Comparing low volume saphenous-obturator block with placebo and femoral-obturator block for anterior cruciate ligament reconstruction: a randomized controlled trial

BACKGROUND: Anterior cruciate ligament reconstruction (ACL-RC) is often associated with moderate to severe postoperative pain even with a multimodal analgesic regimen. We aimed to compare the analgesic efficacy of low volume saphenous-obturator block with placebo and femoral-obturator block in patients undergoing ACL-RC. METHODS: In a randomized controlled trial eighty-two patients undergoing ACL-RC with hamstring autograft were allocated to either low volume saphenous-obturator block, placebo block or femoral-obturator block. Ropivacaine 0.75% was used for active blocks and saline for placebo. Primary outcome was pain-scores at rest quantified as area-under-the-curve 0-6 hr postoperatively. Secondary outcomes were postoperative opioid consumption and pain localization in the knee. RESULTS: No statistical difference existed between groups in area-under-the-curve 6 hr pain-scores. However, pain-scores were significantly lower in the two ropivacaine groups compared to placebo at emergence t=0 (P<0.018), at t=5 (P<0.042) and at t=6 hours (P<0.002) postoperatively. Furthermore, ropivacaine blocks exhibited significantly reduced total opioid consumption (15.81 and 18.44 mg) postoperatively compared with placebo (26.38 mg) (P<0.018). Patients receiving ropivacaine blocks localized pain in the posterolateral knee, whereas placebo block patients reported anteromedial and central pain. Other secondary outcomes were similar between groups. CONCLUSIONS: Low volume saphenous-obturator block is significantly more effective than placebo in reducing both early and late pain-scores as well as postoperative opioid consumption in patients undergoing ACL-RC. No statistical difference existed when comparing low volume saphenous-obturator block to femoral-obturator block regarding early and late pain-scores and postoperative opioid consumption.
Pathology and bacteria related to digital dermatitis in dairy cattle in all year round grazing system in Brazil

Digital dermatitis (DD) is one of the main causes of lameness in dairy cattle worldwide, and it is frequently reported in high-yielding, free stall dairy herds from regions with a temperate climate. However, DD is also observed with high prevalence in grazing cattle with a low milk yield in tropical regions. To clarify whether these differences have an impact on the etiology of the disease, we studied DD lesions from all year round grazing cattle of mixed breed in Brazil using high-throughput 16S rRNA gene sequencing and fluorescent in situ hybridization. The study included samples from 66 skin lesions and 5 healthy skins collected from five farms. Both techniques showed Treponema spp. to be the most abundant bacteria, present in all but one of the samples with minimal epidermal alterations. We identified eleven different Treponema strains belonging to the six major phyotypes of Treponema which have all previously been identified in DD lesions. Furthermore, we identify Dichelobacter nodosus in DD lesions by gene sequencing and also by fluorescent in situ hybridization in almost half of biopsy specimens in areas with mild epithelial damage and together with Treponema. The present data support the hypothesis that Treponema constitutes the main pathogen responsible for DD, independent of the environment and region where cows are kept, and it further suggests D. nodosus as another potentially important pathogen.
Characterization of Campylobacter jejuni and Campylobacter coli Broiler Isolates by Whole-Genome Sequencing

Campylobacter has been the most commonly reported cause of bacterial diarrheal disease in humans in the European Union since 2005. Most broiler batches at slaughter are colonized with Campylobacter, and the major source of infection is contaminated poultry meat. The aim of this study was to characterize a selection of Campylobacter jejuni and Campylobacter coli isolates from broilers through whole-genome sequencing (WGS). A total of 16 isolates (C. jejuni = 12 and C. coli = 4) from five broiler farms from Catalonia (northeastern Spain) were analyzed. A phylogenetic analysis based on 8420 single-nucleotide polymorphisms showed two main cluster grouping strains by species. Phenotypic resistances to quinolones (100%), tetracycline (81%), streptomycin (75%), erythromycin (56%), and gentamicin (13%) were found. All the isolates carried the C257T point mutation in the subunit A of the DNA gyrase gene (Thr86Ile) conferring resistance to quinolones, while all the isolates showing resistance to tetracycline carried the tet(O) gene. The genes aph(3′)-III and aadE conferring resistance to aminoglycosides were identified in the two isolates (one C. jejuni and one C. coli) resistant to streptomycin and gentamicin. The point mutation A2075G on the 23S rDNA conferring high resistance to macrolides was detected in three C. coli isolates. The CmeABC multidrug efflux pump was also detected, both in C. jejuni and C. coli isolates. All C. jejuni and C. coli isolates were positive for most of the 34 virulence-associated genes studied related to motility, chemotaxis, adhesion, and invasion. Interestingly, the wlaN gene involved in the Guillain–Barré syndrome was found in two isolates. The results underline the power of WGS for investigation of virulence, clonality, and antimicrobial resistance in Campylobacter.

