

February 26, 2008

NOAA Attn: Alternative Feed Initiative

# Re: Recommendations on Alternative Feeds for Aquaculture

This letter is in response to your request for recommendations and ideas pertaining to alternative feeds and feed ingredients for aquaculture. For the past eight months, Neptune Industries, Inc. has pioneered next-generation feed research through a cooperative relationship with Mississippi State University. The research is focused on development of a sustainable, high protein meal derived from the commercial mass production of select insect species. Such insects would be grown, harvested, dried, ground and packaged for use in fish, shrimp, and livestock diets. Fishmeal dependency represents the most significant bottleneck to achieving projected industry growth over the next 20 years and beyond.

To date, three phases of research have been completed, through the self-funding efforts of Neptune Industries. The results to date have been very encouraging. A number of viable insect species have been identified based upon a litany of production, nutritional, and logistical parameters required for mass production. Two week feeding trials on hybrid striped bass were completed in October, 2007, with 100% fishmeal replacement. Control and experimental fish were subjected to blind taste testing at MSU's Food Science and Technology Dept. A blind panel actually preferred the taste of fish fed the insect based diet.

A 60 day growth trial is now underway to assess digestibility and food conversion relative to traditional fishmeal based diets. The results should be completed in April, 2008.

This letter is brief, as I am departing in two days for Bangkok, Thailand to present our research findings at the Aquafeed Horizons conference there. For informational purposes, and in the essence of time, I have included via email a copy of a paper that I wrote as a written summation of my presentation for this conference. This should provide further perspective as to the enormous potential of this product in our industry.

In summary, much work needs to be done on insect diet development, blending of different species, habitats, quality control, processing, etc. etc. We have initiated this exciting research, however bringing it to fruition will require support. This effort cannot be expected to be self-funded in its entirety. I sincerely hope that this review panel will consider the enormous industry, environmental, ecological, and employment implications that this research holds, not only on a national level, but globally. I remain

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available to discuss our progress, and future needs following my return on March 10, 2008.

I look forward to your reply and consideration for future grant funding opportunities.

Sincerely,

Ernest D. Papadoyianis, President

# Aquaculture Independence Sustainable Production of a High Quality Fishmeal Substitute from Insects Ernest D. Papadoyianis, President, Neptune Industries, Inc.

The global aquaculture industrv currently accounts for over 45% of all seafood consumed. That figure has been projected to increase to 75% over the next 20 years. While the industry is truly on a dynamic growth path, it is nonetheless dangerously dependent upon fishmeal as a key protein constituent in fish and shrimp diets. aquaculture The

|      |                           |   |  |   | Jan. Oct   | Jan- Oct   |
|------|---------------------------|---|--|---|--|--|
| 2002 | 2003                      | 2004  | 2005   | 2006  | 2006   | 2007   |
|      | (10                       | 00 tonnes   | s)   |   |  |  |
| 834  | 667                       | 935   | 815  | 776   | 683  | 643  |
| 1929 | 1219                      | 1983  | 2126   | 1456  | 903  | 937  |
| 227  | 196                       | 212   | 154  | 176   | 206  | 131  |
| 311  | 246                       | 259   | 222  | 213   | 135  | 119  |
| 300  | 271                       | 204   | 179  | 162   | 161  | 148  |
|      | 834<br>1929<br>227<br>311 | (10<br>834 667<br>1929 1219<br>227 196<br>311 246 | (1000 tonnes)<br>834 667 935<br>1929 1219 1983<br>227 196 212<br>311 246 259 | (1000 tonnes)<br>834 667 935 815<br>1929 1219 1983 2126<br>227 196 212 154<br>311 246 259 222 | (1000 tonnes)<br>834 667 935 815 776<br>1929 1219 1983 2126 1456<br>227 196 212 154 176<br>311 246 259 222 213 | (1000 tonnes)<br>834 667 935 815 776 683<br>1929 1219 1983 2126 1456 903<br>227 196 212 154 176 206<br>311 246 259 222 213 135 |

industry is not alone however, as cattle, poultry, hog and mink producers utilize fishmeal as the primary protein source in their diets as well. Currently ten countries produce 80% of all world fishmeal supply, and three of those suppliers are net importers of product, thereby reducing supply, not increasing it. These include the U.S. and China. Production of fishmeal decreased 20% in 2006, and another 5% in 2007. Prices rose from \$750/metric tonne to over \$1400/metric tonne in 2006 prior to retreating slightly to \$1000 currently. China's tremendous demand has had a significant impact on these prices. China's stockpiling of fishmeal in 2006 caused much of the dramatic increase on world prices. Likewise, China's relative absence in the market in later 2007 allowed prices to retreat to current levels. China's anticipated buying in 2008 may again see fishmeal prices soar.



