some of these regulators, in association with a short hydrophobic peptide (SHP) playing the role of pheromone, are involved in a new quorum-sensing mechanism. The latter has been deciphered in detail in *Streptococcus thermophilus* where we showed that the activity of Rgg is positively controlled by a direct interaction with SHP. The construction of a phylogenetic tree of all Rgg proteins found in Gram-positive bacteria, highlighted 68 shp/rgg systems only present in streptococci. They were classified into three groups using the following criteria: the amino acid sequence of the SHP and the genetic organization of both shp and rgg genes. We also identified a conserved Rgg DNA binding site specific to each SHP/Rgg group. Furthermore, we have detected similar SHP/Rgg systems in different streptococci species, different SHP/Rgg systems in a streptococci species and different SHP/Rgg systems inside a strain. These findings raised the question of crosstalk, i.e. cross-activation or cross-inhibition between different species of streptococci, between different strains of the same species or inside a strain, when several SHP/Rgg sytems are present. Using biochemical and genetic approaches, we have studied and expanded the functionality of the SHP/Rgg systems to two shp/rgg loci of pathogenic streptococci (*S. agalactiae* and *S. mutans*). We have assessed the specificity of the SHP/Rgg pairs by studying three pairs chosen among different species and groups (*thermophilus, agalactiae and mutans*) and by performing functional complementations using synthetic SHPs. We have demonstrated that cross-activation occur with specific pheromones between species. We are currently investigating cross-inhibition phenomena. Our results concerning peptide-based quorum sensing mechanisms in streptococci open new perspectives on the control of relevant genes in streptococci.

1.14 THE EFFECT OF DIETARY CAPRYLIC ACID ON THE *SALMONELLA* SPP. SHEDDING IN EXPERIMENTALLY INFECTED BROILER CHICKENS

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Fatty acids have been studied for their antibacterial properties in a few past decades, indicating a clear inhibitory effect of unsaturated, medium-chain fatty acids in particular (C8 – C12). In our previous *in vitro* experiments, caprylic acid (C8:0) was found the most effective fatty acid against salmonellas. The aim of the present study was to evaluate the effect of caprylic acid on counts of salmonellas in chickens experimentally infected with *Salmonella enteritidis*. Fourteen day old Ross 308 male chickens were housed individually, divided into four groups: positive control, negative control, and two treatment groups. Control animals were fed a commercially available diet. Treatment groups received a diet supplemented with 0.25 % and 0.5 % of caprylic acid. The feed of treated birds and those of the positive control was infected with 5 ml of overnight-grown bacterial culture per one kilogram of feed. On the eighth day of the experiment, chickens were slaughtered and crop and cloaca contents sampled for microbiological analyses. Differences between control and treated samples were evaluated. Caprylic acid at both concentrations significantly decreased counts of salmonellas, the effect of caprylic acid in the crop contents, however, was more pronounced. Antibacterial activity of caprylic acid was dose-dependent. It can be concluded that caprylic acid is able to reduce numbers of salmonellas in the gastrointestinal tract of chickens and has a potential to improve health status of infected animals.

1.15 TARGETING MOTILITY PROPERTIES OF BACTERIA IN THE DEVELOPMENT OF PROBIOTIC CULTURES AGAINST *CAMPYLOBACTER*

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Campylobacter is a leading cause of bacterial foodborne illness and is associated with the consumption of poultry products. *Campylobacter* is commonly present in the intestinal tract of poultry and one strategy to reduce enteric colonization is the use of probiotic cultures. These cultures consist of beneficial bacteria which may displace enteric pathogens. Although probiotic cultures have been successfully used to reduce enteric *Salmonella* colonization, their use has been met with limited success against *Campylobacter*. In an effort to improve the efficacy of probiotic cultures, we developed a novel in vitro screening technique, a rigorous selection of poultry bacterial isolates based on motility and flagella characteristics. The theory is that motility selected bacteria have the marked ability to exclude *Campylobacter* because of their ability to reach the same environmental niche in the intestinal crypts of poultry species. Cecal contents from healthy young chickens were collected and bacterial isolates were identified. Multiple passes were conducted and colonies with enhanced motility were selected at each pass. Strains with the greatest motility and the ability to inhibit *Campylobacter* growth *in vitro* were evaluated in two trials. Day of hatch chicks were administered these isolates alone or in combination and chicks were challenged with a mixture of four different strains of *Campylobacter* by oral gavage on day 7. One isolate reduced *Campylobacter* colonization in both trials. A follow-up study compared this isolate before or after selection for enhanced motility. The enhanced isolate was more effective than the unenhanced isolate in reducing *Campylobacter* colonization in separate trials. These findings indicate that selecting for enhanced motility after multiple passes improved the abilities of these bacteria to compete with *Campylobacter* and may provide a strategy for reduction of *Campylobacter* in preharvest poultry. As alternatives to antibiotics are needed both for conventional and organic poultry production, improved probiotics provide a strategy to reduce enteric pathogens that could be utilized by producers. Funded in part by USDA OREI Program 2011-01955.

1.16 PHAGE-THERAPY TO CONTROL INFECTIONS CAUSED BY *BACILLUS LICHENIFORMIS* IN THE CULTURE OF THE PACIFIC WHITE SHRIMP *LITOPENAEUS VANNAMEI*

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Bacillus licheniformis is a facultative anaerobic, saprophytic Gram-positive bacterium that is found widespread in nature. Unfortunately, it was isolated by researchers at CENIACUA, Colombia, because it was responsible for the mortality of the Pacific white shrimp *Litopenaeus vannamei*, which is the major aquaculture product in Colombia. However, the use of antibiotics for the control of bacterial pathogens is not a viable option because of the legislation that regulates the presence of these antimicrobials in human foodstuffs. For these reasons, phage therapy is an interesting alternative treatment. *Bacillus licheniformis* CEB1 was isolated from juveniles' haemolymphs. A bacteriophage specific for *B. licheniformis* CEB1 was isolated from shrimp pond water; its potential use for the control of *B. licheniformis* CBE1 was tested *in vitro* and *in vivo. In vitro,* the efficacy of the phage was assayed by infection curves. The results showed a decrease of ca. 2 logarithmic units in the bacterial population within 24 hours after the inoculation of the phage. The *in vivo* tests of shrimp inoculated with bacteria and phage simultaneously showed 75-90% survival, whereas positive control assays inoculated with bacteria only, showed a 10% survival. Another *in vivo* test was conducted in symptomatic *L. vannamei* shrimp with vibriosis. In this experiment a 90% survival rate was also achieved. These results of the *in vivo* tests showed that the phage was effective not only against *B. licheniformis* CEB1 but also influences positively the survival of the shrimp affected by other diseases.

All previously mentioned *in vivo* experiments were conducted administering the phage through reverse gastric tube injection. This way of administration serves to prove the potential for the control of the infection on the animal model; nonetheless, it is not viable in a commercial production facility. Therefore, we developed experiments administering the phage in the shrimp's food. To prepare the phage-supplemented feed, regular shrimp food was submerged in a phage suspension; then, the impregnated food was dried and coated with flavorless gelatin. The end product was given to infected *L.vannamei* shrimp with *B. licheniformis* and to healthy *L.vannamei* shrimp.

This work presents a step ahead in the application of bacteriophages as an antibiotic alternative for *B. licheniformis* infections for the aquaculture industry in Colombia.

1.17 PEPTIDE-BASED QUORUM SENSING MECHANISM IN *STREPTOCOCCUS AGALACTIAE* AS A NEW APPROACH TO CONTROL GENE EXPRESSION

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Quorum sensing (QS) is a cell-to-cell communication process used by bacteria to control gene expression at the population level. Recently, a new QS mechanism has been described in streptococci involving a transcriptional regulator belonging to the Rgg family and a small hydrophobic peptide (SHP), playing the role of a signaling molecule. SHP is first synthesized, processed and secreted in medium, then, imported back into the cell and further, intracellularly detected by Rgg. The interaction between SHP and Rgg makes it possible to positively control the expression of target genes among which is its own shp gene (1). In most streptococci, one copy of rgg/shp loci is present in *Streptococcus agalactiae* (GBS). This bacterium is an asymptomatic commensal inhabitant of the human gastrointestinal and genitourinary tract, but also a leading cause of devastating infections in newborns and immunocompromised adults. Samen et al. described that Rgg is involved in the regulation of several virulence factors of GBS. However, this study was performed in rich medium (2), and we have shown that, in this condition, SHP is not expressed. Consequently, we believe that Rgg relevance in the pathogeny of GBS has been underestimated. To study in-depth the function of Rgg in GBS, we analysed this QS mechanism using a transcriptional fusion between the promoter of shp and lacZ, and a rgg deletional mutant in GBS strain NEM316. We have shown that this fusion is not expressed in the rgg mutant, and, in the same way, is stimulated by the addition of synthetic SHP. These results indicate that shp expression is positively controlled by Rgg and SHP and confirm that the QS mechanism is functional in GBS. Using a label-free proteomic approach combining SDS-PAGE with LC-MS, we are looking for Rgg targets and we have already identified at least one secreted protein. We will focus our study more specifically on targets that could be potentially related with the pathogeny of this bacterium. All results together suggest that the addition of some analogous synthetic peptides, which can compete with SHP, might diminish the expression of the SHP/Rgg targets, opening possible new approaches to decrease the virulence of GBS.

1. Fleuchot, B., et al., Rgg proteins associated with internalized small hydrophobic peptides: a new quorum-sensing mechanism in *streptococci*. Mol. Microbiol., 2011. 80: 1102-1119.
2. Samen, U.M., et al, The transcriptional regulator RovS controls the attachment of *Streptococcus agalactiae* to human epithelial cells and the expression of virulence genes. Infect. Immun., 2006. 74: 5625-5635.

1.18 MINIMAL INHIBITORY CONCENTRATION OF A NOVEL PLANT EXTRACT ON GROWTH OF BACTERIAL ISOLATES WITH VETERINARY IMPORTANCE

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LiveLeaf GRAZIX is an extract of green tea (*Camellia sinensis*) and pomegranate (*Punica granatu*). Plant antimicrobial function is based on a different mechanism than antibiotics or chemical sanitizers. Such natural plant compounds have a long history of use as bacterial inhibitors. However, commercially available plant-based bacteriostat products have remained expensive and have failed to achieve broad spectrum potency without formulation, user preference, or toxicity problems. The objective of this study was to subject Gram-negative and Gram-positive bacteria to serial dilutions of the extract in order to define its MIC. The plant extract GRAZIX was diluted in sterile distilled water to achieve concentrations of 500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.9, 1.95, 0.98, and 0.49 µL GRAZIX per mL of water. Strains of Gram-negative (*Bordetella bronchiseptica*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica* serovar *Typhimurium*) and Gram-positive (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*) bacteria were isolated from different pathological samples originating from swine and poultry in a veterinary diagnostic bacteriological laboratory (Veterinary Diagnostic Directorate, NFCSO, and Department of Microbiology and Infectious Diseases, SZIU-FVS, Budapest, Hungary) and cultured in Trypticase Soy Broth (TSB) for several hours until the resulting culture's viable bacterial cell count was between 10^4 and 10^5 CFU/mL. The GRAZIX dilutions were added to tubes containing Mueller Hinton Broth followed by the addition of 10 µL of bacterial suspension; as a control, sterile water was used in place of dilutions of the plant extract. This mixture was incubated at 37°C for 24 hours and then the degree of inhibition of bacterial growth was evaluated visually, with the MIC values determined by either growth visible (the presence of turbidity in the tube) or no growth visible (no turbidity). All controls exhibited bacterial growth; all but two bacterial isolates were inhibited by the GRAZIX solutions diluted to 3.9 µl/mL or 7.8 µl/mL. One Gram-positive and one Gram-negative isolate were not inhibited until 15.6 µl/mL and 31.3 µl/mL solutions, respectively, were applied. These results demonstrate the novel plant extract, GRAZIX, has antibacterial activity against various pathogens of veterinary importance.

1.19 INHIBITION OF INTESTINAL PATHOGEN ADHERENCE BY *PICHIA GUILLIERMONDII* IN AN IN VITRO MODEL

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The European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) reported a high resistance for commonly used antimicrobials such as ampicillin and tetracyclines. In animals and food, a very high proportion of campylobacteria is resistant to ciprofloxacin, particularly in chicken but also in pigs and cattle. In humans, animals, and food a high proportion of salmonellae and indicator *Escherichia coli* is resistant to common antimicrobials (www.efsa.europa.eu/en/efsajournal/pub/2598.htm). Despite the EU-wide ban of antibiotic growth promoters in feed, significant levels of AGPs are still used in the livestock industry and the search for alternatives is high on the agenda. Yeast preparations are a primary ingredient in this context since they contain manno-oligosaccharides (MOS) and ß-glucans, oligosaccharides of the yeast cell wall that may exert specific functions in supporting the immune system and fend off pathogen invasion already in the intestines of the host. The capacity to bind certain pathogenic bacteria is a well documented characteristic of many *Saccharomyces cerevisiae* derived products (Mirelman et al. 1080; Perez-Sotelo et al. 2005). For *Pichia guilliermondii* such information was lacking. In the present study, mucus from two-week old broiler chickens and piglets two weeks after weaning was used to determine the inhibition of pathogen (*E. coli* F4+ and *Salmonella enterica* serovar *Enteritidis*) adherence by the yeast. Treatments were negative control (no addition), positive control (mannose) and *P. guilliermondii* (untreated and treated with gastric juice or heat). Doses applied ranged from 0.5 to 2.5%. The pathogenic strain of *E. coli* adhered at equal efficiency to mucus from piglets and broiler chickens. The pathogenic strain of *S. enterica*, however, had a higher affinity to mucus from broiler chickens than piglets. *P. guilliermondii* inclusion inhibited the binding of both tested pathogens dose dependently. In the broiler chicken model the highest dose of yeast inhibited adherence by > 90% and in the piglet model by > 80% and 90% for the strain of *E. coli* and *S. enterica*, respectively. The same dose of the positive control inhibited the binding of pathogens by 40 to 65%, respectively. Gastric and heat treatment of the yeast further improved the inhibitory effect of *P. guilliermondii* significantly, suggesting that the inhibitory effect of the product *in vivo* may significantly increase in situ, distal to stomach.

1.20 IDENTIFICATION AND CHARACTERIZATION OF BACTERIOPHAGES INFECTING *SALMONELLA ENTERICA*

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Salmonella enterica causes various salmonellosis in swine and poultry. Use of antibiotics for such diseases is becoming forbidden in many countries due to the emergence of antibiotic resistant strains. Bacteriophages are good alternatives to antibiotics. They infect specific host strains and effectively lyse them. Phages have presented in nature for a very long time and served to control bacterial balance in nature. We have isolated three novel bacteriophages infecting *S. enterica* from rivers and sewage treatment facilities. Among many isolates of bacteriophages, the three phages were chosen for novelty using analysis of major capsid proteins by MALDI-TOF mass spectrometry. Phage number 4 had a major capsid protein of 37 KDa, number 12 had a major capsid protein of 25 KDa, and number 19 had a major capsid protein of 35 KDa. All the phages had DNA genomes as determined by RNase sensitivity assay. Based on morphology observed under transmission electron microscope, they belonged to myoviridae and siphoviridae. Their host range included *S. enteritidis, S. typhymurium, S. gallinarum*, and *S. pullorum*. They did not infect *Escherichia coli*. Their burst size was between 90 and 150 depending on the host strain. All three phages were stable at temperatures between 37°C and 53°C for two hours. All three phages were stable when exposed to pH ranged from 4 to 9 for one hour. Efficacy *in vivo* was tested in mice model. Mice challenged with bacteria were treated with three independent phages or cocktail of the three. Bacteria recovered from feces and cecum of mice decreased up to 5X10-4. The decrease was most prominent when phage cocktail was used.

1.21 GARLIC IMPAIRS *ACTINOBACILLUS PLEUROPNEUMONIAE IN VITRO* AND ALLEVIATES PLEUROPNEUMONIA IN A PIG MODEL

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A huge number of diverse sulfurous compounds have been identified in garlic preparations, and many of them are associated with health-supporting properties. Digestion products of sulfurous compounds from garlic, the most stable volatile one being allyl methyl sulfide (AMS), are to a certain extent excreted via the lungs and could therefore have an effect on the course of pneumonia in pigs. Hence, the objectives of this study were (i) to test the susceptibility of the pig pathogen *Actinobacillus pleuropneumoniae* to AMS in *in vitro* experiments, and (ii) to assess the impact of garlic on systemic blood AMS levels and on clinical and pathological symptoms in the lungs of pigs experimentally infected with *A. pleuropneumoniae*. In *in vitro* experiments, the effect of AMS on the growth of *A. pleuropneumoniae* serotype 9 was examined in closed bottles equipped with a photometer tube. The bottles were incubated at 37°C and the growth of *A. pleuropneumoniae* was monitored as optical density at 600 nm. In an *in vivo* challenge trial, 15 seven-week-old pigs, which received a diet with 5% of a commercial garlic feed component, and a control group of 15 pigs, which received a diet without garlic, were infected with *A. pleuropneumoniae* serotype 2 by exposure to an aerosol, and subsequently followed for 4 days. In the *in vitro* experiments, AMS was shown to exhibit an antibacterial effect against *A. pleuropneumoniae* serotype 9. At 1.1 mM, AMS impaired the growth rate of *A. pleuropneumoniae* by 8% compared to unimpeded growth. Although causing a delay in the growth of *A. pleuropneumoniae* when compared to unaffected growth in medium, AMS did not lower the stationary phase yield of *A. pleuropneumoniae*. In the *in vivo* challenge trial, blood AMS in the garlic-fed group amounted to 0.32 ± 0.13 μM at the day of the challenge, whereas in the control group no AMS was detected. At the end of the experiment, the occurrence of characteristic pleuropneumonia lesions in 47% of the lungs of the control group and in 27% of the lungs of the garlic-fed group, in combination with a near to significant ($P = 0.06$) lower relative lung weight in the garlic-fed group, indicated a beneficial, alleviating effect of garlic on the course and severity of the *A. pleuropneumoniae* infection (Becker et al., 2012). Reference: Becker et al. (2012) Vet. Microbiol. 154, 316-324.

1.22 EVALUATION OF ALLICIN AS ANTIBACTERIAL AGENT AGAINST *CAMPYLOBACTER JEJUNI* IN *IN VITRO* EXPERIMENTS AND IN A BROILER SEEDER EXPERIMENT

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Campylobacteriosis is a gastrointestinal disease mainly caused by consumption of *Campylobacter jejuni* contaminated broiler meat. Currently it is the most reported bacterial foodborne disease in the EU. A risk assessment study at our institute showed that lowering the *C. jejuni* excretion and external contamination of broilers prior to slaughter by 1 to 3 log colony forming units can lead to an average reduction of campylobacteriosis cases in Belgium by 60% to 96%, respectively. Allicin, one of the active phytochemicals of freshly crushed garlic, has, in its pure form, already demonstrated antimicrobial activity. The main antimicrobial effect of allicin is due to its chemical reaction with the thiol groups of various enzymes, e.g. RNA polymerase. Allicin, its precursor alliin and several allicin-derived molecules (allyl-sulfide and garlic oil blend, a mixture of diallyl disulfide, diallyl trisulfide an allyl disulfide) were tested *in vitro* for anti-*Campylobacter* activity. *C. jejuni* growth was completely inhibited after 24h by allicin concentrations as low as 7.5 ppm. The allicin-derived molecules also inhibited growth completely after 24 to 48h, at a concentration of 50 ppm (no lower concentrations tested), while alliin had no anti-*Campylobacter* effect. Subsequently, controlled batch fermentations, simulating the broiler cecal environment *in vitro* using cecal background flora, were performed with different concentrations of filter sterilized allicin (50, 25, and 10 ppm). Two different experimental designs were used: 1) inoculation of *C. jejuni* and addition of allicin at the same time, or 2) addition of allicin, followed 24 hours later by *C. jejuni* inoculation. Results indicate that allicin concentrations of 50 ppm inhibit *C. jejuni* growth completely after 24h in both in vitro designs. Allicin concentrations of 25 ppm inhibit *C. jejuni* growth in the first 24h, but growth resumes after 48h. No *C. jejuni* inhibition was detected when an allicin concentration of 10 ppm was tested. Finally, allicin was used in an *in vivo* seeder model. Broiler chicks in three groups were given 25 ppm of allicin in the drinking water, a concentration which was tolerated by the chicks. At 15 days old, two birds per group were inoculated with *C. jejuni* KC40. Allicin was unable to reduce cecal *Campylobacter* colonization in this trial. It can be concluded that allicin is a promising phytochemical against *C. jejuni* in broilers provided that an effective application dosage and formulation can be found.

1.23 BACTERIOCINS AND BACTERIOPHAGE LYTIC PROTEINS AS ALTERNATIVES TO ANTIBIOTICS FROM RUSSIAN FEDERATION AND USA COLLABORATIONS

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Novel anti-microbial peptides (bacteriocins) were isolated and characterized during collaborative research between Poultry Microbiological Safety Research Unit (PMSRU), ARS-USDA scientists and representatives of the State Research Center for Applied Microbiology and Biotechnology (SRCAMB) in Obolensk, Russian Federation. The bacteriocins are effective against several bacterial pathogens. Treatment of chickens by feeding bacteriocins consistently reduced *Campylobacte*r levels in their gastrointestinal system as compared with levels found in untreated birds. Five patents have been issued describing this alternative to antibiotic treatment for bacterial infection and technology transfer is on-going. Screening of bacteriophages lytic for *Clostridium perfringens* was completed utilizing filtered samples obtained from poultry (intestinal material), soil, sewage, and poultry processing drainage water. From the collections highly lytic viruses were isolated and the double-stranded deoxyribonucleic acid (DNA) genomes of the bacteriophages were sequenced to completion. DNA sequencing of six bacteriophage genomes completed at PMSRU and four genomes in collaboration with Russian investigators resulted in identification of unique amidases as well as phage encoded proteins that potentially contain lysozyme and endopeptidase activities. Two recombinant bacteriophage lytic enzyme genes encoding putative amidases have been cloned, their proteins expressed as recombinants and isolated to homogeneity, then demonstrated to lyse *C. perfringens*. Patent applications have been submitted as a result of the bacteriophage research. These bacteriocins and phage lytic enzymes may have possibilities for use in agriculture and medical applications as potential replacements for current antibiotics that may have diminished activity.

1.24 *BACILLUS SUBTILIS* PB6, A POTENTIAL ALTERNATIVE TO ANTIBIOTICS

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Bacillus subtilis PB6 (ATCC PTA6737) is known to have a spectrum of activity against pathogenic bacterial species, such as *Clostridium perfringens*. PB6 is also known to have immunomodulatory properties, reducing negative inflammatory effects during intestinal disorders. The objective of this study was to demonstrate the efficacy of PB6 in decreasing the severity of experimentally induced necrotic enteritis (NE) in broiler chicks and the reduction of related production losses. This study had 6 treatments, with 8 chicks each replicated eight times. Chicks were raised in battery brooder pens from 0 to 27 days of age. The NE model used, consisted of infecting all birds at day 14 with a mixed coccidial challenge containing ca. 25,000 oocysts of *Eimeria acervulina* per bird and 5,000 oocysts of *E. maxima* per bird. Three control treatments were included for comparison: an unmedicated, uninfected positive control; an unmedicated, infected negative control and an infected, antibiotic control receiving 50g Bacitracin MD (BMD) per ton of feed. On days 19, 20, and 21, all birds, with the exception of the positive controls received a dose of 1×10^8 cfu of a *Clostridium perfringens* strain proven to induce NE. Birds and feed were weighed on days 0, 19, 22, and 27 for body weight (BW) and feed conversion ratio (FCR) records. Mortality was necropsied and all NE deaths recorded. Three birds per pen were killed at 22 days for intestinal NE lesions evaluation. The 0 to 22 day BW of broilers fed PB6 at 1×10^9 cfu/ton was higher ($P < 0.05$) *vs*. the non-medicated, infected negative control. By day 27 PB6 proved to be as efficacious in protecting against experimentally induced NE as BMD at 50g/ton and not different from the uninfected positive controls. The FCR of negative controls in the 0 to 27 day observation period was higher ($P < 0.05$) than those of all other treatment means, which in turn, did not differ ($P > 0.05$) from one another. Apparently the most severe period of NE disease had passed before the intestinal lesions were scored resulting in erroneously low scores with no comparative value. Nonetheless, NE mortality proved that there was a strong NE disease induced by the model despite the lack of lesion scores. Mortality due to NE of the BMD antibiotic controls was not different ($P > 0.05$) from the PB6 treatment. Based on these results, PB6 proved to be effective in reducing NE mortality and related production losses. Active microbials, such as PB6 are considered more natural than antibiotics, making them a possible and real alternative to antibiotics.

