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Cover photo: CSIRO—Fresh barramundi at market in Vietnam
Get more out of your increasingly expensive Tilapia feed

By Giovani Sampaio Gonçalves¹, Manoel Joaquim Peres Ribeiro¹, Diogo Villaça², Peter Coutteau³

¹Instituto de Pesca, São José do Rio Preto, SP, Brazil; ²Nutriad Brazil, Campinas, SP, Brazil; ³Nutriad International, Belgium

Feed represents the largest production cost in tilapia (Oreochromis niloticus). As a result of ever increasing raw material prices, nutritionists are under continuous pressure to reduce formulation cost and search for cheaper, alternative ingredients. The use of feed additives to improve the digestibility of nutrients is an important tool to improve cost-efficiency in intensive production of tilapia. The current study compares the effect of different application strategies of a digestibility enhancing feed additive on productivity and profitability of tilapia production in cages in Brazil. Reserving a limited budget in the feed formula (often only a fraction of the increase of budget for standard ingredients) for performance enhancing feed additives seems a sound strategy to improve feed cost efficiency, particularly in situations where ingredients prices reach new historic records.
Digestibility enhancing additives that are compatible with the digestive physiology of each fish species, have the potential to improve nutrient utilization from cheap ingredients. Furthermore, they can stimulate the conversion of nutrients into meat gain and reduce the fat accumulation in muscle and viscera. Previous work has revealed the potential of synergistic blends of digestive phytobiotics, natural emulsifying agents and co-factors of digestion to improve feed efficiency and growth, and to reduce visceral depositions in Nile tilapia under lab conditions (Ceulemans et al., 2009). The optimal application of novel feed additives requires field evaluations to provide information on optimal dosage at different life stages of the fish, and their effects on farm economics and processing qualities of the fish. The optimal application of a digestibility enhancer for pond production of Pangasius in Vietnam resulted in economic gains both for the farmer (up to 2.4% reduction of feed cost per kg of whole fish produced and 16.4% shortening of the production cycle) as well as for the fish processor (up to 7.5 % improvement in filleting yield) (van Halteren et al, 2009). The present study aimed at the evaluation of different application dosages of a digestibility enhancer as well as the effect of the basal feed quality on the cost efficiency during a production cycle of Nile tilapia farmed in cages in Brazil.

THE CAGE TRIAL

The study evaluated the entire production cycle of tilapia, including the processing of commercial size fish, and was carried out by the Instituto de Pesca in collaboration with a commercial tilapia integration in the Sao Paulo region, Brazil. The trial was performed during two consecutive phases in 7 m³ cages: phase 1 (from 28g to approx. 170g) and phase 2 (from 170 g to 750 g, i.e. commercial size). In Phase 1, 880 juveniles of Nile tilapia (GIFT strain, initial weight 28 g) were stocked per cage. 10 replicate cages were fed the control feed, consisting of a commercial feed (36% crude protein). The treatment feed consisted of the control feed supplemented with a digestibility enhancing feed additive (Aquagest® OMF, Nutriad; 3 kg per MT of feed). The treatment group consisted of 15 replicate cages.

In Phase 2, the stocking density was 800 fish per cage and 4 different treatments were run with 5 replicate cages per treatment: (1) Control: fed a commercial feed with 32% crude protein; (2) AG3: control feed supplemented with the feed additive at 3 kg/MT throughout the entire cycle; (3): AG 3/1.5: control
feed supplemented with the feed additive at 3 kg/MT until 350g and subsequently at 1,5 kg/MT until the end of the trial; (4) LC-AG2: low cost feed, formulated with protein of lower digestibility and poorer amino acid profile, supplemented with 2 kg/MT of the feed additive (7% reduced formula cost compared to the control feed). During Phase 2, the control group was stocked with fish originating from the control group during phase 1; whereas the other treatments were recruited from fish receiving the additive during phase 1.

Feeds were produced in a commercial extrusion line and the additive was included directly in the mixer with all other ingredients prior to extrusion. Feed distribution was based on feeding tables four (Phase 1) and three times per day (Phase 2). At the end of Phase 1, all fish were weighed, average daily weight gain and feed conversion were determined. At the end of Phase 2, all fish from each experimental cage were weighed and counted. At harvest, 5% of the population per cage were processed for fileting. The evaluated parameters included survival, daily weight gain, feed conversion, filleting yield, viscera weight, liver weight and visceral fat weight.