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Authors: Cantero, G. (Ekstern), Correa-Fiz, F. (Ekstern), Ronco, T. (Intern), Strube, M. L. (Intern), Cerdà-Cuéllar, M. (Ekstern), Pedersen, K. (Intern)
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Charaterization of C. jejuni and C. coli broiler isolates by whole genome sequencing

Campylobacter is the most commonly reported cause of bacterial diarrhoeal disease in humans in the EU since 2005. The major source of infection is contaminated poultry meat with most broiler batches at slaughter colonized with Campylobacter. C. jejuni and C. coli are responsible for the isolates vast majority of infections, which may subsequently lead to serious neuropathologies such as Guillain-Barré syndrome. The aim of this study was to take advantage of whole genome sequencing (WGS) to in-depth characterize a subset of 16 C. jejuni and C. coli isolates from broilers from five farms.

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Authors: Cantero, G. (Ekstern), Correa-Fiz, F. (Ekstern), Ronco, T. (Intern), Strube, M. L. (Intern), Cerdà-Cuéllar, M. (Ekstern), Pedersen, K. (Intern)
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Characterization of C. jejuni and C. coli broiler isolates by whole genome sequencing

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Organisations: National Veterinary Institute, Bacteriology & Parasitology, CReSA - Centre for Animal Health Research, Centre de Recerca en Sanitat Animal
Authors: Guillermo, C. (Ekstern), Florencia, C. (Ekstern), Ronco, T. (Intern), Strube, M. L. (Intern), Cerdà-Cuéllar, M. (Ekstern), Pedersen, K. (Intern)
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Microbiota analysis of environmental slurry and its potential role as a reservoir of bovine digital dermatitis pathogens

At present, very little information exists regarding what role the environmental slurry may play as an infection reservoir and/or route of transmission for bovine digital dermatitis (DD), a disease which is a global problem in dairy herds. To investigate, if DD-related bacteria belong to the indigenous microbiota of the dairy herd environment, we used deep amplicon sequencing of the 16S rRNA gene in 135 slurry samples collected from different sites in 22 dairy farms, with and without DD-infected cows. Both the general bacterial populations as well as digital dermatitis-associated Treponema were targeted in this study. The results revealed significant differences in the bacterial communities between the herds, with only 12 bacterial taxa shared across at least 80% of all the individual samples. These differences in the herd microbiota appeared to reflect mainly between-herd variation. Not surprisingly, the slurry was dominated by ubiquitous gastrointestinal bacteria, such as Ruminococcaceae and Lachnospiraceae. Despite the low relative abundance of spirochetes, which ranged from 0 to 0.6%, we were able to detect small amounts of bacterial DNA from DD-associated treponemes in the slurry. However, the DD-associated Treponema spp. were only detected in samples from herds with reported problems of DD. These data indicate that treponemes involved in the pathogenesis of DD are not part of the normal environmental microflora in dairy herds without clinical DD and, consequently, that slurry is not a primary reservoir of infection.

Importance Bovine digital dermatitis (DD), a dermal disease which causes lameness in dairy cattle, is a serious problem worldwide. To control this disease, the infection reservoirs and transmission routes of DD pathogens need to be clarified. The dairy herd slurry may be a possible pathogen reservoir of DD-associated bacteria. The rationale for the present study was, therefore, to examine whether DD-associated bacteria are always present in slurry or if they are only found in DD-afflicted herds. The results strongly indicated that DD Treponema are not part of the indigenous slurry and, therefore, do not comprise an infection reservoir in healthy herds. This study applied next-generation sequencing technology to decipher the microbial compositions of environmental slurry of dairy herds with and without digital dermatitis.
Selection of Bacillus species for targeted in situ release of prebiotic galacto-rhamnogalacturonan from potato pulp in piglets