1.25 A SIMPLE HPLC METHOD FOR THE DETERMINATION OF FIVE BIOACTIVE COMPONENTS AND FINGERPRINT ANALYSIS OF *PUERARIA LOBATA*

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A simple and sensitive high performance liquid chromatography method with photodiode array detection (HPLC-PDA) was developed for simultaneous determination of five bioactive constituents (puerarin, genistin, genistein, dadizin, daidzein) in the root of *Pueraria lobata* and its traditional Korean herbal preparations Galgen Ex San by optimizing the extraction, separation, and analytical conditions of HPLC-PDA. The chemical fingerprint of *P. lobata* was established using raw materials of 20 different origins in Korea. The chromatographic separations were obtained by YMC Pro C18 reversed-phase column (250 mm x 4.6 mm i.d., μm) using gradient elution with water-acetic acid (100:0.1, v/v) and acetonitrile, at a flow rate of 0.5 mL min-1, an operation temperature of 35°C, and a wavelength of 260nm. The new method was validated and successfully applied to simultaneous determination of components in five batches of Galgen Ex San. The results indicated that this multi-component determination method in combination with chromatographic fingerprint analysis is suitable for quantitative analysis and quality control of *P. lobata.*

1.26 USE OF PYROSEQUENCING TO INVESTIGATE THE INHIBITORY EFFECT OF DIETARY PHYTONUTRIENTS ON THE PROLIFERATION OF *EIMERIA MAXIMA* IN BROILERS

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Avian coccidiosis and necrotic enteritis (NE) are among the most economically significant diseases affecting the poultry industry worldwide. As an alternative control strategy for these diseases without using chemotherapeutic agents, we have investigated the efficacy of dietary phytonutrient mixture, XT-6930® (carvacol, cinnamaldehyde, and Capsicum oleoresin) in broiler chickens co-infected with *Eimeria maxima* and *Clostridium perfringens*. One-day-old Cobb and Hubbard broiler chickens were orally infected with 1.0 x 10^4 *E. maxima* at 14 days post-hatch and 1.0 x 10^9 CFU/ml of *C. perfringens* 4 days later. The birds were randomly assigned to 5 groups (15 birds/group): C (control); EM (*E. maxima* infected); CP (*C. perfringens* infected); NE (co-infected with EM and CP); XT (NE + XT-6930®). At 2 days post-CP infection, 5 birds from each group were killed and total genomic DNA extracted from ileal content was subjected to pyrosequencing for sequencing pooled amplicons of the V1 toV3 regions of the bacterial 16S rRNA gene. More than 60,000 partial 16S rDNA sequences obtained from 50 ileal samples were analyzed. Individual pyrosequencing reads corresponded to a specific operational taxonomic unit (OTU) and was assigned at the phylum, genus, and species level by homology comparison. The number of reads per OTU allowed us to determine the relative abundance of each bacterial group comprising gastrointestinal microflora as well as the infected *E. maxima* strain for the challenge study. Regardless of the breed of broiler, pyrosequencing revealed the absence of *E. maxima* in their ileal contents from C and CP groups. In addition, relative abundance of *E. maxima* was significantly increased in NE group compared with EM group, suggesting that *C. perfringens* infection may have a potential role in *E. maxima* life cycle in their host. Interestingly, dietary supplementation with XT-6930® significantly decreased the relative abundance of *E. maxima* in their intestinal tract of both Cobb and Hubbard. In conclusion, this is the first report on the enumeration of *E. maxima* by pyrosequencing instead of manual counting of fecal oocysts. The results of this study support the idea that phytonutrients provide significant protection against *Eimeria* protozoa infection and further studies will be needed to elucidate the underlying mechanisms.

1.27 DEVELOPMENT OF A COMBINED PREPARATION OF BACTERIOPHAGES FOR THE PREVENTION AND TREATMENT OF SALMONELLOSIS IN POULTRY

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Salmonellosis is the major single cause of economic losses in poultry farming. It is known that pathogenic *Salmonella* can be transmitted to humans through chicken meat and eggs. In many countries this path of human infection with the pathogen is the predominant. Pathogenic *Salmonella* are usually resistant to many antibiotics. Resistance to antibiotics set forward the need to seek for alternative treatments of salmonellosis. Virulent bacteriophage preparations seemed to be one of those. The present study is aimed at the development of effective phage preparation for the prevention and treatment of salmonellosis in poultry.

Materials and Methods. Bacteriophages were carefully studied looking into their biological, immunochemical, and physico-chemical characteristics in accordance with the recommendations of the International Committee on Taxonomy of Viruses (Ackermann, Dubow, 1987). *Salmonella* pathogens (*Salmonella enteritidis*, *Salmonella Typhimurium* and *Salmonella infantis*) used as reference strains were isolated from sick chickens taken from Russian farms.

Results. A set of highly effective phages for therapeutic and preventive medicine has been isolated using specially developed in-house techniques. The product should meet the following criteria: 1) each selected virulent phage possesses a broad lytic spectrum against *Salmonella* strains of the appropriate serotype, 2)the phage does not interact with normal poultry bacterium flora, 3) the phage rapidly lyses the pathogen cells with a high yield of the secondary phage particles, 4) the phage is resistant to adverse physical and chemical factors (heat, pH variations, etc.), 5) the preparation includes phages with various adsorption mechanisms vis-a-vis the host cell. This helps to eliminate the probability of generating phage-resistant forms in the population of *Salmonella*. As a result of this work, a preparation containing nine types of phages of morphotypes A, B, and C (three species for pathogen *S. enteritidis*, *S. Typhimurium* and *S. infa*ntis), has been developed. Efficacy of the drug in the treatment of chickens with experimental *Salmonella* infection has been tested. Data confirm that the phage preparation eliminates *Salmonella* from chicken organism rapidly and completely. The phage preparation has been successfully tested in three poultry farms in Russia. S&PC "MicroWorld" has started to market its phage preparation on the Russian market and is prepared to discuss its implementation elsewhere in the world. The Center is able to develop phage preparations for the prevention and treatment of any bacterial infectious disease in poultry and animals.

1.28 SELECTION AND EVALUATION OF CANDIDATE *BACILLUS*-BASED DIRECT-FED MICROBIALS FOR USE IN COMMERCIAL POULTRY PRODUCTION

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Increasing pressure from consumers and government regulatory agencies has led an ever increasing number of U.S. poultry producers to reduce or eliminate the use of antibiotics in their operations. This has directly resulted in reduced overall performance and an increase in flock health issues. One possible alternative may be an effective *Bacillus*-based direct-fed microbial that reduces enteric health issues and improves overall production parameters. To select for potential direct-fed microbial isolates belonging to the genus *Bacillus*, environmental samples were pasteurized, plated, and evaluated for anti-microbial activity using soft agar overlays containing target bacterial pathogens. Colonies which produced anti-*Salmonella* activity were isolated and then evaluated for in vitro anti-clostridial and anti-*Campylobacter* activity using similar soft agar overlays under appropriate atmospheres. Polyvalent isolates were speciated and both nonpathogenic and/or GRAS species were further evaluated for resistance to high temperatures and for the ability to grow to high numbers with high sporulation efficiency (1010 spores per gram or greater) in a solid state media. Isolates PHL-MM65 and PHL-NP122 (a *Bacillus laterosporus* and *Bacillus subtilis* respectively) were further evaluated using poults raised under commercial conditions. After 7d of conventional brooding, 480 poults from within the house were tagged, weighted, and placed into one of four replicate pens for each treatment group: negative control, nitarsone (an organic arsenical), PHL-MM65 106 spores/g feed, or PHL-NP122 106 spores/g feed. After 23 days the poults were weighed and body weight was calculated for each group, PHL-NP122 (853g), and nitarsone (852 g) were found to be heavier ($p \leq .05$) than the negative control (784g), while PHL-MM65 (794g) was not significantly heavier. Also at day 23 of the trial, the ceca were aseptically removed from 10 euthanized poults per pen and cultured for recovery of *Salmonella*. Treatment with *Bacillus* isolates PHL-NP122 and PHLMM65 resulted in a significant reduction ($p \leq .05$) in the percentage of poults colonized by *Salmonella* (17.5% and 23.3% respectively) as compared to the negative control (47.5%). These data may suggest that this method of screening and evaluation could lead to commercially useful *Bacillus*-based probiotics.

1.29 THE EFFECT OF THE APPLICATION OF MONO-LAURIC ACID WITH GLYCEROL MONO-LAURATE IN WEANED PIGLETS, ON THE USE OF ANTIMICROBIALS IN SOW HERDS

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The use of antimicrobials in pigs in The Netherlands is relatively high in comparison with the use in some other European countries. This caused a public debate, which led the Minister of Agriculture to demand that the use of antimicrobials should be reduced by 50% by 2013 from the pig sector. This has triggered the use of alternative substances to improve pig health. Mono-lauric acid (MLA) is a medium chain fatty acid (MCFA) with antimicrobial properties that can be used as an additive through the feed of weaned piglets, to improve their health and as a result reduce the use of antimicrobials. MLA was continuously used in the feed of weaned piglets for periods of 4 – 6 months. Data on the use of antimicrobials per herd was extracted from the sales records of VP "Lintjeshof" for 33 sow herds that used MLA and 30 herds that did not. Used amounts of antimicrobials were transformed to Animal Daily Dose (ADD) according to the nationally accepted rules established by the chemistry of the Faculty of Veterinary Medicine in Utrecht. Data were used from an equal period before the application of the MLA and for the period during which MLA was used. Data for the control herds was extracted for comparable periods. The change in ADD (delta-DDA) was calculated by ADDbefore – ADDduring. Statistical analysis was done at the AHS using the Two-Sample Wilcoxon rank-sum (Mann-Whitney) test in STATA/SE 11.0 for Windows. P-values of < 0.05 were considered significant. Mean, median and SD of the ADD for the control and test group were -1.7, 1.8, 18.2 and -9.8, -8.2, 10.6 respectively. The difference in delta-ADD between test and control group was significant. From these data we conclude that the addition of MLA to the feed of weaned piglets significantly reduced the ADD in the treated herds.

1.30 BIO-MOS® REDUCES THE EFFECT OF ENTERTOXIX *E.COLI* K88 (ETEC) IN PIGLETS

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The objective of this study was to determine the effect of Bio-Mos, fed at 2.5 kg/T, on the prevention of the development of diarrhea in piglets after an artificial ETEC challenge was administered 7 days post weaning. Two groups of 12 individually housed piglets were fed ad libitum after weaning at 22 d. One group was fed the control feed, while the other received the control feed supplemented with Bio-Mos at 2.5 kg/T. Seven days post weaning an ETEC challenge (*E. coli* K88) was administered to the piglets. Feed intake and faecal consistency (score 0: normal faeces to score 3: liquid faeces) was monitored until 20 d post weaning. Due to the ETEC challenge, feed intake in the control group dropped by 45%, which was linked to fever development. The inclusion of Bio-Mos in the treatment group led to a less negative impact of the ETEC challenge on feed intake (drop of only 18%). As a consequence of the ETEC challenge, the piglets developed severe diarrhea in the control group (score increased from 0.2 to 1.48). However when Bio-Mos was added to the feed, the increase in diarrhea score was quite low (scores increased from 0.2 to 0.6). In addition, 14 days post infection, the diarrhea score had already decreased to below the original level group (0.08) in the Bio-Mos, while the score for the control group fell to only 0.5. This reveals that a faster recovery was achieved when Bio-Mos was used. This trial clearly demonstrates the mode of action of Bio-Mos: by blocking the adhesion of *E. coli* to the gut wall, *E. coli* is hindered in its potential to colonize the gut and thereby damage the gut wall. As a consequence, the incidence of reduced feed intake and diarrhea is largely reduced.

1.31 ARTILYSINS: ANTIBACTERIAL ENZYMES THAT ATTACK BACTERIAL SURFACE STRUCTURES

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Increasing antibiotic resistance of bacteria provides a clear need for novel ways to combat bacterial pathogens. Artilysins are novel designed recombinant polypeptides that are modified specifically to provide the activities needed to kill bacterial pathogens. Artilysins combine an efficient enzyme with membrane penetrating activities. Upon contact from the outside, Artilysins efficiently disrupt surface structures of both Gram-negative and Gram-positive target bacteria. MIC data, as well as infection experiments, show that using an enzymatic mechanism Artilysins are efficiently killing e.g. strains of *Pseudomonas aeruginosa* and MRSA independent whether or not these strains are antibiotic resistant. Furthermore Artilysins are also active in reducing biofilm formation of both bacterial species. In contrast to most classic antibiotics, Artilysins are not metabolized and are attacking highly conserved structures on the bacterial surface. Thus bacteria will hardly be able to adapt to this new mode of action provided by Artilysins and, thus, the risk of the development of resistances by bacteria against Artilysins is significantly low. Thus Artilysins are an efficient tool to combat pathogenic bacteria.

1.32 EFFICACY OF BACTERIOPHAGE THERAPY IN EXPERIMENTAL SEPSIS AND MENINGITIS CAUSED BY O25b:H4-ST131 *E.COLI* STRAIN PRODUCING CTX-M-15

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We evaluated phage therapy in experimental infections due to *S242*, a fatal neonatal meningitis *Escherichia coli* strain, belonging to the worldwide distributed O25b:H4-ST131 clone that produces extended spectrum beta-lactamase CTX-M-15. A lytic phage, $EC200^{PP}$, active against S242, was isolated from environmental water. After determining *in vitro* and *ex vivo* stabilities and pharmacokinetic properties of $EC200^{PP}$ in rat pups, we assessed therapeutic efficacy of a single dose of 10^8 PFU using models of sepsis and meningitis in which fatality was 100%. $EC200^{PP}$ was partially neutralized by human serum. In contrast to the high concentration of phage in the spleen and the kidney, low titres in urine and the central nervous system were observed. Nevertheless, the sepsis model, $EC200^{PP}$ administered 7h or 24h post infection resulted in 100% and 50% pup survival, respectively. In meningitis model, $EC200^{PP}$ administered 1h or 7h post-infection rescued 100% of the animals. The most delayed treatments were associated with the selection of phage-resistant S242 mutants. However, a representative mutant was highly sensitive to killing serum activity and avirulent in an animal model. $EC200^{PP}$ is a potential therapeutic agent for sepsis and meningitis caused by the widespread *E. coli* O25:H4-ST131 multidrug resistant clone.

1.33 ANTIMICROBIAL AND IMMUNOMODULATORY ACTIVITIES OF PR-39 DERIVED PEPTIDES

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Host defence peptides (HDPs) are considered an interesting alternative to antibiotics. They play an important role in the innate immune system and exhibit broad spectrum activity against Gram-positive and Gram-negative bacteria. PR-39 is a porcine HDP with a high content of proline and arginine residues. In order to find the core elements involved in activity of this peptide, both N-, and C-terminally truncated peptides were produced. Antibacterial activity against both Gram-positive and Gram-negative bacteria was contained within the first 15 amino acids of PR-39. However, the C-terminal 20 amino acids also still maintained antibacterial activity. Interestingly, smaller peptides seemed more susceptible to inhibition by high salt concentrations than larger peptides. PR-39 showed low cytotoxicity towards porcine epithelial cells and porcine macrophages and reducing the size of the peptide did not affect cytotoxicity. Finally, PR-39 induced IL-8 production in the porcine macrophage cell line 3D4/31, indicative of an additional, more immunomodulatory role for this peptide. None of the truncated forms induced IL-8 production, showing that contrary to pure antibacterial activity, immunomodulation requires the full length peptide.

1.34 REDUCED INCIDENCE OF *CLOSTRIDIUM PERFRINGENS*-ASSOCIATED LESIONS AND IMPROVED BIRD PERFORMANCE IN BROILER CHICKENS WITH THE COMPETITIVE EXCLUSION PRODUCT BROILACT®

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Necrotic enteritis (NE) is a disease of poultry occurring predominantly in broiler chickens when they are from 2 to 4 weeks of age. *Clostridium perfringens* (CP) is known to play a significant etiologic role in NE. The ability of normal intestinal microflora (competitive exclusion) to reduce the counts of CP was proved by Barnes *et al.* already in 1980. In a pilot-scale trial the first commercial competitive exclusion product Broilact® was shown to decrease mortality due to necrotic enteritis (NE) and hepatitis and reduce the counts of *Clostridium perfringens* (CP) (Elwinger *et al.*, 1992). To examine if such an effect could be seen in the field, a study was conducted. Two flocks from each of four houses at a farm having problems with high condemnation rates at slaughter because of NE-related liver lesions were selected for the trial. Altogether eight flocks were included in the study. One flock from each house was treated on the day-of-hatch with Broilact® by spray application, and one flock was left as untreated control. A total of 135, 800 day-old birds, were included in the study, of them 50.6% were treated with Broilact® and 49.4% were left as untreated controls. In both treatment groups one flock was started in August, two flocks in October and one flock in December. All birds were given starter and grower feed without growth promoting antibiotics but with 70 ppm of the anticoccidial agent narasin. Finisher feed containing neither anticoccidial nor growth promoting additives, was used at least five days before slaughter. The average ages at slaughter were 35.25 and 35.75 days for treated and untreated flocks, respectively. From each flock 10 birds were sampled at an age of approx. 2, 3, 4, and 5 weeks, altogether 40 birds per flock. A total of 320 birds were sampled during the study period. Each bird was scored (0 to 3) for intestinal lesions. From each bird, 0.5 g of caecal contents was sampled and pooled with the corresponding specimens from the other birds sampled on the same day. Pooled samples were examined quantitatively for CP. Condemnation rates due to NE-associated liver changes were recorded at slaughter. Broilact® reduced the mean mortality rate by 1.5%, induced a reduction in CP numbers by 3 logs at 3-4 weeks of age, reduced the mean condemnation rate by 1.7% and reduced the condemnations due to CP- associated hepatic change by 1.5%. Further, Broilact® treatment was associated with increased income per bird to the farmer.

1.35 REDUCING *SALMONELLA* SPP. ATTACHED TO THE SURFACES USING BACTERIOPHAGES

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Rendered animal meals, as a major ingredient of animal feeds, may be contaminated with foodborne pathogen *Salmonella*, especially those antibiotic resistant serotypes. The objective of this study was to determine if bacteriophages can be an alternative treatment to reduce the risk of cross-contamination of *Salmonella* on environmental surfaces found within a rendering facility. Bacteriophages were isolated and purified for five *Salmonella* serotypes (*Enteritidis*, *Typhimurium*, *Mbandak*a, *Johannesbur*g, and *Idikan*), and characterized by host range, restriction digestion, and transmission electronic microscope. A five-strain cocktail of bacteriophages was then optimized and applied to a variety of surfaces (steel, plastic, cement, and rubber) attached by *Salmonella*. Bacteriophage treatment of the surface materials with attached *Salmonella* resulted in up to 2 log decreases in the *Salmonella* population at 40° and 30°C for all surface materials. Bacteriophage treatment was also effective to reduce 2~3 or 1.5~2.0 logs of *S. Enteritidis* single-species or double species biofilms at 30°C. These results demonstrated that a bacteriophage cocktail significantly reduces levels of *Salmonella* contamination on environmental surfaces, and may potentially decrease the incidence of cross-contamination in rendering facilities.

1.36 MICROBIAL CHANGES IN THE ILEAL AND CAECAL DIGESTA OF BROILERS FED LEMON PEEL AND ORANGE PEEL EXTRACTS AND *CURCUMA XANTHORRHIZA* ESSENTIAL OIL, AND SUBJECTED TO CHRONIC HEAT STRESS

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Heat stress exerts deleterious effects on animal health and productivity. Release of neurohormones in the intestinal tract associated with heat stress can increase growth and virulence factor expression in harmful microbes within the lumen. Several strategies e.g. feed additives have been evaluated for their efficacy to optimize the intestinal microbiota in challenging conditions. Antibiotics have been used in poultry feed for improving growth performance, however, their use in animal feeds have been banned recently due to potential development of antibiotic resistant human pathogenic bacteria. Nowadays, the possibility of using natural alternative additives instead of antibiotics in animal diets is being researched. One such alternative is plant extracts. *Curcuma Xanthorrhiza* essential oil (CXEO) isolated from java turmeric (*Curcuma xanthorrhiza* Roxb.) has been reported as a phenolic-rich product. Orange peel *(Citrus aurantium)* and lemon peel *(Citrus limon)* which are common by-products of both food and agriculture and some studies showed that are rich in phenolics. The main phenolic compound of Orange Peel Extract (OPE) and Lemon Peel Extract (LPE) was protocatchic (approximately 80%). Xanthorrhizol (> 30%) and ar-curcumene (> 30%) were the major compounds in CXEO. Studies on bioactive compounds showed that single phenolic compounds or their combination resulted in growth inhibition of different bacterial strains. Their antimicrobial ability may modulate the gut ecosystem to affect feed efficacy. We therefore conducted an experiment to study the performance and intestinal microflora of broiler chickens fed these plant extracts under heat stress. A total of 336 Ross 308 broilers were randomly allocated to 7 dietary treatments (4 pens per treatment), consisting of a basal diet and the same diet supplemented with either OPE, LPE and CXEO at two levels (200 and 400 mg/kg). Diets were fed from 25 to 38 days of age. From day 28, the basal ambient temperature was set at 22^{o}C and this was increased daily to 34°C with 50% relative humidity for 5 hours to induce heat stress. At day 38 of age, ileal and caecal contents were collected (4 animals per pen) for microbial study. The samples were diluted and plated into selective media to identify coliforms, *Lactobacillus* spp., and total aerobic count. Dietary extracts didn't affect the chicken performances. The results showed significantly lower counts for coliforms in ileum of chickens fed with 400 mg/kg LPE (3.50 $\log_{10}$ CFU/g) or CXEO (3.42 $\log_{10}$ CFU/g) diets as compared to control (3.93 $\log_{10}$ CFU/g) ($P<0.05$). In caecal digesta, only for treatment CXEO at 400 mg/kg there was reduction of coliforms. For both intestinal sections, similar counts of *Lactobacillus* spp. and total aerobic counts across treatment groups were found. This study shows that plant extracts, in particular CXEO and LPE reduced the number of pathogenic bacteria in the distal part of the gut. It could be speculated that CXEO and LPE could be of value to replace antibiotics in poultry diets.

1.37 CAPRYLIC ACID REDUCES ENTERIC *SALMONELLA ENTERITIDIS* AND *CAMPYLOBACTER JEJUNI* COLONIZATION IN POULTRY WITH PROPHYLACTIC AND THERAPEUTIC EFFICACY

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Salmonella Enteritidis (SE) and *Campylobacter* are the most commonly reported bacterial causes of human food-borne illness, and epidemiological evidence indicates poultry and poultry products as significant sources of human infections. Caprylic acid (CA) is an 8-carbon fatty acid, naturally found in caprinae milk and coconut oil, and a food-grade compound with bactericidal properties against several microbial pathogens, *in vitro*. A series of studies were conducted to determine if CA reduced SE or *Campylobacter* in birds before being infected (prophylactic efficacy) or after colonization (therapeutic efficacy). Efficacy was tested with 0.7 % or 1% CA against SE and 0.7% or 1.4% CA against *Campylobacter* in day old chicks supplemented with CA for up to 20 days, and for market aged birds fed CA for the last 3, 5 or 7 days before slaughter. Results revealed that prophylactic and therapeutic supplementation of CA in feed significantly reduced SE and *Campylobacter* populations in the cecum of treated chickens, compared to the control birds. For example, CA at 0.7% in feed decreased SE and *Campylobacter* in the cecum by 3.0 log CFU/g compared to control in therapeutic treatment of market aged birds ($P < 0.05$). Cell culture and gene expression studies to elucidate the potential molecular mechanisms of action of CA revealed reduced invasion of *Salmonella* in avian intestinal epithelial cells by down-regulating *Salmonella* invasion genes *hilA* and *hilD* ($P < 0.05$). Feeding of CA did not adversely affect the body weight, feed intake, pH, or endogenous cecal bacterial population in treated chickens, compared to the negative controls. Caprylic acid is a natural and relatively inexpensive compound and its supplementation through feed represents a practical and economical strategy for poultry farmers for reducing SE and *Campylobacter* carriage in chickens. Caprylic acid could potentially be used as a natural and safe feed additive to reduce these significant human pathogens in poultry.

1.38 INTRACELLULAR REPLICATION INHIBITORY EFFECTS OF *GALLA RHOIS* ETHANOL EXTRACT FOR *BRUCELLA ABORTUS* INFECTION

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Galla Rhois (GR) has long been applied in traditional Korean and Oriental medicine. Although GR has an anti-bacterial effect, the anti-bacterial mechanism and therapeutic efficiency of GR for intracellular parasitic *Brucella* infection are still unclear. The objective of this study was to investigate the antibacterial and therapeutic effects of GR ethanol extract (GRE), which is a natural antibacterial component for the treatment of *B. abortus* infection.

The antibacterial activity of GRE towards *B. abortus* was evaluated by incubating *B. abortus* with GRE. Following treatment with GRE, *B. abortus* adherence, uptake, intracellular growth, and intracellular trafficking in macrophages were monitored. Mice were infected intraperitoneally with *B. abortus* and treated orally with GRE for 14 days, and then the weight and CFUs from each spleen were monitored. The viability of *B. abortus* was markedly decreased in a dose-dependent manner. Moreover, *B. abortus* internalization and intracellular growth within macrophages were reduced in GRE-treated cells. The number of bacteria that adhered to GRE-pretreated cells was significantly lower than that of untreated cells. With regards to intracellular trafficking, treatment with GRE augmented the colocalization of *B. abortus*-containing phagosomes with LAMP-1. GRE-treated mice showed considerably decreased weight and bacterial burdens in the spleen compared to untreated mice. GRE exhibits antibacterial and protective effects on *B. abortus in vitro* and *in vivo*. These results highlight the beneficial effects of GRE in the prevention and treatment of brucellosis.

1.39 BACTERIOPHAGES TO CONTROL *CAMPYLOBACTER* CONTAMINATION: ISOLATION, CHARACTERIZATION AND INTERACTIONS WITH HOST BACTERIA

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In last ten years the number of EU citizens becoming infected with foodborne pathogens *Campylobacter jejuni* and *C. coli* is steadily increasing and was believed to reach 9 mil cases in 2008. Implementation of efficient control measures in the food processing would decrease frequency of human illnesses. Therefore, phages are being explored to control number of campylobacters in poultry production. We have isolated a set of Campylobacter phages from environmental samples. They all belong to *Myoviridae* family and are able to lyse *C. jejuni* and *C. coli* isolates of human, animal and enviromental origin. We examined also their genomic DNA restriction endonuclease profiles. For molecular characterization of interaction between phages and bacteria we used two C. jejuni strains, NCTC 11168 and LBA65. A gene involved in the transport of the capsule through the membrane (*kpsM*) and a gene encoding flagellin transport protein (*flhA*) were inactivated in above strains by marker rescue technique. We determined the significance of mutation by screening the strains' ability to synthesize flagellin and capsule by immunoblotting and Alcian blue staining, motility test and electron microscopy. Phenotypes of defined mutant strains were compared to wild type strains with regard to their ability to resist infection by isolated phages. Acapsular mutant was completely resistant to all phages, while aflagellar showed reduced sensitvity to certain phages. In order to confirm role of inactivated structures *in trans* complementation of *flhA* and *kpsM* was done by inserting entire gene and its downstream genes between the structural genes of the ribosomal gene cluster. Complementation of *flhA* and *kpsM* restored sensitivity to tested phages to the wild type level or higher.

