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PHASE I - The Effect of PrimaLac on Disease Resistance of Common Carp (*Cyprinus carpio*)

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EXPERIMENTAL GOALS

I – GENERAL HEALTH:

- 1) Presence of disease causing organisms among treated & non-treated populations
- 2) Morbidity rates
- 3) Mortality rate

II - EFFECT OF PRIMALAC ON “ULCERATIVE DISEASE” OF COMMON CARP.

Experimental Protocol

Challenge against “ulcerative disease of Carp” (*Aeromonas spp.*)

- 6 groups of Common Carp (*Cyprinus carpio*) were stocked for a period of 2 months in the Aquavet Facility.
- Three groups of fish, (**Control vat # 1, 2 & 3**) were fed with M.R.C. Common Carp feed.
- Three groups of fish (**PrimaLac vat # 1, 2 & 3**) were fed with M.R.C. Common Carp feed, supplemented with **PrimaLac** at the recommended dose.
- All the fish will be stocked at the 5-10 Gram range.
- Every vat will be stocked with 150 fish. (Total of 900 fish)
- **PrimaLac vats # 1, 2 and 3** were challenged by cohabitation with clinically symptomatic (ulcerated) Common Carp and Koi.
- Presence of *Aeromonas spp.*, the causative agent of this disease in these fish was confirmed in our bacteriology lab.
- **Control vats # 1, 2 and 3** were also challenged by cohabitation with clinically symptomatic (ulcerated) Common Carp and Koi.
- During the next 6 weeks, Common Carp populations in the **Control vats and the PrimaLac vats** were closely monitored for the development of clinical symptoms of ulcerative disease.

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- Bacteriology samples were part of this monitoring program and were conducted every 2 weeks. Dead fish were confirmed for presence of *Aeromonas spp.*



Ulcerative Disease of Common Carp (*Cyprinus carpio*)

RESULTS:

As indicated in the clinical trial experimental protocol, 30 clinically symptomatic Koi (*Cyprinus carpio*) were introduced into the vats of all 6 populations (Control # 1, 2, 3 and PrimaLac 1, 2, 3). Total of 5 sick fish were introduced to each vat.

Koi population was confirmed for presence of *Aeromonas Spp.*, the causative agent of Ulcerative Disease.



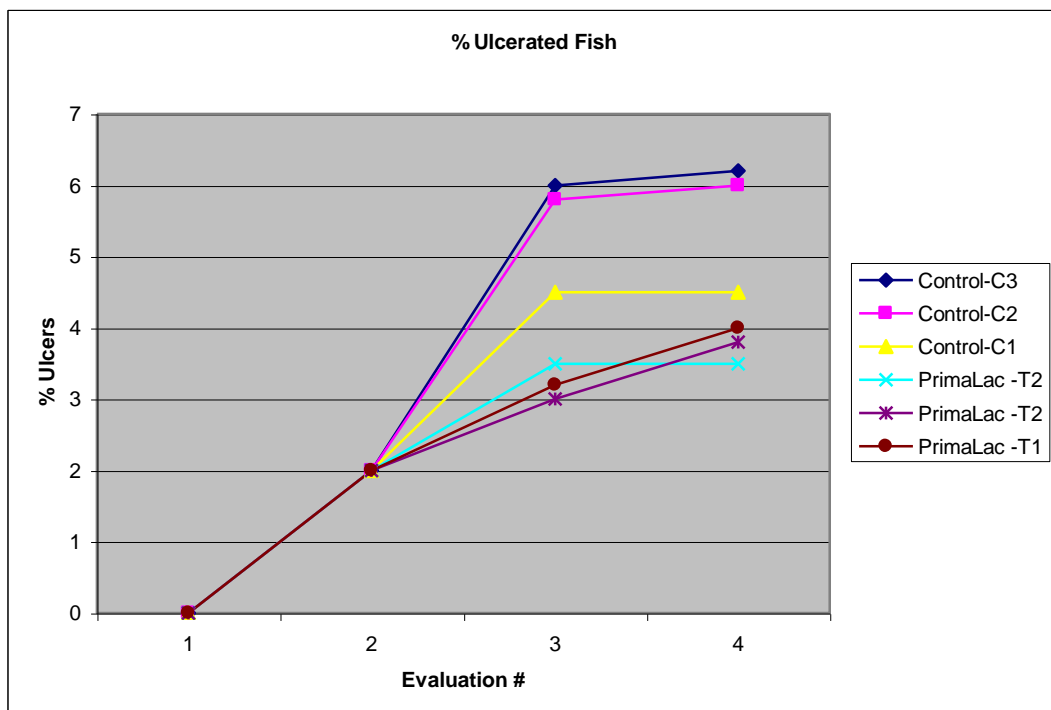
***Aeromonas Spp.* (from Koi) Grown on B.H.I. Agar**

RESULTS: cont.