RESULTS

At the end of Phase 1, fish supplemented with the feed additive showed significant improvements on performance compared to the control group, i.e. 12% better daily weight gain, 5% better survival and 6% better feed conversion (Fig. 1; Table 1).

Table 1: Performance of Nile tilapia (from approx. 170 g to commercial size) fed different diets during phase 1. Averages from 10 and 15 cages for control and treatment group, respectively.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>AQUAGEST OMF 3 kg/MT</th>
<th>difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>92.5</td>
<td>97.2</td>
<td>5%</td>
<td>0.00618</td>
</tr>
<tr>
<td>Initial Weight (g)</td>
<td>28.0</td>
<td>28.0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Final Weight (g)</td>
<td>168.6</td>
<td>186.1</td>
<td>10%</td>
<td>0.00029</td>
</tr>
<tr>
<td>Weight gain (g/day)</td>
<td>1.65</td>
<td>1.86</td>
<td>12%</td>
<td>0.00029</td>
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<tr>
<td>Feed Intake (kg)</td>
<td>177.0</td>
<td>177.0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>FCR</td>
<td>1.54</td>
<td>1.44</td>
<td>-6%</td>
<td>0.00204</td>
</tr>
</tbody>
</table>

Fig. 1: Daily weight gain and feed conversion of juvenile Nile tilapia during phase 1 (from 28 to approx. 170 g) fed the control diet with or without the supplementation of a digestibility enhancing additive indicate significant differences (P<0.05) and deviations from control are shown as percentages in red.
During phase 2, the best results were obtained by supplementing the control feed throughout the production cycle with 3 kg/MT of the feed additive (treatment AG3); resulting in a significantly better final weight and reduced amount of feed consumption at the end of the cycle (Table 2; Fig. 2). Relatively compared to the control group, treatment AG3 showed improved survival (+2.8%), daily weight gain (+5%), feed conversion (-6.4%), filleting yield (+1.5%), visceral fat deposition (-9.9%), hepatosomatic index (-22%), and viscerosomatic index (-10.7%). Reducing the additive inclusion from 3 to 1.5 kg/MT during phase 2, still resulted in interesting benefits on growth, feed conversion and visceral fat but did not affect filleting yield.

Table 2: Fish performance and processing parameters (filleting yield, visceral fat, hepatosomatic index HSI, viscerosomatic index VSI) of Nile tilapia (from approx. 170 g to commercial size) fed different diets during phase 2. Different letters indicate significant differences (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>CONTROL + AQUAGEST OMF 3kg</th>
<th>CONTROL + AQUAGEST OMF 3-1.5 kg</th>
<th>LC FEED + AQUAGEST OMF 2kg</th>
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<tbody>
<tr>
<td><strong>Fish performance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Survival (%)</td>
<td>92.1 ab</td>
<td>94.7 a</td>
<td>93.0 a</td>
<td>95.6 b</td>
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<tr>
<td>Initial Weight (g)</td>
<td>166.9 b</td>
<td>177.4 a</td>
<td>166.7</td>
<td>169.8 n.s.</td>
</tr>
<tr>
<td>Final Weight (g)</td>
<td>714.2 b</td>
<td>751.1 a</td>
<td>730.1 ab</td>
<td>739.2 ab</td>
</tr>
<tr>
<td>Growth (g/day)</td>
<td>4.93 b</td>
<td>5.17 a</td>
<td>5.08</td>
<td>5.21 n.s.</td>
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<tr>
<td>Feed Intake (kg/cage)</td>
<td>676.4 b</td>
<td>644.5 a</td>
<td>655.25 ab</td>
<td>662 ab</td>
</tr>
<tr>
<td>Fins (% ABW/d)</td>
<td>3.08 ab</td>
<td>2.81 a</td>
<td>2.83 a</td>
<td>3.18 b</td>
</tr>
<tr>
<td>FCR</td>
<td>1.72 ab</td>
<td>1.61 a</td>
<td>1.63 a</td>
<td>1.77 b</td>
</tr>
<tr>
<td><strong>Processing parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Filleting yield (%)</td>
<td>33.4 a</td>
<td>33.9 a</td>
<td>33.3 a</td>
<td>31.5 b</td>
</tr>
<tr>
<td>Visceral Fat (%)</td>
<td>4.76 ab</td>
<td>4.29 a</td>
<td>4.17</td>
<td>5.00 n.s.</td>
</tr>
<tr>
<td>HSI (%)</td>
<td>1.07 ab</td>
<td>0.83 a</td>
<td>0.96</td>
<td>0.84 n.s.</td>
</tr>
<tr>
<td>VSI (%)</td>
<td>8.84 ab</td>
<td>7.89 a</td>
<td>8.18</td>
<td>8.67 n.s.</td>
</tr>
</tbody>
</table>