1.40 CHEMICAL AND ANTIMICROBIAL STUDIES OF MONOTERPENE: CITRAL

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Cymbopogon citratus (DC) (Gramineae) is an herb worldwide known as lemongrass. The tea made from its leaves is popularly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative. The volatile oil obtained from fresh leaves of this plant is widely used by the perfumes, cosmetics industries and in traditional medicine for various purposes. Citral is the major component of lemongrass oil which was extracted from its leaves, present at levels of, approximately, 65–85%. Citral (3,7-dimethyl-2,6-octadienal) geranial, geranylacetate and myrcene. A number of dietary monoterpenes have been shown to act effectively in chemoprevention and chemotherapy of different cancers in animal models, at cellular level, and in human clinical trials. Unsaturated trepenes are capable of trapping activated oxygen species in vivo to give intermediate epoxides, which can alkylate DNAs, proteins, and other biomolecules. 6,7-Citral-epoxy derivative (a mixture of E and Z isomers with respect to the C2 = C3 double bond) can react with DNA base producing a major adduct. The mixture of epoxides was condensed with 2 mol of cytosine to give the adduct through condensation between aldehyde and amino groups. Antifungal and antibacterial studies were carried out on citral and citral epoxide. Studies on the antifungal especially *Penicillium italicum* and *Rhizopus stolonifer* showed that citral and citral-epoxide have good antibacterial action. Antimicrobial studies of *P. italicum* and *R. stolonifer* explained also that citral and citral-epoxide have good antimicrobial activity. Citral epoxide shows high activity against the growth of bacteria methicillin resistant *Staphylococcus aureus* (MRSA) and fungi comparing by citral. The epoxide shows antibacterial activity more than the antibiotics nalidixic acid (NA) and ampicillin (AP) and nitrofurantoin (NI). The results revealed that these complexes are most effective against MRSA.

Session 2
Immune Modulation Approaches to Enhance Disease Resistance and Treat Animal Infections

Oral Presentations

2.1 SELECTIVE MODULATORS OF INNATE IMMUNITY FOR ANTI-INFECTIVE THERAPY TO SUPPLEMENT OR REPLACE ANTIBIOTICS

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Antibiotics are the underpinning of all modern medicine, but are being undermined by an explosion of (multidrug) resistance, and a dearth of new antibiotics. We have proposed that manipulation of natural innate immunity will serve as a new therapeutic strategy against antibiotic-resistant infections. Cationic host defence (antimicrobial) peptides are produced by virtually all organisms, ranging from plants and insects to humans, as a major part of their immediate, relatively non-specific (innate) immune defences against infection. Although originally noted for their modest direct antimicrobial activity, it was recently demonstrated that host defence peptides profoundly modulate innate immunity. Cell based assays, transcriptomics, sophisticated bioinformatics, as well as pathway and transcription factor studies have demonstrated that these peptides stimulate innate immunity in a unique fashion, boosting protective immunity while suppressing potentially harmful inflammation/sepsis. Using the principle of selective boosting of innate immunity we have developed novel small innate defence regulator peptides with no direct antibacterial activity, that are nevertheless able to protect against many different microbial infections in animal models, including antibiotic resistant infections and cerebral malaria, as well as inflammatory diseases, providing a new concept in anti-infective therapy. Systems approaches have helped considerably in understanding the mechanistic basis for protection by these agents. These agents are currently being developed pre-clinically to treat diseases of animals and man.

2.2 NOVEL ANTI-INFECTIVE MOLECULE FROM INNATE IMMUNE CELLS AS AN ANTIBIOTIC-ALTERNATIVE TO CONTROL INFECTIONS CAUSED BY *APICOMPLEXA*

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With increasing needs for the global animal industry to address the regulatory restrictions on the use of antibiotic growth promoters (AGPs) in animal production, there is much interest to find alternatives to AGPs. To develop alternatives to antibiotics against major poultry parasitic diseases, we have identified a chicken gene which encodes an antimicrobial peptide, NK lysin, from an EST cDNA library that we prepared from *Eimeria*-infected chicken intestine. The contig 171 (NK-lysin like sequence), composed of 87 ESTs, occurred with the highest prevalence in an *Eimeria*-induced intestinal cDNA library. Chicken NK lysin showed less than 20% identity to granulysin and other mammalian NK-lysins. Although NK-lysin in humans showed anti-microbial activity against numerous targets including Gram-positive and Gram-negative bacteria, as well as protozoan parasites, chicken NK-lysin shows exclusive activity against apicomplexan parasites. This presentation will report the expression of chicken NK-lysin in various expression vectors, its efficacy in ameliorating clinical signs of avian coccidiosis, and identification of lytic peptide (cNK-2) sequence derived from NK lysin which has a direct killing activity against multiple *Eimeria* species as well as against other apicomplexa parasites including Neospora and Cryptosporidia. The results demonstrate that chicken NK-lysin can be an antibiotic alternative to mitigate the intestinal damages due to protozoan parasites in poultry.

2.3 CONTROL AND PREVENTION OF ANTIBIOTIC-RESISTANT INFECTIONS BY HOST DEFENSE PEPTIDE THROUGH MODULATION OF INNATE IMMUNITY

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Widespread emergence of antibiotic-resistant pathogens worldwide demands the development of novel antimicrobial agents with a less likelihood of triggering resistance. With concomitant antimicrobial, endotoxin-neutralizing and immunomodulatory activities, cationic host defense peptides (HDPs) represent a new class of antimicrobials to combat resistant pathogens. We previously discovered three cathelicidin HDPs in the chicken. Among them is fowlicidin-1, adopting a largely α-helical structure. We have shown that this peptide possesses potent antibacterial activity, but also displays considerable toxicity toward mammalian cells. To further identify fowlicidin-1 analog(s) with enhanced therapeutic potential, a series of amino-terminal deletion analogs were synthesized and functionally evaluated. Fowlicidin-1(6-26), an analog with omission of the first five amino acid residues of the parent peptide, retained the antibacterial potency against a range of Gram-negative and Gram-positive bacteria including antibiotic-resistant strains, with the minimum inhibitory concentrations ranging from 1 to 4 μM. Desirably, this analog showed a ≥4-fold reduction in toxicity to human erythrocytes and colonic epithelial cells, as compared to the parent peptide. In addition, intraperitoneal administration of the carboxyl-terminal amidated form of fowlicidin-1(6-26) together with a lethal dose of methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591 in neutropenic mice resulted in a 50% increase in 7-day survival, concomitant with a 1-2 log reduction in bacterial titer in both spleens and peritoneal fluids. Furthermore, pre-treatment with a single dose of fowlicidin-1(6-26)-NH2 1-2 days prior to infection completely protected mice from an otherwise lethal MRSA challenge. Such protection was found to be mediated at least in part through neutrophil-chemotactic activity of the peptide. Therefore, the availability of these short HDPs may have potential for further development as alternatives to antibiotics for both food animal and human applications.

2.4 EVALUATION OF AN INTERLEUKIN-2 TREATMENT FOR PREVENTION OF INTRAMAMMARY INFECTIONS IN COWS AFTER CALVING

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A low-dose treatment based on interleukin (IL)-2 spontaneously secreted by the gibbon cell line MLA 144 was investigated for preventing mastitis in dairy cows. The treatment consisted of a single 800-picogram IL-2 dose injected into the skin region drained by the supramammary lymph node 3-5 days after calving. The study included 45 cows (23 treated and 22 controls) of three commercial dairy herds. The results showed that the treatment had no side effects. Indeed, most of the different blood markers assessed did not show any significant variation due to the treatment, supporting the safety for the cow. A significant increase of milk somatic cell counts (SCC) in treated cows was observed, as expected, but in both control and treated group the SCC values were below the level of log 5 (100,000 cells/ml) by day 4 after calving, and therefore without any consequence on milk quality. The presence of a local effect due to the treatment with IL-2 was confirmed by the significant increase of several milk markers related to leukocyte and epithelial cell functions, i.e. SCC, serum amyloid A (SAA), lactoferrin, and NAGase. The increased concentration of the above milk markers suggested an activity of IL-2 on epithelial cells, resulting in a higher resistance to invading pathogens. Indeed, the increased efficiency of cells in the udder was confirmed by the frequency of significantly higher healthy udder quarters observed until day 17-19 after calving in the treated group, compared with the control one. Although these results should be confirmed by further large-scale field studies, they nevertheless provide important evidence as to how a targeted and site-specific modulation of the local immune response could be an efficient strategy for mastitis control in dairy cattle, conducive to a lesser requirement for antibiotics in dairy farms.

2.5 BIOTHERAPEUTICS AS ALTERNATIVES TO ANTIBIOTICS: EFFECTS OF IFN-α AND G-CSF ON INNATE AND ADAPTIVE IMMUNITY IN SWINE

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Acceptable alternatives to the use of antibiotics in food animal practice need to be explored. The use of immunomodulators is a promising area for therapeutic, prophylactic, and metaphylactic use to prevent and combat infectious disease during periods of peak disease incidence. We developed a method to circumvent the need for production of a recombinant cytokine by using a replication-defective adenovirus vector that expresses interferon-α (IFN-α) or porcine granulocyte colony-stimulating factor (G-CSF). Type I interferons, such as IFN-α, contribute to innate antiviral immunity by promoting production of antiviral mediators and also play a role in the adaptive immune response. G-CSF enhances neutrophil production and release from the bone marrow and is already licensed for use in humans for treatment of neutropenia and prevention of infections in those with compromised immunity such as chemotherapy patients. Its prophylactic use has also been experimentally shown to reduce the incidence of coliform and staphylococcal mastitis in cows. Porcine reproductive and respiratory syndrome virus (PRRSV) causes one of the most devastating and costly diseases to the swine industry world-wide and has been shown to induce a meager interferon IFN-α response. Pigs administered the vector expressing porcine IFN-α and challenged with PRRSV had lower febrile responses and decreased percentage of lung involvement. Viremia was delayed and there was a decrease in viral load in the sera of pigs. In addition, there was an increase in the number of virus-specific IFN-γ secreting cells, as well as an altered cytokine profile in the lung 14 days post-infection, indicating that the presence of IFN-α at the time of infection can alter innate and adaptive immune responses to PRRSV. These results indicate that IFN-α can have protective effects if present during the time of infection with PRRSV. Intramuscular administration of the vector expressing porcine G-CSF was found to elicit a substantial persistent neutrophilia of at least 3 weeks duration. These findings provide evidence that it is possible to deliver G-CSF in order to have a sustained increase in circulating neutrophil numbers in pigs that may be a useful alternative to antibiotics for prevention or treatment of infectious disease, especially during typical times of stress and pathogen exposure such as postweaning and post partum.

Session 2

Poster Presentations

2.6 USE OF INTERFERON TREATMENT TO PROTECT CHICKENS AGAINST HIGHLY PATHOGENIC AVIAN INFLUENZA

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Avian influenza (AI) is a significant public health concern and serious economic threat to the commercial poultry industry worldwide. While properly matched vaccines can be effective at limiting morbidity and mortality, the use of therapeutics in veterinary animals to combat this disease are relatively non-existent. Interferons (IFNs) are a group of polypeptides that are secreted from most all eukaryotic cells in response to external signals. They are classified into three groups, designated type I, type II and type III. Type I IFN (alpha and beta), are expressed rapidly after viral infection, and represent a first line of defense initiated by the innate immune response. Induction of IFN-alpha results in an antiviral state which can decrease morbidity and mortality following viral infection. Immediately following infection with AI, host cells begin to express proinflammatory cytokines, including interleukin (IL)-1beta and IL-6, and type I IFN genes, which results in a general antiviral response through the activation of a broad range of effector molecules, including Myxovirus (Mx) resistance gene 1, RNA-activated protein kinase and 2',5'-oligoadenylate synthetases. Unlike mammals, chickens have a single Mx gene with multiple alleles. The original evaluation of chicken Mx indicated the encoded protein lacked antiviral activity, however, more recent reports have determined that the chicken Mx1 gene is highly polymorphic, and cDNAs of some, but not all Mx1 alleles, transfected into mouse 3T3 cells conferred protection against highly pathogenic avian influenza (HPAI) *in vitro*. According to that report, chicken Mx1 variants encoding Asn at position 631 have antiviral activity, whereas variants with Ser at 631 lack activity. We have previously demonstrated the protective potential of IFN-alpha applied to poultry against low pathogenic avian influenza viruses. In those studies, intranasal application of IFN-alpha during infection reduced clinical signs of disease and the incidence of viral shedding. In the present studies, we evaluated protection of chickens against HPAI in birds with different Mx during IFN-alpha application. We observed >90 percent protection from mortality that was dependent on Mx-631 allele. Birds with the Mx-Asn631 (White Leghorn) were resistant to disease whereas Mx-Ser631 birds (White Rock) were susceptible to HPAIV. Taken together, these studies show that IFN-alpha can protect chickens from disease associated with HPAIV and that the Mx-631 allele may contribute to that protection.

2.7 DISEASE SPECIFIC ANTIBODIES AS EFFECTIVE ALTERNATIVE TO ANTIBIOTICS FOR ANIMAL PRODUCTION

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The aim of efficient pork production is to maximize lean meat yield while minimizing production cost. It has been established that two important factors contributing to the efficient pork production are improving health status and promoting their growth performance. Enterotoxigenic *Escherichia coli* (ETEC) K-88 are a major cause of diarrhea and death in pigs, resulting in major economic loss to the pork industry. Feed antibiotics have been used as growth promoter for animal production. However, due to the concerns that antibiotic-resistant pathogens may transmit to humans, public pressure to discontinue the use of feed antibiotics for animal production is building. Therefore, effective agents that could be used in swine diets for prevention of ETEC disease and to enhance their growth performance, particularly during their critical period of life, nursery phases, are essentially needed. The objective of the study was to evaluate the effectiveness of ETEC–specific egg antibodies for improving growth performance and prevention of ETEC disease in post-weaning piglets. A number of experimental studies were done using post-wean piglets that were fed a diet supplemented with different doses of egg antibodies for 14 days, when the control animals were fed with either antibiotics or control egg powder without ETEC-specific antibodies. The results indicated that inclusion of K-88 -specific antibodies at 0.2-04% into a standard piglet diet improved their growth performance and reduced morbidity. Moreover, a statistically significant level of improvement was achieved (P-value <0.05), when piglets were fed with 0.4% antibodies in diet in the absence of antibiotics. To further confirm the effect of K-88 specific antibodies on prevention of ETEC disease, and improvement of growth performance, a study was performed with piglets of same age groups, which were fed with 0.1-0.4% of avian egg antibodies, followed by ETEC K-88 infection on day 7 post-treatment. A significant level ($P<0.01$) of growth improvement and prevention of ETEC infection were achieved when piglets were fed with 0.2-0.4% K-88-specific antibodies. Furthermore, results from the *in vitro* studies confirmed that anti-ETEC avian antibodies are capable of inhibiting proliferation of ETEC K-88 and ETEC F-18 *in vitro,* using the piglet intestinal epithelial cell lines, IPEC-1 and IPEC-J2.

Thus, orally administered specific antibodies provide the advantage to prevent enteric disease as well as improvement of growth performance, offering effective and sustainable replacements of antibiotics for animal production.

2.8 INFLUENCE OF CINNAMALDEHYDE AND SELECTED ORGANIC ACIDS ON EXPRESSION OF IMMUNE RELATED GENES IN IPEC-J2 CELLS EXPOSED TO *SALMONELLA* TYPHIMURIUM OR *ESCHERICHIA COLI* K88.

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Introduction: Plant-derived compounds are increasingly used to replace antibiotics in the feed of farm animals and exert an effect on the gut microbiota. Cinnamaldehyde, cinnamic acid and propionic acid inhibit attachment of porcine pathogen *Escherichia coli* K88 and invasion of *Salmonella enterica* serotype Typhimurium into intestinal epithelial cells when present at sub-lethal concentrations that do not limit bacterial growth. Lactic acid does not have this effect. We wanted to investigate effects on host (porcine) gut cells. The aim of this study was to investigate the effects of cinnamaldehyde and organic acids on selected immune related genes in porcine jejunum epithelial (IPEC-J2) cells when exposed to *S.* Typhimurium or *E. coli* K88 to find out whether such effects could contribute to reduction of virulence.

Materials and Methods: IPEC-J2 cells were grown to confluence and exposed to *E. coli* O149:K91:K88 strain 498 or *S.* Typhimurium ATCC 14028 for 1 h in the presence and absence of cinnamaldehyde, cinnamic, lactic, or propionic acid. RNA lysis buffer was added to each well and cell samples were frozen. qRT-PCR analysis was carried out using primers for immune related genes as follows: IkBa (a marker for the crucial inflammatory mediator NFkB), Heat shock proteins Hsp70, Hsp70.2, Hsp27, hypoxia inducible factor HIF-1a and Nrf2. Expression levels were normalized to untreated control cells.

Results and Discussion: Expression levels of IkBa were raised (2-fold) by the presence of bacteria, indicating that the inflammatory response had been initiated. HIF-1a expression was raised by *E. coli* (3-fold) but not by *S.* Typhimurium. The increase in expression levels of IkBa in response to bacteria were further increased by cinnamic and propionic acids (from 2-fold to 4-fold). In contrast, IkBa was suppressed by cinnamaldehyde whether bacteria were present or not. Expression of Hsp70 and Hsp70.2 was not changed by cinnamic, lactic, or propionic acids but was very high in the presence of cinnamaldehyde (>5-fold increase) in both presence and absence of bacteria. The expression of Hsp27 and Nrf2 were unchanged in all cases.

Conclusions: Cinnamic and propionic acids stimulate the IkBa response to bacterial attack in IPEC-J2 cells and cinnamaldehyde suppresses inflammation via IkBa route but induces Hsp's highly in IPEC-J2 cells. These changes may contribute to the observed reduction in bacterial attachment/invasion of IPEC-J2 cells.

2.9 SPRAY-DRIED PORCINE PLASMA IMPROVES WEANER PIG RESILIENCE TO ENTERIC CHALLENGE

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Spray-dried porcine plasma (SDPP), a food grade slaughterhouse co-product, may at least partially protect weaned pigs from the consequences of sub-clinical post weaning colibacillosis. We tested whether SDPP inclusion in typical UK weaner pig diets increases resilience to sub-clinical post weaning colibacillosis and reduces inflammation. A total of 128 pigs, weaned at 28.7±0.3 days of age and weighing 9.4±0.1 kg, were divided in 32 pens with 2 males and 2 females. Pigs were fed one of two iso-energetic diets (16.9 MJ DE/kg), i.e. a commercial UK diet with dried skim milk powder at 50 g/kg and a test diet where SDPP replaced milk powder. Lactose levels were kept constant and pure amino acids were used to balance at 16.7 g lysine/kg. Pigs were fed *ad libitum* for two weeks post weaning, and were either kept uninfected, or trickle infected with 10^9 cfu enterotoxigenic *Escherichia coli* (ETEC) per pig on 5 occasions through inoculated foods. Pigs were fed commercial diets for three weeks further to assess carry-over effects. Feed refusals were taken to calculate average daily feed intake. Pigs were weighed weekly to calculate averaged daily weight gain. Feed conversion ratio was calculated (feed intake/weight gain). On days 7 and 14 post weaning, a male pig from each pen was blood sampled to assess acute phase proteins. Data was analysed using a 2 x 2 factorial ANOVA, with pen as experimental unit. SDPP inclusion increased feed intake and weight gain by 9% ($P<0.001$), the latter especially in presence of challenge (+14%; $P=0.093$) without significantly affecting feed conversion ratio ($P>0.10$). Pigs previously fed SDPP diets had higher intakes on follow-on commercial diets (+5%; $P=0.004$) without impact on feed conversion ratio ($P>0.10$). SDPP inclusion resulted in 27% lower serum haptoglobin, 37% lower serum C-reactive protein and 50% lower serum amyloid A ($P<0.05$). These effects were independent of ETEC exposure. Our data support the view that feeding SDPP increases pig performance, especially during sub-clinical post weaning colibacilosis. Its performance benefits may be mediated through reduced systemic inflammatory responses. As per EU directive 1292/2005, food grade SDPP of non-ruminant origin is permitted as feedstuff for monogastric farm animals. Thus, subject to authorisation, registration, permission, and safety requirements under UK regulations for feedstuff use, SPDD may be an alternative protein source for newly weaned pigs, whilst their health benefits may result in reduced reliance on antibiotics.

2.10 DEVELOPMENT OF DRUG-ALTERNATIVE STRATEGY AGAINST COCCIDIOSIS: ENHANCEMENT OF EIMERIA PROFILIN-INDUCED VACCINAL IMMUNITY BY MONTANIDETM ADJUVANTS IN BROILER CHICKENS

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Avian coccidiosis is an economically important disease caused by infection of the intestine by protozoan parasites from the genus *Eimeria*. Whereas prophylactic medication is the predominant method used to suppress flock infections, new disease control strategies are needed due to the emergence of drug-resistant strains of *Eimeria* and increasing consumer demands for drug-free poultry meat. Live coccidia vaccines are commercially available, but cross protection against heterologous *Eimeria* spp. is poor. The profilin 3-1E protein is a highly conserved apicomplexa ligand of toll-like receptors that stimulates broad-spectrum immunity. Here we show that recombinant protein vaccines derived from profilin combined with Montanide™ vaccine adjuvants increase protective immunity in broiler chickens against infection with *Eimeria* spp. In the first study, chickens were immunized subcutaneously with a purified *E. acervulina* recombinant profilin protein, either alone or mixed with Montanide™ ISA 71 VG (ISA 71). Body weight gain and fecal oocyst shedding were evaluated following oral challenge infection with live *E. acervulina* or *E. tenella* oocysts. In both cases, vaccination with profiling plus ISA 71 reduced oocyst shedding compared with animals immunized with profilin alone or not immunized animals. In a second study, broiler chicks were vaccinated twice with an *Eimeria* recombinant profilin protein alone or mixed with Montanide™ IMS 1313 VG (IMS 1313, oral administration) or ISA 71 (subcutaneously) prior to infection with *E. acervulina* oocysts. Birds vaccinated with profilin plus ISA 71 had increased body weight gains and IgY levels compared with the profilin-only and non immunized control groups, and equivalent to vaccination with Coccivac-B commercial live vaccine. Immunization with profilin plus IMS 1313 or ISA 71 reduced fecal oocysts shedding, and increased intestinal sIgA levels, compared with profiling alone and control groups. Birds vaccinated with profilin plus IMS 1313 or ISA 71 had higher percentages of CD4+, CD8+, and TCR1+, but not TCR2+, intestinal IELs compared with the control group. These results indicate that injectable or oral immunization of chickens with recombinant profilin subunit adjuvanted vaccines increases protective immunity against experimental *Eimeria* spp. infection and this strategy can work as a non-antibiotic alternative for coccidiosis control.

2.11 DEVELOPMENT OF IMMUNE BOOSTING DIETARY SUPPLEMENTS AS ALTERNATIVES TO ANTIBIOTICS

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Routine use of antibiotics in animal feed at sub-therapeutic dose for growth promotion and disease prevention is suspected to be a major driving force for rapid emergence of antibiotic-resistant pathogens, which have become a serious threat to public health worldwide. To ensure public health and a stable and safe supply of animal food products, alternative approaches to disease control are urgently needed. Through a comprehensive screening process, we discovered several dietary supplements to be highly effective in enhancing host innate immunity and disease resistance without triggering proinflammatory response. Of particular interest is short-chain fatty acids (SCFAs) produced naturally by intestinal commensal bacteria. We found that SCFAs strongly induces the expression of multiple genes for endogenous antimicrobial host defense peptides (HDPs), which possess potent immunomodulatory and broad-spectrum antimicrobial activities. In addition, dietary supplementation of SCFAs reduced the titer of *Salmonella enteritidis* in the chicken cecum following experimental infections. We further revealed that the induction of HDP gene expression is inversely correlated with the length of the aliphatic carbon chain of free fatty acids in chicken HD11 macrophages and primary monocytes, with SCFAs being the most potent, medium-chain fatty acids moderate, and long-chain fatty acids largely ineffective. Moreover, we observed a strong synergy in inducing HDP synthesis among SCFAs and between SCFAs and a botanical extract. Therefore, dietary supplementation of immune boosting SCFAs or SCFA/botanic extracts may have potential for further development as a promising antibiotic alternative approach to disease control and prevention. In addition to poultry, such an immunostimulatory approach is expected to be broadly applicable to all other animal species including humans, offering great potential for enhancing production efficiency, and food safety, while minimizing the use of antibiotics and emergence of drug-resistant pathogens.

2.12 DIRECT-FED MICROBIALS AS DRUG ALTERNATIVES TO MITIGATE GUT DAMAGE DUE TO INTESTINAL PARASITES IN COMMERCIAL BROILER CHICKENS

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Direct-fed microbials (DFMs) are live microorganisms which confer a health benefit to the host. The mode of action of DFMs involves multiple mechanisms, including direct inhibition of enteric pathogens and indirectly through competitive exclusion of pathogens by the normal gut microbiota. Additionally, our recent studies showed that DFMs promote cross talk between host innate immunity and gut microflora. Commensal bacteria on the intestinal mucosa contain many probiotics ligands which can communicate with PRRs inducing downstream signaling pathways that eventually lead to probiotic (health-promoting) effects. *Bacillus subtilis* (*B. subtilis*) has long been considered a non-pathogenic, spore-forming, soil microorganism that is recognized by toll-like receptors. We have recently evaluated several field isolates of *B. subtilis* strains by continuous feeding of young broiler chickens with the spore-supplemented standard poultry diet to investigate the probiotic effects of *Bacillus* strains. Depending on the *B. subtilis* strain, feeding diets supplemented with *B. subtilis* spores increased various intestinal intraepithelial T cell subpopulations, cytokine mRNA levels, and macrophage function. Feeding of young broiler chickens with *B. subtilis*-based DFMs also enhanced NO production and phagocytosis of peripheral blood-derived macrophages. Following an *Eimeria maxima* challenge infection, DFM-fed chickens showed enhanced disease resistance with higher body weight gain and decreased intestinal lesions compared with uninfected control birds. Detailed immune pathways that were affected by *Bacillus* treatment were further examined using a high-throughput gene expression analysis using 45K avian chip. Differential gene expression by microarray hybridization identified 453 transcripts whose levels were significantly altered in intestinal lymphocytes of *B. subtilis*-fed birds compared with non-supplemented controls. Biological pathway analysis identified the altered transcripts as belonging to the category of "Molecular and Cellular Function". The most significant function identified was "Cell-to-Cell Signaling and Interaction". This new information documents the immunologic and genomic changes that occur in chickens following *B. subtilis* supplementation. These results provide a rational scientific basis for future studies to investigate DFMs as drug alternatives to enhance host protective immunity against enteric pathogens in broilers chickens.