A staggering 25% of all world fish production goes into fishmeal and fish oil ! Due to the fact that world supply is so isolated geographically, every tonne of fishmeal travels an average of 5000 km before it reaches the end user. This has enormous economic implications in supplying the global markets.

Clearly the future demand for fishmeal is on a rapidly increasing track. With world fish stocks, and baitfish stocks in particular, in decline, the stage is set for a bottleneck that could severely limit industry growth. In order to head off such a bottleneck, the industry must eliminate its dependence

upon fishmeal (and fish oil) and develop sustainable dietary protein sources that can be commercially produced with all natural and organic products.

# Alternative Protein Sources

#### Plant

Plant-based proteins have been around for many years. Grains such as soybean, wheat, and corn are commonly incorporated in small amounts in many fish diets. Plant-based by-products such as the dried distilled grains (DDG's) from ethanol production, and other similar products from other bio-diesel are now becoming more available, however are generally, lower in protein that the original grains themselves.

Seaweeds and algae have also been used on a limited basis in fish and shrimp diets, and further research is underway to evaluate the quality and quantity of different sources.

In general, plant-based proteins are inferior in quality and amino acid profiles to animal proteins and therefore to date have not proven to be adequate substitutes in carnivorous fish/shrimp diets, as the digestibility and feed conversion ratios are generally lower. Diets of omnivorous species such as catfish and tilapia however may incorporate plant proteins and achieve reasonable conversion ratios.

# **Bacterial**

Bacteria-based proteins are currently being researched and while the initial data on quality of the proteins appears favorable, the economics are in question.

### <u>Animal</u>

Animal proteins typically used in fish diets include poultry meal, feather meal, blood meal, and with less frequency, beef heart, collagen protein, etc. Animal proteins have superior amino acid profiles to plant proteins, as well as higher overall protein content. While superior to plant protein, these animal proteins are not foods naturally eaten by fish, and therefore have certain nutritional shortcomings relative to fishmeal. Limiting amino acids such as Methionine, Lysine, and Arginine as well as omega 3 and 6 fatty acids are critical to fish growth, health and development.

# How do they Compare to Fishmeal ?

# <u>Plant</u>

- May provide adequate protein quality for omnivorous species such as tilapia and catfish.
- By-product waste generally lower in protein composition and quality than processed grains.
- Seaweeds and Algae while high in minerals are typically lower in protein

# Table 1. Limited amino acid comparison of Soybean Meal vs Fishmeal (as % of sample)

|                | Fishmeal* | Soybean meal ^ | Ento-protein** | Poultry meal <sup>^</sup> |
|----------------|-----------|----------------|----------------|---------------------------|
| Crude Protein  | 62-67     | 47             | 41.58 - 62.47  | 67                        |
| Fat            | 8-12      | 1.56           | 20.21 – 51.48  | 10.87                     |
| Ash            | 16-21     | 5.80           | 2.41 – 9.03    | 13.98                     |
| Omega 6        | 0.89      | 0.40           | 3.90 - 10.74   | 2.00                      |
| Omega 3        | 2.02      | 0.05           | 0.15 - 0.39    | 0.10                      |
| Limiting Amino |           |                |                |                           |
| Acid (%)       |           |                |                |                           |
| Methionine     | 1.75      | 0.68           | 0.55 – 1.02    | 0.86 - 1.03               |
| Lysine         | 4.88      | 3.03           | 2.01 – 3.60    | 2.65 – 2.81               |
| Arginine       | 4.24      | 3.51           | 1.94 - 3.68    | 2.28 - 3.69               |

\* Menhaden meal analysis from Eurofin Scientific, 4/2007

\*\* Initial analysis of 4 selected spp. from Eurofin Scientific, 4/2007

^ Analysis courtesy of Zeigler Bros.

#### **Bacterial**

- May provide higher grade protein than plants.
- Experimental at this time.
- Can commercial production be economical ?

Animal - Poultry, Blood and Feather (see Table 1.)

- Good supplemental protein source but lacks amino acid profile to fully replace fish meal.
- Many seafood buyers & chefs shun the use of mammalian/avian products in the diets.
- Will they qualify for organic certification ?

#### Animal - Invertebrates.

- Broad diversity of species.
- May hold the greatest potential for sustainable production.
- Many have good amino acid and digestibility profiles.
- Provide natural food sources in the wild.
- Extensive research is needed.
- Will commercial production be economical ?

One class of invertebrates has drawn particular attention – Insects. Neptune Industries, Inc. (OTC.BB - NPDI) has filed a process patent on the production protocol for a product called Ento-Protein<sup>™</sup>. Ento-Protein<sup>™</sup> is a high quality dry protein meal created from commercially raised and processed insects. Through a cooperative research effort with Mississippi State University (MSU), Neptune is in the beginning stages of assessing Ento-Protein's <sup>™</sup> commercial feasibility. MSU was the pioneer in insect rearing methodology over 30

years ago, and remains one of the few Entomology programs worldwide to specialize in insect rearing. Research efforts began in April, 2007 with the first of three critical R & D stages.

