O-001.

Effect of a phytogenic feed additive on the growth performance and immunity of pacific white leg shrimp, *Litopenaeus vannamei*, fed a low fishmeal diet

J. Kesselring [1], *C. Gruber* [1,#], *B. Standen* [1], *S. Wein* [1].

[1] Biomin Holding GmbH, *Erber Campus 1*, 3131 Getzersdorf, *Austria*

Abstract
Fishmeal has long been one of the most important ingredients in formulated aquafeeds, due to its high protein content, excellent composition of essential amino acids, and high digestibility. Increasing economic and ecological concerns regarding the use of fishmeal have encouraged the development of replacement strategies. Plant-derived products have been suggested to partially replace fishmeal in aquafeeds, however often at decreased growth performance, inflammatory responses, and increased susceptibility to diseases. This study assessed the effects of the commercial phytogenic feed additive Digestarom® PEP MGE on the growth, nutritional performance, and immune response of *Litopenaeus vannamei*. Juvenile shrimp (N=540) were stocked in 36 tanks (V = 100 L) for 63 days and fed one of the four experimental diets: i) standard formulation (control, 24% fishmeal), ii) low fishmeal diet (5%), iii) low fishmeal diet plus 0.2 g/kg Digestarom® PEP MGE, and iv) low fishmeal diet plus 0.4 g/kg Digestarom® PEP MGE. The results obtained after 63 days of feed supplementation suggest that the blend of essential oils tested compensated for the negative performance and health consequences of the low fishmeal diet. Particularly, the survival, FCR, total hemocyte count, and respiratory burst of the shrimp fed a low fishmeal diet supplemented with this phytogenic improved up to the levels recorded for shrimp fed a high fishmeal diet. Overall, results suggest that Digestarom® PEP MGE can be incorporated into shrimp low fishmeal diets to compensate for the negative performance and immunological effects of partially replacing fishmeal with plant-based protein.
Keywords: phytogenics, low fishmeal, white leg shrimp, growth performance, immunity

Corresponding author.
E-mail address: christina.gruber@biomin.net (C. Gruber).

O-002.

Membrane associated protein flotillin in *Litopenaeus vannamei* plays a role in WSSV infection

Hong Shi [1,#], *Guangran Guo* [1,2], *Sujie Li* [1], *Rongdiao Liu* [1,2], *Xun Xu* [1,2], *Lingwei Ruan* [1].

[1] State Key Laboratory Breeding Base of Marine Genetic Resources, Key Laboratory of Marine Genetic Resources of ministry of natural resources, Third Institute of Oceanography, Ministry of Natural Resources, Key Laboratory of Marine Genetic Resources of Fujian Province, South China Sea Bio-Resource Exploitation and Utilization Collaborative Innovation Center, Xiamen, 361005, PR China
[2] School of Life Science, Xiamen University, Xiamen 361005, PR China

Abstract
Flotillin, an important protein of vesicular endocytosis, plays an essential role in a large number of cellular processes, including viruses and pathogen infection. In the present study, a *flotillin-2* homolog in *Litopenaeus vannamei*, designed as *Lvflotillin-2*, was cloned and characterized. To analyze the putative role of Lvflotillin-2 during white spot syndrome virus (WSSV) infection, real-time quantitative PCR was performed. The result showed that the transcriptional level of *Lvflotillin-2* was up-regulated significantly after virus challenge. Furthermore, upon WSSV stimulation, Lvflotillin-2 in shrimp cells could translocate from the plasma membrane to intracellular compartments, and unexpectedly, also into nucleus. Additionally, depletion of *Lvflotillin-2* inhibited WSSV gene *ie1* transcription. These observations indicated that Lvflotillin-2 was involved in viral infection and WSSV stimulation resulted in its dynamic localization.
Keywords: Shrimp, flotillin-2, WSSV, endocytosis

Corresponding author.
E-mail address: shihong@tio.org.cn (H. Shi).

O-003.

Complexity of *Penaeus monodon* Dscam gene structure occurs in both extracellular region and cytoplasmic tail

H.C. Wang [1,2,#], *K. Apitanyasai* [1], *C.F. Lo* [1,2], *H.T. Yu* [3].