We have previously shown that galacto-rhamnogalacturonan fibers can be enzymatically extracted from potato pulp and that these fibers have potential for exerting a prebiotic effect in piglets. The spore-forming Bacillus species are widely used as probiotics in feed supplements for pigs. In this study, we evaluated the option for further functionalizing Bacillus feed supplements by selecting strains possessing the enzymes required for extraction of the potentially prebiotic fibers. We established that it would require production and secretion of pectin lyase and/or polygalacturonase but no or limited...
secretion of galactanase and β-galactosidase. By screening a library of 158 Bacillus species isolated from feces and soil, we demonstrated that especially strains of Bacillus amyloliquefaciens, Bacillus subtilis, and Bacillus mojavensis have the necessary enzyme profile and thus the capability to degrade polygalacturonan. Using an in vitro porcine gastrointestinal model system, we revealed that specifically strains of B. mojavensis were able to efficiently release galactorhamnogalacturonan from potato pulp under simulated gastrointestinal conditions. The work thus demonstrated the feasibility of producing prebiotic fibers via a feed containing Bacillus spores and potato pulp and identified candidates for future in vivo evaluation in piglets.
Sequence-Based Genotyping of Expressed Swine Leukocyte Antigen Class I Alleles by Next-Generation Sequencing Reveal Novel Swine Leukocyte Antigen Class I Haplotypes and Alleles in Belgian, Danish, and Kenyan Fattening Pigs and Göttingen Minipigs

The need for typing of the swine leukocyte antigen (SLA) is increasing with the expanded use of pigs as models for human diseases and organ-transplantation experiments, their use in infection studies, and for design of veterinary vaccines. Knowledge of SLA sequences is furthermore a prerequisite for the prediction of epitope binding in pigs. The low number of known SLA class I alleles and the limited knowledge of their prevalence in different pig breeds emphasizes the need for efficient SLA typing methods. This study utilizes an SLA class I-typing method based on next-generation sequencing of barcoded PCR amplicons. The amplicons were generated with universal primers and predicted to resolve 68-88% of all known SLA class I alleles dependent on amplicon size. We analyzed the SLA profiles of 72 pigs from four different pig populations; Göttingen minipigs and Belgian, Kenyan, and Danish fattening pigs. We identified 67 alleles, nine previously described haplotypes and 15 novel haplotypes. The highest variation in SLA class I profiles was observed in the Danish pigs and the lowest among the Göttingen minipig population, which also have the highest percentage of homozygote individuals. Highlighting the fact that there are still numerous unknown SLA class I alleles to be discovered, a total of 12 novel SLA class I alleles were identified. Overall, we present new information about known and novel alleles and haplotypes and their prevalence in the tested pig populations.
Atlantic salmon (Salmo salar) protein hydrolysate in diets for weaning piglets – effect on growth performance, intestinal morphology and microbiota composition

Salmon protein hydrolysates (SPH) from two different rest raw materials were evaluated in diets for weaning piglets. Four experimental diets were included in the study: a diet based on plant protein with soy protein as the main protein source (Diet PP), a diet based on fishmeal in exchange for soy protein (Diet FM) and two diets in which different SPH replaced fishmeal in the FM diet. The experimental diets were fed to piglets from the day of weaning until 32 d postweaning. In addition to the record of performance data, an intestinal sampling for mucosal morphometry and microbiota 16S rRNA gene sequencing were performed at day 11 on a subset of the animals. The duodenal villi absorption area was significantly larger in piglets receiving Diets SPH compared with Diet PP (p < 0.02). A significant positive correlation between duodenal villi height and average daily gain during the first 11 d postweaning was detected. Only small differences in intestinal microbiota community and no differences in growth performance were detected between the experimental diets. To conclude, SPH seem to be an interesting novel protein source in weanling piglets.

Bibliographical note
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Natural Pig Plasma Immunoglobulins Have Anti-Bacterial Effects: Potential for Use as Feed Supplement for Treatment of Intestinal Infections in Pigs

There is an increasing demand for non-antibiotics solutions to control infectious disease in intensive pig production. Here, one such alternative, namely pig antibodies purified from slaughterhouse blood was investigated in order to elucidate its potential usability to control post-weaning diarrhoea (PWD), which is one of the top indications for antibiotics usage in the pig production. A very cost-efficient and rapid one-step expanded bed adsorption (EBA) chromatography procedure was used to purify pig immunoglobulin G from slaughterhouse pig plasma (more than 100 litres), resulting in >85% pure pig IgG (pplG). The pplG thus comprised natural pig immunoglobulins and was subsequently shown to contain activity towards four pig-relevant bacterial strains (three different types of Escherichia coli and one type of Salmonella enterica) but not towards a fish pathogen (Yersinia ruckeri), and was demonstrated to inhibit the binding of the four pig relevant bacteria to a pig intestinal cell line (IPEC-J2). Finally it was demonstrated in an in vivo weaning piglet model for intestinal colonization with an E. coli F4+ challenge strain that pplG given in the feed significantly reduced shedding of the challenge strain, reduced the proportion of the bacterial family Enterobacteriaceae, increased the proportion of families Enterococcaceae and Streptococcaceae and generally increased ileal microbiota diversity. Conclusively, our data support the idea that natural IgG directly purified from pig plasma and given as a feed supplement can be used in modern swine
production as an efficient and cost-effective means for reducing both occurrence of PWD and antibiotics usage and with a potential for the prevention and treatment of other intestinal infectious diseases even if the causative agent might not be known.