#### Phase I a.

*Species Identification* – From an initial pool of 15 species candidates, the research team at MSU narrowed the field down to 4 species that met a detailed list of production criteria which included:

- Short life cycle
- High survival
- Gregarious
- Self-harvesting
- High fecundity
- Large body mass
- High dry protein to body mass ratio
- Adequate amino acid and fatty acid profiles
- Void of toxins or off-flavor components
- Consumes diet of processing by-products

In May, 2007, initial results of the 4 species selected were obtained, and appear very promising. The results are expressed in Table 1a. below in a comparison with fishmeal, as well as soybean and poultry meals.

|                            | Fishmeal* | Ento-protein™** | Soybean meal ^ | Poultry meal <sup>^</sup> |
|----------------------------|-----------|-----------------|----------------|---------------------------|
| Crude Protein              | 62-67     | 41.58 - 62.47   | 47             | 67                        |
| Fat                        | 8-12      | 20.21 – 51.48   | 1.56           | 10.87                     |
| Ash                        | 16-21     | 2.41 – 9.03     | 5.80           | 13.98                     |
| Omega 6                    | 0.89      | 3.90 – 10.74    | 0.40           | 2.00                      |
| Omega 3                    | 2.02      | 0.15 - 0.39     | 0.05           | 0.10                      |
| Limiting Amino<br>Acid (%) |           |                 |                |                           |
| Methionine                 | 1.75      | 0.55 – 1.02     | 0.68           | 0.86 - 1.03               |
| Lysine                     | 4.88      | 2.01 - 3.60     | 3.03           | 2.65 – 2.81               |
| Arginine                   | 4.24      | 1.94 - 3.68     | 3.51           | 2.28 - 3.69               |

#### Table 1a. Limited amino acid comparison of Ento-protein<sup>™</sup> vs Fishmeal (as % of sample)

\* Menhaden meal analysis from Eurofin Scientific, 4/2007

\*\* Initial analysis of 4 selected spp. from Eurofin Scientific, 4/2007

^ Analysis courtesy of Zeigler Bros.

#### Phase I b.

Feed Acceptability and Off-flavor Testing - Phase I (b.) research completed feeding trials on juvenile hybrid striped bass at the Dept. of Wildlife and Fisheries at MSU. A standard 40% protein and 10% fat diet contains 20-23% fishmeal by dry weight. In the experimental diet, 100% of the fishmeal was replaced with Ento-Protein<sup>™</sup> of one or two of the insect species selected. Throughout the three week trial, diet acceptability was evaluated. No noticeable differences in diet acceptability were seen. An independent panel at MSU's Food Science and Technology Dept. evaluated fish fed both control and experimental diets, for off-flavor. The results were very encouraging, as the panelists reported no significant differences in taste or texture within the trials. It was noted in the reports however that more panelists actually preferred the fish fed the Ento-Protein<sup>™</sup> based diet over the control fishmeal diet. The reasons stated the fish had a sweeter, milder taste than those fed with standard fishmeal diets.

#### Phase II

Diet Digestibility and Growth Trials – Phase II research began in January, 2008 and is currently underway. A 60 day growth trial and accompanying digestibility analyses will be completed on juvenile hybrid striped bass. Food conversion ratios of both control and experimental diets will be assessed upon completion in March, 2008. The control diet is a standard 44% protein and 12% fat production diet that includes 20% fishmeal. Experimental diets are evaluating 100% fishmeal replacement with Ento-Protein<sup>™</sup>. Four treatments are being evaluated including 100% fishmeal replacement with Ento-Protein<sup>™</sup> (spp "A".); 100% fishmeal replacement with Ento-Protein<sup>™</sup> (blend of two spp.); and 100% fishmeal replacement with Ento-Protein<sup>™</sup> (blend of two spp.); and 100% fishmeal replacement with Ento-Protein<sup>™</sup> in carnivorous fish diets.

#### Road to Commercial Development

Once the research has provided sufficient data to support fish growth and health, Neptune Industries is prepared to move toward pilot scale development. The Company has already begun sourcing sites based upon favorable economic and climate conditions. The primary goal of pilot facility will be to supply feed mills, and researchers with adequate quantities of Ento-Protein<sup>™</sup> to allow extensive trials to be conducted on a global basis with a variety of freshwater, marine, warm and coldwater species.

Following the completion of the pilot facility, Neptune Industries anticipates developing the world's first and largest insect production facility of its kind to produce over 750,000,000 insects per week, or over 320 metric tonnes of high protein meal per month. A design and engineering team is already being assembled to map out the first production facility of its kind in the world.

