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

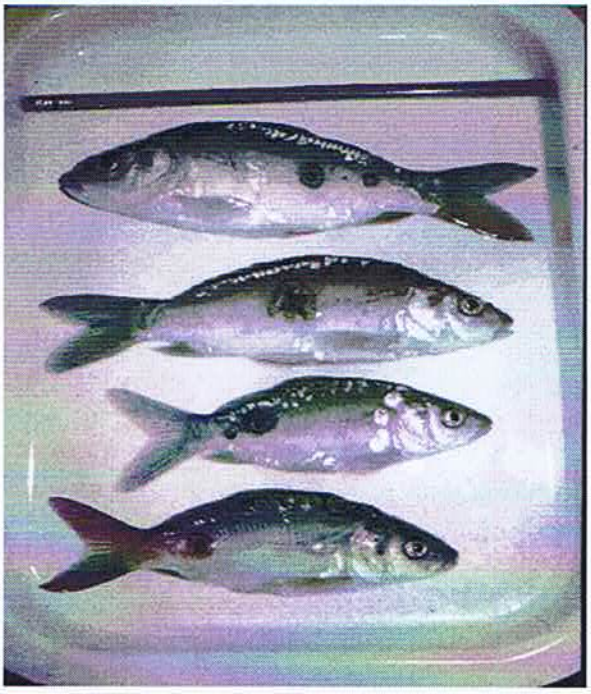
II - EFFECT OF PRIMALAC ON "ULCERATIVE DISEASE" OF COMMON CARP.

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.

- 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.

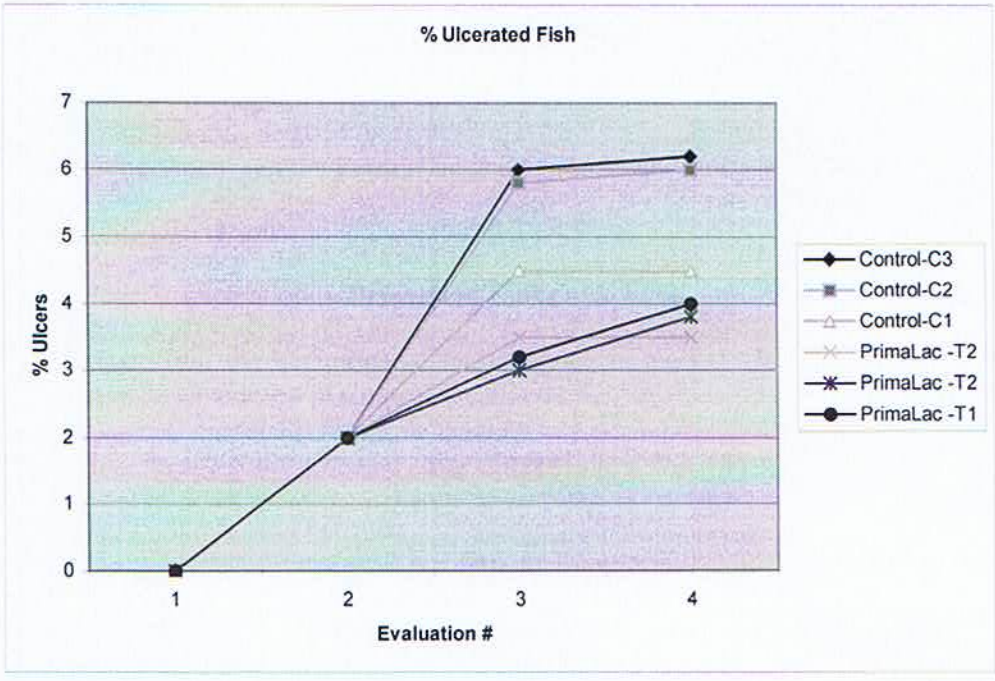
***Aeromonas Spp.* (from Koi) Grown on B.H.I. Agar**

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 %.

