pathogens which is evidenced from the decrease in optical density in haemolymph supplemented broth for crab and shrimp respectively for (*A. hydrophila* - 0.31, 1.06, 1.18, 1.71, 1.78 & 0.32, 1.25, 1.32, 1.98, 2.04; *P. aeruginosa* - 0.12, 0.31, 0.77, 1.53, 1.62 & 0.30, 0.45, 1.05, 1.52, 1.94) compared to control both for (*A. hydrophila* - 1.27, 1.50, 1.72, 2.16, 2.38 & 1.07, 1.31, 1.53, 2.26, 2.31*; P. aeruginosa*-0.82, 1.18, 1.54, 1.74, 1.94 & 0.62, 1.09, 1.36, 1.68, 2.05). The probable reason may be lysis of bacterial cells by the antimicrobial proteins present in haemolymph. Crustacean serum protein profiling was carried out using SDS-PAGE to detect antimicrobial proteins. In SDS-PAGE, the antibacterial proteins found in shrimp haemolymph were 20, 25, 40 and 60 kDa, whereas in crab haemolymph they were 20 and 25 kDa. In conclusion, crustacean haemolymph has the ability to clear the external pathogens from the haemolymph.

AH PO 10

Antimicrobial activity of fish blood against three pathogenic bacteria and detection of serum proteins

K. VIDYA, N. RANJIT KUMAR, G.K. SIVARAMAN, V. MURUGADAS, M.M. PRASAD*

ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India; *prasadmm@hotmail.com*

Fish blood contains several antibacterial proteins that play very important role in the immune responses especially innate immune system which has garnered lesser attention when compared to higher vertebrates. The present study reports the antimicrobial property of fish blood on commonly occurring fish pathogens. The reference cultures employed in this study included *Pseudomonas aeruginosa* (ATCC 10145), *Edwardsiella tarda* (ATCC 15947) and *Aeromonas hydrophila* (ATCC 35654). Fish blood was aseptically drawn from the caudal vein using 23 gauge sterile needle. The bacteria were inoculated into sterile Brain-heart infusion (BHI) broth and 1% blood was added to the broth. After inoculation, broth was incubated at 30^0C for 48 h with observations on the bacterial density using spectrophotometer at 4, 8, 16, 24 and 48 h intervals. For comparison, readings of two sets of control i.e. one with BHI broth & blood and another BHI broth with bacteria (without blood) were taken. The optical density was measured at 4, 8, 16, 24 and 48 h in blood supplemented broth for *A. hydrophila* (0.69, 1.28, 1.50, 1.50, 1.54); *P. aeruginosa* (0.51, 0.73, 0.92, 0.96, 1.03) and *E. tarda* (0.60, 0.90, 1.23, 1.25, 1.32), whereas increase in optical density for *A. hydrophila* (1.27, 1.50, 1.72, 2.16, 2.38); *P. aeruginosa* (0.82, 1.18, 1.54, 1.74, 1.94) and *E. tarda* (1.03, 1.74, 1.97, 2.14, 2.17) in control broth (without blood) was noticed at same time periods. The clear inhibition of growth was noticed for the three pathogens in BHI broth supplemented with fish blood when compared to control broths. The presence of antimicrobial substances in the fish blood is responsible for the inhibition of growth. The antimicrobial substances present in the fish serum was analysed with 10% SDS-PAGE, which revealed proteins of 20, 22, 23, 33, 34, 44, 45, 46 and 65 kDa. In conclusion, growth of fish pathogenic bacteria is inhibited by the presences of antibacterial proteins in fish blood.

AH PO 11

Haematological, biochemical and antibacterial response of andrographolide in *Labeo rohita* fingerlings

K. AHAMED BASHA[1]*, N. RANJIT KUMAR[1], S. EZHIL NILAVAN[1], R.P. RAMAN[2], K. SOURAV[2], K. KUNDAN[2], K. PANI PRASAD[2], M.M. PRASAD[1]

[1]ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India; [2]ICAR- Central Institute of Fisheries Education, Mumbai, Maharashtra, India; **ahamedfishco@gmail.com*

Herbs and their active ingredients application in aquaculture sector have been an alternative strategy to counter chemotherapy. Andrographolide (EC 50%), a diterpenoid lactone derived from the herb, *Andrographis paniculata* was used in the present study to evaluate its efficacy on haematological, biochemical profiles in *Labeo rohita* fingerlings. Fishes were fed with a diet containing 0.0 g kg^{-1} (control), 1.0 $g.kg^{-1}$ (T_1), 2.0 $g.kg^{-1}$ (T_2), 4.0 $g.kg^{-1}$(T_3) and 8.0 $g.kg^{-1}$ (T_4) of andrographolide (EC 50%) and were sampled on 14th, 28th, 42nd and 56th day for haematological and biochemical analysis. On 56th day blood samples were taken for analysis of haematological, biochemical parameters and challenge study was conducted by injecting *Aeromonas hydrophila*. *In vitro* antibacterial test was performed by using 0 mg L^{-1} (T_0), 250 $mg.L^{-1}$ (T_1), 500 $mg.L^{-1}$ (T_2) and 750 $mg.L^{-1}$ (T_3) of andrographolide (EC 50%) through standard agar well diffusion method against fish pathogens such as *P. aeruginosa*, *A. hydrophila* and *E. tarda*. Biochemical and haematological parameters at the end of 42 days trial indicated that Andrographolide (EC 50%) administered through feed significantly ($p<0.05$) enhanced serum total protein, globulin, total erythrocyte counts and haemoglobin content. The survival rate was significantly ($p<0.05$) high in experimental diet fed groups T_2 followed by T_1 and T_3 when compared with control. The post challenge total leukocyte count, total protein, and globulin were higher in treated groups as compared to control group. The highest zone of inhibition (mm) 17, 11, 13 against *P. aeruginosa*, *A. hydrophlila* and *E. tarda* was recorded with 750 mg L^{-1} andrographolide (EC 50%). The results suggested that andrographolide (EC 50%) at the level of 2 $g.kg^{-1}$ exhibited stimulatory effect on haematological and biochemical parameters with increased disease resistance in *L. rohita* fingerlings against *A. hydrophila* infection.

AH PO 12

Molecular identification of *Aeromonas hydrophila* from C*larias batrachus* (Burchell, 1822)

P.J. SARLIN*, M. MEGHA, A.T. JENNIFER

Fatima Mata National College, Kollam, Kerala, India; **sarlinpoly@yahoo.com*

The parasitic infestation in the commercially important fish *Clarias batrachus* was investigated. The study based on examination of *Clarias batrachus* under aseptic conditions collected from a commercial farm at Kollam, Kerala revealed the bacterial flora of the catfish to be fairly rich comprising *Aeromonas hydrophila*. Identification of Aeromonas species is known to be troublesome due to their phenotypic and genotypic heterogeneity. The gut region samples were serially diluted, incubated and plated. The edited sequence (16S rRNA sequence) were then used for similarity searches using BLAST (Basic Local Alignment Search Tool) program in the NCBI Genbank DNA database for identifying the sample. The 16S rRNA gene was identical and exhibited 100% sequence similarity with the other known isolates of *A. hydrophila* available in the GenBank. *Aeromonas hydrophila* is a heterotrophic, Gram-negative, rod-shaped bacterium mainly found in areas with a warm climate. This bacterium can be found in fresh or brackish water. It can survive in
aerobic and anaerobic environments, and