2.13 FEEDING LAYING HENS DIETS WITH SUPPLEMENTAL CHELATED TRACE MINERALS IMPROVES IMMUNE RESPONSE, SHELL QUALITY, AND TIBIA BREAKING STRENGTH

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Feeding highly bioavailable forms of trace minerals can support essential physiological functions necessary to animal health and structural integrity. Birds fed HMTBa-chelated Zn, Cu and Mn exhibit better bone strength, skin integrity, and immune response. Evidence these trace minerals play a key role in egg shell formation suggest supplementing hens with HMTBa-chelates of Zn, Cu and Mn will support the production of quality eggs across the laying period. A 56 wk (24 to 80 wk of age) study was conducted to determine the long term effects of feeding MINTREX® (metal methionine hydroxy analogue chelate) vs. ITMs (inorganic trace minerals) in layers on performance, egg shell quality, tibia breaking strength, and immune response. A total of 216 Hy-Line W-36 laying hens were assigned to 6 treatments with 36 pens/treatment and 1 hen/cage. The study was carried out under a randomized complete block design. The data were analyzed using both 1-way ANOVA (including all 6 treatments) and 2x2 factorial design with 2 sources (chelated vs. ITMs) and 2 levels (20-5-20 vs. 40-10-40ppm of Zn-Cu-Mn) of supplemental minerals. The treatments consisted of: 0-0-0ppm supplemental Zn-Cu-Mn, T1; 20-5-20ppm Zn-Cu-Mn as sulphates, T2; 20-5-20ppm Zn-Cu-Mn as chelates, T3; 40-10-40ppm Zn-Cu-Mn as sulphates, T4, 40-10-40ppm Zn-Cu-Mn as chelates, T5; 80-10-80ppm Zn-Cu-Mn as sulphates, T6. Overall results (1-way ANOVA) indicate a significant treatment effect ($P<0.05$) only for shell thickness at wk 74. Factorial analysis data indicates a significant improvement in shell breaking strength (Source effect, $P<0.05$ at wk 68), shell thickness (Source effect, $P=0.08$ at wk 68; $P=0.03$ at wk 74), and Ab titers (Source effect, $P<0.05$ at wk 63) to SRBCs (sheep red blood cells) for hens fed chelated trace minerals compared to ITMs. Supplementing increased levels (Level effect, $P=0.07$ at wk 80) of dietary minerals or chelates (Source effect, $P=0.19$ at wk 80) increasing tibia breaking strength. In summary, feeding laying hens the diets with supplemental chelated trace minerals compared to ITMs improved egg shell strength and thickness, tibia breaking strength, and immune response.

2.14 BENEFITS OF A CHELATED TRACE MINERAL BLEND (MINTREX®) ON IMMUNE FUNCTION IN GILTS

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Trace minerals are required for proper immune development and function. Deficiencies in trace minerals can cause decreased antibody responses to vaccination, which could be very costly in sow production. The objective of this trial was to test the benefit of a chelated trace mineral blend on immune function and reproduction performance in gilts. Replacement gilts (50 per treatment) were fed diets supplemented with 165 ppm zinc, 16 ppm copper, and 38 ppm manganese, either as inorganic trace minerals (ITMs) or an equal mixture of ITMs and HMTBa-chelated minerals (MINTREX®, Novus International Inc). The pigs were vaccinated with a commercial vaccine for *Mycoplasma hyopneumoniae* (Myco Silencer Once-Intervet) on weeks 0 and 2 postweaning, and bled for antibody titers on weeks 0, 2, 4, 8, and 12. Titers were measured by a commercially-available ELISA. Log titers below 2.8 are considered to be negative titers according to the kit instructions. While both groups of pigs achieved a similar titer by 12 weeks, the gilts supplemented with the chelates reached a positive titer 8 weeks prior to the gilts fed the control diet. A large scale follow-up study suggested that gilt removal rate was reduced 10% with MINTREX supplementation with 8.0% vs. 8.8% for MINTREX and ITMs, respectively (P=0.04). Mortality rate was 1.52% and 2.12% for MINTREX and ITMs, respectively (P=0.001). In addition, gilts fed MINTREX had a better walking/leg score than the ITMs group evaluated around 100 kg body weight. These data suggest that for those eight weeks, the replacement gilts fed ITMs were not as protected against *M. hyopneumoniae* as the gilts fed the HMTBa-chelated minerals were. Consequently, gilts fed MINTREX were in better health status and were better prepared for reproduction.

2.15 IMMUNOMODULATORY ACTIVITIES OF CHICKEN CATH-2 DERIVED PEPTIDES

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Large quantities of conventional antibiotics are used to prevent infection in farm animals, in particular in poultry and swine. Host Defense Peptide (HDP)-based anti-infectives may be an alternative to antibiotics in veterinary medicine. These peptides have direct antimicrobial activity and are immunomodulatory. Previously we identified a chicken cathelicidin (CATH-2) and reported the antimicrobial properties of this peptide. In addition, using mammalian cells, we could demonstrate that CATH-2 has immunomodulatory properties. Next, we wished to investigate the effects of CATH-2 on avian cells. Effects of CATH-2 derived peptides on avian immune cells were examined using a chicken macrophage cell line (HD11). HD11 cells were stimulated for 4 or 24 h with peptides and/or lipopolysaccharides (LPS). After incubation, supernatants were used to determine nitric oxide levels (Griess assay). Isolated RNA was transcribed and used for QPCR analysis of cytokine expression levels. Here we report that full-length CATH-2 peptide, C(1-26), dose-dependently induces transcription of the chemokines CXCLi2/IL-8, MCP-3 and CCLi4/RANTES, but not of pro-inflammatory cytokine IL-1β, in a chicken macrophage cell line (HD11). In addition, peptide C(1-26) effectively inhibits IL-1β transcription and nitric oxide production induced by LPS from different sources. N-terminal truncated peptides as small as 15 residues still have the capacity to selectively induce chemokine transcription, but lack LPS-neutralizing capacity. Substitution of Phe- by Trp-residues introduces endotoxin neutralization capacity in previously inactive truncated CATH-2 derived peptides. Phe/Tyr substitutions result in abrogation of endotoxin neutralization and support a pivotal role for Phe and Trp residues in peptide-mediated endotoxin neutralisation. We conclude that peptides can be designed, based on CATH-2, that selectively modulate chemokine transcription and could serve as new leads for the design of HDP-based antimicrobials with tailor-made immunomodulatory activities.

2.16 THE IMPACT OF A BIOACTIVE FRACTION DERIVED FROM YEAST CELLWALL ON FECAL AND SALIVA IGA LEVELS IN BOVINE CALVES

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Yeast abstracts, mannan-oligosaccharides and bioactive components of yeast have been studied extensively for their relationship with intestinal function and their role in modulating gut micro flora. An experiment was conducted to study the influence of a yeast derived bioactive fraction, on adaptive immunity in neonatal dairy calves. In this study, two groups of 30 heifer calves were randomly assigned to treatment at birth and were fed milk replacer (12% of body weight/day; 20% protein, 20% fat) and calf starter grain. In addition to the control group, a group fed the same diets with the addition of 1 gm/day/calf of a unique bioactive fraction derived from yeast cell walls (Actigen™; Alltech, Inc, Nicholasville, KY, USA) was fed in the milk replacer, half in each feeding per day. Calves were fed and observed for 6 weeks in relation to feed intake, health, and growth, as well as initially ingested levels of colostrum immunoglobulin G and immunoglobulin A (IgA). IgA levels in feces and saliva were analyzed over the first 20 days of age using an ELISA analysis (Bethyl Laboratories, Montgomery, TX, USA). Body weights were statistically similar between control and treatment groups at the start and end of the study, with some greater growth on the treated group. Feed intake was slightly lower on the treated group allowing for a greater feed to gain ratio ($P<.05$) on the Actigen fed group. Fecal and saliva IgA secretion showed some residual colostrum IgA in the first 4-6 days of life. After that time period, fecal IgA showed significant increases at 8 -10 days, earlier than observed in saliva. At 12-14 days of age, saliva IgA levels were increasing in both groups and greater in the Actigen fed calves over the control calves. At 16 days of age the Actigen fed calves had significantly greater ($P < .01$) IgA levels than control calves and it remained that way for the period of time where measurements were taken (20 days). Similar to saliva we found significant increases ($P < .01$) of IgA in the Actigen fed calves over the controls by 13 days of age and this difference remained throughout the period of measurement. Saliva and fecal IgA were beneficially elevated earlier in life with the addition of Actigen for these calves. We also conclude that saliva IgA can be an indicator of fecal IgA, however not as sensitive as fecal IgA. It may however allow us an alternative measure of mucosal IgA levels in the calf.

2.17 DISCOVERY OF NOVEL PEPTIDES OF AVIAN B-DEFENSINS AS ANTIBIOTICS ALTERNATIVES TO CONTROL NECROTIC ENTERITIS IN COMMERCIAL BROILER CHICKENS

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In the United States, necrotic enteritis (NE) is among the most important infectious diseases in chickens. Globally, the economic loss due to NE is estimated to cost the United States $2 billion annually largely due to medical treatments and impaired growth performance. Recently, NE has re-emerged as a significant problem as a result of restricted use of in-feed antibiotics, high-density housing conditions, and re-use of litter. Thus, there is an urgent need to develop rational, and alternative β-defensin management strategies not only to control, but also to prevent NE. β-defensins represent important effector molecules of host innate immunity in poultry, and they have been isolated from leukocytes and epithelial cells of skin, gastrointestinal, and respiratory tracts. In chickens, 14 β-defensin genes (AvBD1 to 14) have been identified in the leukocytes, epithelial cells, or expressed sequence tags (EST) of chicken genome. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are known to be potent inducers and up-regulators of defensins such as human beta defensin-2, hBD-2. However, there have been no studies showing the expression of β-defensins in NE in chickens yet. Therefore, we examined the expression profiles of AvBD transcripts in three different tissues to compare AvBD involvement in NE in two commercial broiler chicken strains showing disparate NE disease susceptibility with a long term goal of using defensins in immunotherapeutics. Among the 14 AvBD types examined, there was a tissue-specific expression of AvBD transcripts: AvBD1, AvBD7, and AvBD9 were expressed in the crop, and AvBD8, AvBD10, and AvBD13 were expressed in the intestine. The two different commercial broiler chicken lines also showed differential gene expression patterns of AvBD transcripts following NE, with R line chickens generally showing higher expression levels than the C strain. Both chicken strains showed enhanced gene expression levels of pro-inflammatory cytokines such as IL-1β, IL-6, IL-17F and TNFSF15 in spleen, and TNFSF15 in intestine, whereas IL-17F was significantly increased only in the intestine of R line chickens following NE infection. Although the exact nature of interactions between defensins and cytokines in determining the outcome of host innate immune responses to the pathogens of NE remains to be investigated, the differences in gene expression levels of β-defensins and pro-inflammatory cytokines in the intestine, crop, and spleen could explain the predisposed disease resistance and susceptibility to NE in the two commercial broiler chicken lines (This project was supported by the Next-Generation BioGreen 21 No. PJ008084, RDA, Korea).

2.18 THE SYNERGISTIC EFFECTS OF PLANT-DERIVED NUTRITIONAL MIXTURES ON RECOMBINANT ANTIGEN VACCINATION AGAINST AVIAN COCCIDIOSIS

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The present study was conducted to examine immunomodulatory effects of commercially available dietary plant-derived phytonutrient mixture, (XT on adaptive host immune response against avian coccidiosis. XT is a nutritional mixture of 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin. Vaccination against avian coccidiosis was carried out by subcutaneous immunization of young broiler chickens using recombinant *Eimeria* profilin protein at day 7 after hatch and challenge infection was given by an oral inoculation using live sporulated oocysts of *E. tenella* (ET) (2.0 × 104) at 17 days of age. Four groups of broiler chickens (12 birds/group) were continuously fed a standard diet without (CON) or with profilin vaccine (CON-V) or standard diet supplemented with XT with profilin vaccine for 23 days. Changes in body weights were measured at 9 days post-infection (DPI) and fecal oocyst outputs were assessed in individual samples collected from 5 through 9 DPI. Cell-mediated immunity was assessed by evaluating the cecal cytokine transcript levels of IFN-γ, IL-6, IL-17, and TL1A by quantitative real time-PCR at 0 DPI. The XT-V group showed a 20% increase in body weight ($P<0.05$) in comparison to the CON group after ET challenge infection. Fecal oocyst shedding was significantly reduced by 35% in XT-V group compared with the infected CON group. Furthermore, IFN-γ, IL-6, IL-17, and TL1A cytokines were significantly decreased in the XT-V group in comparison to the CON group. This study demonstrates that molecular and cellular changes were affected by XT- nutritional immunomodulation with enhanced vaccine-induced protective immunity against avian coccidiosis. This vaccination strategy against avian coccidiosis will facilitate the development of a new antibiotics-free alternative for enteric parasites in commercial broiler chickens.

2.19 INFLUENCE OF PHYTONUTRIENT “VITASTIM” ON CHICKEN MUCOSAL IMMUNITY AFTER INFECTION WITH LOW-PATHOGENIC AVIAN INFLUENZA VIRUS

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Poultry production is now rapidly developing and needs modern veterinary software. The presence of accelerated evolutionary processes has resulted in a complication of the epizootic situation, increasing the pathogenic properties of the pathogens and the spread of infectious diseases. Immunostimulation is widely used in infectious diseases. Adjuvants of different origins are a valuable way to improve the immune status of the avian organism and enhance the immune response during vaccination. Search for new immunostimulating preparations continues to view ever-increasing requirements regarding their safety, effectiveness, and accessibility. The aim of our study was to investigate the immunostimulatory effect of phytonutrient "Vitastim” on the immune response after avian infection with low-pathogenic avian influnza virus. We investigated the immune response of chickens after infection with a highly pathogenic avian influenza virus, A/mallard/Ukraine/2007 H5N2, followed by drinking of the phytonutrient "Vitastim” using an immunohistochemical method (LSAB). The dynamics of CD4, CD8, IgM, IgG, and IgA accumulation in spleen, caeca, trachea, lung on 1st, 3rd, 5th, 7th, 10th, 14th, and 21st dpi was studied. From the results of the immunohistochemical research on the influence of immunostimulating phytonutrient “Vitastim” on chickens it was determined that given preparation more actively influence on humoral immune response in normal and at a low dose of pathogenic avian influenza (caeca, trachea, lungs) that testified more intensive formation and accumulation of B-lymphocytes which produce immunoglobulins. In the spleen there was an increase in the amplified proliferation of T-lymphocytes, macrophages that characterize the activation of cell immune reaction. We then investigated 7 clusters of immunocompetent cells that determine immune response on the stage of early direct influence on agents –CD8, on the stage of cooperation and transmission of antigenic products –CD4, on the stage of its processing and presenting – macrophages and IgG, IgM, and IgA. We have established an immunostimulating effect of phytonutrient "Vitastim" that can be recommended to apply it in order to stimulate the immune response of poultry, as well as the drug-support for animal immunization against infectious diseases.

2.20 EFFECT OF HYPERIMMUNE EGG YOLK IMMUNOGLOBULIN Y IN BROILER CHICKENS

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Avian coccidiosis is an intestinal disease caused by several distinct species of *Eimeria* protozoa and is the most economically significant parasitic infection of the poultry industry worldwide (Lillehoj). Different control methods are needed due to increasing concerns with drug use and high cost of vaccines; the use of hyperimmune egg yolk immunoglobulin Y as an alternative control is the passive immunization using parasite specific antibodies (Lillehoj and Lee). In this study 69,000 broilers were fed during 5 weeks with a normal standard diet containing hyperimmune IgY antibodies and supplemented with the same hyperimmune IgY antibodies in water during the third week. Body weight gains and oocyst numbers were measured. In this study, the protective effect of oral IgY from eggs of hens hyperimmunized with mixed *Eimeria* oocyst was evaluated. After the administration, broilers exhibited increased body weight gains, 40 grams compared with the historical weights in the same farm; and after the third week reduced fecal oocyst shedding. There was not a coccidiosis problem in the farm. This study demonstrated beneficial effect of using an immune enhancing supplement like hyperimmune IgY antibodies to passively provide significant protection against avian coccidiosis.

2.21 RAPID INDUCTION OF ENTERIC CYTOKINE CHANGES BY AN EFFECTIVE LACTIC ACID BACTERIA-BASED CULTURE FOR POULTRY

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During the last decade, significant efforts in our laboratory have been directed toward development and understanding of effective probiotics and direct fed microbials (DFM) for control of both food-borne pathogens and for use as antibiotic alternatives in poultry. One culture (laboratory designation B11) is a specific combination of lactic acid bacteria that were developed by both *in vitro* assay and by extensive testing of individual candidate isolates and specific combinations *in vivo* using a *Salmonella* challenge model in our laboratory. This specific culture (B11) has been demonstrated to have tremendous efficacy for both prophylactic and therapeutic reductions of enteric *Salmonella* loads, comparable reductions in alpha-toxin expressing *Clostridium perfringens* levels, clinical necrotic enteritis (comparable to bacitracin at 50g/ton), and enhance performance (comparable to organic arsenicals) in more than 20 refereed publications in both laboratory and field trial settings. Using microarray analysis, we recently identified several alterations suggestive of reduced inflammatory cytokine pathways, within 24h post-administration of B11, in a recently published study. Very recently, using rtPCR, we have evaluated the effects of B11 administration one hour after *Salmonella enteritis* challenge (104 cfu) on day-of-hatch. At 24h sampling, cecal mucosal mRNA expressing for chicken TNFa, and IL8 were significantly reduced, but no changes in IL-4, iNOS, or IL-2 were observed. At 72h post-treatment, a small but significant change in Il-4, IL-8 and iNOS were observed, but a marked and significant increase in IL-2 (5-fold) was observed in B11-treated chicks. These observations may be consistent with reported reductions in gut-inflammatory response in mammalian models caused by effective lactic acid bacterial cultures and may be consistent with the reduction of necrotic enteritis, as many investigators have indicated that inflammatory insult is necessary for *Clostridium perfringens* blooms associated with onset and progression of necrotic enteritis. An interesting but unproven hypothesis that reduced inflammation may be associated with some of the observed AGP-like performance enhancement previously reported. Elevated IL-2 responses have been associated with enhanced acquired immune function in probiotic treated rats. Ongoing research in this area relates to direct markers of inflammation in B11-treated control and challenged chicks.

2.22 PLASMA PROTEINS ARE A NATURAL ALTERNATIVE TO ANTIBIOTICS IN FEED FOR WEANLING PIGS

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Weaning is characterized by a period of anorexia that leads to gut barrier dysfunction associated with intestinal inflammation, increased intestinal permeability and as a result increased sensitivity to enteric infections and diarrhea. Traditionally, sub-therapeutical antibiotics (ATB) were included in feed as antimicrobial growth promoters (AGP) to reduce the harmful effects of enteric pathogenic bacteria. However, AGP were banned in the EU in 2006 due to the concern that their use in feed may contribute to the development of microbial resistance to ATB used in both animal and human medicine. But, contrary to EU regulatory intentions, the use of prescription diets with therapeutic levels of ATB has increased since the ban, resulting in greater risk for development of antimicrobial resistant bacteria. Therefore, alternatives to the use of sub-therapeutic antibiotics are increasingly important for all phases of food animal nutrition. Significant research has demonstrated the benefits of using spray-dried plasma (SDP) as an alternative to ATB in feed for weanling pigs. The mechanisms by which SDP benefits animal well being are not fully understood but past research has suggested the naturally occurring antibodies in SDP offer protection against pathogens. In addition, recent research indicates that dietary SDP supported and maintained gut barrier function during intestinal inflammation induced by intraperitoneal injection of an antigen, *Staphylococcus aureus* enterotoxin B, which by-passed the potential for an antigen-antibody interaction in the gut lumen. These authors concluded that the preventative effect of SDP on intestinal inflammation involved modulation of intestinal cytokines that was characterized by expression of anti-inflammatory cytokine. Further research has demonstrated that dietary SDP impacts a similar modulation of pulmonary cytokines that were again shown to have increased expression of anti-inflammatory cytokine. These results suggest that dietary SDP impacts the common mucosal systems, not just the local mucosal system in the gut. Recent publications suggested that diets supplemented with SDP is probably one of the best ways to prevent post-weaning gut disorders and that SDP can be safely used as an alternative to ATB without risk of generating antibiotic resistance bacteria.

2.23 IN VITRO IMMUNOMODULATORY AND IN VIVO ANTI-INFLAMMATORY PROPERTIES OF BACILLUS SUBTILIS STRAIN PB6

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Chronic intestinal inflammation is characterized by deregulation of several pro- and anti-inflammatory cytokines. Probiotic mediated immunomodulation represents an attractive approach to help manage this inflammatory process. Not all probiotic strains will be equally efficient due to differences in survival and persistence in the harsh conditions of the gastrointestinal tract and to differences in immunomodulatory performance. This paper will address an *in vitro* and *in vivo* study set up to address the anti-inflammatory potential of a *Bacillus subtilis* strain PB6 (ATCC – PTA6737, PB6). For the *in vitro* study, the immunomodulatory properties of PB6 were characterized using human peripheral blood monocytes. The cytokines monitored were IL-12, TNF-α, IFN-γ, and IL- 10. For the *in vivo* study, the anti-inflammatory capacity of PB6 was assessed in an acute mouse model of trinitrobenzenesulfonic acid (TNBS)-induced colitis. The protective effect mediated by feeding PB6 was compared with that of the drug prednisolone based on blinded colon wall macroscopic and histological scores, colon myeloperoxidase, and blood inflammatory markers IL-6 and serum amyloid A protein. Results show that stimulation of immunocompetent cells with PB6 causes a substantial induction of cytokine IL-10. The levels of the pro-inflammatory cytokines IL-12, TNF-α, and IFN-γ remained very low. Macroscopic and histological evaluation of the colon after induction of colitis showed that PB6 exhibits significant protective effects and markedly minimizes the severity of inflammation. The relative level of protection was 52%, higher than the protection level observed with the anti-inflammatory corticoid drug prednisolone (39%). Colon myeloperoxidase activity, a marker for neutrophils infiltration, and levels of blood inflammatory markers IL-6 and serum amyloid A protein were considerably reduced. The anti-inflammatory properties of PB6 strain result in both local and systemic effects. At the colon, the site of induction of inflammation, macroscopic and histological observations clearly showed that the severity of the inflammation was reduced. Serum levels of IL-6 and SAA, two important systemic markers of inflammation, were substantially lower. Strategies targeting IL-6 and IL-6 signaling have led to effective prevention and treatment of models of rheumatoid arthritis and other chronic inflammatory diseases. It is an important observation therefore that PB6 *in vivo* is able to help balance these first-line mediators of inflammation.

2.24 A NOVEL PLANT EXTRACT MIX, GRAZIX™, IS CAPABLE OF BINDING ENDOTOXIN

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Phytobiotics are a group of feed additives that consist of plant-derived ingredients for promoting livestock health and well-being and improving livestock growth and production efficiency. The mechanisms of phytobiotics have not been entirely understood but their benefits to overall health in animals have been noted. The objective of the present study was to determine if a novel plant extract mix (GRAZIX™, LiveLeaf Bioscience, San Carlos, CA) could bind polysaccharide (LPS), the major component of endotoxin. Endotoxin is present in Gram-negative bacterial outer membrane and can contribute to life-threatening inflammatory reactions, diarrhea, and shock. A novel Polymyxin B/LPS-fluorescein isothiocyanate (FITC) binding inhibition assay was developed to determine the binding ability of GRAZIX™ to LPS. GRAZIX™ and a purified polyphenol compound Tannin (with the same dry weight as GRAZIX™) were diluted into 5 different strengths, 1000x, 100x, 10x, 1x, and 0.1x, in distilled water, respectively. Diluted compound was mixed with LPS-FITC and incubated at room temperature (RT). Polymyxin B was then added and incubated at RT. The supernatants containing compounds free in the supernatants and compounds bound by LPS-FITC were discarded after centrifugation. The pellets containing Polymyxin B bound by LPS-FITC (Polymyxin B/LPS-FITC) were re-suspended in distilled water. The fluorescence of the suspension mixture was measured in a microplate reader. The percentage (%) of Polymyxin B/LPS-FITC binding inhibition is calculated by using 100% minus the percentage of Polymyxin B/LPS-FITC binding detected. GRAZIX™ was shown to have 97.9 and 85.4% of Polymyxin B/LPS-FITC binding inhibition when diluted to 100x and 10x, respectively, while the polyphenol Tannin compound had 98.2, 97.8% of Polymyxin B/LPS-FITC binding inhibition when diluted to 100x and 10x, respectively. The results clearly indicated that GRAZIX™ can bind LPS by their near 100% inhibition of the binding between Polymyxin B and LPS; thus, GRAZIX™, a plant extract mix containing polyphenols, is capable of binding endotoxin. This is likely one of the mechanisms that GRAZIX™ improves performance and health of livestock.

