



January 12, 2010

## **INTERKOI REFERENCE REPORT ON CHRISAL PIP:**

Interkoi-Arendonk is an international company specializing in high quality Japanese Koi fish. Our facilities cover ten tank basins holding a quarter of a million liters of water. These are both inside and outside tanks, most of which are heated. Individual Koi fish can run many thousands of Euros each, with some in the many tens of thousands of Euros, and so the health and well being of each fish is of great importance and no risk can be taken with these valuable fish.

In February, 2008 we were approached by Chrisal NV from Lommel, Belgium, who specialize in microbial (probiotic) healthcare and cleaning products, to test Chrisal's new product for fish farms and ponds, the PIP (Probiotics In Progress) Pond Plus that was also used for animal slurry ponds.

The idea behind the PIP product being that fish ponds, especially heavily or overpopulated ones, often contain large amounts of pathogenic bacteria and other contaminants and factors that threaten the health and life of the fish populations. This is of particular concern when dealing with our facility's very expensive and illness-susceptible koi.

When Interkoi studied the results of the use and testing of Chrisal's products in other facilities, we found that Chrisal's PIP Plus Pond was of particular interest to us because this product was developed as a solution for the three main goals for fish farms and aquarium operations: (a) the optimum removal of organic matter/dirt, (b) to support and optimize the nitrogen cycle and (c) to establish and the maintenance of healthy and hygienic water conditions, as well as to greatly reduces the risk of infections.

The testing of the use of Chrisal's PIP Pond Plus was performed on two of the facility's outside basins (tanks), one (heated) inside basin and, of great special interest, the quarantine facilities with sick fish. The testing started on the 6<sup>th</sup> of March, 2008 with the full support of Dr. Robin Temmerman and B.sc. Guy Vossen. Water analyses were made before the introduction of the Chrisal products and then ongoing analyses were done to document the progress of the testing.

The microbiological analyses showed that at the start of the test period, right before the addition of the PIP Pond Plus to the fish tanks, there were considerable amounts of *Aeromonas hydrophila* and *Escherichia coli* in existence in the water. The presences of these bacteria are considered to establish a real danger for infections.

Then after the initial testing to set a base standard for the testing, all the aforementioned basins were treated with PIP Pond Plus. After just the first week, testing already showed a 10% decrease in these pathogenic bacteria. After two weeks the microbiology in the

pond water showed a considerable improvement and the numbers of pathogenic bacteria further decreased. At this time, some dominance of the *Chr. Bacillus* bacteria could be documented. After three weeks, the levels of *Aeromonas hydrophila* and *Escherichia coli* were down by 30%. Also of great importance was that the quality of the pond water in the tanks were improved a great deal and the difference seen was actually noted with the word 'spectacular'. In addition, it was also noted that the fish in the PIP treated tanks were now swimming in a "much more lively way" – and most important, was that the ill fish that were in the quarantined facilities were significantly improved.

After six weeks of using PIP Pond Plus, the number of pathogenic bacteria had been reduced to a very low level and the quality of the pond water now was listed as good to very good. Microbiological laboratory counts showed that numbers of pathogenic bacteria had been reduced by 80% of the original count that was present at the start of the test when PIP was introduced. What was also most interesting was that once the PIP Pond Plus took control of the environment in the tanks, the fish now performed excellently. This was documented in both the outside tanks as well as in the inside basins.

What was also of special interest was that It could be seen that the wounds and damages on the hurt and ill fish in the quarantine facilities had entered into a solid healing process, and that this had accomplished in a far faster time period than could be normally expected.

**SPECIFIC LEVELS:** by the end of the initial testing and use period, one of the key results were that in the water tanks first tested without using Chrisal PIP, we experienced the normal growth of a great deal of pathogenic bacteria (approx 2200 per millimetre). However, once our facility started using PIP Pond Plus, the growth was greatly reduced (to approx 440 per millimetre).

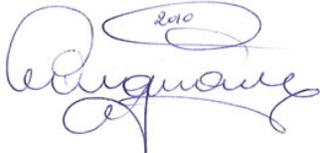
After the clear and excellent results of this well documented and expansive series of testing, we have, of course, continued to use the Chrisal PIP products in our own facilities over the last two years.

In addition, to then further safeguard the fish we sold to all our customers, we also started to provide the PIP products to our customers to assure the health and wellbeing of the fish we sell (and therefore assure our customer's satisfaction). It has

been rewarding that since we started these sales, through to today, there have not been one single negative and we have only received very favorable comments.

We are pleased to be able to share these results with others in our field.

Sincerely,



BORGMAANS H.L.A.

Luc Borgmans,  
Managing Director,