During the next 60 days, Common Carp populations were evaluated for development and presence of ulcers and/or other clinical signs characteristic of Ulcerative Disease. Early signs of Ulcerative Disease were first observed during the second evaluation, 30 days from the beginning of the cohabitation period. At that time, 2% of the population (in all 6 tanks) was diagnosed as symptomatic for Ulcerative disease.

The Common Carp population in the vats was confirmed for presence of *Aeromonas Spp.*, the causative agent of Ulcerative Disease regardless of the absence of clinical symptomology. Forty-Five Days (45d) from the beginning of the cohabitation period, differences between the **PrimaLac** and Control vats were clearly evident.

- Average Ulceration rate in the Control Tanks was 5.4 %.
- Average % Ulceration in **PrimaLac** Tanks was 3.2%.
- 60 Days from the beginning of the cohabitation period, (last day of experiment) average % Ulceration in Control Tanks was 5.5 %.
- Average % Ulceration on Day 60 in **PrimaLac** Tanks was 3.7 %.

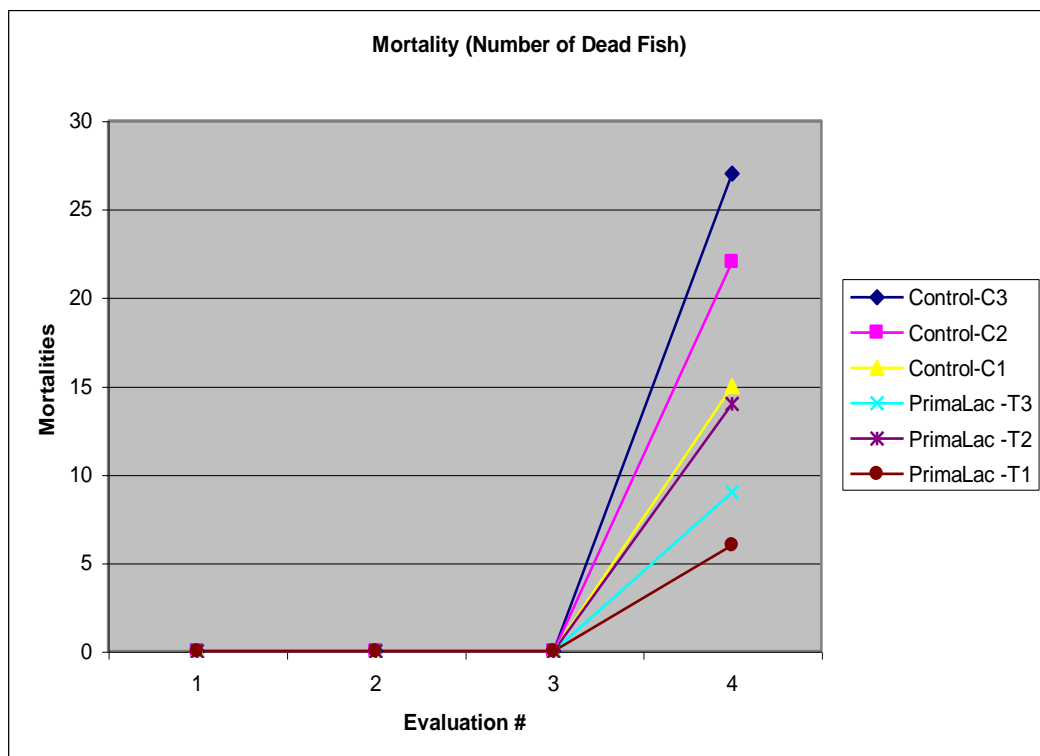


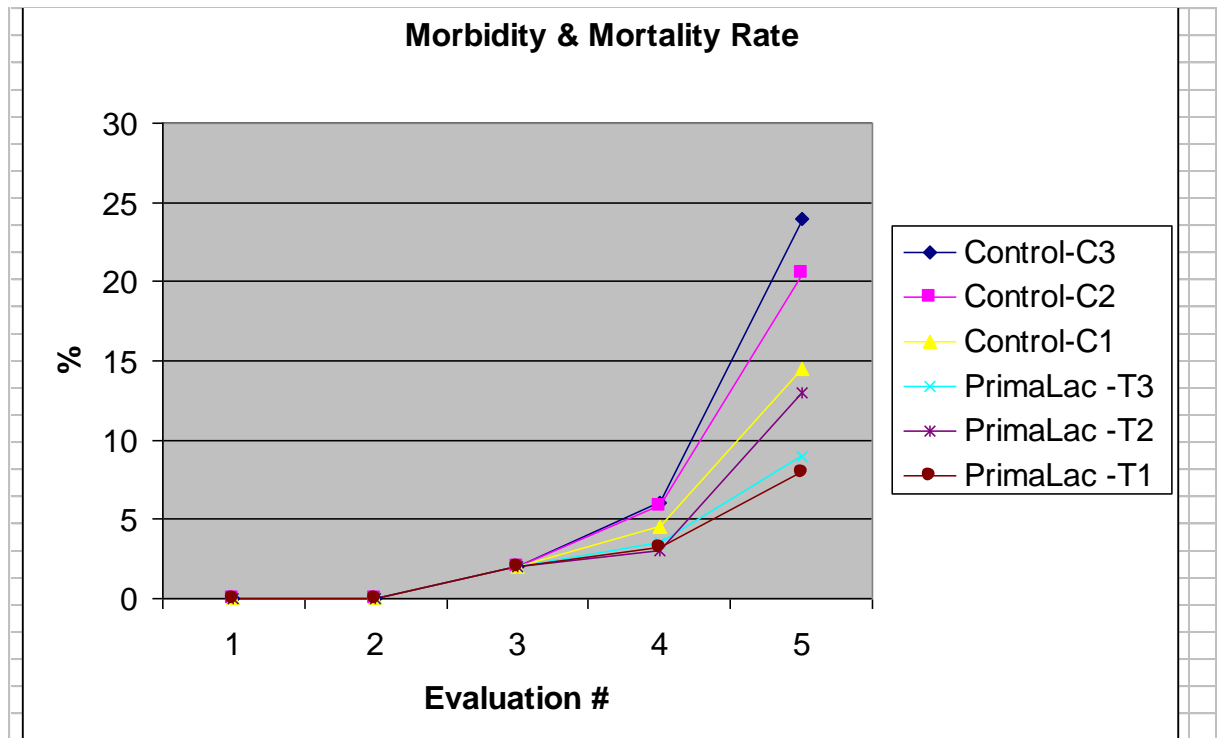
RESULTS: cont.

Mortality due to systemic infection of *Aeromonas Spp.* was clearly evident during the last 15 days of this trial. Ulcerative Disease was further complicated (as expected) by the presence of *Mycobacterium Spp.*, a common secondary opportunistic bacterium.

- 60 Days from the beginning of the cohabitation period, (last day of experiment) average % morbidity and mortality rate in Control Tanks was 19.6 %.
- Average % morbidity and mortality on Day 60 in **PrimaLac** Tanks was 10 %.

As such, total number of ulcerated and/or dead fish in Control vats was nearly 100% higher in comparison to the **PrimaLac** supplemented vats.