2.25 REDUCING SCOUR IN COMMERCIAL PIG FARMS WITH A NOVEL PLANT EXTRACT— RESULTS OF A VETERINARIAN'S FIELD TRIALS

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The Dutch government has asked commercial pig farmers to reduce their use of antimicrobials by 50% before 2013, as well as cease the use of 3rd and 4th generation cephalosporines and quinolones immediately. In addition, there are concerns about use of colistine for prevention/treatment of neonatal diarrhea in the farrowing unit. Such increasing restrictions have resulted in the proliferation of plant-based antimicrobials to improve/maintain the health status of growing pigs. A novel but different approach to such products is GRAZIX™ (LiveLeaf Bioscience, San Carlos, California, USA). This material, provided in liquid form, has demonstrated reduction of diarrhea in pigs in laboratory settings and in a few commercial large farms. Its mode of action is hypothesized to be a modifying agent of the innate immune response in regions of the gastrointestinal tract that are stressed or injured by pathogens associated with the diarrheal response. In this assessment, 20 farms (units ranging from 400 to 1000 sows) agreed to administer the GRAZIX solution to individual piglets upon first observation of scouring. If required, a repeat application of the solution was provided 6 to 8 hours after the initial administration. At 4 to 6 weeks after providing the GRAZIX solution, it was noted that the mortality rate of piglets who had consumed the solution was 50% lower than the mortality rate on these same farms in immediately preceding herds. Piglets that had consumed the solution developed 50% fewer infections and 75% fewer episodes of scour. During the time that the GRAZIX solution was available, these farmers required little to no use of antimicrobial agents and after they had used the volume of solution provided, requested additional units of the solution in order to maintain the results. This field trial demonstrated that administration of this novel plant extract reduced the need for antimicrobial agents in order to maintain the health of piglets, which may be a means of meeting the Dutch government's mandate prior to 2013. More rigorous testing is needed to determine whether this response can be replicated but the results noted on these farms are encouraging.

2.26 A SYSTEMATIC APPROACH TO REDUCING ANTIBIOTIC DEPENDENCE IN INTENSIVELY FARMED ANIMALS

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Logically, one of the simplest approaches to decrease dependence on routine antibiotic regimes in animal production is to control the bacterial challenges that the antibiotics are modulating. This leads to the idea of specific pathogen freedom but this is often very difficult to implement at the production level due to economic barriers, existing infrastructure, lack of insurance, and current antibiotic usage. Pragmatically, apart from pathogen freedom (with the problem of maintenance of associated totally susceptible populations) development of alternative solutions has not been systematically approached but some empirical experience now suggests an approach. To take a simple system – cage egg layer production in temperate climates; the main bacterial problem controlled by routine antibiotic administration was thought to be Mycoplasmosis and therefore probably *Mycoplasma gallisepticum* (MG) infection. Enteric bacterial (and coccidial) infections are controlled in the cage system by reducing access to faeces. In Australia the development of an effective (live) MG vaccine (ts-11) greatly decreased antibiotic dependence of the poultry industries but *M. synoviae* emerged as a problem and possibly *Brachyspira* spp. (Effective control of *Avibacterium paragallinarum* with a killed vaccine was also important initially). As MG control developed, luckily the *M. synoviae* vaccine was available or the industry may have gone back to antibiotics. These live mycoplasma vaccines differed from previous generations of vaccines in that they have displaced wild strains from farms (not always predicted in the laboratory where they can be overwhelmed in some challenge systems). *Brachyspira* species could be controlled by a short treatment of amoxicillin or acidification of water. Currently antibiotic treatment of industrial poultry in Australia is very rare. Different animal production systems have different problems but a systematic approach can be implemented. First is to analyse what infections are being controlled by antibiotics and not to look at antibiotics as a non-specific performance enhancing factor. Then look for a solution to each problem and make sure that the solution will not interfere with the solution to other problems. Bacterial vaccines that interfere with wild strain spread and maintenance will be more useful than ones that just ameliorate clinical signs (probably vaccines that induce useful mucosal immunity rather than predominately humoral antibody) especially if some other producers are unwilling to participate in pathogen control programs and their populations then become significant pathogen reservoirs. The control of mycoplasma infections appears central to this approach as these are chronic and increase the effect of other bacterial (and viral) infections.

2.27 AMELIORATE EFFECT OF CALIBRIN®-Z ENTEROSORBENT ON SERUM REPRODUCTIVE HORMONE, IMMUNOGLOBULIN, ANTIBODY TITER IN YOUNG PIGS FED PURIFIED ZEARALENONE

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Zearalenone (ZEA) is a mycotoxin that mimics estrogen; causing infertility, abortion, and other reproductive problems, especially in swine. Besides its estrogenic effects, the toxin can also negatively impact the immune response. *Calibrin-Z (CAZ)*, a highly-refined montmorillonite sorbent mineral, has high affinity and capacity to sequester a wide range of mycotoxins found in feed grains. The objective of this study was to evaluate different dosages of CAZ on the effects of ZEA on serum hormone and immune response in piglets. A total of 36 pigs with initial body weight of 8.9 ± 0.2 kg were allotted to 6 treatments (TRT). The 6 TRT were: 1) Control (Con); 2) Con + 0.1% CAZ; 3) Con + 1 ppm ZEA; 4) Con + 1 ppm ZEA + 0.1% CAZ; 5) Con + 1ppm ZEA + 0.2% CAZ; 6) Con + 1 ppm ZEA + 0.4% CAZ. Data were analyzed using the GLM procedure of SAS with individual pig as the basis for analysis. Each pig received classical swine fever (CSF) vaccine 1-d before the study, serum samples were collected weekly for CSF antibody titer determination. On d 21, blood and spleen samples were collected for hormone, immunoglobulin, interleukin (IL) cytokine analyses, and lymphocyte proliferation rate (LPR). Gilts fed TRT 3 had lower ($P < 0.05$) progesterone (75% of 1 and 2), and testosterone (74% of 1 and 2) levels than TRT 1 and 2. Adding CAZ improved serum hormone levels and the elevated hormone was CAZ dosage dependent. Male pigs fed TRT 3 showed similar results as gilts except serum estrodial was not different. Serum IgA and IgM were not different; however, IgG level was reduced ($P < 0.01$) in TRT 3 (77% of 1 and 2) compared with TRT 1 and 2. It increased linearly ($P < 0.05$) in TRT 4, 5, and 6 but remained lower ($P < 0.05$) than TRT 1 and 2 (84% of 1 and 2). The LPR from blood and spleen cells followed a trend similar to IgG. Serum IL-2 followed results similar to those of the hormones; pigs fed TRT 3 had the lowest IL-2 but an addition of 0.4% clay restored the levels equal to TRT 1 and 2. There was no difference on d-7 or d-14 on CFS titers. On d-21, pigs fed TRT 2 had the highest titer against CSF and greater ($P < 0.01$) than pigs fed TRT 3, 4, 5, and 6; but not different than TRT 1. Feeding 1 ppm of purified ZEA caused adverse effects on immunity in pigs; which were ameliorated by CAZ. Feeding CAZ without ZEA had the greatest antibody titer production, implying that the Calibrin-Z may add value to feed with or without a mycotoxin challenge.

2.28 BOVINE COLOSTRUM WITH HYPERIMMUNITY AGAINST CLOSTRIDIA

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Bacteria from the *Clostridia* groups are associated with gastrointestinal diseases in both infants and adults. Intestinal overgrowth with *Clostridium perfringens* is commonly associated with necrotizing enterocolitis (NEC) in preterm infants and pigs, and *Clostridium difficile* plays a key role in antibiotics-associated diarrhea in adult patients. Earlier studies in preterm pigs suggest that bovine colostrum reduces the risk and severity of NEC. Here, we investigate the effect hyperimmune bovine colostrum having activity against *C. perfringens*. The hyperimmune colostrum was prepared by immunizing late gestation pregnant cows (n = 5) with COVEXIN® 8A (targeting *C. perfringens* Type A, C and D toxoid; *C. Chauvoei*; *C. novyi* (*oedematiens* type B) toxoid; *C. septicum* toxoid; and *C. tetani* toxoid) and collecting colostrum at the first milking (HYPER-COLOS). This was compared with colostrum from non-immunized control cows (COLOS, n = 5). Western Blot was used to confirm the presence of immunoreactivity in colostrum from immunized cows, using antigens extracted from *C. perfringens* NCTC 10240 and *C. difficile* O27 as positive controls, and E. coli ATCC 25922 and L. sakei DMS 20017 as negative controls. We confirmed hyper-immunity of the colostrum towards *C. perfringens*, but no clear difference in band intensity or number of bands was observed towards *C. difficile*, *E. coli* and *L. sakei*. The results show that we are able to produce hyper-immune bovine colostrum towards *C. perfringens*. Such a product could serve as a dietary supplement for preterm neonates during the weeks after birth to protect against the damaging toxins of *C. perfringens*.

2.29 ENABLING PASSIVE IMMUNIZATION AS AN ALTERNATIVE TO ANTIBIOTICS FOR CONTROLLING ENTERIC INFECTIONS IN PRODUCTION ANIMALS

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Enteric infections cause major problems in most intensive animal production sectors, including poultry, pigs and cattle, leading to disease, reduced production and compromised welfare. In addition some of these infections are zoonotic, and they are to a large extent responsible for the continued massive use of antibiotics in food animals. Thus there is a pressing need for economically feasible, efficient, non-antibiotics based means for controlling the problem. Passive immunization has been known for decades as an efficient way of endowing humans or animals with short-term (weeks) immunity. To control enteric infections by passive immunization a bolus of immunoglobulin may simply be administered orally. For this to work, large amounts of active immunoglobulins are needed. To be a real alternative to antibiotics the price of the immunoglobulin product needs to be low. We combined an efficient and mild high-capacity method for extracting immunoglobulins directly from raw materials like milk, whey and blood plasma with a novel method for stabilizing activity. In a first experiment a total of 15 kg unstabilized bovine immunoglobulin was purified from whey (35.000 liters) and administered to colostrum-deprived calves (225-300 grams per calf during the first 24 hours after birth). No difference in resulting immunoglobulin serum concentration, weight gain or disease frequency were seen in this group of calves compared to a control group given full access to high-quality colostrum. The effect of orally administered bovine immunoglobulin is currently being tested in a calf herd with persistent diarrhea problems. Furthermore, it was shown in a *Campylobacter* challenge model in chickens that caecal and faecal counts of *Campylobacter* were between 0.5 and 1.0 logs lower in birds when given 200 mg avian immunoglobulins orally together with the challenge (at day 21 of age) compared to a placebo group receiving immunoglobulin with no reactivity against *Campylobacter*. While clearly preliminary, these results show that immunoglobulin can be produced from renewable sources at a price enabling passive immunization as a viable strategy for control of infectious diseases in the intensive animal production, with the potential to significantly reduce antibiotics consumption.

2.30 PROBIOTICS FOR NILE TILAPIAS, *OREOCHROMIS NILOTICUS* SUBMITTED TO CHALLENGE WITH *AEROMONAS HYDROPHILA* AND *STREPTOCOCCUS INIAE*

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The evaluation of the mixture composed of three microorganisms (*Bacillus subtilis, Aspergillus oryzae* and *Saccharomyces cerevisiae*) as potential probiotic was performed and studied for their effect on immune parameters and protection against infectious challenges with *Aeromonas hydrophila* and *Streptococcus iniae*. Analysis of hematological parameters, such as MCV and MCHM did not show changes during the experiment. Increase in plasma glucose and cortisol was observed in fishes of the control group, and as a consequence, increase in RBC was observed, showing that probiotics suppressed the physiologic deleterious effects of the experiment´s stress. In addition, the probiotic supplementation assured to erythrocytes increase in resistance to hemolysis. The fish that received the probiotic had higher taxes of reactive oxygen species activity, which assured them an increase in innate immunity. Higher mortality rates were observed in fish of the control group challenged with experimental infection against *A. hydrophila* and *S. iniae*. Doses of 5 and 10 g.kg-1 of probiotic ensured the highest survival rates for tilapia against *A. hydrophila*, while 10 g.kg-1 of probiotic promoted higher survival tax against *S. iniae*. The supply with probiotic containing *Bacillus subtilis, Aspergillus oryzae* and *Saccharomyces cerevisiae* is safe for fish food, and promote an increase in nonspecific immunity against bacterial diseases.

Session 3
The Gut Microbiome and Immune Development, Health and Disease

Oral Presentations

3.1 THE ROLE OF MICROBIOTA IN ENTERIC AND ALLERGIC DISEASES

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Recent work in our lab has begun to explore the role of the microbiota in experimental asthma and infectious diarrhea. We are finding that the microbiota play central roles in these diseases. We have been studying the role of the microbiota in enteric infectious diseases using pathogenic *Escherichia coli* and *Salmonella* murine models. It is becoming apparent that specific components of the microbiota play a critical role in immune development and host responses, and the establishment and outcome of infectious enteric diseases. The microbiome also impacts the host susceptibility to disease, and even affects the host metabolome during infection. We are also finding that specific members of the microbiota affect the outcome of experimental asthma. Results probing these aspects will be discussed in the context of these diseases, as will potential mechanisms involved.

3.2 THE RUMINAL MICROBIOME AND ANIMAL HEALTH

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Analysis of the ruminal microbiome, in terms of the species present, is advancing to be a mature topic thanks to the advances in DNA sequencing technologies. Its application to understanding its relation to rumen metabolic function and animal health and disease is, in contrast, in its infancy. The rumen is home to ca. 20 species of ciliate protozoa, which fall into two categories within which the different species are relatively closely related. The other main eukaryotes, the ruminal fungi, comprise about six genera, again closely related. The bacteria are the most diverse group, with at least 300 different species present in the rumen of any single animal. Nevertheless, the great majority of the bacteria are derived from a relatively narrow grouping of the bacterial kingdom, namely the *Bacteroidetes* and *Firmicutes*, with lower numbers of α-proteobacteria. Three main groups of archaea, comprising mainly *Methanobrevibacter* spp., produce methane from H_2 and CO_2. Key metabolic functions of the rumen microbiota include fiber breakdown, protein breakdown, and methane formation. Although spectacular amounts of information about cellulases and related enzymes are being generated from the analysis of metagenomic sequences, for example >27,000 putative glycosyl hydrolases, surprisingly there is so far no hint of how the information can be used to enhance ruminal fiber digestion. The rumen microbial community impacts animal health in both negative and positive ways. Dysfunctions include lactic acidosis, sub-acute ruminal acidosis (SARA), and bloat. The microbial community changes that cause lactic acidosis are already well known (although yet to be confirmed by microbiome analysis). SARA is a more difficult problem, as it can be difficult to diagnose in the live animal. Microbiome analysis has recently helped to identify certain strains of *Escherichia coli* most closely associated with SARA. Bloat is a microbially derived pathology that prevents the release of fermentation gases from the rumen. Once again, no microbiomic or metagenomic analyses have yet been carried out to describe accompanying community changes. The rumen also protects the animal from exposure to antinutritional compounds present in certain plants. Ruminal bacteria detoxify these compounds, and experimentation is under way to understand how antinutritional compounds influence the microbiome and *vice versa*.One might speculate that future investigations will attempt to find links, or the absence of links, between the ruminal microbiome and other performance and health indicators, such as laminitis, pulmonary disease, and perhaps even fertility.

3.3 THE RUMINAL VIROME

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Viruses have been shown to be a driving factor in the evolution of microbial communities in various environments. They play important roles in controlling the numbers of microbes in an ecosystem, naturally selecting phage-resistant microbes, and facilitating horizontal gene transfer. In the rumen, gene exchange between bacteria and bacteriophage has been implicated in the spread of antibiotic resistance genes. Although bacteriophage are abundant in rumen environments, little is known about the types of viruses present or their interaction with the rumen microbiome. An indication of phage-bacteria interactions is the presence of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins in the microbial populations. The CRISPR/Cas system in bacteria and archaea provides a means of adaptive, sequence-specific resistance to the bacteriophages. We used a metagenomic approach to investigate the virus-enriched metagenome (virome) of the rumen to uncover the phage types that are present in a typical rumen environment, and determine how they vary among individuals and interact with the bacterial populations. A high level of diversity was observed with up to 28,000 different viral genotypes obtained from each environment. The majority (~78%) of sequences did not match any previously described virus. Prophages outnumbered lytic phages approximately 2:1 with the most abundant bacteriophage and prophage types being associated with members of the dominant rumen phyla (Firmicutes and Proteobacteria). CRISPRs were detected suggesting previous interactions between viral and microbial communities. Understanding how these CRISPRs adapt may lead to approaches for preventing the spread of antibiotic resistance, by reducing the load of antibiotic resistance plasmids that are already present.

3.4 THE CHICKEN INTESTINAL MICROBIOME AS A TARGET FOR IMPROVING PRODUCTIVITY

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The historical approach to improving production efficiency has involved changing the animal's genetics by selecting new animals with specific characteristics, improving the environment to reduce energy waste from thermoregulation, and improving the quality and digestibility of the diet. Germ-free animals have poor feed conversion compared to animals with a complex intestinal microbiome illustrating that the intestinal microbial community plays an important role in the energy partition of the host. With the exception of growth promoting antibiotics, attempts to manipulate the microbiome towards better feed efficiency have been inconsistent. Feed additives, such as prebiotics, enzymes, antibiotics, and organic acids can improve the feed:weight gain ratio assumedly by improving digestibility, absorption, or modifying the intestinal microbiota. In order to use bacterial communities as agents to stimulate feed efficiency, we must elucidate how components of the intestinal microbiome shape the host absorptive system. Emerging research technologies signal a convergence of molecular ecology and cell biology, providing new insights about the dialog between the host intestine and its microbiome and how these interactions have shaped their co-evolution.

3.5 IMPACT OF AGE AND INTESTINAL MICROBIOTA ON THE EXPRESSION OF AVIAN DEFENSINS IN THE CHICKEN GUT

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Defensins of birds belong to the large family of antimicrobial peptides that are key components of mucosal innate immunity. Gene expression of two major avian defensins (AvBD1 and AvBD2) in the chicken intestinal tissue is linked to the host protection against *Salmonella* colonisation. These antimicrobial peptides can be produced by granulocytes and by epithelial cells. They can be purified from chicken bone marrow and are active against a large panel of Gram+ and Gram- bacterial species. While intestinal expression of AvBD1 and AvBD2 can be observed at birth, how it evolves with age remains unclear. In order to assess the influence of the gut microbiota, we compared defensins genes expression profiles in conventional and axenic chicken intestinal tissues during the first two weeks of life. Kinetics of expression of AvBD1 and AvBD2 were different, independently of the microbiological status of the chicken gut. Interestingly, AvBDs expression level appeared lower in the small intestine of axenic chicken by comparison to conventional birds. The presence of a flora seems thus to positively influence the level of expression of AvBDs in the chicken gut. Future work will be devoted to the identification of commensal bacterial species that are beneficial for these antimicrobial peptides expression.

Session 3

Poster Presentations

3.6 EFFECTS OF A NOVEL PLANT EXTRACT ADMINISTERED THROUGH DRINKING WATER ON THE POST-WEANING GUT HEALTH OF PIGLETS AFTER EXPOSURE TO *E. COLI*

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The objective of the present study was to determine the effects of a novel plant extract (Grazix, LiveLeaf Bioscience, San Carlos, CA, USA) derived from common food plants on the performance and health of weaned piglets fed a mixed diet and then challenged with *Escherichia coli*. At weaning, a total of 144 piglets were allocated to two post-weaning rooms; half of the piglets received the novel plant extract (PE) in their water, the other half did not (control). On day 9 of the trial, half of the piglets were given a 4 mL solution containing 109 colony forming units of *E. coli* orally. The piglets' growth performance and fecal scores were recorded weekly. On days 0, 14, and 35, fecal samples were collected for microbiological analysis, while on days 0, 6, 19, and 35, blood samples were obtained from one pig per pen. At the end of the trial (day 35), 24 animals (12 from the control group and 12 from the PE group) were slaughtered and their distal ileum collected and examined in order to assess the ileal micro-anatomical structure, to perform histometry and immunohistochemistry, and to measure intestinal inflammatory parameters. When the data were analyzed, piglets given the PE supplement had an increased average daily gain during the last week of the study ($P=0.007$) and reduced feed conversion rate during the second ($P=0.009$) and last weeks ($P=0.04$), and over the entire study period ($P=0.01$) when compared to piglets in the control group. Also a lower fecal score was observed in the piglets given the PE solution ($P<0.01$). On day 35, fecal *E. coli* and Entrobacteriaceae concentrations were lower in animals given the PE when compared to the controls ($P=0.02$ and $P=0.009$, respectively). Ileal crypts from piglets in the PE group were deeper in *E. coli* challenged animals than in non-challenged ones ($P<0.05$), while the number of mucosal macrophages was higher in control piglets challenged with *E. coli* ($P<0.05$). The number of mucosal macrophages present in PE piglets challenged with *E. coli* was comparable to the number present in piglets that were not exposed to *E. coli*. Use of the PE supplement increased glutathione peroxidase plasma concentration at day 6 ($P=0.02$), lowered malondialdehyde value at day 6 ($P=0.07$), and increased total antioxygenic capability value at the end of the trial ($P=0.07$). In conclusion, the use of novel plant extract (Grazix) improved the gut lining and increased innate immune response.

3.7 EFFECTS OF CERAMIDE PRODUCED FROM PLANT GLUCOSYLCERAMIDE TO THE ALLEVIATION OF INFLAMMATORY BOWEL DISEASE AND GUT MICROFLORA

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a prevalent colonic disorder. Patients with UC and CD are at increased risk for developing colorectal cancer. Ceramide is an important bioactive substance to exert various effects on animals and is considered as the central hub of sphingolipid metabolism. Ceramide has been reported to modulate immune system pathways, and to induce apoptosis in cell lines derived from human colon cancer. Thus, ceramide might alleviate colon inflammation. In this study, we examined the effects of dietary ceramide on colitis induced by dextran sodium sulfate (DSS) in mice and gut microflora. To test the hypothesis described above, we initially prepared large quantities of ceramide from glucosylceramide (GluCer) by using the novel strain isolated in this laboratory (unpublished observation). GluCer is a precursor of ceramide and is found in plant food material. Ceramide was hydrolyzed by the isolated novel strain from partially purified GluCer from maize germ (Tsuji Oil Mill co., Ltd, Mie, Japan). Four-week-old male Jcl:ICR mice were used for this experiment. Mice were housed in plastic cages with wire tops, and permitted free access to sphingolipid-free diet. After three days of feeding, mice were fed sphingolipid-free diet supplemented with 0.1 % (wt/wt) ceramide for 14 days. Three days after the start of ceramide administration, DSS was added to drinking water for 11 days at a concentration of 2 % (wt/v). Ten mice were used in each group. After 14 days, mice were sacrificed by cervical dislocation under ether anesthesia for autopsy. Although the severity of IBD as expressed by the disease activity index (DAI) markedly increased with DSS administration, feeding a diet containing ceramide lowered the DAI value significantly. Myeloperoxidase (MPO) activity in colonic tissue also increased with DSS administration, suggesting the development of inflammation. Simultaneous administration of ceramide with DSS prevented the MPO activity increase, suggesting that ceramide could suppress the development of inflammation. DGGE analysis showed that the great shift in caecum bacterial populations was caused by the DSS addition. When ceramide and DSS were fed, the bacterial population pattern was different from those in the control or DSS addition, suggesting that dietary ceramide might affect gut microflora composition. These results suggest that dietary ceramide supplementation can alleviate the symptoms of IBD in mice.

3.8 LIVE BACTERIAL VECTORS FOR THE DELIVERY OF THERAPEUTIC PROTEINS TO THE GASTROINTESTINAL TRACT OF CHICKENS

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Antibiotics have been used in animal feed to control subclinical infections and improve productivity. It is desirable to have alternative ways of achieving these outcomes. One possible approach is to use compounds that provide the positive impacts of in-feed antibiotics without the potential negative impacts, such as development of antibiotic resistance in human microbiota. Effective delivery of alternatives, such as antimicrobial proteins and vaccine antigens for pathogen control and enzymes for productivity enhancement, can sometimes be problematic. We are addressing the issue by developing bacterial strains that can be used to deliver a diverse range of recombinant proteins to the gastrointestinal tract (GIT) of chickens. We have isolated and characterized commensal strains of bacteria that can be used to deliver therapeutic proteins, such as antigens, antimicrobial proteins, enzymes, cytokines and single chain antibodies, to the chicken GIT. One of our aims has been to develop strains that could be used for long-term delivery of therapeutics throughout the life of a chicken. Our approach has been to isolate a diverse collection of lactic acid bacteria and *Escherichia coli* strains from healthy chickens and then use the ability of the strains to reliably recolonize the GIT of innoculated chickens as a primary screen for the identification of potentially useful strains. Surprisingly the great majority of strains isolated from chickens are not able to consistently recolonize the GIT. From the hundreds of isolates tested we have selected just a few *E. coli* and *Lactobacillus* strains that can reliably colonize and persist, therefore have good potential as live vectors for prolonged delivery of therapeutics. The *Lactobacillus* strains efficiently colonize the upper GIT wheras the *E. coli* isolates more effectively colonize the lower GIT. We have demonstrated the utility of these vectors by delivering recombinant versions of chicken interleukin-6 (IL-6) and the antimicrobial protein Piscicolin 126 (P126) to the gut of chickens. IL-6 delivery resulted in an increase in immunoglobulin A secreting cells in the gut and P126 delivery reduced colonization of pathogenic *Clostridium perfringens*. The further development of such strategies offers the potential to provide effective alternatives to the use of in-feed antibiotics and may lead to the development of rationally designed probiotic strains.