At the end of the trial, fish were processed and evaluated for filleting yield, viscera fat weight, somatic liver index and somatic viscera index.
The low cost feed performed significantly worse compared to the control feed in most parameters, particularly in terms of survival, feed conversion and fileting yield. The addition of 2 kg/MT of the feed additive was only capable of improving growth but the nutritional impact of reducing the protein digestibility and amino acid balance in this feed heavily affected the overall performance, particularly FCR and survival. The trials results showed that tilapia are highly sensitive to reducing the quality of the dietary protein in the feed. The digestibility enhancer was not capable of rectifying the effect of inferior nutritional specifications, which finally resulted in a less cost-efficient feed for the producer.

Considering the cost efficiency of the different feeds, the optimal additive treatment (3 kg/MT throughout the production cycle) improved farm revenues with 17% compared to the unsupplemented control.
group and showed a return on investment (ROI) of 3.8:1 (Fig. 3). Reducing the additive inclusion to 1.5 kg/MT of feed once fish reach 350 g still resulted in 7% improved revenues compared to the unsupplemented control group and a ROI of 2.1:1. The application of the low cost feed supplemented with 2 kg/MT of the additive resulted in important economic losses (10% reduced revenues compared to control).

This study clearly indicated the potential of improving cost efficiency of tilapia feeds through the use of digestibility enhancing additives. The results showed the importance of maintaining the nutritional balance in the feed in order to maximize the benefits of a digestibility enhancing concept. It is important to note that the economic impacts of performance enhancing feed additives (having a relatively stable cost, independent from standard commodity ingredients) increases dramatically with increasing ingredient cost.

Fig. 3: Economic evaluation of different application strategies for a feed additive in tilapia farming. Feed ingredient costs used for this study dated early 2012. Data show change of farm revenues and return of investment, relative to the non-supplemented control group. Treatment groups differ in inclusion of the feed additive and formulation: AG3 (control feed + 3 kg/MT throughout the production cycle), AG3/1.5 (control feed + 3 kg/MT till 350 g; followed by 1.5 kg/MT till harvest), LC/AG2 (low cost feed + 2 kg/MT).

For more information, please contact Diogo Villaca, Nutriad Brazil, Campinas, SP, Brazil, or Peter Coutteau, Nutriad International, Belgium.
HUMIC ACIDS in aquafeeds

The performance improvements and health benefits of humic and fulvic acids in animal diets are well documented and the research database on this topic continues to grow each year. Improvements in growth, immune system stimulation, and detoxification are among the advantages observed when humic acids are implemented in farmed fish feed. This article provides a brief overview of the use of humic acids in aquaculture.

By Landon Bench, Manager, Humatech, Inc., Houston, Texas, USA.

ACUTE AND CHRONIC STRESS

The acute stress of grading and the chronic stress of transportation are ubiquitous and constant factors in aquaculture environments. The effects of acute and chronic stress on the fish are:

- suppressed immune system,
- decreased growth and
- decreased reproduction (Wendelaar Bonga, 97).

These effects lead to infection and disease within the aqua environment and are typically treated with chemicals such as formaldehyde, methylene blue, malachite green, acriflavine, and trichlorfon. The problems with these typical medical treatments for secondary infections and diseases are:

- Few chemical substances are approved for use in aquaculture and not all of these are effective against all pathogens.
Typical medical treatments have a small application range between effective treatment and toxicity.

All chemicals approved for use in aquaculture are suspected to be or know to be mutagenic or carcinogenic. (Steinberg, 2003)

HUMIC ACIDS IN AQUACULTURE

Research shows that humic acids present a viable solution to the above-mentioned consequences of stress and typical medical treatments in aquaculture.

Researchers have found the following benefits of humic acids in aquaculture feed:

- Improvement in growth and food utilization.
- Stimulation of defense mechanisms
- Detoxification of harmful metals and xenobiotics in water.
- Increased brood yield.
- Improvement in condition, strength and resistance to disease, wellness and vitality of culture, particularly during transportation.
- Faster healing of ectoparasite infected fish.
- Suppression of secondary infection.
- Inhibition of outbreaks of primary infection.
- Humic acids healed fish infected by natural and artificial skin lesions.