[1] Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan
[2] International Center for the Scientific Development of Shrimp Aquaculture, National Cheng Kung University, Tainan 701, Taiwan
[3] Department of Life Science, National Taiwan University, Taipei, Taiwan

Abstract
In pancrustaceans, the Down syndrome cell adhesion molecule (Dscam) is an extraordinarily labile gene; thousands of isoforms can be generated by combining alternatively spliced exons from a single-locus gene. In insects, Dscam is involved in immunity as a hypervariable immune receptor. Similarly, we reported that Dscam was a hypervariable immune receptor in shrimp and crayfish. Interestingly, a unique tail-less Dscam identified in shrimp lacked a transmembrane domain and cytoplasmic tail. However, the mechanism to produce this unique tail-less Dscam is unknown. Here, we determined that the *P. monodon* Dscam (*Pm*Dscam) genome was ~250 kbp. The extracellular region had 10 immunoglobulin domains and six fibronectin III domains, i.e., [Ig1-Ig9]-[FNIII 1-FNIII 4]-[Ig10]-[FNIII 5-FNIII 6], with half of the second and third Ig domains and the entire Ig7 domain encoded by exon duplication. There were 26, 81, and 26 alternatively spliced exons in the Ig2, Ig3, and Ig7 domains, respectively, with potential to generate >54,000 protein isomorphs in the extracellular region of *Pm*Dscam. A very complex cytoplasmic tail structure was retrieved from this gene organization analysis. We identified three stop codon sites on the single gene sequence; furthermore, several exons encoded for cytoplasmic tail have also been identified. Taken together, *Pm*Dscam has potential to generate >21,000,000 unique isoforms via alternative splicing of both extracellular region and cytoplasmic tail, the highest potential number of isoforms among those crustaceans. In conclusion, we inferred that shrimp Dscam can use an alternative splicing event to produce selective isoforms against pathogens. Furthermore, Dscam may mediate specific immune responses in shrimp.
Keywords: Dscam, gene structure, genome, immunoglobulin family, shrimp

Corresponding author.
E-mail address: wanghc@mail.ncku.edu.tw (H.C. Wang).

O-004.

Artemia bioencapsulation delivers sulfated galactans to tissues and activates the expression of immune genes in shrimp

T. Rudtanatip [1,#], *K. Wongprasert* [2].

[1] Department of Anatomy, Faculty of Medicine, Khon Kaen University, Mittraphap Road, Muang District, Khon Kaen, 40002, Thailand
[2] Department of Anatomy, Faculty of Science, Mahidol University, Rama 6th Road, Rajdhevi, Bangkok, 10400, Thailand

Abstract
Live food has been used for feeding and delivery of the compounds to larval stages of aquatic animals. *Artemia* can be used as a possible vector for the delivery of different substances such as nutrients, probiotics and immune-stimulants. Sulfated galactans (SG) from *Gracilaria fisheri* has been demonstrated to stimulate immune activity and against pathogenic infection in shrimp. In vitro study revealed that SG bound with shrimp haemocyte and activated the expression of immune related genes in haemocyte culture. The present study was carried out to investigate the bioencapsulation of *Artemia* with SG for delivery to tissues and activating the expression of immune genes in shrimp. SG conjugated with fluorescein isothiocyanate (FITC-SG) showed no significant toxic in *Artemia*. Bioencapsulation of *Artemia* with FITC-SG (0-100 μg/ml) revealed that FITC-SG was located in *Artemia* gut lumen with a time and dose dependent manners. Tracking of SG in shrimp, shrimp fed with *Artemia* bioencapsulated with FITC-SG demonstrated that FITC-SG presented in shrimp gut until 2 h after feeding. Twenty-four hours after feeding, shrimp tissues were collected and the distribution of FITC-SG was observed in gill, hepatopancreas and haemolymph. FITC-SG was also found to bind with shrimp haemocyte. In addition, shrimp continuously fed with *Artemia* bioencapsulated with SG for 3 days showed activated the expression of immune genes including IMD, IKKβ, dicer and proPO-I, and prolonged high expression levels for a week. These results indicated that *Artemia* bioencapsulation could deliver the SG passed through gut to tissues and bound with haemocyte, and subsequently activated expression of immune genes in shrimp.
Keywords: *Artemia* bioencapsulation; Sulfated galactans; FITC-SG; Shrimp haemocyte; Immunity

Corresponding author.
E-mail address: tawut@kku.ac.th (T. Rudtanatip).

O-005.

Vaccination strategies and IgM responses against PKD in rainbow trout

M.N. Faber [1,#], J.W. Holland [2], C.J. Secombes [1].