3.9 YEAST DERIVATIVES AS NATURAL FEED ADDITIVES

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Yeast and its different derivatives have been used as natural feed additives for a long time. Different modes of action contribute to an improvement of animal health and performance. Several examples (pathogen binding, influence on gut morphology, immune modulation, and prebiotic effects of yeast in the rumen) will be illustrated by our experimental results. The recent development of a quantitative *in vitro* bacterial binding assay for yeast derivatives facilitated the optimization of yeast downstream processing to maximize binding capacity for pathogens. An autolysate of *S. cerevisiae*, enriched in cell wall fragments, represents a promising preparation with a high binding capacity for *Escherichia coli* and *Salmonella typhimurium* and was tested for its efficacy in broilers. Performance parameters were improved (10% higher body weight on d 35, P=0.05). In addition, a statistically significant increase (41%, P = 0.05) in goblet cell density in the jejunum was observed. The mucin produced by goblet cells is an important defense mechanism against invading pathogens. A different, nucleotide enriched yeast derivative also increased broiler performance (4% increase of weight on d 14 and of average daily weight gain d 1-14, P< 0.05). In vitro experiments suggest possible modes of action: nucleotides led to an increased transepithelial resistance of IPEC-J2 cells, supporting a positive influence on gut barrier function. Moreover, the NO production of a murine macrophage cell line in response to a bacterial lipopolysaccharide challenge was decreased, suggesting an anti-inflammatory effect. Finally, a simple in vitro rumen fermentation model was employed to compare four different yeast products (autolysed and hydrolyzed yeast, yeast culture, and live yeast). The autolysate led to a two-fold increase (albeit not statistically significant) in the number of total anaerobic colony forming units, suggesting a prebiotic effect on rumen microorganisms. Feeding the autolysate in 35 d periods to nine rumen cannulated heifers in three concentrations in a 3x3 Latin Square design resulted in a dose-dependent, statistically significant increase of urinary allantoin levels up to 45% (P= 0.04). In addition, the rates of in situ degradation of Tifton dry matter and neutral detergent fibre were increased (up to 33% P=0.07 and 48% P=0.06, respectively). Taken together, positive effects of different yeast derivatives on monogastric animals as well as on ruminants are shown *in vivo* and possible modes of actions are suggested by histology as well as in vitro experiments.

3.10 MICROBIOTA COMPOSITION DIFFERS ACCORDING TO CLASSIFICATION OF BROILER BY CAECAL SALMONELLA NUMBERS

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Introduction: In previous trials with experimentally *Salmonella* infected broilers we observed repeatedly that up to 20% of animals carry much less caecal *Salmonella* than the majority of birds. We hypothesized that initial variation of microbiota composition between individuals might contribute at least to some extend to the observed colonization inhibition.

Material & Methods: One group of 14 day old broiler chicks remained uninfected, while the second group was experimentally infected with a *Salmonella enterica* strain. Animals were sacrificed 7 days post infection (p. i.). Birds with the highest and lowest caecal *Salmonella* counts were assigned to a so called "moderate" and "low" *Salmonella* group (5.3 vs. 3.1 log cfu/g), respectively. Short chain fatty acid (SCFA) profile was determined, quantitative PCR was performed, %G+C profile analyzed and subsequently four characteristic %G+C fractions used for partial sequencing of 16S rRNA genes. In addition, comparable pools were subjected to an analysis by the CHICKChip, a newly developed chicken microbiota array.

Results: Challenged birds had significantly higher concentration of total SCFAs than the unchallenged birds. In the low *Salmonella* group propionic acid was significantly elevated, whereas in the moderate *Salmonella* group butyric acid was elevated. Quantitative PCR revealed that the number of 16S rRNA gene copies was not significantly different between the groups of birds as well as the total lactobacilli and clostridial cluster IV were significantly elevated in the challenged birds. In the unchallenged birds the major bacterial peak was at 46% G+C, whereas it was at a significantly higher and lower %G+C position in the low *Salmonella* and in the moderate *Salmonella* group, respectively. The majority of bacteria in the middle %G+C fractions belonged to the *Clostridium* cluster XIV, with no difference in abundance between the three bird groups. Nevertheless, the low *Salmonella* group showed a higher abundance of *Clostridium* cluster IV sequences and of three subgroups within cluster XIVa than the other two groups or birds. CHICKChip microarray profiling confirmed the enhanced relative abundance of cluster IV in the low *Salmonella* group.

Conclusions: We demonstrated a correlation between the microbial community structure and the *Salmonella* abundance. Future studies have to reveal whether the changed *Salmonella* numbers are due to changed microbiota composition and function or whether the extent of *Salmonella* colonization leads to these changes.

3.11 INVESTIGATION OF THE EFFECT OF ORGANIC ACID WATER TREATMENT ON COLONIZATION OF BROILER CHICKENS WITH CAMPYLOBACTER SPP DURING REARING AND THINNING

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A reduction in the contamination of poultry meat with *Campylobacter* spp. is a major objective of food safety authorities in Europe and elsewhere. The bacteria are non-pathogenic in poultry but some strains cause acute enterocolitis in humans. Although it is still unclear as to the routes of infection in poultry, increased numbers in the gastrointestinal tract are associated with the practice of 'thinning' birds during the latter stages of broiler production. During this process infection may be introduced into the sheds on the clothes, shoes, and hands of the catchers and on the transport crates. Colonization of the digestive tract by environmental sources of *Campylobacter* spp. relies upon the passage of the bacteria through the upper digestive tract to the large intestine, caecum, and cloaca. Evidence that *Campylobacter* spp. may be sensitive to particular organic acids suggested that the provision of these acids in the drinking water around the time of 'thinning' could aid in reducing colonization in the gut at this time. The trial was run in four identical broiler sheds on a single site. The birds in two of the sheds were treated with water containing a blend of organic acids (methionine hydroxyl analogue, formic acid, and propionic acid, Activate WD Max®) starting at 22 days until the end of the trial (Day 46). Thinning was carried out at Day 36. The other two sheds acted as 'control' with no dosing in the water. Apart from the organic acid treatment all birds were reared with the same management regime. *Campylobacter* spp. were monitored by taking bootsocks from the litter of all houses before 'thinning' and by sampling the caecal contents of 20 birds per shed at the abattoir at 'thinning' and at final clearance. None of the birds in any of the houses was colonized by *Campylobacter* spp. at thinning (< 10 cfu/g caecal contents) and all bootsocks were negative. At final clearance all 20 samples of caecal contents from each of the two control sheds showed very high numbers of *Campylobacter* spp. (log10 7.6-9.3 cfu/g). There was no significant difference between the two control sheds. Numbers of *Campylobacter* spp. in the caecal samples from the two treated sheds were inversely all below the level of detection (<10 cfu/g). These preliminary results indicate that the use of Activate WD Max® in drinking water over the 'thinning' period can reduce the incidence of colonization of the broiler large intestine by *Campylobacter* spp. resulting in a lower risk of transfer of the pathogen into the food chain.

3.12 FOOD AND FEED-RELATED PATHOGEN AND TOXIN BINDERS FOR AN IMPROVED GUT HEALTH

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Activated carbon is commonly used in the treatment of diarrhea and for detoxification purposes, because of its high absorption capacity. However, activated carbon does not discriminate between beneficial and harmful compounds and cells. Hence, one of the objectives of this study was to find dietary fiber-related, specific binders for enteropathogens and toxins to promote gut health. To study the adhesive capacity of different food and feed ingredients, miniaturized adhesion tests were developed for bacterial cells and AB5 toxins, such as the diarrhea-causing *Eschericia coli* heat-labile toxin LT and cholera toxin. The binding capacity of natural substances for bacterial cells was tested by allowing bacteria to adhere to different fibrous materials supplied as well coatings in microtitration plates. The amount of bacteria retained on the materials was determined in an automated way as growth after addition of liquid medium. The test principle was based on an inverse relationship between initial cell densities and the appearance of growth: The higher adhering cell numbers are, the shorter the detection times of growth. The interfering efficiency of natural substances with binding of the diarrhea-causing LT toxin and cholera toxin to the host receptor ganglioside GM1 was tested using an adapted GM1-coated microtiter-well ELISA. With growth as a measurement for bacterial adhesion, a simple, high-throughput method was developed for the screening of huge numbers of different binding matrices and bacterial species. The adhesion screening of different food and feed components for bacteria resulted in highly discriminating product rankings. Konjac gum, for example, was a good binding matrix for *Salmonella* strains, *E. coli* K88 adhered well to yeast cell wall material, and *E. coli* K99 to coffee grounds. Host receptor binding of LT and cholera toxin was most efficiently counteracted by skim milk powder and ground fenugreek seed. Employing the adhesion tests, we were also able to show that pea hulls bind *E. coli* K88 and bean hulls bind the ETEC's toxin LT, after a small-intestinal segment perfusion experiment with ETEC K88ac-challenged piglets had indicated that both pea and bean hulls have the potential for successful application in diarrhea prophylaxis and treatment.

3.13 SUCCESSFUL CONTROL OF SALMONELLA AND A MINIMIZED USE OF ANTIBIOTICS IN SWEDISH BROILER PRODUCTION BY LONG TERM IMPLEMENTATION OF DISEASE PREVENTATIVE METHODS WITH SPECIAL REFERENCE TO THE USE OF COMPETITIVE EXCLUSION (CE)

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The use of antimicrobials and the isolation of *Salmonella* are rare events in the Swedish broiler production. This is result of a long term implementation of disease preventive measures as alternatives to antibiotics for health management, in particular in the absence of antimicrobial growth promoters, and for the control of *Salmonella*. Apart from coccidiostats, antimicrobials, was e.g. in 2011 only used in 6 (0.02%) of 3185 commercial broiler flocks (approx.70 mill. birds) and during the last 16 years the average annual incidence of *Salmonella* infected flocks when tested before slaughter, was 0.2%, and 0.03% of *Salmonella* contaminated carcasses when tested after slaughter. The control of *Salmonella* in food animals was initiated some 60 years ago due to severe *Salmonella* epidemics including one of world's largest known outbreaks, in 1953 which involved more than 9000 sick and the death of 90 persons. Due to similar reasons, in 1970 producers initiated a specific control for broilers and since 1984 this is mandatory for all producers, who also pay its cost with the aid of insurance. Detailed rules for hygiene and management procedures and testing for *Salmonella* are formulated and the highest demands apply to hatcheries. Essential elements include prevention of introduction of *Salmonella* through feed and breeding animals as well as a high level of biosecurity at the farm level, and for e.g. during 1982-1988 only 12 out of 39 (30.8%) flocks of broiler-GP to be imported were found to be *Salmonella* infected. Since 1972, all broiler feed must be heat treated. HACCP based controls are in place in all feed plants, and corrective actions taken whenever *Salmonella* is isolated in the weekly samples. Compliance is ensured by bacteriological testing of all flocks 2 weeks before slaughter which is intended to detect a flock prevalence of *Salmonella* infected birds of > 5%. If any *Salmonella*, irrespective of serotype, is detected the flock is destroyed. In addition meat products contaminated by any serovar of *Salmonella* since 1971 are declared unfit for human consumption. Antibiotics have never been used to control or eliminate *Salmonella* infections in poultry or other food animals. CE was used during a critical period for the buildup of the current favorable *Salmonella* status. CE-culture (Broilact®) was found to be a valuable tool in particular to avoid reinfection in units where preceding flocks had been *Salmonella* infected and during periods when the probability for *Salmonella* contamination of feed was considered high. During 1981-1990, CE-culture was thus given on arrival of the chicks of 179 flocks (3.82 mill. chickens) in their initial drinking water. Only one of the flocks was found to be *Salmonella* infected. The virtually *Salmonella* free status of the hatcheries in Sweden are assumed to be contributing to this good result. However, a specific assessment of the salmonella controlling effect during a period when *Salmonella* was spread by contaminated feed, demonstrated that the CE-culture had an effect also under these conditions. During recent years the CE- culture has been administrated as a spray to chickens directly after hatch to prevent the spread of possible *Salmonella* infection from breeders and interesting studies are underway to assess its possible effect for reducing the spread of *Enterobacteriaceae* with transmissible resistance against extended-spectrum cephalosporins.

3.14 ALGINATE-WHEY PROTEIN MICROENCAPSULATION FOR TARGET DELIVERY OF HYDROPHOBIC ANTIMICROBIALS TO THE PIG INTESTINE

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The objective of current study was to develop a novel encapsulation technique for targeted delivery of hydrophobic antimicrobial agents, such as carvacrol, to the lower region of the small intestine of pigs. Carvacrol was encapsulated in alginate-whey protein microcapsules by an emulsion-extrusion technique. Response surface analysis was used to optimize encapsulation formulation. The release profiles of the microcapsules were tested in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and ileal digesta (ID) from growing pigs at 37°C. The carvacrol retention rate was over 98% and average carvacrol content of dry microcapsules was 71.8% ±0.5% (w/w). Depending on the encapsulation formulation, a wide spectrum of release profiles was obtained. All the microcapsules remained intact after 1 hour incubation in SGF, but a small amount of carvacrol, ranging from 10.7± 0.9% to l7.8 ±0.4% depending on the formulation, was released. However, the encapsulated carvacrol was completely released within two hours incubation in SIF. When incubated with ID, no intact microcapsule was found after 210 minutes, indicating a complete release of carvacrol. An *in vivo* study was then conducted to investigate the release characteristics in the pig gastrointestinal track. An encapsulated carvacrol having a medium release rate was selected and thirty purebred Yorkshire pigs (male:female=1:1; initial body weight 11.9±0.9 kg) were used for the experiment. The free form (as a control) and encapsulated carvacrol were mixed with the feed at 1,500 ppm of carvacrol. Ti_2O_3 (0.2% w/w) was added to the feed as an indigestible marker to monitor the absorption of carvacrol, i.e. cumulative carvacrol disappearance from the gastro-intestinal lumen. The pigs were fed the experimental diet for 7 days prior to sampling. On day 8, pigs were fed 800 g of experiment diets in a single meal after a 16 hour fast. Animals were euthanized at 4, 5, and 6 hour postprandially. Cumulative absorption of free carvacrol at these three sampling points were 58.1±8.2%, 63.2 ±10.0%, 82.4±4.0% in the stomach and 66.2±21.1%, 70.6±12.2%, 95.1±0.2% in the duodenum. In contrast, encapsulation reduced the cumulative absorption in the stomach to 22.8±13.8%, 25.0±8.5%, and 48.5±2.7%, and in the duodenum to 28.7 ± 9.1%, 31.8 ±12.7% and 53.8±8.7%, respectively, which are significantly lower than the values of free carvacrol ($P<0.0001$, $P<0.0001$, $P=0.0011$ for 4, 5, 6 hour respectively). The cumulative absorptions of carvacrol were 43.6±14.4%, 48.9±3.8% and 75.7±3.8% in the jejunum for encapsulated carvacrol treatment. The current study indicates that microencapsulation is a potential tool to increase the amount of hydrophobic antimicrobial agents to be delivered to the lower region of the intestine of pigs to protect their antimicrobial activity. Further research is undertaken to improve the encapsulation technique for maximizing the delivery of carvacrol to the lower intestine region.

3.15 INFLUENCE OF AN ACIDIFIER ON CECAL CONTENT MICROFLORA AND BROILER GROWTH PERFORMANCE

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Antibiotic growth promoters (AGPs) have been used in animal production worldwide since 1946 when their positive effects were first observed. AGPs have been added to animal feed at subtherapeutic levels to increase growth, improve feed efficiency, and decrease the incidence of diseases caused by bacterial pressure, which has resulted in the emergence of antibiotic resistance. The development of alternative products is therefore necessary to eliminate the use of AGPs while achieving the same productivity. Organic acids as alternatives to AGPs have increasingly and successfully been supplemented in feed in broiler production. However, the efficacy of organic acids can be improved by combining them with phytochemicals and permeabilizing substances (PS). For example, the phytochemical cinnamaldehyde (CA) targets the FtsZ protein, which plays an important role in the cell division of pathogenic bacteria, whereas permeabilizing substances damage the outer membrane of Gram-negative bacteria, thereby boosting the combined antimicrobial effect of organic acids and the phytochemical. An experiment was conducted to study the effects of dietary supplementation with a natural growth promoter (NGP) consisting of a blend of organic acids, CA and PS (Biotronic® Top3, BIOMIN, Austria) in a diet based on corn-soybean meal on growth performance and cecum microflora. The trial was conducted in a commercial broiler farm in Jiangsu Province, China. Four hundred day-old healthy AA broiler chicks were randomly assigned to two treatments with four replicates in each treatment and fifty broilers (half male and half female) in each replicate. A negative control group received no APG or NGP, whereas a trial group received 1 kg NGP based on formic, propionic and acetic acids, CA and PS, per ton of feed. The feeding trial lasted for 42 days. Eight animals from each group were randomly selected and slaughtered at 21 and 42 days of age. The counts of *E. coli, Salmonella, Clostridium perfringens*, and *Lactobacilli* in cecal content were analyzed. The results showed that NGP significantly increased the final weight and average daily gain and decreased the feed conversion ratio ($P<0.05$). The NGP significantly increased the amount of *Lactobacillus* ($P<0.01$), and decreased the amount of *E. coli, Salmonella* and *Clostridium perfringens* in the cecum ($P<0.05$). These results indicated that the compound acidifiers improved growth performance in broilers by changing the intestinal micro-ecological environment.

3.16 FERMENTATION COMBINED WITH ENZYME SUPPLEMENTATION – A STRATEGY TO IMPROVE BOTH NUTRITIONAL VALUE OF RAPESEED CAKE AND GASTROINTESTINAL HEALTH OF PIGS?

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Fermented liquid feed (FLF) has been used in the pig industry for many years. Due to its antimicrobial properties, FLF has gained interest in relation to the search for alternatives to antibiotic growth promoters. The antimicrobial properties of FLF are obtained during the fermentation process. In the initial phase of fermentation, a bloom in the number of *Enterobacteriaceae* occurs, which is replaced by a proliferation of lactic acid bacteria. The latter results in an increased lactic acid concentration with a consequent low pH in the FLF. The combination of these two factors reduces the number of *Enterobacteriaceae* in the mixture. Rapeseed is an important oil- and protein crop in Denmark, and its production is expected to expand in the coming years due to the political agenda in EU on increasing biofuels in the transport sector. Rapeseed cake contains protein of high nutritional quality. However, there is a limit to its use in animal feed due to the content of anti-nutrients such as glucosinolates, phytate and dietary fibres. Addition of enzymes (phytases and carbohydrases) during fermentation of liquid feed based on rapeseed cake was tested as a strategy for improving the nutritional value of this feed ingredient, and at the same time keeping the antimicrobial properties of FLF. *In vitro* experiments with rapeseed cake were carried out in bioreactors with a volume of 1L to test the impact of fermentation and addition of various enzymes on the content of total-, soluble- and insoluble non-starch polysaccharides (NSP), protein solubility and glucosinolates.
The results showed that some enzymes increased protein solubility during the initial 48 h of fermentation. Also some of the enzymes reduced the level of insoluble NSP of rapeseed cake. The enzyme mixture (glucanase+xylanase) had the clearest effects on fibre degradation including a reduced recovery of total NSP. This enzyme mixture was selected and used in an *in vivo* digestibility study with pigs. Eight pigs were cannulated at the distal *ileum* and fed a complex diet rich in rapeseed cake fermented and non-fermented. Results on microbial composition in *ileum* and *fece*s showed lower *Enterobacteriaceae* numbers in both sites ($P < 0.05$) of pigs fed the FLF compared to those fed non-FLF, indicating a beneficial effect of FLF on gastrointestinal health of the animals.

3.17 SPORE FORMING PROBIOTIC *BACILLUS SUBTILIS* C-3102 IN *PIG* AND *POULTRY* DIETS – A REVIEW

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Since the EU-ban of antimicrobial growth promoters the interest in the use of probiotics to support the micro biota is increasing. Probiotics are living cultures of non pathogenic strains of bacteria and yeasts which are able to influence the microbiota in the intestine of the host animal in a positive way (Fuller 1989). Most known probiotics however, are not able to survive applied technology in feed production, e.g. heat administration. For practical use compatibility with feed supplements such as organic acids and coccidiostats is important too. One way to overcome these problems is the use of spore forming probiotics, particularly *Bacillus subtilis*. For the use of *B. subtilis* in monogastric diets various modes of action are underlying. *B. subtilis* consumes oxygen in the digestive tract and produces also various enzymes such as catalase and subtilisin. As a result the environmental conditions for beneficial bacteria such as lactic acid bacteria improve. These colonize the intestinal wall and block the binding sites for pathogenic bacteria, a process called competitive inhibition. In addition, lactobacilli produce lactic acid, which acts against salmonella, E. coli, campylobacter and clostridia (Hosoi et al. 2000). The effects of B. subtilis C-3102 (Calsporin) on pathogens in broilers have been demonstrated in several trials with the outcome of a reduction of salmonella, campylobacter, E. coli and Clostridium perfringens (Maruta et al. 1996; Fritts et al. 2000; La Ragione, Woodward 2003).
In broilers a reduction of positive birds for campylobacter infections was reported (100% in control to 40% of birds fed *B. subtilis*) and when B. subtilis was fed longer infection rate was reduced to only 16% positive birds (Maruta et al. 1996). In turkey *B. subtilis* C-3102 resulted in significantly reduced ammonia concentrations in the faeces (7.80 vs. 25.2 ppm in the control (Blair et al. 2004). In a piglet challenge trial the effect of *B. subtilis* C-3102 on gut microbiota was compared with an antibiotic treatment after challenging the piglets with *E.coli* K88. The results show that antibiotics lower numerically the amount of *E. coli*, but at the same time also lower the amount of lactic acid bacteria. The *B. subtilis* C-3102 group also numerically lowered the amount of *E. coli* but increased the level of lactic acid bacteria (significantly higher compared to antibiotic). Both the antibiotic group and the *B. subtilis* C-3102 group showed improved faeces score and lower mortality in comparison to control group (Bhandari, 2008). EU efficacy studies in both piglets, broilers and turkeys show that inclusion of *B. subtilis* C-3102 significantly improves performance compared to a control treatment (piglets 30ppm inclusion: ADG +3.8%, FCR -5%; broilers 50 ppm inclusion: ADG +1.6%, FCR -2.6%) . These results are confirmed in practical trials with broilers all over Europe (total 9 trials in 5 different countries with in total approximately 2.000.000 birds). Summarized, spore forming probiotics can survive heat treatment and are compatible with feed agents like organic acids and coccidiostats. *B. subtilis* C-3102 can be successfully used in pig and poultry diets to ensure good gut health and improve performance.

Session 4
Alternatives to Antibiotics to Promote Growth in Livestock, Poultry, and Aquaculture Production

Oral Presentations

4.1 PERSPECTIVES FROM OUTSIDE THE BOX: THE USE OF PHYTONUTRIENTS FOR OPTIMIZING GUT HEALTH AND PRODUCTIVE EFFICIENCY OF LIVESTOCK AND POULTRY ANIMALS

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Considering the predicted increase in the human population worldwide, it is not surprising that the role of animal nutrition has become a critical focus in the development of strategies for ensuring that sufficient amounts of food from animal origin can be produced. Although nutritional interventions have proven highly successful in improving production efficiency, there have been some major changes in the animal industry during the past decade that has forced producers and scientists to develop alternative approaches. For example, governmental restrictions on the use of antibiotic growth promoters in animal production have presented new challenges with respect to animal growth, health, and production efficiency, as well as food security for the human population. Agricultural scientists in animal health and production must continue to develop alternative strategies to improve production efficiency while also mitigating the negative effects of infectious diseases. This has prompted an interest in the development of drug-independent growth promoting strategies, such as phytonutrient-based feed additives. However, these alternative approaches are often received with much skepticism due to limited understanding on their mode of action.

The objectives of this presentation are to present the results of field studies examining the efficacy of selected phytonutrients, and to describe the current state of knowledge regarding the basic mechanisms by which phytonutrients elicit changes in animal health and production. For the first objective, a meta analysis from 13 selected field trials showed that phytonutrients elicit a consistent improvement in animal growth and production efficiency compared with conventional antibiotic growth promoters, and revealed that the degree to which phytonutrients improve animal performance is highly dependent on the environmental conditions in which they are used. With regards to the second objective, to better understand the mechanism by which phytonutrients exert their effects, it is necessary to recognize the intestine as a highly complex communicating organ capable of nutrient sensing by enteroendocrine cells and the enteric nervous system. Further, there exists a continuous cross-talk between the gut mucosal immune system and the gut microbiota, which is a major driver of host health and homeostasis. Therefore, the current view of the gut needs to be updated so that it is described not simply as a digestive and absorptive tube, but as an organ that regulates growth and production efficiency. As we improve our understanding of the role the gut plays in animal production, we can begin to think outside the box as we develop novel strategies and technologies for improving gut function, and consequent animal health and production.

4.2 ALTERNATIVES TO ANTIBIOTICS AS GROWTH PROMOTANTS FOR DAIRY AND BEEF CATTLE: MECHANISMS OF ACTION AND FIELD PERFORMANCE

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The use of antibiotics in ruminant diets has been very effective in improving nutrient utilization, reducing emission of pollutants to the environment, and controlling the incidence of acidosis and bloat in cattle. However, regulatory restriction of in-feed antibiotics growth promoters (AGPs) in the EU (Directive 1831/2003/CEE) resulted in a 3.5-5.0% increase in production costs and industry needs AGP alternatives. The objectives of rumen modulation are to increase propionate production and reduce acetate, methane, lactic acid, and ammonia-N production. There are two main strategies available: a) To promote the growth of specific bacteria with direct fed microbials (DFM) or organic acids. DFM are live or cultures of *Saccharomyces cerevisiae* or *Aspergillus oryzae*. Most of these products increase the total number of anaerobic, cellulolytic, fungi, and lactic acid utilizing bacteria, resulting in the stabilization of rumen fermentation. The increase in DM intake fully justifies the increase in fat corrected milk (average around 0.7 to 1.0 kg/animal/day). Some organic acids (malic acid) also induce changes in ruminal pH, methane production, and/or VFA profile through the stimulation of specific ruminal bacterial species. *In vivo* studies have shown some beneficial effects on rumen fermentation and pH, and improvements in performance in dairy and beef cattle, but this method is not economical due to high dose requirement, and b) To inhibit the growth of specific bacterial groups with plant derived products or polyclonal antibodies. Some active components of plant extracts are lipophylic molecules with antimicrobial activity and, therefore, the careful selection of those with desired effects is critical. *In vitro* studies have proved their effectiveness, but only few products have shown effects *in vivo*, including garlic oil derivatives, cinnamaldehyde, eugenol, capsaicin, oregano, and anise oil. Data supporting their production performance is still limited, and further research in this area is warranted. A different approach has been the use of immunization with antigens of rumen bacteria. Immunoglobulins are transferred to the rumen through saliva where these bacteria are neutralized by antibodies. A vaccine against S*treptococcus bovis* and *Lactobacillus* was tested for its efficacy to control lactic acidosis in cattle. The vaccination decreased bacterial counts and rumen lactate concentration. Oral treatment of polyclonal antibodies against *S. bovis* and *Fusobacterium nechrophorum* reduced bacterial counts and improved ruminal pH, average daily gain and feed efficiency. Many new alternatives to antibiotics are becoming available to regulate rumen function. The wise selection of different additives with synergistic activities may further enhance these effects. However, any effect on rumen microbial fermentation will only justify its use when animal performance studies are conducted. The number of published research on the effects of these additives on cattle performance is surprisingly low.