(Meinelt et al., 2001, 2002; Schreckenbach, 1994, Steinberg 2003)

RESEARCH OVERVIEW

Several important research findings in aquaculture show that humic acids:

- Improve the physiological status of farmed fish
- Increase production performance
- Effectively combat fungal and parasitic infections

Humic Acids

From a chemistry standpoint, Humic and Fulvic acids (referred to collectively as humic acids) are categorized as organic acids. Humic acids are formed when changing ocean levels inundated ancient swamps and peat bogs and then deposited sediment over top. The plant matter contained in the swamps and bogs decomposed over time while buried under additional sedimentary layers. During decomposition humic acids are formed through the chemical and biological humification of the plant matter.

It is the chemical nature of humic acids that give the molecules certain properties that are beneficial when applied to animal diets. Numerous exchange sites on the molecule allow humic acids to bind with nearly any other substance. This chelation capacity supports toxin neutralization and improves nutrient uptake in the digestive tract of the animal. Additionally, humic acids support a healthy gut environments and a strong immune system.

Humatech, Inc. has been mining, formulating, processing and shipping humic acid-based products for over 33 years. Humatech, Inc. holds a self-affirmed GRAS (Generally Regarded As Safe) determination for Dried Aged Peat, sold under the commercial name DPX 9902. This puts Humatech, Inc. in the position of being the only company in the US legally able to sell an identifiable humic substance unique in its formulation and proven to meet certain specifications as a nutritive carrier for animal feeds.
A summary of these research pieces follows.

The Physiological Effect of Humic Acids on Fish (Meinelt et. al, 2003)

**Trial:** Researchers exposed young swordtails to varying dosages of humic acids.

**Dosage:** 0mg/L, 5 mg/L, 30mg/L, 180mg/L.

**Results:** Fish exposed to humic acids were significantly longer and heavier than the control group. All the treatment groups exposed to humic acids continued steady growth after stress (2 weeks of daily nettings).

**Discussion:** Researchers found that the fish exposed to humic acids were able to compensate for stress very quickly. It is known that humic acids have a disinfectant and wound healing effect on skin lesions as well as bacteriostatic, virostatic and astringent and antiphlogistic effect. As seen in terrestrial animals, humic acids stabilize the mucosa, the first fundamental barrier against pathogens. In fact, research has shown that the amount of mucosa cells increased in humic acids-exposed fish (Schreckenbach, 1996).

PRODUCTION PERFORMANCE IMPROVED BY FULVIC ACID
(De Wet & Visagie, 2009)

**Background and Rationale:** Antibiotic growth promoters (AGPs) improve the production results of meat-producing monogastric animals. However, the use of low levels of these antibiotics in animal feeds possesses the possibility to transfer bacterial immunity to species pathogenic in animals and humans.

Humic acids have found application in aquafeeds due to their role in reducing or eliminating growth suppressing microorganisms in the gut of fish, hence optimizing production performance.

**Trial:** Evaluate the effect of a fulvic acid on production performance of Mozambique Tilapia.

**Dosage:** 0.5 g/kg, 0.1g/kg, 5g/kg, 10g/kg

**Results:** Significant differences (P<0.05) were observed for a 0.1 g/kg fulvic acid inclusion (delivering 0.04 g/kg fulvic acid) for both final body length and weight. This represents an increase in final body length and weight of 8% and 22% respectively.
HUMIC ACIDS—AQUAFEED AUTUMN 2012

IMPACT OF HUMIC ACIDS ON FUNGAL INFECTIONS

Scope of Problem: Fungal infections in fish farms are dominated by *Saprolegnia* sp. and *Achlya* sp. These ubiquitous fish pathogens lead to typical secondary infections of the skin and the surface of fish eggs, with spores and fungal hyphae encroaching from damaged tissue to healthy fish and eggs. The economic losses caused by pathogenic fungi affecting farmed fish are enormous (Steinberg, 2003).

Research Findings From Multiple Trials:

- No significant damage to rainbow trout eggs from mycosis over 4 days, following a one-hour exposure to 5 mg/L humic acids (Steinberg, 2003).
- With the application of humic acids there was no direct inhibition of fungal growth, but a striking protective effect against *Saprolegnia* when humic acids were applied prophylactically, even with artificially induced infection by *Saprolegnia*, compared to non-humic acids controls. This suggests a stimulation of the defense mechanisms of fish skin (Schreckenbach et al., 1991).
- Eggs and larvae of rainbow trout were artificially infected with spores of *Saprolegnia* sp. The presence of humic acids significantly reduced mortality due to fungal infection. Additionally, a significant increase in the hatching rate was observed across all dosages of humic acids as compared to control groups (Schreckenbach et al., 1994).