**General information**
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Authors: Hedegaard, C. J. (Intern), Strube, M. L. (Intern), Hansen, M. B. (Ekstern), Lindved, B. K. (Ekstern), Lihme, A. (Ekstern), Boye, M. (Intern), Heegaard, P. M. H. (Intern)
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Scopus rating (2009): SJR 2.614 SNIP 1.046
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Potential bacterial core species associated with digital dermatitis in cattle herds identified by molecular profiling of interdigital skin samples

Although treponemes are consistently identified in tissue from bovine digital dermatitis (DD) lesions, the definitive etiology of this debilitating polymicrobial disease is still unresolved. To study the microbiomes of 27 DD-infected and 10 healthy interdigital skin samples, we used a combination of different molecular methods. Deep sequencing of the 16S rRNA gene variable regions V1–V2 showed that Treponema, Mycoplasma, Fusobacterium and Porphyromonas were the genera best differentiating the DD samples from the controls. Additional deep sequencing analysis of the most abundant genus, Treponema, targeting another variable region of the 16S rRNA gene, V3–V4, identified 15 different phylotypes, among which Treponema phagedenis-like and Treponema refringens-like species were the most abundant. Although the presence of Treponema spp., Fusobacterium necrophorum and Porphyromonas levi was confirmed by fluorescence in situ hybridization (FISH), the results for Mycoplasma spp. were inconclusive. Extensive treponemal epidermal infiltration, constituting more than 90% of the total bacterial population, was observed in 24 of the 27 DD samples. F. necrophorum and P. levi were superficially located in the epidermal lesions and were present in only a subset of samples. RT-qPCR analysis showed that treponemes were also actively expressing a panel of virulence factors at the site of infection. Our results further support the hypothesis that species belonging to the genus Treponema are major pathogens of DD and also provide sufficient clues to motivate additional research into the role of M. fermentans, F. necrophorum and P. levi in the etiology of DD.

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Web of Science (2014): Indexed yes
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Sequence-based genotyping of expressed SLA class I alleles by Next Generation Sequencing reveal novel SLA class I haplotypes and alleles in Belgian, Danish and Kenyan fattening pigs and Göttingen minipigs

The need for typing of the swine leukocyte antigen (SLA) is increasing with the expanded use of pigs as models for human diseases and organ-transplantation experiments, their use in infection studies, and for design of veterinary vaccines. Knowledge of SLA sequences is furthermore a prerequisite for the prediction of CTL epitopes based on predicted MHC binding in pigs. The low number of known SLA class I alleles and the limited knowledge of their prevalence in different pig breeds, emphasizes the need for efficient SLA typing methods.

Here we obtain SLA class I–typing and –expression based on Illumina MiSeq Next Generation Sequencing of barcoded PCR amplicons. Universal primers were designed to generate amplicons spanning exon 2 and exon 3 of the SLA class I genes and predicted to resolve 68% to 88% of all known SLA class I alleles dependent on amplicon size. Based on whole blood mRNA we analyzed the cDNA SLA profiles of 72 pigs from four different pig populations: Göttingen minipigs and
Belgian, Kenyan, and Danish fattening pigs. We identified 67 different allele sequences, including 12 novel alleles, and observed 24 haplotypes of which 15 have not been described before. The highest variation in SLA class I profiles was observed in the Danish pigs and the lowest among the Göttingen minipig population, which also had the highest percentage of homozygous individuals. The identification of 12 novel SLA class I alleles from only 72 pigs highlight the fact that there are still numerous unknown SLA class I alleles to be discovered.

In conclusion, we present an NGS-based method to obtain sequence-based high-resolution SLA class I typing based on expressed alleles and provide new information about known and novel alleles and haplotypes and their prevalence in the tested pig populations.

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology, International Livestock Research Institute, Technical University of Denmark
Authors: Sørensen, M. R. (Intern), Ilsøe, M. (Ekstern), Strube, M. L. (Intern), Bishop, R. (Ekstern), Jungersen, G. (Intern)
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The impact of dietary swine plasma immunoglobulins on intestinal microbiota and general health in weaner piglets

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Authors: Hedegaard, C. J. (Intern), Strube, M. L. (Intern), Jensen, T. K. (Intern), Heegaard, P. M. H. (Intern)
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Tracking the elusive cytotoxic T cell response in pigs

Quantitative and qualitative assessment of antigen-specific cytotoxic T cell (CTL) responses in pigs is not a straightforward process. Through the years we have developed a series of reagents, tools and protocols to characterize peptide-specific CTL responses in pigs.