[1] School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland, United Kingdom
[2] Institute of Medical Sciences, University of Aberdeen, Scotland, United Kingdom

Abstract
PKD is one of the most serious diseases affecting trout aquaculture in the UK. Caused by the myxozoan parasite, *Tetracapsuloides bryosalmonae*, PKD is elicited by the temperature-dependent development of parasite spore sacs in colonial bryozoans. Since recovered fish are known to exhibit protective immunity to re-infection, a successful treatment, based on the reduction of kidney pathology and parasite burden, could markedly reduce fish mortalities leading to improved productivity and fish welfare. Our investigations have focused on the selection of putative *T. bryosalmonae* virulence factors to unravel host immune evasion mechanisms exploited by the parasite, whilst shortlisting candidates for vaccine studies.
Here we report the results of three DNA-vaccination field trials using selected antigens administered individually or in combination. Some vaccine groups were found to have a partial protective effect in reducing PKD-associated kidney pathology whilst decreasing parasite load**.** Pathology reduction was improved in successive trials by improving the vaccination strategy.
We have functionally characterized the most promising antigen from our vaccine studies, which represents a novel micro-exon gene (*Tb*-MEG1). MEGs, until now, were thought to be unique to helminth parasites. In schistosomes, they exhibit extensive antigenic variability that is thought to enable greater plasticity in host protein targeting as a mechanism of host immune subversion over the course of infection. Using a validated anti *Tb*-MEG1 MoAb, we have demonstrated the protein to be expressed in and on the surface of parasites and a subset of immune cells within the kidneys of infected fish. We have also demonstrated potent *Tb*-MEG1-specific IgM responses in sera from parasite-infected (farmed) rainbow trout and have successfully induced a specific IgM response after protein vaccination.
DNA vaccines encoding molecules homologous to proteins involved in nutrition acquisition, cell-cell interactions or of unknown function, have shown promise towards the development of a future PKD vaccine, that may also be applicable to the generation of vaccines against other fish parasites. The discovery and characterization of *T. bryosalmonae* antigens has provided valuable insights into host immune evasion by myxozoan parasites, with the present *Tb*-MEG1 studies also having major implications towards understanding the evolution of antigenic variability in metazoan parasites.
Keywords: Vaccination, myxozoan, parasite, antigen, igm response

Presenting author
E-mail address: Marc.Faber@abdn.ac.uk (M.N. Faber).

O-006.

Demonstration of herd immunity effects in dna vaccinated rainbow trout

N. Lorenzen [1,#], E. Lorenzen [1], J.S. Rasmussen [1], T.E. Kjaer [1], K. Einer-Jensen [2].

[1] Technical University of Denmark, Lyngby, Denmark
[2] Qiagen-Aarhus, Aarhus C, Denmark

Abstract
While DNA vaccination by intramuscular injection is known to provide highly protective immunity against viral haemorrhagic septicaemia (VHS) in rainbow trout at an individual level, we here aimed at examination of heard immunity effects. Induction of clinical disease following experimental challenge by waterborne exposure of the fish to the pathogens represents a hurdle in vaccine potency testing for many pathogens, but in the case of VHS virus, challenge can be done efficiently by immersion. However, progression and level of mortality depends on the challenge dose. This has some implications for the experimental design and interpretation of the results. Initial experiments indicated that while mortality following a high challenge dose was independent on whether the fish were kept together in one aquarium or in individual aquaria, indirect infection by virus released from primary infected fish seemed to play an important role at low challenge doses. In experimental vaccination trials, it may be assumed that challenge of cohabitant naïve and vaccinated fish represents a harder test of vaccine potency compared to challenging the vaccinated fish separately due to secretion of high amounts of virus from the naïve fish. In challenge trials with high inoculum of virus, this seemed to be true for VHSV challenge of DNA vaccinated rainbow trout as illustrated by increased mortality among vaccinated fish cohabitated with naïve fish. However, at lower inoculum, the effect tended to be opposite in the sense that mortality among the naïve fish was reduced. Implications of the results for experimental vaccine testing will be discussed.
Keywords: Fish rhabdovirus, DNA vaccine, challenge setup, vaccine testing, herd immunity

Corresponding author.
E-mail address: nilo@aqua.dtu.dk (N. Lorenzen).

O-007.

Exposure to antibiotics affects saponin immersion induced immune stimulation and shift in microbial composition in zebrafish larvae

A. López Nadal [1], D. Peggs [2], G. Wiegertjes [3], S. Brugman [1,#].

[1] Cell Biology and Immunology, Animal Sciences Group, Wageningen University and Research, Wageningen, Netherlands