4.3 INTESTINAL MICROBIOTA ASSOCIATED WITH HIGH FEED CONVERSION EFFICIENCY IN CHICKENS

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Low-dose in-feed antibiotics have been used in poultry industries as “growth promoters” to improve flock health and productivity. The use of pre- and probiotics may provide effective and more acceptable treatments to promote heath and productivity. To develop such treatments in a rational manner it is useful to have a greater understanding of the microbiota present in the gastrointestinal tract of production chickens. This study investigated microbiota that was associated with differences in feed conversion ratios (FCR) of broiler chickens. FCR is a very important indicator of production efficiency and profitability. Our experimental approach was to measure the FCR of individual chickens within a single flock and then correlate differences in microbiota between the high and low performance birds. The structure of the microbiota of each bird was assessed by pyrosequencing of 16S rRNA amplicons. Over a series of four trials, carried out at different times in the same facility, we aimed to replicate the same feeding and environmental conditions for the different flocks. Notably, we found that the intestinal microbiota was significantly different across the four flocks and even within a flock there was a surprising level of bird to bird variation in the population structure of the microbiota. We postulate that the high degree of difference in microbiota between trials, and even within a trial, is due to the dominant role that the initial bacterial colonization process, immediately after hatch, has on the lifetime structure of the microbiota. In each flock we found that specific bacterial groups were in differential abundance between the high and low performance birds. Some of these bacteria correlated with differences in performance across several trials, despite the significant differences in overall microbiota structure between trials. It may be possible to develop bacterial isolates associated with highly efficient energy use as probiotics to enhance bird productivity.

4.4 CINNAMALDEHYDE ENHANCES IN VITRO PARAMETERS IF IMMUNITY AND AUGMENTS IN VIVO PROTECTION AGAINST AVIAN COCCIDIOSIS

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Cinnamaldehyde (CINN) is a constituent of cinnamon (*Cinnamomum cassia* Presl (Lauraceae)) that is widely used as a flavoring compound and, to a lesser extent, has been traditionally used to treat human diseases, including dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases. CINN was reported to possess antifungal, antipyretic, antioxidant, antimicrobial, and larvicidal activities, as well as to modulate T-cell differentiation. At the physiological level, CINN protects the intestinal microvilli, which are responsible for the absorption of nutrients. Dietary feeding of CINN along with carvacrol and capsicum improved chicken ileal and fecal digestibility, but on the other hand did not improve body weight gain or feed efficiency. Our previous microarray study showed that feeding of CINN to chickens altered the expression of 62 genes (31 upregulated, 31 downregulated) in intestinal intraepithelial lymphocytes. Therefore, the current investigation was performed to evaluate the effects of CINN on *in vitro* parameters of immunity and to assess its ability to enhance protection against avian coccidiosis *in vivo*. *In vitro* stimulation of chicken spleen lymphocytes with CINN induced greater cell proliferation compared with the media control. CINN activated cultured macrophages to produce higher levels of nitric oxide, inhibited the growth of chicken tumor cells, and reduced the viability of *Eimeria* parasites compared with media controls. *In vivo* experiments demonstrated that CINN-fed chickens showed 10-30% increased body weight gains following challenge infection with live parasites of *E. acervulina*, *E. maxima*, or *E. tenella* compared with birds fed a standard diet alone. CINN-fed chickens produced higher levels of IgY serum antibodies against coccidia parasites compared with the control group. Finally, the levels of IL-1β, IL-6, IL-15, and IFN-γ transcripts produced by intestinal lymphocytes were 2- to 10-fold higher in CINN-fed chickens compared with controls. This study provides the first evidence that CINN enhances immunity and protects chickens against experimental coccidiosis.

4.5 EFFECT OF DIETARY PROTEIN AND PROTEASE SUPPLEMENTATION ON PERFORMANCE AND GUT HEALTH OF BROILER CHICKS

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The effect of dietary protein level and protease supplementation on performance and gut health was evaluated in two studies. For study 1, 288 broilers were used to examine the effect of normal crude protein (CP) vs high CP (22% vs 30%) without or with protease (CIBENZATM DP100, Novus International Inc.) supplementation in a 2 X 2 factorial arrangement. Each test diet was fed to 9 replicate pens of 8 birds from 0 to 28 days. All diets contained 20% rye and 25% wheat, high CP diets had 14% poultry meal, and all birds were given a cocci challenge (10X immunizing dose) on day 7. In the absence of protease, increasing dietary CP increased ileal *Clostridium perfringens* by 2 logs (2.35 vs 4.34) and with protease supplementation no CP effect was seen (2.09 and 2.30 for normal and high CP), accounting for a significant interaction. Protease was also associated with increased growth efficiency in the gut and reduced systemic inflammation demonstrated by improved crypt villus ratio and lower serum α-1 glycoprotein level. For study 2, three corn soy DDGS based diets - normal CP, low CP (7% less), and low CP + protease, were fed to birds under two stress conditions- normal (21 birds per pen) and stress (8-hr feed outage on day 0 and day 14, 25 birds per pen). Each test diet was fed to 8 replicate pens. Under the normal condition, body weight was not affected by dietary CP at d 14 and 27 whereas under the stress condition, birds fed normal CP actually weighed less. Under the normal condition, broilers on normal CP had better 0-14 days FCR than those on low CP, but under the stress condition, no significant difference was observed. Protease improved FCR throughout the trial and the response tended to be greater under the stress condition from 0 to 14 days. In summary, undigested protein in the gut either from excessive CP supply or comprised gut function by stress could cause gut dysbacteriosis by promoting *Clostridium perfringens* growth and reduce performance and protease can alleviate these negative effects through improving protein digestion in young broilers.

4.6 IDENTIFICATION OF BILE SALT HYDROLASE INHIBITORS, PROMISING ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTORS

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Antibiotic growth promoters (AGP) have been used as feed additives to improve average body weight gain and feed efficiency in food animals for more than five decades. However, there is a worldwide trend to limit AGP use to protect food safety and public health, raising an urgent need to discover effective alternatives to AGP. Previous studies have shown that the growth promoting effect of AGP is highly correlated with the decreased activity of intestinal bile salt hydrolase (BSH), an enzyme that is produced by various gut microflora and involved in host lipid metabolism. Thus, BSH inhibitors are likely promising feed additive to replace AGP for improving animal growth performance. In this study, the genome of *Lactobacillus salivarius* NRRL B-30514, a BSH-producing strain isolated from chicken, was sequenced by 454 GS FLX sequencer. Sequence analysis identified two putative bsh genes. His-tagged recombinant BSH of one bsh was produced for enzymatic analyses. The BSH displayed hydrolysis activity for both glycoconjugated and tauroconjugated bile salts. The optimal pH and temperature for the BSH activity were 5.5 and 41^{o}C, respectively. Screening of a panel of dietary compounds identified some potent BSH inhibitors, such as copper, that has recently been demonstrated to promote feed digestion and body weight gain of different food animals. Together, this study identified and characterized a BSH with broad substrate specificity from a chicken *L. salivarius* strain, and strongly supported our hypothesis that BSH inhibitors are promising alternatives to AGP for enhancing the productivity and sustainability of food animals.

Session 4

Poster Presentations

4.7 POTENTIAL OF BUTYRATE GLYCERIDES AS AN ALTERNATIVE TO DIETARY ANTIBIOTICS: A MECHANISTIC STUDY WITH BROILERS

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Butyrate plays an important role in animal gut health and has been shown to reduce *Salmonella* infection to broilers. Although very efficacious and possible to substitute for dietary antibiotics, pure butyrate has obvious limitations of smell and handling and is virtually absorbed in the upper digestive tract. Butyrate glycerides have no such limitations and their butyrate can be released by lipase in the small intestine, thus providing a novel delivery system to the chicken gut. Recently, we reported the inhibition of butyrate glycerides to the growth of *Salmonella* and *Clostridium perfringens in vitro*. We also observed through a chicken feeding study that dietary supplementation with 3,000 ppm of butyrate glycerides was able to significantly improve the feed efficiency ($P< 0.05$) towards young birds (up to 20 d) compared to both non-medicated birds and those fed virginiamycin. We speculated that the effect was achieved through energy redistribution. To verify, we have conducted a second chicken trial with a similar design, but reduced numbers of chickens. Forty newly hatched chicks were equally divided into two groups: 1) on a basal diet (Control); 2) on a basal diet supplemented with butyrate glycerides (3,000 ppm each; 0-7 d: Baby C4 + Mono C4, 7-20 d: Mono C4; SILO, Industria Zootecnica). On d 21, all chickens were weighed before feeding and then euthanized. The synthesis of abdominal and mesenteric fat as well as the weight and length of small intestine were measured. RNAseq analysis was used to determine the gene expression profiles of the liver and jejunum. The dietary butyrate glycerides increased the body weight, the ratio of body weight to abdominal and mesenteric fat, small intestine weight, and small intestine length by 15.12%, 11.80%, 29.84%, and 15.37% ($P< 0.05$), respectively. The data from the transcriptome analysis showed that 88 and 231 genes were differentially expressed in the liver and jejunum, respectively, in response to the treatment of butyrate glycerides. Among them, 9 genes in the liver and 23 genes in the jejunum were involved in the signaling pathways relating to lipid and carbohydrate metabolism. These results suggest that butyrate glycerides can regulate energy redistribution and beneficially reduce lipid deposition in young chickens.

4.8 EFFECTS OF ALL-LAC™, ACID-PAK™, AND ACTIGEN™ ON BROILER CHICKS CHALLENGED WITH INTESTINAL HOMOGENATE OR LITTER FROM RUNTING-STUNTING SYNDROME POSITIVE BROILERS

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Six consecutive 12-day experiments were run to determine whether a combined treatment (All-Lac XCL 5X at 0.3 mL orally per chick, Acid-Pak 4Way 2X 2 g/L water for 5 days, Actigen 400 g/metric ton of feed) could affect the outcome of runting-stunting syndrome (RSS) during the starter period. Day-old broiler chicks were treated or not treated and either inoculated with intestinal homogenate from RSS-positive (RSS+) broilers, or exposed to RSS+ contaminated litter, or not (2x2 factorial arrangement of treatments; individual birds were experimental units). Exp. 1, 2, and 3 were identical with 150 chicks in each of the 4 colony houses and simply repeated the protocol consecutively on either fresh or used (RSS+) litter. Mean 12-day body weights in grams (100 birds/colony house) for the first 3 trials were (LSD; $P<0.001$): RSS-/untreated 239.7^{b}, RSS-/treated 262.0^{a}, RSS+/untreated 166.8^{d}, and RSS+/treated 188.6^{c}. This indicated beneficial effects due to treatment. Following Exp. 3, 30 birds per treatment were continued to market age, and chickens in the RSS+/treated group had significantly lower ($P=0.006$) body weight at 91% whereas the chickens in the RSS+/untreated group had 87% of the weight of the RSS-/untreated control birds ($P<0.001$). The RSS+/untreated group had 25/89 birds with intestinal cysts (mean 2.95 cysts) whereas the RSS+/treated group had 18/90 birds with intestinal cysts (mean 2.44 cysts). In the second series of three 12-day experiments, 150 chicks were placed in each of 3 of the same colony houses with either fresh wood shavings, RSS+/Exp. 1-3 treated (Exp. 4-6 untreated), or RSS+/Exp. 1-3 untreated (Exp. 4-6 treated) birds and litter. Recycling RSS+ infected litter during Exp. 4, 5, and 6 appeared to overwhelm the treatments which had been beneficial during the first 3 trials. The 12-day mean body weights in grams for experiments 4-6 were ($P<0.001$): RSS- 252.6^{a}, RSS+/Exp. 1-3 treated (Exp. 4-6 untreated) 165.3^{b}, and RSS+/Exp. 1-3 untreated (Exp. 4-6 treated) 153.3^{c}. Birds with intestinal cysts and mean number of cysts by treatment were 3/90 (0.67), 32/92 (2.29), and 38/90 (2.33). The 42-day mean body weights in grams of 30 birds per treatment were $2{,}541^{a}$, $2{,}161^{b}$, and $2{,}188^{b}$, respectively. An intervention strategy by treatment during an RSS outbreak without changing the husbandry but using the combination treatment was not effective after 4 to 6 flocks on RSS+ litter but did give significant body weight improvement during the first 1 to 3 flocks.

4.9 EFFECTS OF ACTIGEN™ ON PERFORMANCE AND THE OCCURRENCE OF ANTIBIOTIC RESISTANCE IN FATTENING PIGS

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The aim of this study was to investigate the effect of Actigen (unique bioactive fraction derived from yeast cell wall) on the performance of growing pigs and on antimicrobial resistance patterns of fecal *Escherichia coli* isolates. The trial was conducted under commercial conditions on five farms and the inclusion level of Actigen was 400g/T. Three groups of animals were used in the evaluation: 1) control: finishing herds (3 months before starting the use of the dietary treatment); 2) Actigen partial: finishing herds (3 months after starting the use of Actigen); and 3) Actigen: finishing herds (4 - 7 months after starting the use of Actigen). Body weight gain, feed intake, feed conversion ratio (FCR), and mortality were evaluated. In addition, *E. coli* and *Salmonella* sensitivity to antibiotics was followed over time. The feeding of Actigen led to a numeric increase in average daily gain (ADG) from 796 g to 835 g. The FCR was 2.70 in the control group and 2.67 in group 3, Actigen. On 4 out of 5 farms the Actigen treatment led to a strong reduction in the percentage of Colistin and Enrofocacin resistant *E. coli* strains. However, on one farm this reduction was not observed. Overall the dietary treatment improved the *Salmonella* status of the farms. While at the beginning of the experiment all farms were *Salmonella* positive, at the end only one farm was positive. The observed reduction in antibiotic resistant isolates of *E.coli* is of great interest and merits more detailed investigations.

4.10 THE EFFECT OF ACTIGEN™ ON POST-WEAN PIG PERFORMANCE COMPARED WITH AN ANTIBIOTIC GROWTH PROMOTER

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This study was done to determine the effect of Actigen compared with Colistin-Amoxicillin on the performance of newly-weaned pigs. A total of 120 mixed sex, Large White-Landrace x Pietrain- Duroc pigs averaging 7.01±0.57 kg were randomly assigned to either Colistin-Amoxicillin or Actigen groups. The feeding duration was for 34 days. Performance data were analyzed using MS Excel Statistical Analysis package. Final weight, growth rate, daily feed intake, mortality %, and scouring % was the same for both treatments ($P>0.05$). The cost of injectable medication was the same for both groups. However, FCR in the Actigen group tended to be better than in the Colistin-Amoxicillin group ($P=0.07$). In-feed medication cost per pig was lower with Actigen group compared with the control ($0.10 vs $0.78, resp.) This study shows that Actigen performs similarly with colistin-amoxicillin in post-wean pigs up to 64 days of age and is a better option cost-wise.

4.11 META-ANALYSIS SUMMARY OF BROILER CHICKEN PEN TRIALS WITH DIETARY ACTIGEN™ (2009-2011)

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Statistical meta-analyses of results from broiler trials in 2009-2011 using dietary Actigen were conducted to estimate beneficial effects on live performance. Actigen is a unique, second-generation bioactive fraction derived from yeast cell wall considered to be a "growth permitter" through its roles in immune modulation and improved intestinal health. Parameters evaluated were dietary inclusion rates for Actigen, age of birds, body weight, feed conversion ratio or feed/gain ratio, and mortality %. Nine reports were collected allowing 15 comparisons of negative control diets and Actigen supplemented diets fed during the entire trials. Similarly, 9 reports were collected allowing 11 comparisons of positive control (antibiotic supplemented) diets and Actigen supplemented diets. When added to basal diets, Actigen at average inclusion rates by phases of 520/400/347 g/metric ton (n=15) and broiler age of 41.87 days (n=15) significantly improved body weight by +0.129 kg (+5.41%), feed conversion ratio or feed/gain ratio by -0.046 (-2.54%), and mortality % by -0.76 (-10.5% relative to negative control). Compared with positive control (antibiotic) results, dietary Actigen at average inclusion rates by phases of 535/331/238 g/metric ton (n=11) and broiler age of 43.64 days (n=11) nonsignificantly changed body weight by +0.016 kg (+0.62%), feed conversion ratio or feed/gain ratio by -0.003 (-0.17%), and mortality % by +0.57 (+7.97% relative to positive control). Broiler live performance results for antibiotic or Actigen supplemented diets were statistically equivalent. Comparison of these Actigen meta-analysis results with holo- (82 comparisons) and meta-analysis (44 comparisons) results obtained previously using data from feeding trials with the yeast cell wall product Bio-Mos® from which it was derived suggest that the second generation product Actigen may be more effective in terms of growth promotion.

4.12 EFFECT OF THE DIETARY SWEET WORMWOOD (*ARTEMISIA ANNUA*) ON IRON (FE) STATUS IN WEANED PIGLETS

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The aim of this study was to evaluate the effect of sweet wormwood supplements on iron status in weaned piglets. Sweet wormwood (*Artemisia annua*) is an herbaceous plant from the family of Asteraceae/Compositae. Besides the main active substance, artemisine, the sweet wormwood is also a natural antibiotic. An experiment was conducted on 39 weaned Landrace × Large White piglets, in the presence of a mineral deficit. The chemical composition of the sweet wormwood was established and it was used in piglet's diets in a dried form. The piglets were assigned to 3 groups (C, E1 and E2) and received the same corn-sunflower soybean meal diet, however, with different mineral premixes: the diet for C group contained 1% vitamin-mineral premix IBNA Balotesti. The diet for group E1 contained 1% vitamin-mineral premix in which the salts of Cu and Zn were reduced by 50 compared to the standard formulation (C) plus 1% sweet wormwood. The diet for group E2 contained the same premix as E1 plus 2% sweet wormwood. The Fe content was determined by FAAS in the samples (weekly samples/piglet) of ingesta, faeces, and urine. The Fe concentrations in feeds: C – 271.22 mg/kg; E1 – 239.00 mg / kg; 253.53 mg / kg. Animal performance indicators recorded at the end of the experiment (final weight: C – 28.89 g/day; E1 – 29.11 g/day; E2 – 30.15 g/day) and the blood count (haemoglobin concentration: C – 9.3 g/dL; E1 - 9.04 g/dL; E2 - 9.14 g/dL; haematocrit concentration: C - 34.28%; E1- 33.92%; E2 – 34.72%) showed that the dietary sweet wormwood (with antimicrobial action) replaced the deficit of Fe, maintaining the physiological state of the animals within the normal parameters for that particular category.

4.13 ZOOTECHNICAL IMPROVEMENT IN WEANED PIGS BY APPLICATION OF THE PHYTOGENIC FEED ADDITIVE FRESTA®F

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Phytogenic feed additives are plant-derived products, comprised of herbs and spices and their extracts. They are increasingly used in animal nutrition because of their beneficial biological effects, such as support of digestive and immune functions, which lead to improved performance in livestock production. Recently, FRESTA® F (Delacon, Austria) was registered as the first phytogenic, zootechnical feed additive for piglets according to EC directive 1831/2003. FRESTA® F, a mixture of essential oils of caraway and lemon, spices and herbs (carvone content 0.35%), is designed for the special needs of piglets after weaning. The product enhances nutrient digestion by stimulating the secretion of digestive enzymes and juices. Meta-analysis of five performance trials, with a total of >1,000 piglets, shows that application of the additive positively influenced feed intake of piglets after weaning. High feed intake after weaning is crucial to retain gut health and integrity, allowing for efficient nutrient digestion and reducing the risk of postweaning diarrhea. Piglets fed FRESTA® F showed significantly improved feed conversion ratio (1.35 vs. 1.54 kg/kg) compared with controls. Higher feed intake and better feed efficiency resulted in improved weight gain and FRESTA® F fed piglets showed 10.6% higher final weight than controls. Safety of the additive was tested with piglets fed 5-fold the maximum recommended dosage. Blood analysis showed that the additive is safe for the target animal (weaned piglets). For decades, active substances of FRESTA® F appear in form of spices and herbs in human diets and were generally accepted to be safe for human consumption. Nevertheless, consumer safety was confirmed with blood and meat analyses of piglets that were fed up to 10-fold the maximum recommended dosage. Analyses in meat and blood did not show carvone concentrations above the limit of detection of 0.04 ppm. It can therefore be concluded that consumers are not exposed to carvone from piglets fed FRESTA® F. The results of the presented meta-analysis clearly demonstrate the potential of phytogenic feed additives as an alternative for antibiotic growth promoters. Zootechnical registered phytogenic feed additives have proven performance enhancing effects and should be used for safe and effective support of livestock production.

4.14 IMPROVEMENT OF NUTRIENT DIGESTION AND PRODUCTION PERFORMANCE IN BROILER CHICKENS BY PHYTOGENIC FEED ADDITIVE

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Despite the ban of antibiotic growth promoters in 2006, application of antibiotics in livestock production is still enormous due to an increased application of therapeutics. The industry is intensively looking for alternative, zootechnical feed additives which are proven to be safe and efficient, in order to fulfill consumer and legislative demands. BIOSTRONG® 510 is a phytogenic feed additive, whose active components are approved in the EU under Directive 70/524 as flavours, aromatic and appetising substances for all species and animal categories, with no maximum feed inclusion limit, and without a time limit, now subject to re-evaluation under Regulation (EC) No 1831/2003. The product is a standardised mixture of essential oils (minimum 7.4%) of thyme and star anise in an excipient based on mixed, dried herbs and spices, and other bulking and anti-caking agents. The additive is pending EU registration as zootechnical feed additive according to directive EC 1831/2003. Statistical meta-analyses were performed in order to evaluate short-term effects on nutrient digestibility (six trials up to 21 days) and long-term effects on animal performance (five trials up to 35 or 42 days), respectively. Application of the additive significantly improved caecal digestibility of crude protein by 4.2%, of crude fat by 2.7%, and of crude ash by 4.7%. Better digestion of nutrients was well reflected in the performance of the chickens, resulting in relative improvement of daily gain by 3.3% and feed efficiency by 3.1%. Similar results were obtained in performance trials, where average daily gain was numerically increased by 1.2% and feed efficiency was significantly improved by 2.9%. It is concluded from the eleven trials that the addition of BIOSTRONG® 510 to broiler diets improves zootechnical performance, especially feed efficiency, and thus, economical efficacy in poultry production. The positive effects can be explained by improved digestion of dietary nutrients. The additive is therefore suitable as digestibility and performance enhancer in broiler chickens.

4.15 GALLIPRO® IMPROVES CHICKEN PRODUCTION PERFORMANCE ABOVE THE LEVEL OF ANTIBIOTICS

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Chickens are hatched under strictly clean conditions without influence of microorganisms from other animals or from the environment. These conditions make the chicken gut very vulnerable and unprotected against pathogens making it prone to inflammation. Antibiotics can be used to eliminate pathogens however it does not cure the inflammation. Probiotics are supportive in the colonization of a beneficial micro flora in the immature gut and furthermore has a positive influence on the immune system and animal health. Two independent studies with chickens aimed at examining the influence of a *Bacillus* based probiotic (GalliPro®) on weight gain and feed conversion ratio (FCR) in contrast to antibiotics hypothesizing that GalliPro® would perform in line with antibiotics. The first trial (Universidade Federal de Viscosa, Brazil) was performed with 504 Cobb chickens divided into three groups, 0-42 days of age. The chickens were fed a corn-soy based diet differing in additives: 1) control without additives, 2) GalliPro® 8x105 CFU/g feed and 3) Bacitracin Methylene Disalicylate (BMD) 50 ppm in week 1-5. The feed conversion ratio was significantly improved ($P<0.05$) in the GalliPro® fed group compared to both the BMD and the control group. Furthermore, weight gain was numerically higher in GalliPro® fed chickens compared to the other groups. The second trial (Cooperative Central Aurora – Research Facility, Chapecó, Brazil) included 1680 Ross chickens divided into three groups, 0-45 days of age: 1) control without additives, 2) GalliPro® 8x105 CFU/g feed and 3) Avilamycin 10ppm. Both FCR and weight gain was significantly improved ($P<0.05$) in the GalliPro® fed chickens compared to Avilamycin and the control group. In conclusion, the results from the two trials show that probiotics supplied to chickens improved performance parameters above the level of antibiotics. This indicates that probiotics promote a higher general health status than is obtained by use of antibiotics. The results show that probiotics qualify as an unquestionable alternative to antibiotics and furthermore have the potential to increase production performance in chickens in contrast to a feed without additives.

4.16 EFFECTS OF A PATENTED ACTIVATED CLAY ON LAYING HENS PERFORMANCES

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Antibiotics have been used for decades in animal production at low levels during long period for their performance enhancement effect. With the increase of microbe resistance, the use of antibiotics for non-clinical reasons is nowadays decreasing worldwide. As a consequence, there is a need for alternative products able to maintain production performances without antibiotics. In this context, a trial was conducted on Bovans laying hens to evaluate the effects of a patented activated clay, commercially named B-Safe, in comparison with a negative control and a positive control containing zinc bacitracine (50 ppm). Each diet was tested on 60 hens housed in cages, in 5 replicates of 12 animals. The performances were followed during 8 weeks, between 52 and 59 weeks of production. The following data were registered: number of eggs produced daily, average weight of the eggs, daily feed consumption, feed conversion ratio, number of downgraded eggs (broken, dirty, shell problem). All data were subjected to analysis of variance procedure with diet, time, and cage nested in diet as the 3 fixed factors of the model. Statistically different means were separated using Duncan's multiple range tests ($P< 0.05$). Zinc bacitracine numerically improved laying percentage in comparison with the negative control and significantly improved average egg weight (+0.8%, $P<0.001$) and egg mass (+2.4%, $P<0.001$). B-Safe significantly improved laying percentage (+2.7 pt, $P<0.05$), average egg weight (+1.7%, $P<0.001$) and egg mass (+4.7%, $p<0.001$) in comparison with the negative control. Feed conversion ratio was also significantly improved (-4.1%, $P<0.001$). No significant difference was observed on the number of downgraded eggs with any of the treatments. In the conditions of this trial, B-Safe significantly improved laying hens performance in comparison with negative control and with zinc bacitracine treatment. As a consequence, B-Safe can be considered as a promising alternative to zinc bacitracine for egg production. Further works are currently ongoing to evaluate the ability of microorganisms to develop resistance to B-Safe.