The most common recombinant SLA heavy chains were produced and peptide binding motifs were determined by assays measuring the affinity and stability of the peptide-SLA complex (pSLA) interaction. These results have been used to train neural networks to predict the binding of any pSLA (http://www.cbs.dtu.dk/services/). Recombinant SLA molecules complexed with verified binding peptides can be assembled to SLA multimers for staining of peptide-specific CTLs, and measured by flow cytometry, as we have shown with FMDV and influenza. This, however, requires SLA-matched pigs for which we have developed two methods: a sequence-based, high-resolution SLA genotyping method by standard PCR for specific detection of eight in-house SLA molecules; and a next-generation sequencing method for parallel detection of up to 50 samples of barcoded cDNA PCR products spanning exon 2 and 3. The latter for a wider characterization of expressed alleles in candidate pigs.

The in vivo generation of CTL responses to antigens following peptide immunizations is thought to require cross-presentation in appropriate dendritic cells (DC). In mice this was linked to targeting of CD103+DCs recruited after intraperitoneal immunizations. We have therefore developed a protocol for intraperitoneal delivery of peptides formulated in poly(I:C)/MMG-decorated liposomes (CAF09) to investigate the influence of peptide dose on the generation of CTL vs. antibody responses. Finally, the induced CTL killing was assessed by an in vivo cytotoxicity assay, where purified autologous PBMCs, fluorescently labeled and pulsed with target peptides, were reinjected into the donor. The in vivo killing of peptide-pulsed cells was measured by flow cytometry relative to non-pulsed PBMCs at different time points after cell transfer.

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Virology, Section for Bacteriology, Pathology and Parasitology, Department of Systems Biology, Center for Biological Sequence Analysis, Department of Bio and Health Informatics, University of Copenhagen, United States Department of Agriculture
Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea

Background: In recent years, new neonatal porcine diarrhoea (NNPD) of unknown aetiology has emerged in Denmark. NNPD affects piglets during the first week of life and results in impaired welfare, decreased weight gain, and in the worst-case scenario death. Commonly used preventative interventions such as vaccination or treatment with antibiotics, have a limited effect on NNPD. Previous studies have investigated the clinical manifestations, histopathology, and to some extent, microbiological findings; however, these studies were either inconclusive or suggested that Enterococci, possibly in interaction with Escherichia coli, contribute to the aetiology of NNPD. This study examined ileal and colonic luminal contents of 50 control piglets and 52 NNPD piglets by means of the qPCR-based Gut Microbiotassay and 16 samples by 454 sequencing to study the composition of the bacterial gut microbiota in relation to NNPD. Results: NNPD was associated with a diminished quantity of bacteria from the phyla Actinobacteria and Firmicutes while genus Enterococcus was more than 24 times more abundant in diarrhoeic piglets. The number of bacteria from the phylum Fusobacteria was also doubled in piglets suffering from diarrhoea. With increasing age, the gut microbiota of NNPD affected piglet and control piglets became more diverse. Independent of diarrhoeic status, piglets from first parity sows (gilts) possessed significantly more bacteria from family Enterobacteriaceae and species E. coli, and fewer bacteria from phylum Firmicutes. Piglets born to gilts had 25 times higher odds of having NNPD compared with piglets born to multiparous sows. Finally, the co-occurrence of genus Enterococcus and species E. coli contributed to the risk of having NNPD. Conclusion: The results of this study support previous findings that points towards genus Enterococcus and species E. coli to be involved in the pathogenesis of NNPD. Moreover, the results indicate that NNPD is associated with a disturbed bacterial composition and larger variation between the diarrhoeic piglets.

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In situ prebiotics: enzymatic release of galacto-rhamnogalacturonan from potato pulp in vivo in the gastrointestinal tract of the weaning piglet

Prebiotics may be efficient for prevention of intestinal infections in humans and animals by increasing the levels of beneficial bacteria and thereby improving gut health. Using purified prebiotics may however not be cost-effective in the livestock production industry. Instead, prebiotic fibres may be released directly in the gastro-intestinal tract by feeding enzymes with a suitable substrate and allowing the prebiotics to be produced in situ. Using low doses, 0.03 % enzyme-to-substrate ratio, of the enzymes pectin lyase and polygalacturonase in combination with potato pulp, a low-value industrial by-product, we show that high molecular weight galacto-rhamnogalacturonan can be solubilized in the stomach of weaning piglets. The release of this fiber is in the order of 22–38 % of the theoretical amount, achieved within 20 min. The catalysis takes place mainly in the stomach of the animal and is then followed by distribution through the small intestines. To our knowledge, this is the first paper describing targeted production of prebiotics in an animal model.
In situ prebiotics for weaning piglets: In vitro production and fermentation of potato galactorhamnogalacturonan