#### Initial Insect Comparison to Fishmeal

Table 2. below compares an analysis of the four selected insect species with three varieties of fishmeal in a nutritional profile. The preliminary results of these analyses were very encouraging. Crude protein levels, fatty acids, and limiting essential amino acids were very comparable with fishmeal. Crude protein was highest in Species A, which was most comparable to fishmeal. In terms of limiting essential amino acids, all four insect species compared very well in Arginine levels. Lysine, Cystine, and Methionine levels were slightly lower than fishmeal, however were well within a range that could provide a high growth diet. The actual feeding trials will provide the most useful data as to the growth potential of the Ento-Protein<sup>™</sup> based diets, as lab analyses do not take into consideration the synergistic effect of the diet, as well as the combination of elements and there overall affect on growth. Although Methionine levels are thought to be most limiting, this has not always proven to be the case in fish diets.

|          |               | Minerals (M, %) |           | Lipids          |                   | Crude              | Amino<br>(AA, % PR) |                | Acids          |
|----------|---------------|-----------------|-----------|-----------------|-------------------|--------------------|---------------------|----------------|----------------|
| Species  | Ash<br>(A, %) | Ca              | Р         | Total<br>(L, %) | Linoleic<br>(% L) | Protein<br>(CP, %) | Arg                 | Met+<br>Cys    | Lys            |
| Α        | 4.7(0.2)      | 0.2(0.0)        | 0.9 (0.0) | 21(1)           | 34(-)             | 66(1) <sup>a</sup> | 7.1(-)              | 2.7(-)         | 6.3(-)         |
| В        | 3.1(0.2)      | 0.06(-)         | 0.7(-)    | 34(2)           | 29(-)             | 49(1)              | 5.9(-)              | 2.4(-)         | 6.2(-)         |
| С        | 16(1)         | 5.2(0.2)        | 1.2(0.3)  | 32(3)           | 3.3(-)            | 43(1)              | 5.1(-)              | 3.3(-)         | 6.4(-)         |
| D        | 2.3(0.5)      | 0.04(-)         | 0.4(-)    | 57(2)           | 6.0(-)            | 36(2)              | 5.6(-)              | 2.6(-)         | 6.2(-)         |
| Menhaden | 20(-)         | 5.7(-)          | 3.3(-)    | 10.2(-)         | 1.1(-)            | 68(-)              | 5.9 <b>(-)</b>      | 3.8 <b>(-)</b> | 7.7 <b>(-)</b> |
| Herring  | c             | 2.6(-)          | 1.9(-)    | 9.9(-)          | 1.5(-)            | 73(-)              | 5.9 <b>(-)</b>      | 4.0 <b>(-)</b> | 8.0 <b>(-)</b> |
| Anchovy  | 17(-)         | 4.3(-)          | 2.8(-)    | 8.6(-)          | 3.4(-)            | 70(-)              | 5.7 <b>(-)</b>      | 4.0 <b>(-)</b> | 7.9 <b>(-)</b> |

# Table 2. Comparison of the nutritional characteristics of selected insect species with common fish meals.

All entries are Mean(SEM) with N = 1-4 based on dry weights; SEM missing for N =1.

<sup>a</sup>Bold format:  $0.75 \text{ MIN}[\text{fish meals}] \leq \text{Mean} \leq 1.25 \text{ MAX}[\text{fish meals}].$ 

<sup>b</sup>Italic format: Estimated.

<sup>c</sup>No data.

#### Keys to Success

Developing a new, revolutionary product involves many important steps. The thorough completion of research and analysis of data is critical to the success of commercial operations. Identification of potential bottlenecks and anticipated solutions should be mapped and included in the business development strategy. Clearly the economics of mass insect production will play a major role in the commercial viability. Utilizing low-cost land, labor, and feed will be key components. As well, technology and automation of facilities to reduce manpower and increase production efficiency will inevitability influence cost.

#### Benefits to the Aquaculture Industry

As an industry, we must react now to head off a significant bottleneck in projected growth and market competitiveness. Sustainability of feed is critical to allowing unhindered growth of our industry, as well as to protect and nourish our world fisheries stocks.

All-natural and organic foods are producing 20%+ growth per year in the food industry. As more and more countries come on-line with Organic standards and certification, the aquaculture industry should see a genuine growth spurt. Once again, the use of sustainable, high quality, commercially produced protein, that can also be certified organic, will be critical to allowing farmed seafood to compete in this market.

The broad diversity and body composition within the class of insects should allow dietary recipes to be created to generate species specific diets. It is anticipated that Ento-Protein<sup>™</sup> will initially provide a necessary solution to sustainable, all-natural, and organic based fish and animal production. As the production process reaches full stride globally, it is anticipated that the market for sustainable Ento-Protein<sup>™</sup> will expand to include other fish, shrimp, livestock, and eventually human diets.