4.17 EFFECT OF ACTIGEN™ ON TECHNICAL RESULTS AND FOOT PAD LESIONS IN BROILERS

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The objective of this study was to determine the effect of Actigen, a unique bioactive fraction derived from yeast cell wall, on performance and foot pad lesions using a pen trial combined with a large barn trial. Pen trial: six pens per treatment with 60 broilers per pen, grown in the commercial broiler house in which the barn trial was also conducted. The feeds were wheat soy based and fed ad libitum: starter (0-7 days), grower (8-28 days) and finisher (29 -34 days). There were two treatments: (a) control feed and (b) control feed + Actigen at 800 g/T in starter, 500 g/T in grower and 300 g/T in finisher. Barn trial: 1 broiler house, one half containing 21,800 broilers (control), and other half containing 27,000 broilers (Actigen). Feeds and treatments were the same as the pen trial. The measurements in both trials were growth, feed intake, feed conversion ratio (FCR), and mortality. Also foot pad lesion scoring was conducted in the barn trial at 32 days of age using the official Danish method. This includes scoring of 100 birds per treatment attributing points: (i) no lesions: 0 point; (ii) minor/superficial damage: 0.5 point; and (iii) severe damage: 2 points. Results showed that the technical results in the barn trial were quite similar to the pen trial achieving > 2,100 g LW at 34 days with a FCR of approximately 1.6. Results from the pen trial showed that, despite the absence of statistical differences, that Actigen led to an improved end weight of +35 g (+ 1 g/a/d), which was mainly due to strong growth during the final 6 days of the trial (+ 3.8 g/d). FCR in the pen trial was improved by 3 points. In the barn trial, Actigen reduced FCR by 5 points, while mortality was reduced by 0.6%. Total foot pad lesion scores were 45 for the control group and only 3 for the Actigen group. This indicates that Actigen reduced wet litter and as a result little or no damage of the foot pads or hocks occurred. Economic calculation revealed a profit of €25.80 (pen trial) and €27.80 (barn trial) per 1,000 broilers produced. It can be concluded from this trial that Actigen improved end weight by 17-35 g at 34 days of age, reduced overall FCR by 3 to 5 points; reduced mortality by 0.6%; and dramatically reduced the foot pad lesion score. This demonstrates that Actigen has a positive effect on gut health, allowing for a reduction in the use of antibiotics to maintain performance.

4.18 CAN WE SUBSTITUTE AVILAMYCINE BY A NON MEDICATED SOLUTION IN BROILER PRODUCTION?

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Antibiotics have been used for decades in broiler chicken production for their gut microflora regulation effect, resulting in growth promotion. All antibiotics growth promoters were step by step banned in European countries but are still widely used in other parts of the world. Governments and consumers are becoming more and more aware of the consequences of the routine use of antibiotics, and as a consequence, there is a growing need for farmers to find alternatives without losing profitability. In this context, a patented activated clay, commercially named B-Safe, was compared to avilamycine in a field study. It was conducted on 468,000 broiler chickens of Ross 308 genotype housed in 24 buildings of 19,500 birds each. The buildings were all located on the same farm and managed in the same way regarding prophylaxis, raising conditions, bird's origin and feeding sequence. 12 of the buildings were randomly selected as the control group, which received 10 ppm of avilamycine during the whole study (from 0 to 39 days of age) in accordance with common practice of the farm. The 12 other buildings received the same feed as the control group but the avilamycine was replaced by B-Safe. All zootechnical data were registered on a building basis (global feed intake, final body weight, feed conversion ratio, mortality) and intestinal integrity was evaluated at 28 and 35 days of age on 5 birds per building by the HTSi methodology (Elanco). Mortality was very similar between avilamycine and B-Safe groups (respectively 4.8% and 4.5%). Animals receiving B-Safe consumed a little less feed (-1.8%) but had a slightly better growth (+1.3%) because of a better feed conversion (+2.9%) compared to animals receiving avilamycine. Regarding intestinal integrity, avilamycine and B-Safe groups were also very similar; they respectively obtained a score of 86.6 and 86.0 at 28 days and of 89.2 and 89.2 at 35 days. In the conditions of this study, B-Safe enabled similar or slightly better zootechnical performance and intestinal integrity than avilamycine. Moreover profitability was preserved for the integrator. As a consequence, B-Safe can be considered as a promising alternative to avilamycine for broiler production. Further works are currently ongoing to evaluate the ability of microorganisms to develop resistance to B-Safe.

4.19 MINAPIG – A MULTI-COUNTRY PROJECT TO EVALUATE ALTERNATIVES TO ANTIMICROBIAL USAGE IN PIG PRODUCTION

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Katharina DC Stärk for MINAPIG Consortium Statistics on the use of antimicrobials in pig farming in Europe indicate that the extent of usage may be larger than assumed. It also varies considerably between countries, among prescribing veterinarians, and individual pig farmers. In order to progress towards corrective action, differences in the extent of antimicrobial use and the reasons to use them between farms and countries need to be explained. Antimicrobials are used when livestock are affected by pathogens in an environment that does not prevent disease. "Prevention is better than cure," is the European Commission's motto in its animal health strategy. Because many management factors can have an impact on antimicrobial usage on a farm, it is difficult to identify individual factors that are consistently and strongly correlated with reduced antimicrobial use. Important factors determining prescription and usage patterns in farmers and veterinarians may be different knowledge levels, beliefs, attitudes, and perceived risks. There are indications that differences between countries, e.g., health status, ethical values, and financial concerns, influence prescribing or usage behaviour. In order to effectively implement policies on limited antimicrobial use, a thorough understanding of factors affecting behaviour will be critical. Therefore, MINAPIG research pursues a vision of sustained animal health by investigating strategies that promote pig health and thus lead to a reduced need for antimicrobial use. The research activities will be presented here. Participating researchers from Belgium, Denmark, France, Germany, Sweden, and Switzerland aim to evaluate strategies in agriculture that will reduce the need for antimicrobials while assuring the health and welfare of pigs and sustainable solutions for farmers. The objectives of the project are to investigate the efficacy and effectiveness of specific and unspecific technical alternatives to antimicrobial usage in pig production, and to identify drivers impacting the choices of farmers and veterinarians between alternative strategies. Field studies are conducted to investigate and compare different farming practices with the amount of antimicrobials used. Economical evaluations will establish the relative costs and benefits from alternative strategies. Attitudes of farmers and veterinarians towards antimicrobial usage and alternative preventive strategies are investigated to compare between farmers and veterinarians within and between countries. MINAPIG research will provide the foundation for an integrated understanding of technical and psychological factors driving decisions of farmers and veterinarians about pig health and production and the consequential interventions, particularly the use of antimicrobials across different pig production practices in Europe. MINAPIG is funded by the Era-Net programme Emida.

4.20 ZOOTECHNICAL AND ECONOMICAL EVALUATION OF THE USE OF A LIVE ANTICOCCIDIAL VACCINE IN ROTATION WITH ANTICOCCIDIAL PRODUCTS IN BROILER CHICKENS: RESULTS OF A SET OF FIELD TRIALS FROM BELGIUM AND THE NETHERLANDS

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Designing a preventive program for controlling coccidiosis is one of the most important decisions in order to safeguard or improve zootechnical and financial results. Two field trials have been performed, using two or three consecutive cycles of a live anticoccidial vaccine (Hipracox®), rotating from a non-rotational shuttle program using nicarbazin/narasin and salinomycin as anticoccidial products (ACP). During and after vaccination, performance was calculated. Mortality, average daily gain (ADG), live slaughter weight, feed conversion rate (FCR), and European Production Efficiency Factors (EPEF) of consecutive cycles were calculated. Oocyst per gram (OPG) counts and lesion scoring were performed. Impact antibiotic use was compared. Finally, the return of investment of implementing live anticoccidial vaccines as rotational tool was calculated. Impact of vaccination on ADG after returning to ACP was 2.21 gram (58.39 gram cycles before vaccination (CBV), 60.60 gram ADG cycles after vaccination (CAV)). Cycles during vaccination (CDV) had 15% lower mortality (3.13% CBV, 2.67% CDV). Body weight at average slaughter age of 41 days was average 91 gram higher CAV (2491 gram) compared to CBV (2409 gram). FCR2000 was improved with 2 points CDV (1.54) and 8 points CAV (1.48) compared to CBV (1.56). EPEF was improved from 362 CBV to 370 CDV (+8 points) and 399 CAV (+ 37 points). Financially, birds of CAV had 8.33 eurocent improved income, compared with CBV. These trials demonstrate the level of impact an improved coccidiosis control can have on performance of broilers and the level of financial impact this has on broiler production.

4.21 ENTEROSORPTION THERAPY PROVIDED BY CALIBRIN®-Z ENTEROSORBENT AFTER PIGS WERE INTOXICATED BY ZEARALENONE.

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Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* fungi. Its estrogenic effects cause infertility, abortion and other breeding problems, especially in swine. The excretion of absorbed ZEA and its metabolites are mainly through bile from liver and they can be re-absorbed via enterohepatic circulation. *Calibrin-Z (CAZ)* is a highly-refined montmorillonite sorbent mineral that has high affinity and capacity to sequester a wide range mycotoxins found in feed grains. CAZ has been shown to prevent dietary ZEA absorption in pigs. However, it has not been demonstrated to prevent ZEA and its metabolites re-absorption in the small intestine. Therefore, the objective of current study was to evaluate the effect of CAZ feeding after pigs were intoxicated by pre-fed different levels of ZEA. A total of 64 female pigs were fed 0, 200, 400, or 800 ppb of cultured ZEA (provided by University of Missouri, Columbia) contaminated diets for 28 d prior to the start of the current study. Sixteen pigs from each pre-fed ZEA treatment were randomly divided into half (4 replicate pens with 2 pigs each) and fed either a control diet (no detectable mycotoxins) or control + 0.2% CAZ for 18 d to form 8 treatments in a 4 x 2 factorial arrangement. Vulva size was measured at 3 d intervals. Blood samples were collected on d-9 and d-18; serum malondialdehyde (MDA), superoxide dismutase (SOD), and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) were analyzed. Pigs previously fed diets containing ZEA at 400 and 800 ppb had reduced ($P < 0.05$) ADG in the post-ZEA period compared with pigs previously fed a non-ZEA diet. Supplementation with 0.2% CAZ increased ($P < 0.05$) ADG in pigs pre-fed 200 (651 vs. 734 g), 400 (615 vs. 686 g), or 800 (617 vs. 690 g) ppb ZEA diets as compared with the pigs pre-fed the same ZEA diets. However, improved FE ($P < 0.05$) from CAZ feeding was observed in pigs pre-fed the 400 (757 vs. 832) ppb treatments, only. Pigs fed 0.2% CAZ reduced ($P < 0.05$) average vulva size through-out the 18 d period as compared with those pigs fed the control diet regardless of pre-fed ZEA dosage. The rate of vulva size reduction was significantly improved in pigs fed the CAZ diet, over those fed the control diet. Serum MDA was reduced ($P < 0.05$) in pigs fed CAZ supplementation on d-18 but not different on d-9; SOD increased ($P < 0.05$) in those pigs fed CAZ on d-9 but not different on d-18. In general, serum liver enzymes were not different between control and CAZ feeding, except the AP was lower ($P < 0.05$) in pigs pre-fed 800 ppb ZEA and continued on the control diet. The results suggested that Calibrin-Z may increase detoxification of ZEA in pigs possibly by preventing re-absorption of ZEA and its metabolites in the small intestine originating from bile (enterohepatic circulation); and it may be used to accelerate the recovery from ZEA intoxicated pigs.

4.22 ENTEROTHERAPY OF CALIBRIN®-Z AND MODIFIED CALIBRIN®-Z AGAINST FUMONISIN EFFECTS IN GROWING PIGS

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Fumonisin (FUM) is a mycotoxin that produced by *Fusarium* fungi. Clinical symptoms of the toxin have been reported in both animals and humans. These include pulmonary edema and neural tube defects in pig, leukoencephalomalacia in horse, and abdominal pain and diarrhea in humans. *Calibrin-Z (CAZ)* is a highly-refined sorbent mineral with high affinity and capacity to sequester a wide range of the mycotoxins found in feed grains. The objective of this study was to investigate the protection of CAZ and modified CAZ against the negative effects of FUM in pigs. Seventy-two pigs with initial body weight of 11.8 kg were used in a 42 d study to evaluate the ability of 3 anti-mycotoxin additives (AMA), CAZ, CAZ + organic compound (COC), and CAZ + charcoal carbon (CCC), at increasing concentrations to reduce the effects of fumonisin B1 (FB1). There were 12 treatments (TRT) in the study with 6 replications per TRT. Individual pig was the experimental unit and data was analyzed using the Tukey test (difference = $P \leq 0.05$). The 12 TRT were: 1) a control diet with no detectable mycotoxins (CON); 2) CON + 0.5% CAZ; 3) CON + 0.7% COC ; 4) CON + 0.7% CCC; 5) CON + 50 ppm FB1 (FUM50); 6) FUM50 + 0.25% CAZ; 7) FUM50 +0.5% CAZ; 8) FUM50 + 0.2% COC; 9) FUM50 + 0.5% COC; 10) FUM50 + 0.7% COC; 11) FUM50 + 0.5% CCC; 12) FUM50 + 0.7% CCC. Over the 42 d period ADFI was lower for pigs fed diets that contained FB1 and no AMA compared to those fed the CON diet. Adding CAZ, COC, or CCC, at any level, to the FB1 contaminated diet improved ADFI compared to the diet containing only FB1. Starting at week 4 of the experiment both ADG and body weight decreased when diets were contaminated with FB1 compared to those receiving the CON diet. Pigs fed the TRT with the highest level of the three AMAs had higher ADG and body weight than those fed TRT FUM50 and equal to those fed the uncontaminated CON. Pigs fed the lower level of AMAs had ADG and body weight between those fed the CON diet and the FUM50 TRT. There was no difference in the relative weight of the livers, feed conversion, or total plasma proteins between the TRT. The ratio between sphinganine and sphingosine (SA/SO) was higher in animals which received FB1 in the diet even with the inclusion of AMAs. Overall, fumonisin addition decreased feed intake and weight gain while adding the highest level of the Calibrin-Z and modified Calibrin-Z protected these measures to the level of the uncontaminated control. Relative liver weight was not affected by FB1 but the SA/SO ratio increased with FB1 contamination and was not affected by AMA addition.

4.23 NURSERY PIG GROWTH AND HEALTH ARE IMPROVED WHEN SUPPLEMENTED WITH A MICROBIAL FERMENTATION PROTOTYPE FEED ADDITIVE

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Previous research demonstrated increased bacterial production of butyrate when a *Saccharomyces cerevisiae* fermentation product (Diamond V Original XPC) or a fermentation prototype was evaluated in an in vitro assay using fresh fecal inoculum from pigs. These results led to an in vivo trial in pigs to test these feed additives. The objective of the study was to evaluate the impact of feeding XPC and the newly developed *Lactobacillus acidophilus*-based microbial fermentation prototype (LAFP) on nursery pig growth and health. A total of 120 pigs weaned at 19 d of age and weighing 6.7 kg were use in this study. Pigs were allotted to 1 of 4 treatments and housed 2 pigs/pen with 15 replications/treatment. The treatments were Control, XPC (1 g/kg), and LAFP at 1 g/kg and 2 g/kg. The Control diet contained antibiotics and pharmacological levels of copper and zinc supplementation. The XPC and LAFP treatments were supplemented to the Control diet and treatments were fed in two dietary phases (Phase 1: d 1 to 9 and Phase 2: d 10 to 21). Pig body weight and feed intake were recorded, as well as the number of injectable medications administered to the pigs to treat health problems during Phase 1 and 2. Pigs supplemented with XPC or LAFP had significantly greater growth rate and feed intake than pigs fed the Control diet during Phase 1 ($P < 0.05$). Pig weights at the end of Phase 1 for Control, XPC, and LAFP at 1 g/kg and 2 g/kg were 7.03, 7.37, 7.67, and 7.58 kg, respectively, with the weights of LAFP fed pigs being significantly greater than Control ($P < 0.05$). Pigs supplemented with LAFP did not require any injectable antibiotics during Phase 1, while pigs fed Control and XPC required 24 and 15 injections, respectively ($P = 0.27$). During Phase 2, growth rate in pigs supplemented with LAFP at 2 g/kg was significantly greater than Control ($P < 0.05$). In addition, pigs supplemented with LAFP at both inclusion rates had significantly greater feed intake than Control during Phase 2 ($P < 0.01$). At the end of Phase 2, pigs receiving LAFP at 1 g/kg and 2 g/kg were 1.16 and 1.29 kg heavier ($P < 0.05$) than Control. The number of injectable medications administered ($P < 0.50$) to the pigs during Phase 2 for Control, XPC, LAFP at 1 g/kg, and LAFP at 2 g/kg were 14, 5, 1, and 2; respectively. The results of this research demonstrate that feeding LAFP, a *Lactobacillus acidophilus*-based microbial fermentation prototype, can improve growth performance and health of pigs. These improvements in animal health and production may be related to improved endogenous butyrate production in the gastrointestinal tract. However, additional research is required to better understand the mode of action.

Session 5
Regulatory Pathways to Enable the Licensing of Alternatives to Antibiotics

Oral Presentations

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

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Antimicrobial resistance development represents a major concern for both human and animal health. Ensuring effective treatements for infectious disease remains among the top concerns for physicians and veterinarians. Finding safe and effective therapeutic interventions that do not propagate resistance is key for sustaining adequate health care for humans and animals. The search for appropriate therapeutic interventions extends well beyond simply finding new antibiotics. The future solutions will depend on new innovative technologies. The development and commercialization of these novel technologies are inherently more difficult and risky for the animal health industry to pursue. Some of this risk lies in the regulatory environment in which these new technologies will be evaluated. The U. S. Food and Drug Administration's Center for Veterinary Medicine (FDA/CVM) has developed a new approach to the development and evaluation of novel technologies intended for use as animal drugs. This presentation will discuss the efforts underway by FDA/CVM to meet the challenges presented with these novel technologies.

5.2 EUROPEAN APPROACH TO AUTHORIZATION OF NOVEL TECHNOLOGIES WITH PARTICULAR EMPHASIS ON ALTERNATIVES TO ANTIBIOTICS

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Research into the development of alternatives to antibiotics and their rapid access to market is a policy priority for the European Commission and its agencies, particularly the European Medicines Agency (EMA). When considered as medicines, such products are treated as any other innovative medicinal product and a number of measures are already in place to assist applicants through the regulatory process including scientific advice, assistance to small to medium size enterprises and the MUMS (minor use/minor species) scheme for relevant products. Due to the high profile that the threat of antimicrobial resistance in man and animals represents, the development of alternatives has recently attained a new urgency. Particular focus is therefore now being given by the EMA to cooperating at an international level with the FDA and other international regulators to resolve challenges that are perceived as common. Some of the main regulatory challenges will be described in this presentation.

5.3 APPROACH TO AUTHORIZATION OF NOVEL TECHNOLOGIES ON ALTERNATIVES TO ANTIBIOTICS IN CHINA

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Over 90% of antibiotics consumption are used for feed in the world, and 50% of the world feed antibiotics were consumed in China. Many countries have a comprehensive ban of antibiotics in feed. However, feed antibiotics are still a meaningful option to prevention of animal diseases. Therefore, intensive researches are focused on the development of alternative strategies with the aim of maintenance of animal health and performance.

The management for feed ingredients and feed additives are under the animal husbandry department, the Ministry of Agriculture of China (MOA). Any new feed additives have to be approved by the feed evaluation committee, MOA. Recently China has published a feed additive book “the use guide for feed additive and feed ingredient”.

Various natural materials, many of which are commercially available, have been investigated as efficient alternatives to antibiotic. 1) Probiotics are widely used in feed mills and animal farms in China. Its output is about 50000 tons a year. Recent research of animal micro ecological preparation has focused on three categories, *Bacillus*, Lactic acid bacteria and Yeasts. 2) Most of studies on prebiotic are focused on fructooligosaccharides, mannanoligosaccharides, arabinoxylo-oligosaccharides and xylo-oligosaccharide. Oligosaccharides can be available on the market. Some of the feed mills start to use this feed additive. 3) Enzymes preparations developed fast in recent 10 years. In general, 12 kinds feed enzyme preparations are used in China. 4) AMPs are another major group of promising novel alternatives to antibiotics based on their effectiveness, safety, and enormous diversity. This is a large family of naturally occurring peptides from diverse sources, having diverse structures and functionalities. We have focus on the research on the *Lactoferrin* and *Plectasin*. 5) It is well proven that herbal medicine, which is used as feed additives for a long time in China, can promote appetite, enhance immunity, prevent and cure diseases. According to incomplete statistics, veterinary medicine and traditional Chinese herbal additives have over 50 kinds. Oregano oil and *Astragalus polysaccharide* are two important represents of the Chinese herbal additives. Some problems of herbal additives application in feed must be solved, such as slow efficacy, large requirement, bad palatability and high cost. 6) Research shows acidifying agent can promote the animal appetite, improve weight gain and feed conversion. So far, acidification agent application in Chinese feed industry is still in initial stage, usage and methods have not yet to be standardized.

5.4 ENSURING ACCESS TO INNOVATIVE THERAPIES: THE CHALLENGES OF MOVING A NEW MOLECULE FROM DISCOVERY TO PRODUCTION AND THROUGH REGULATORY APPROVAL

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Innovator pharmaceutical companies endeavour to develop novel compounds to treat unmet medical needs; to develop novel products from those compounds that have proven safety, efficacy, and product quality for the intended uses; and to ensure return on investment for shareholders. Emerging new drug classes present exciting opportunities for alternatives to antimicrobial drugs, yet also create significant challenges in their development, manufacturing, and regulatory reviews. Commercial accessibility of novel compounds depends on successful new molecule discovery, advancement of the drug candidate through clinical development, through to final approval by the regulatory agency and market launch. Preclinical development programs strive to identify compounds with desirable spectrum of activity including optimal ADME and PK parameters; favourable Animal Safety; demonstrable Human Food Safety; required Efficacy profile; convenience of delivery system; and positive Cost: Benefit ratio for the end-user. In the search for a new antimicrobial compound, or alternative compounds, proof of efficacy is rarely the rate-limiting step. Rather, the animal safety, method of delivery, and economics are the greatest determining factors for advancing candidates through development programs. The novelty of an innovative compound can complicate PK/PD interpretation, and in fact existing models may not be applicable; this in turn complicates the food safety assessment for food animal compounds. Once a compound moves beyond the Discovery and Pre-Clinical phases, the ability to manufacture the product at sufficient scale is key to the commercial viability of the product. In addition to large scale production, GMP requirements and manufacturing facility restrictions can block the transition to full-scale production. Oversight may vary by regulatory jurisdiction, making it difficult to align globally in GMP vs non-GMP environments. Patent lifecycle helps ensure return on R&D investment, and hinges on efficient development, predictable regulatory review, and expedient access to the market. In this regard, early alignment of development plans with regulatory agencies is critical. Examples will be reviewed where innovative and/or “combined” regulatory approaches are needed in order to align the science of the compound with existing regulations. Novel compounds are key to expanding the disease treatment arsenal available to veterinarians. A transparent and science-based regulatory environment will help ensure continued investment by innovator animal health companies, and thus continued accessibility to the marketplace.

5.5 SEEKING REGULATORY APPROVAL FOR A CLAIM NEW TO REGULATORY SCIENCE: THIS PRODUCT REDUCES THE USE OF ANTIBIOTICS

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Bacteriophages are particular viruses that infect bacteria. They are currently spread wordwide and are naturally ingested by humans and animals. Generally speaking, bacteriophages are harmless except for their specific target bacterial host. Bacteriophages are rather host-specific and normally limited only to bacteria species or genera level. As there are major bacterial zoonotic diseases, for instance Salmonellosis and Campylobacteriosis, the potential use of viruses that kill bacteria is a very promising and interesting approach. In theory, the use of bacteriophages may lead to a huge decrease of the amounts of antibiotics currently used in poultry and livestock husbandry, whilst protecting the overall animal and human health.
One of the main milestones for the European Union (EU) industry is to find out the most suitable legislation framework that fits the registration of bacteriophages. As there is neither specific regulation nor guidelines, the registration process shall be assessed in a case-by-case basis. Products consisting of bacteriophages are therefore novel devices in the veterinary field. Unlike the USA, no single product has been registered yet in the EU. This presentation shows the most outstanding registration possibility routes in the EU which are completely conditioned to the mode of use of the bacteriophage candidate, its intrinsic properties and the scope of the specific regulations. According to the way of using bacteriophages in practice, three main routes could be distinguished: 1) Application to surfaces, 2) Usage in foodstuff and 3) Administration to live animals. The application on surfaces may include a wide range of places such as farms, slaughterhouses, vehicles, food processing industries, etc. For such applications, the legislation that could better fit bacteriophages would be the one laid down for biocides. However, if the objective is to use bacteriophages in the food, there is another wide sort of regulations that could be potentially applicable. In this case, the mode of action of bacteriophages and the claims sought for the product are the key points. The possibilities embrace basically regulations concerning food additives, food processing aids, or food “decontaminants”. The third main sort of bacteriophage uses are when these are applied directly to animals. The administration route could be via feed or drinking water. Similarly and according to the mode of action of the bacteriophage and the claims pursued, the registration possibilities would encompass the regulations laid down for feed additive or veterinary medicines.