Post weaning diarrhea (PWD) in pigs is a leading cause of economic loss in pork production worldwide. The current practice of using antibiotics and zinc to treat PWD is unsustainable due to the potential of antibiotic resistance and ecological disturbance, and novel methods are required. In this study, an in vitro model was used to test the possibility of producing prebiotic fiber in situ in the gastro-intestinal tract (GI-tract) of the piglet and the prebiotic activity of the resulting fiber in the terminal ileum. Soluble fiber were successfully produced from potato pulp, an industrial waste product, with a minimal enzyme dose in a simulated upper GI-model extracting 26.9 % of initial dry matter. The fiber was rich in galactose and galacturonic acid and was fermented at 2.5, 5 or 10 g/L in a glucose-free media inoculated with the gut contents of piglet terminal ileum. Fermentations of 5 g/L inulin or 5 g/L of a purified potato fiber were used as controls. The fibers showed high fermentability, evident by a dose-dependent drop in pH and increase in organic acids, with lactate in particularly being increased. Deep sequencing showed a significant increase in Lactobacillus and Veillonella and an insignificant increase in Clostridium as well as a decrease in Streptococcus. Multivariate analysis showed clustering of the treatment groups, with the purified potato fiber being clearly separated from the other groups as the microbiota composition was 60 % Lactobacillus and almost free of Clostridium. For animal studies, a dosage corresponding to the 5 g/L treatment is suggested.
Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhoea: Safety and Preliminary results

General information
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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Section for Bacteriology, Pathology and Parasitology, Section for Virology, Upfront Chromatography A/S, Upfront Chromotography A/S
Authors: Hedegaard, C. J. (Intern), Strube, M. L. (Intern), Bendix Hansen, M. (Ekstern), Kjær Lindved, B. (Ekstern), Larsen, L. E. (Intern), Lihme, A. (Ekstern), Boye, M. (Intern), Heegaard, P. M. H. (Intern)
Publication date: 2015
Event: Poster session presented at 5th European Veterinary Immunology Workshop, Vienna, Austria.
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Swine plasma immunoglobulins for prevention and treatment of post-weaning diarrhoea: Safety and Preliminary results
Post-weaning diarrhoea (PWD) is a common condition in intensive swine production, resulting in reduced welfare of weaners and economic losses for the farmer as a result of illness, death, and treatment costs. It is also one of the main causes of antibiotics- and zinc use in the pig production industry. We aim at developing a sustainable product for protection against PWD based on natural antibodies (immunoglobulins) derived directly from inexpensive raw materials. The availability of such an inexpensive and highly active immunoglobulin product would allow swine producers to reduce expenses and minimize the on antibiotics and zinc usage. Swine immunoglobulins were isolated directly from slaughterhouse swine plasma-waste by expanded bed chromatography. It was shown that the isolated Immunoglobulin fraction bound enterotoxigenic Escherichia coli (ETEC) and Salmonella ssp. and inhibited their adhesion to porcine epithelial cells in vitro. As the immunoglobulin fraction is intended for oral use as a feed supplement, we also tested the safety of feeding 4 grams of natural immunoglobulins to 4-5 week old weaner piglets for 14 days and observed no adverse effects. In an experimental model of E. coli F4+ induced PWD, we observed that piglets given IgG as a feed supplement cleared the E. coli infection significantly faster than control weaner piglets not receiving an immunoglobulin feed supplement. Furthermore, deep sequencing of the ileal microbiota showed a significantly lowered colonization of the family Enterobacteriaceae in immunoglobulin fed piglets as compared to the control group. Thus pig slaughterhouse plasma is indicated as a potential source resource of antibodies for the control of PWD.

General information
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Authors: Hedegaard, C. J. (Intern), Strube, M. L. (Intern), Bendix Hansen, M. (Ekstern), Kjær Lindved, B. (Ekstern), Larsen, L. E. (Intern), Lihme, A. (Ekstern), Boye, M. (Intern), Heegaard, P. M. H. (Intern)
Number of pages: 1
Publication date: 2015
Event: Abstract from 5th European Veterinary Immunology Workshop, Vienna, Austria.
Main Research Area: Technical/natural sciences
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Source: PublicationPreSubmission
Source-ID: 115562559
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2015
Thermostability enhancement of an endo-1,4-β-galactanase from Talaromyces stipitatus by site-directed mutagenesis