CONCLUSION

Based on these findings, we can clearly conclude that Common Carp (*Cyprinus carpio*) which were regularly fed **PrimaLac** formula exhibited increased resistance to bacterial infection when challenged with *Aeromonas spp.*, the causative agents of Ulcerative Disease of Carp.

PHASE II - The Effect of PrimaLac on Disease Resistance of Red *Tilapia aurea*

Conducted in Ein-Hamifratz Fisheries



Ein-Hamifrats Fish Farm

Ein-Hamifratz Fish Farm, one of the most advanced aquaculture facilities in Israel is located along the Northern coast of Israel in the Haifa bay. The farm was constructed over 50 years ago. Surface area of the farm is 60 Ha. It includes semi intensive earthen ponds, a large Intensive rearing unit, a large scale fish hatchery, (mainly of red Tilapia) and a new super Intensive rearing unit for Red *Tilapia aurea* and Sea Bream. (*Sparus aurata*)

The farm's annual production averages 600 metric tons of the following species: Common Carp, Tilapia, Silver Carp, Mullet, Sea Bream and Hybrid Stripped Bass. Of which they produce over 30 Million Male Tilapia Hybrids and Red Tilapia aurea.

For over 50 years the professional team in Ein-Hamifratz has been successful in intensifying its culture units and enhancing production in spite of the ever-growing restrictions on water consumption and the continuous change in the business environment.

The Super Intensive Unit

The super Intensive rearing unit in Ein-Hamifratz Fish Farm has been operational for over 10 Months. This recirculated facility is focused on production of Red Tilapia (*Tilapia aurea*) and Sea Bream (*Sparus Aurata*) in very high densities and with minimal utilization of fresh water.