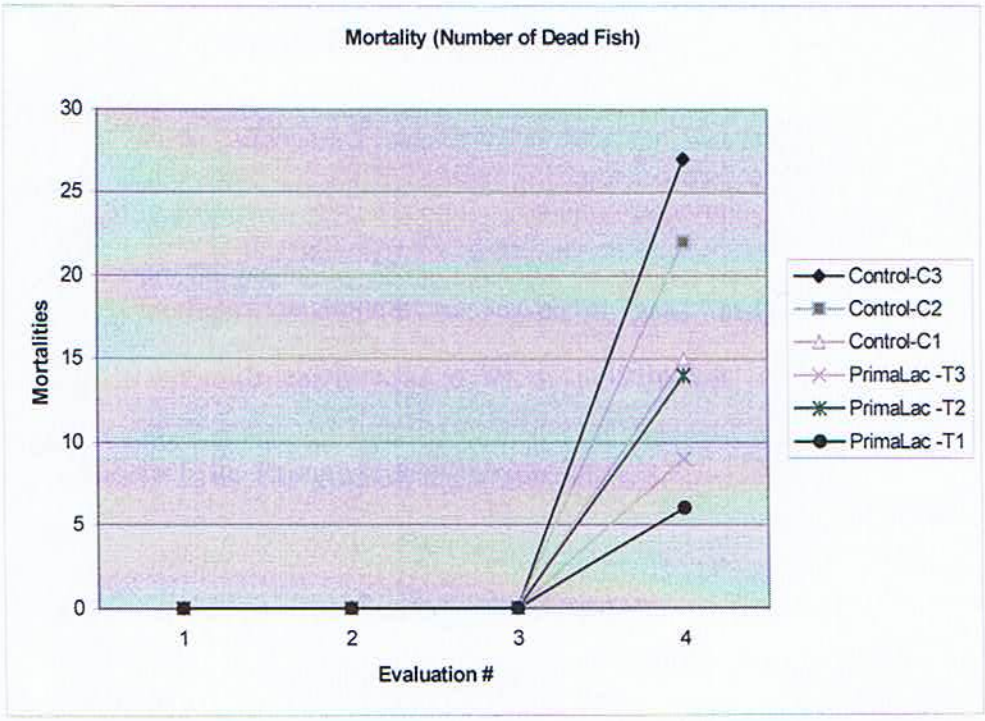


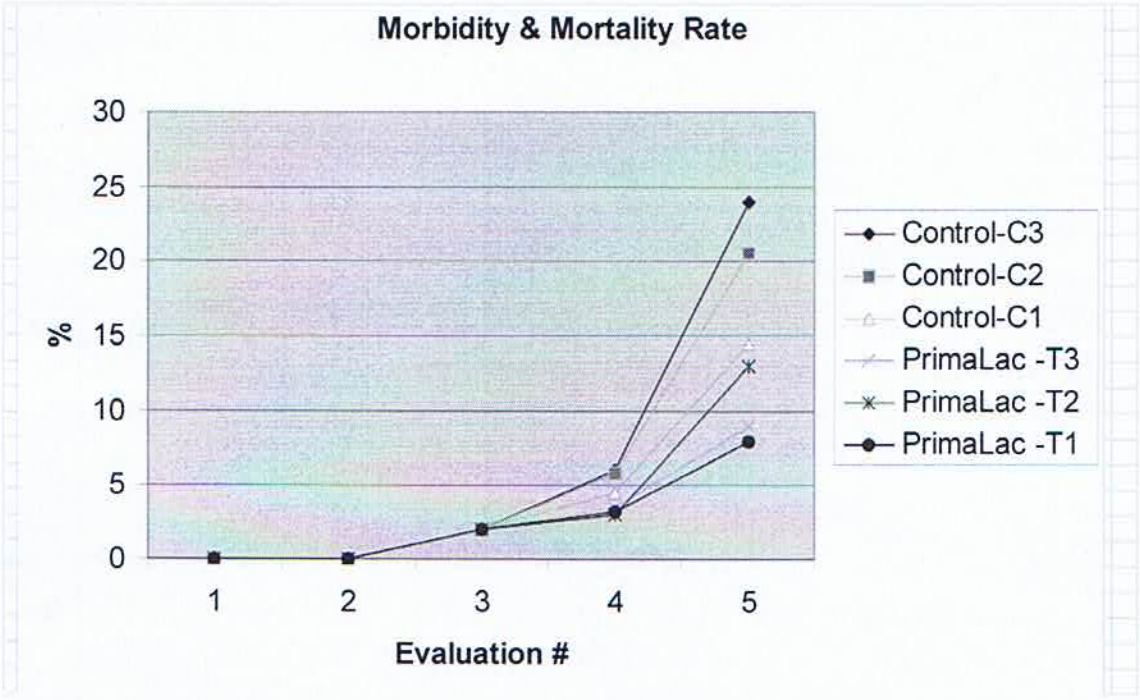
RESULTS: cont.

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.