IMPACT OF HUMIC ACIDS ON PARASITE INFECTIONS

Scope of Problem: Although many parasites are found on and in fish in their normal state, parasitic infections commonly increase following weakening of hosts by stress factors (Steinberg, 2003).

Research Findings From Multiple Trials:

- Application of humic acids can help reduce the number of infections over a long period, as well as healing secondary symptoms of infections such as ulcers and skin necroses (Steinberg, 2003).
- Positive effects observed for humic acids treatment against *Ichthyophthirius*, *Trichodina* and *Costia* are attributed to an improvement in physiological condition of the fish and to increased dermal resistance against invasive stages of the parasites (Steinberg, 2003).

CONCLUSION

Humic acids can be successfully applied to combat the stressors inherent to typical aquaculture environments. The documented benefits include increased fish length and weight, increased immunostimulatory responses and anti-fungal, anti-parasitic and detoxification properties.

For references and more information, please contact Landon Bench, Manager, Humatech, Inc. Houston, Texas, USA
Empyreal® 75 is the first and only protein concentrate made from corn. This high-energy, naturally pure protein source provides the nutrition fish need in a highly digestible ingredient. With superior functionality, Empyreal 75 provides even, consistent expansion in extruded feeds and extraordinary binding capacity in pelleted diet applications. And industry experts are drawn to the fact that Empyreal 75 is manufactured in the U.S., bringing with it superior supply assurance beyond any specialized protein ingredient available to the industry.

To learn more, visit e75aqua.com. And be prepared for a whole new perspective on protein.
Soybean oil supplemented with DHA is an effective alternative to fish oil in juvenile cobia diets

By Jesse Trushenski¹ *, Michael Schwarz², Alexis Bergman¹,³, Artur Rombenso⁴, and Brendan Delbos²

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² Virginia Seafood Agricultural Research and Extension Center, Virginia Tech, Hampton, VA 23669, USA
³ Saluki Scholars Research Opportunity, Southern Illinois University Carbondale, Carbondale, Illinois 62901-6511 USA
⁴ International Initiative for Sustainable and Bio-secure Aquafarming, Norfolk, VA 23503, USA

Fish oil replacement has proven difficult for some marine, carnivorous fish because of their apparent requirements for n-3 long-chain polyunsaturated fatty acids. However, performance can be maintained using fish oil-free feeds if the fish oil alternative is supplemented with a source of essential fatty acids. We evaluated the requirements of juvenile cobia for eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) using soy oil-based feeds, and found their n-3 LC-PUFA requirements can be largely satisfied by DHA, and that EPA, if required, is required only in trace amounts.
The aquaculture industry is increasingly dependent on industrially compounded aquafeeds, particularly for intensive production of high-value species. Fish oil is widely considered the ‘gold standard’ for lipids used in aquafeeds, and demand and prices for this finite commodity continue to increase. The coming bottleneck in fish oil availability has encouraged the research and industry communities to pursue fish oil sparing using alternative sources of energy and essential fatty acids. Replacement has been successful for some omnivorous fishes, but replacing fish oil has proven difficult for many marine carnivores because of their greater demand for lipid, and in particular, long-chain polyunsaturated fatty acids (LC-PUFA) not found in terrestrial-origin oils.

Thus it is difficult to meet the needs of nutritionally demanding fishes while minimizing diet cost and reliance on limited marine resources, mainly because the scarcity of alternative sources of the nutrients provided by marine ingredients. This problem is particularly well-illustrated by the complications which arise when sparing or replacing fish oil in diets for marine carnivores, such as cobia, an emerging species of global aquaculture interest. Cobia growth performance is reduced when fish oil is totally replaced with Figure 1. Juvenile cobia, recirculation aquaculture system, and test diets used in the present work.
soybean oil, presumably as a result of LC-PUFA deficiency. It has been suggested that cobia require both eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids.

**Determination of DHA and EPA requirements**

Accordingly, to facilitate proper diet formulation using fish oil alternatives, we evaluated the growth performance of juvenile cobia cultured in a recirculation aquaculture system and fed varying dietary levels of DHA and EPA (Figure 1). The test diets (Table 1) consisted of a fish oil-based positive control diet (FISH), a soy oil-based negative control diet (SOY), and experimental diets based on soy oil supplemented with EPA, DHA, or both at 50% or 100% of the concentrations typically observed in fish oil (SOY + 50% EPA, SOY + 100% EPA, SOY + 50% DHA, SOY + 100% DHA, SOY + 50% BOTH, SOY + 100% BOTH).