The super Intensive facility in Ein-Hamifratz is currently the most advanced rearing facility in Israel. Regardless, this facility is suffering from chronic presence of infectious disease, mostly of bacterial nature. Bacterial problems are responsible for severe morbidity and mortality rates within this facility. Furthermore, these problems lead to suppressed growth rates and feed efficiency rates.

As result of this situation, this facility is heavily dependent on continues utilization of anti-bacterial medications. These medications cause severe environmental effects in the re-circulated system through their effect on the film of nitrifying bacteria in the biological filter which, in turn, will lead to rapid deterioration in water quality parameters. Such deterioration will lead to additional stress and additional development of secondary disease causing organisms.

Anti-bacterial medications will also effect the marketing program of this facility due to the lengthily withdrawal periods required prior to marketing. In such an industrialized facility, any marketing problem or delay will directly affect the production program and the overall profitability of this facility.

Major Diseases Present within the facility:

Bacterial Diseases

Gram Negative Bacteria:

Tilapias affected by gram negative bacteria in Ein Hamifratz Fisheries are commonly afflicted with a bacterial hemorrhagic septicemic infection. Affected fish will show signs of darkening, exophthalmia, loss of appetite, and hemorrhagic or ulcerated areas at the bases of the pectoral and ventral fins and in the eye region.

Internally, it is common to find pale livers and the presence of hemorrhagic foci.



Bacterial hemorrhagic septicemia (BHS) can break out and lead to losses of 5-20%. Microbiological isolates which were collected during the outbreaks include *Vibrio* spp., *Aeromonas hydrophila* and other species of motile aeromonads.

Treatment involves administration of antimicrobial substances mixed into feed.

Gram Positive Bacteria:

Streptococcus, a gram positive bacterium is now considered to be the most important pathogen in Tilapia operations worldwide. Damages due to Streptococcal infection in Tilapia populations will include very low survival rates (no more than 60%), decreased growth rates, prolonged production cycles and the vast cost of medications and disease control efforts.



In Ein-Hamifratz, streptococcosis usually produces a chronic disease characterized by the presence of granuloma that affects the brain. The affected tilapia show erratic and disorientated swimming movements as a result of the meningoencephalitis that results. In other cases, the animals show clinical signs similar to those of bacterial hemorrhagic septicemia.

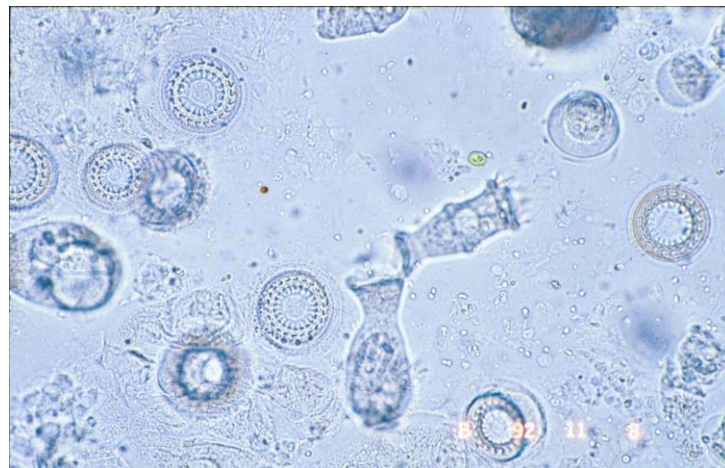
The prevention of streptococcosis is related to appropriate management and hygiene, including the removal and destruction of affected fish and the utilization of anti-bacterial compounds. The use of antimicrobials to control streptococcosis has been made more difficult by the development of resistant strains. The antimicrobials do not destroy all the bacterial cells stored within the macrophages, which gives rise to the reappearance of the disease once antimicrobial therapy has been discontinued.

Parasitic Diseases

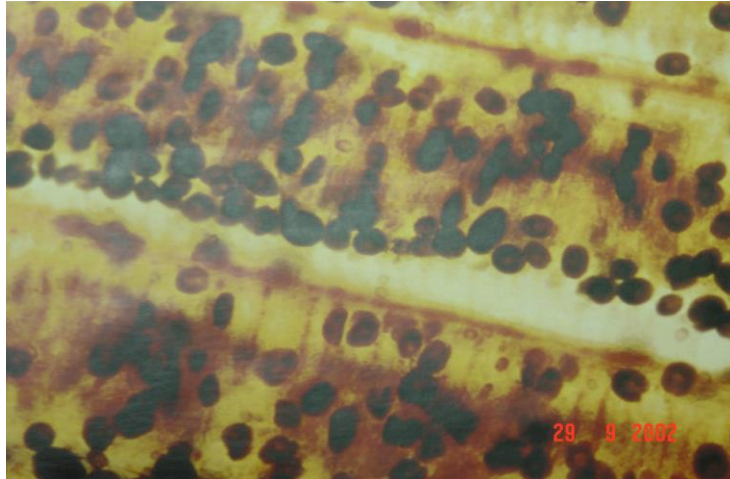
Ciliates

The trichodinids, which include the genera *Trichodina*, *Trichodinella* and *Tripartiella* cause severe parasitism on the gills and skin of Red Tilapia aurea in Ein-Hamifratz Fishery.