Enzymatic conversion of pectinaceous biomasses such as potato and sugar beet pulp at high temperatures is advantageous as it gives rise to lower substrate viscosity, easier mixing, and increased substrate solubility and lowers the risk of contamination. Such high-temperature processing requires development of thermostable enzymes. Talaromyces stipitatus was found to secrete endo-1,4-β-galactanase when grown on sugar beet pectin as sole carbon source. The mature protein contained 353 AA and the MW was estimated to 36.5 kDa. It was subjected to codon optimization and produced in Pichia pastoris in 2 l scale yielding 5.3 g. The optimal reaction condition for the endo-1,4-β-galactanase was determined to be 46 °C at pH 4.5 at which the specific activity was estimated to be 6.93 μmol/min/mg enzyme with half-lives of 13 and 2 min at 55 and 60 °C, respectively. For enhancement of the half-life of TSGAL, nine single amino acid residues were selected for site-directed mutagenesis on the basis of semi-rational design. Of these nine mutants, G305A showed half-lives of 114 min at 55 °C and 15 min at 60 °C, respectively. This is 8.6-fold higher than that of the TSGAL at 55 °C, whereas the other mutants displayed moderate positive to negative changes in their half-lives.

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Volume: 99
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 1.161 SJR 1.182
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.57 SJR 1.2 SNIP 1.182
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.256 SNIP 1.221 CiteScore 3.43
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.332 SNIP 1.448 CiteScore 3.71
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.54 SNIP 1.43 CiteScore 4.3
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.488 SNIP 1.29 CiteScore 4
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.437 SNIP 1.229 CiteScore 3.72
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.389 SNIP 1.233
Diet type dictates the gut microbiota and the immune response against Yersinia ruckeri in rainbow trout (Oncorhynchus mykiss)

This study investigated the influence of the rainbow trout (Oncorhynchus mykiss) commensal intestinal microbiota in connection to an experimental Yersina ruckeri infection, the causative agent of enteric redmouth disease. One marine and one plant diet was administered to two different groups of rainbow trout. The plant-based diet gave rise to an intestinal microbiota dominated by the genera Streptococcus, Leuconostoc and Weissella from phylum Firmicutes whereas phylum Proteobacteria/Bacteroidetes/Actinobacteria dominated the community in the marine fed fish. In connection to the Y. ruckeri bath challenge there was no effect of the diet type on the cumulative survival, but the number of Y. ruckeri positive fish as measured by plate count and the number of fish with a 'high' number of reads belonging to genus Yersinia as measured by 16S rRNA next-generation sequencing was higher for marine diet fed fish. Furthermore, the two experimental groups of fish showed a differential immune response, where Y. ruckeri challenged marine fed fish had a higher transcription of IL-1β and MBL-2 relative to challenged plant diet fed fish. The data suggest that the plant diet gave rise to a prebiotic effect favouring the presence of bacterial taxons proving protective in connection to bath challenge by Y. ruckeri.
Fibre måske et alternativ til antibiotika

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Authors: Strube, M. L. (Intern)
Pages: 38-40
Publication date: 2014
Main Research Area: Technical/natural sciences

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Issue number: September
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Ratings:
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- ISI indexed (2012): ISI indexed no
- ISI indexed (2011): ISI indexed no
Original language: Danish
Source: PublicationPreSubmission
Source-ID: 105563092
Publication: Research › Journal article – Annual report year: 2015

Improving Glucose Tolerance by Reducing Weight Gain in a Polygenic Obese Mouse Model: Use of a High Protein Diet

Diets to decrease body weight have limited success in achieving and importantly maintaining this weight loss long-term. It has recently been suggested that energy intake can be regulated by the amount of protein ingested, termed the protein leverage hypothesis. In this study, we determined whether a high protein diet would be effective in achieving and maintaining weight loss in a genetically obese model, the New Zealand Obese (NZO) mouse. NZO and C57BL/6J (C57) control mice were fed a high protein or chow diet for 5 weeks from weaning (3 weeks of age). Body weight and food intake were determined. Mice on the same diet were bred to produce offspring that were fed either a chow or high protein diet. Body weight, food intake, and glucose tolerance were determined. Feeding NZO and C57 mice a high protein diet for 5 weeks resulted in reduced food intake and consequently energy intake and body weight gain compared with mice on a chow diet. NZO mice fed a high protein diet showed a significant improvement in glucose tolerance compared with their Chow-fed counterparts, while no difference was seen in C57 mice fed chow or protein diet. The offspring of NZO mice that were fed a high protein diet during gestation and weaning were also lighter and displayed improved glucose tolerance compared with chow fed animals. We conclude that a high protein diet is a reasonable strategy to reduce body weight gain and improve glucose tolerance in the NZO mouse, a polygenic model of obesity.