Table 1. Dietary formulation and proximate composition.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>FISH</th>
<th>SOY</th>
<th>SOY + 50% EPA</th>
<th>SOY + 100% EPA</th>
<th>SOY + 50% DHA</th>
<th>SOY + 100% DHA</th>
<th>SOY + 50% BOTH</th>
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**Proximate Composition**

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After eight weeks of culture, the growth performance of fish fed the SOY feed was impaired relative to those fed the FISH diet (Figure 2). Supplementing the soybean oil-based diet with DHA, alone or in combination with EPA, restored performance; whereas EPA supplementation had no restorative effect on growth performance.

The fatty acid composition of cobia tissues varied greatly among dietary treatment groups, largely reflecting the composition of the feeds; feeding diets supplemented with DHA and/or EPA resulted in increased tissue levels of these fatty acids, however, the maximal levels of these fatty acids were observed in the FISH treatment.

Although each of our diets contained trace levels of EPA and DHA, it is clear that the SOY diet was deficient in n-3 LC-PUFA and that only the addition of DHA restored normal performance to fish fed the soy-based diets. Our results are consistent with the typical signs of essential fatty acid deficiency, specifically reduced weight gain, efficiency, and condition (Table 2), and altered hepatic structure, composition, and size. Although it is possible that some attributes other than fatty acid composition, i.e., n-3 LC-PUFA content, rendered the EPA concentrate ineffective (e.g., differences in the chemical form of the fatty acids) or allowed the DHA concentrate to restore growth performance, condition, and HSI (e.g., an unknown growth promoter), it seems most plausible that our results are a direct function of EPA and DHA content of the feeds.

Figure 2.

Weight gain (%) and feed conversion ratio (FCR) of juvenile cobia fed diets containing fish oil (FISH), soy oil (SOY ONLY), or soy oil supplemented with purified sources of eicosapentaenoic acid (SOY + 50% EPA, SOY + 100% EPA), docosahexaenoic acid (SOY + 50% DHA, SOY + 100% DHA), or both (SOY + 50% BOTH, SOY + 100% BOTH) to achieve 50% or 100% of the levels of these fatty acids typically found in fish oil.

Columns represent least square-means (specified as numerals at the base of each column) and error bars represent pooled standard error throughout. P-values generated by one-way ANOVA tests are provided; means with common letters are not significantly different (P > 0.05).

Data illustrate the critical need for DHA supplementation to maintain production performance in juvenile cobia, and the apparent expendability of EPA supplementation in this context.
In conclusion, cobia require intact n-3 LC-PUFA in the diet, specifically DHA. This does not mean that the biosynthetic capacity of these fish to elongate and desaturate fatty acids is nonexistent; however, our work and that of others suggests that cobia do not synthesize LC-PUFA in biologically relevant amounts. Our data suggest that the n-3 LC-PUFA requirement of juvenile cobia can be met exclusively by DHA, and that EPA is largely expendable for this lifestage. By amending soybean oil with supplemental DHA at 50-100% of the levels normally found in fish oil (0.8-1.2% of the diet, dry matter basis), soy-derived lipid can be used to completely replace dietary fish oil without impairing production performance of juvenile cobia.

Dr. Jesse Trushenski is an Assistant Professor with the Center for Fisheries, Aquaculture, and Aquatic Sciences (CFAAS) at Southern Illinois University Carbondale where she heads a research team dedicated to aquaculture nutrition and fish physiology. Holding degrees from Western Washington University (B.S., 2002) and Southern Illinois University Carbondale (Ph.D., 2006), Dr. Trushenski leads her team of undergraduate and graduate students, technicians, and researchers in conducting applied and basic research to develop practical solutions for the aquaculture industry. Recent projects include fish oil and fish meal-sparing studies, experiments to define nutritional requirements and tolerances, and trials to assess and improve the nutritional value and quality of farmed fish. This work involves a number of freshwater and marine species, thanks to collaborators and the extensive and intensive aquaculture facilities available in the CFAAS. The team also pursues a number of interests beyond fish nutrition, including evaluations of fish sedatives and therapeutic drugs, stress tolerance and mitigation in aquaculture, fatty acids as biomarkers in aquatic ecosystems, and fisheries and aquaculture policy. Team Trushenski’s goal is to make aquaculture—in all its forms—economically and environmentally sustainable. The Trushenski lab is also committed to serving its profession and community. Dr. Trushenski maintains an active record of professional service to the American Fisheries Society, and will begin a second term as President of the Fish Culture Section in 2013. Students, technicians, and researchers are also active in professional service, serving roles at different levels in the American Fisheries Society, Fish Culture Section, and U.S. Aquaculture Society.