Their presence is indicative of high population densities and poor water quality.



Amyloodinium ocellatum is a salt and brackish-water parasite found in the Ein-Hamifratz facility. In smaller fish, the infection seems to be limited to the skin, whereas, in larger fish it becomes established on the gill filaments and in the integument. Its control involves baths of copper sulfate.



Monogeneans

The monogeneans are parasites that have foliating action on the skin and gills of Tilapia. They feed on the mucus and epithelium of the body surface, producing external lesions that erode and expose the dermis to infection. As in the case of trichodinids, monogeneans are extremely aggressive organisms. Monogeneans are controlled by baths in formalin and Naled. These parasites (*Gyrodactylus* spp.) can cause heavy mortalities in tilapia within a short period of time.



EXPERIMENTAL GOALS

The goals of this study will be achieved through evaluation of the following parameters:

1. Presence of disease causing organisms:

- Viruses
- Bacteria
- Parasites
- Fungus

2. Morbidity rates

3. Mortality rates

EXPERIMENTAL PROTOCOL

- 6943 Red *Tilapia aurea* were stocked in one vat of the Ein Hamifratz Super Intensive rearing unit.
- Average Weight at stocking was 117 Gram.
- Volume of vat is 16 Cubic meters.
- Total Weight at stocking was 812 Kg. per vat.
- After 35 days, (230 Gram range) fish were separated into two vats (SM. and LG.) for continued growth to market size.
- After an additional 60 Days, large fish were marketed at the 450 Gram range.
- During this period, 2-3 other groups of Tilapia were introduced into the facility.

The performance of these "Control" groups will be compared with the performance of the "**PrimaLac**" groups throughout the period of the clinical evaluation.

- "Control" groups of fish will be fed with M.R.C. Tilapia feed.
- "**PrimaLac**" groups will be fed with M.R.C. Tilapia feed, supplemented with **PrimaLac** at 2 Kg per Ton of Feed.
- Feeding ratio was based on 2 % body weight per day.
- Adjustment of feeding ratio was based on the continuous (every 10 days) evaluation of average weight in each vat.

EXPERIMENTAL PROTOCOL: cont.

Overall performance of the fish in all the vats was monitored by the Aquavet staff and included detailed evaluation of the following parameters:

- Average growth rates.
- Average Wt. Of the fish.
- Length of the fish
- Condition factors
- Feed conversion rates.
- This evaluation was completed on a weekly basis.

Experimental protocol included the daily assessment and of the following water quality parameters:

- Ammonia
- Nitrite
- PH
- Temperature
- Oxygen

In addition, the staff in Ein-Hamifratz conducted a daily assessment of mortality and morbidity rates. Veterinary assessments were conducted on a weekly basis.

Exact diagnosis of disease causing organisms, (parasites, bacteria or fungal infections) treatment protocols and treatment results were collected throughout this period, and are an essential part of the final evaluation.

RESULTS: (Summary)

The super-Intensive facility in Ein-Hamifratz was heavily depended on utilization of anti-microbial compounds. This continued use of antibiotics resulted in drastically increased costs, severe environmental damages and marketing problems due to long withdrawal periods.

Utilization of **PrimaLac** in the treated vats (and to date, within most of the facility) enabled to stop all usage of medication within this facility.

- Furthermore, since the introduction of **PrimaLac** into the feed total morbidity and mortality rates within the facility decreased from an average of 1200 - 1800 dead fish per month to an average of 200 fish per month.
- Fish exhibiting clinical signs of Strep were dramatically decreased.
- Findings of secondary parasitic infections (Trichodina, Gyrodactilus and Amylodium) are negligible.

The inclusion of **PrimaLac** in the diets has not compromised function of the biofilters, which are the “heart” of this facility. **PrimaLac** maybe acting in this facility through their action on pathogenic bacteria (mostly Aeromonas and Strep.) found within the re-circulated environment.

Anorexic periods of the fish caused by presence of sub-optimal environmental conditions have been prevented by the supplementation of **PrimaLac** to the feed.