**General information**
State: Published
Organisations: University of Melbourne
Authors: Blair, A. R. (Ekstern), Strube, M. L. (Intern), Proietto, J. (Ekstern), Andrikopoulos, S. (Ekstern)
Publication date: 2014
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Hormone and Metabolic Research
Volume: 46
Issue number: 11
ISSN (Print): 0018-5043
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- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 1
- Scopus rating (2017): SNIP 0.644 SJR 0.918
Ph.d.-projekt vil skære i antibiotikaforbruget med præbiotiske fibre

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State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
Authors: Strube, M. L. (Intern)
Pages: 42-42
Publication date: 2014
Main Research Area: Technical/natural sciences

Publication information
Journal: Dansk Veterinaertidsskrift
Issue number: 09
ISSN (Print): 0106-6854
Ratings:
BFI (2018): BFI-level 1
BFI (2017): BFI-level 1
BFI (2016): BFI-level 1
BFI (2015): BFI-level 1
The development of the gut microbiota in rainbow trout (Oncorhynchus mykiss) is affected by first feeding and diet type

An influence of the intestinal microbiota in connection to first-feeding of rainbow trout (Oncorhynchus mykiss) fry was demonstrated using Illumina HiSeq sequencing. The period from the end of yolk-sac feeding to seven weeks post first-feeding was examined after administration of either a marine- or plant based diet with or without the probiont Pediococcus acidilactici. Before first feeding the main part of the sequence reads grouped to the genus Sediminibacterium probably originating from the surrounding water. The microbial abundance and diversity increased after first-feeding and the microbiota then changed towards phylum Firmicutes dominance for plant based fed fish and towards dominance of phylum Proteobacteria for the marine fed fish. After first-feeding, there were significantly higher abundances of Streptococcus, Leuconostoc and Weissella in fish fed the plant-based diet. The microbiota clustered separately according to the diet type, but only minor effects were seen from the probiont when using PCA-analysis. The constitutive transcription level of most examined immune genes increased during the ontogenic shift, but the results could not explain the differences in the composition of the microbiota dependent on diet treatment after first-feeding. The results suggest that the intestine of rainbow trout is colonised at an early state, but is guided in new and different directions dependent on the diet type.

General information
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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Danish Genome Institute, University of Copenhagen
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Main Research Area: Technical/natural sciences

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Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): SNIP 1.58 SJR 1.152
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.122 SNIP 1.51
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.107 SNIP 1.256 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.01 SNIP 1.33 CiteScore 2.16
Web of Science (2014): Indexed yes
Mini Review: Basic Physiology and Factors Influencing Exogenous Enzymes Activity in the Porcine Gastrointestinal Tract

The addition of exogenous enzymes to pig feed is used to enhance general nutrient availability and thus increase daily weight gain per feed unit. The enzymes used are mainly beta-glucanase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) and phytase (EC 3.1.3.8). Although in vivo data assessing feed enzyme activity during intestinal transit are few, it is known that the enzymes, being protein molecules, can be negatively affected by the gastrointestinal proteolytic enzymes and the low pH in the stomach ventricle. In this review, the pH-values, endogenous proteases and other factors native to the digestive tract of the adult pig and the piglet are discussed in relation to the stability of exogenous feed enzymes. Development of more consistent assessment methods which acknowledge such factors is warranted both in vitro and in vivo for proper evaluation and prediction of the efficiency of exogenous enzymes in the porcine gastrointestinal tract.
Bovine Abortions Revisited
National Veterinary Institute
Period: 01/04/2016 → 31/03/2019
Number of participants: 4
Phd Student:
Wolf-Jäckel, Godelind (Intern)
Supervisor:
Agerholm, Jørgen Steen (Ekstern)
Strube, Mikael Lenz (Intern)
Main Supervisor:
Jensen, Tim Kåre (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Samfinansieret - Andet
Project: PhD

Prebiotika til hindring af tarmesygdomme hos svin
National Veterinary Institute
Period: 01/07/2012 → 25/11/2015
Number of participants: 6
Phd Student:
Strube, Mikael Lenz (Intern)
Supervisor:
Meyer, Anne S. (Intern)
Main Supervisor:
Boye, Mette (Intern)
Examiner:
Licht, Tine Rask (Intern)
Hotchkiss, Jr., Arland Tillotson (Ekstern)
Thymann, Thomas (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Activities:

In situ production of prebiotics
Period: 10 Jun 2015
Mikael Lenz Strube (Invited speaker)
National Veterinary Institute
Section for Bacteriology, Pathology and Parasitology

Related event
FEMS 2015 meeting
07/06/2015 → …
Maastricht, Netherlands
Activity: Talks and presentations › Conference presentations