This article was derived from a paper recently published in Aquaculture: Trushenski, J.T., M. Schwarz, A. Bergman, A. Rombenzo and B. Delbos. 2012. “DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia Rachycentron canadum”. Aquaculture, doi: 10.1016/j.aquaculture.2011.11.033. For more information, please contact Dr. Trushenski.
Evaluation of Soy Protein Concentrate use for white shrimp *Litopenaeus vannamei*, on commercial diets

By Alberto J.P. Nunes Ph.D., Labomar - Institute of Marine Sciences / Federal University of Ceará, Brazil.

Soy Protein Concentrate (SPC) is a product resulting from soybean processing by alcohol extraction, which increases protein concentration above 60% and reduces anti-nutritional factors, thereby improving digestibility, to provide an excellent amino acid profile. This study aimed to evaluate growth performance of juvenile white shrimp, *Litopenaeus vannamei*, feeding four diets containing increasing levels of fishmeal replacement by SPC, to categorize, in terms of animal production (survival factor, feed conversion ratio, growth, body weight and productivity) and economics, feeding different diets for juveniles of the species under controlled conditions of cultivation.

MATERIALS AND METHODS

The study was conducted at the Nutrition Laboratory of Aquatic Organisms Labomar/UFC, located in the municipality of Eusébio, Ceará, Brazil. The work evaluated four diets produced in a laboratory containing different inclusions of a commercial non-GMO SPC by Sementes Selecta SA (Araguari, Brazil). A total of 20 indoor individual tanks of 500 Liters volume each were used for this study (Figure 1). For each evaluated diet, five replicates

Figure 1. Experimental ponds used in this study— closed circuit with continuous filtration of the water.
were randomly designated (i.e., four cultivation tanks). In the indoor system, shrimp were stocked at a density of 70 shrimp/m² (40 shrimp/tank).

FORMULATION AND PREPARATION OF EXPERIMENTAL DIETS

Four diets were formulated with increasing inclusion levels of SPC, and a diet was used as a positive control (CP00), without SPC.

All diets were designed to contain a minimum of 36.0% crude protein, ether extract 7.9% and a maximum of 12% moisture. For the other diets (CP05, CP07 and CP10), SPC replaced national Brazilian by-catch and fish processing fish-meal; salmon meal, and to a lesser extent, squid meal (Figure 2).

Figure 2. Experimental diets and ingredients tested. CP00 diet, diet without SPC, CP05 diet, diet with 5.00% of SPC; CP07 diet, diet with 7.00% of SPC; CP10 diet, diet with 10.00% of SPC.
MANAGEMENT

Throughout the study, the shrimp were fed twice a day at 07:30 hrs. and at 16:00 hrs. Shrimp were fed under a regime of demand, permitting changes in the amount of food offered for each supply of feed and depending on the appetite of the animals. The uneaten feed was recorded daily in each tank and feeding schedule. If necessary, adjustments were made for meals every feeding time.

RESULTS AND DISCUSSION

All parameters were within the conditions considered ideal (salinity, pH and temperature) for growing shrimp *L. vannamei*. After 73 days of cultivation, there was no statistically significant difference of body weight of shrimp between diets evaluated (P>0.05).

The growth of shrimp reached values close to 1.0 g/week (Fig. 3, P>0.05), considered suitable for the cultivation conditions adopted (high density, natural feed, less nutritional profile of the formulas) and genetic material available in the country.

Fishmeal replacement by SPC in diets CP05 and CP07 was more advantageous in terms of productivity of shrimp than the CP00 control diet without SPC. As the difference between the cost of formulating these diets was less than 0.6%, the use of SPC becomes more economically competitive, even considering the DL

![Figure 3](image3.png)

**Figure 3.** Weekly growth (g) (± standard error) of shrimp (%) after 73 days of cultivation, fed experimental diets in an experimental culture system with clear water. The P value refers to the Analysis of Variance (one-way ANOVA). Initial weight of shrimp from 1.82 ± 0.18 g (n = 800).

![Figure 4](image4.png)

**Figure 4:** Apparent consumption (g / shrimp) and FCA (mean ± standard error) of shrimp after 73 days of cultivation, feed experimental diets in an experimental culture system with clear water. The P value refers to the Analysis of Variance (one-way ANOVA).
-methionine supplementation with fish oil, squid meal and inorganic phosphate. Although in terms of cost, the formulas have remained very similar in value (CV=0.52%), the advantage of using SPC instead of other animal ingredients is its availability and consistent nutritional quality. It is known that fishmeal from different sources can have quality variations (protein content and protein digestibility).

**FEED CONSUMPTION AND FEED CONVERSION RATE (FCR)**

The average daily food consumption by the shrimp reached 17.5 ± 0.01 g/shrimp. There was no detectable difference in food intake of diets (Fig. 4, P> 0.05). The shrimp fed the experimental diets did not differ from each other (P> 0.05).

The FCR averaged 1.81 ± 0.01, consistent with recent results achieved in previous studies performed in the laboratory. With this it can be stated that the inclusion of SPC did not bring deleterious effects on digestibility of dry matter or protein diets.

**ECONOMIC EVALUATION**

![Figure 5: The values of the formulations showed similar values (CV = 0.52%) between the control diet with fish meal (CP00), with inclusion of 5% (CP05), 7% (CP07) and 10% (CP10). Values are expressed in R$/kg.](image)

**CONCLUSION**

Through this study it can be concluded that:

1. In *L. vannamei* fattening diets, soy protein concentrate can be used for additions of up to 70 kg/MT. (natural base) without deleterious effects on survival, body weight gain, growth, feed conversion factor and productivity of species bred in intensive conditions (70 shrimp/m2). It is expected that under semi-intensive farming, higher levels of addition of Soy Protein Concentrate can be adopted without deleterious effects;

2. The above results were achieved by supplementation with dicalcium phosphate, fish oil, DL-methionine and squid meal, which did not cause an economic charge in the formula compared to a control diet containing 60 kg/MT of salmon flour and 90 kg/MT fishmeal national (waste and by-catch fishery).

For more information, please contact Alexandre Wakatsuki, Commercial Manager, Selecta.

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Effect of fish oil aged under different temperatures for different periods on growth performance of juvenile Pacific threadfin (*Polydactylus sexfilis*)

By Dong-Fang Deng¹, Zhi-Yong Ju¹, Lytha D. Conquest¹, Warren G. Dominy¹, Peter J. Bechtel², Scott Smiley³

¹Aquatic Feeds and Nutrition Department, Oceanic Institute, 41-202 Kalaniana‘ole Hwy., Waimanalo, Hawaii 96795, USA, ²USDA Agricultural Research Service, Subarctic Agricultural Research Unit, Fishery Industrial Technology Center, Kodiak, Alaska, USA, ³Fishery Industrial Technology Center, School of Fisheries & Ocean Sciences, University of Alaska, Kodiak, Alaska, USA

A study was conducted to investigate the quality of salmon oil stored under different temperatures (4°C or 30°C) for 6 or 12 months based on a feeding trial on a tropical marine fish, Pacific threadfin. Ethoxyquin was previously added to the fishmeal at a level of 1000 mg/Kg oil. The test diets that contained 7% of the fish oil were fed to the fish for 8 weeks. Results from this study showed that the growth rate, feed efficiency and proximate compositions of juvenile Pacific threadfin were not affected by the diets. This finding indicates that the salmon oil stored under 4°C or 30°C up to 12 months remain good nutritive quality to support fish growth under the current testing conditions.

Fish oil contains high levels of long-chain polyunsaturated fatty acids (PUFA), which is important for optimal growth and health of many most species of fish. Therefore, fish oil is one of important feed ingredients in aquatic feeds, especially for carnivorous fish culture. Polyunsaturated fatty acids in fish oil, such as C20:5n-3 and C22:6n-3, are more susceptible to oxidation due to the unsaturated carbon-carbon bonds in those fatty acids (Ackman and Gunnlaugsdottir, 1992). Lipid oxidation could downgrade the nutritive value of fish oil due to the decreased levels of PUFA. Lipid oxidation also generates products that con-
tribute to an off flavor or rancidity of fish oil (Halliwell and Chirico, 1993). As a result, storage conditions such as period and temperature are very important factors affecting the nutritive quality of fish oil.

Pacific threadfin is a carnivorous marine fish cultured in Hawaii. They are grown in different culture systems such as flow through tanks and race ways. Offshore submerged sea cages have been developed to culture this fish (Kam et al., 2003). Commercial feed developed for other marine species is being used for farming this fish. It takes about 6-8 months to raise this fish to market size. Information on the nutrient requirement of Pacific threadfin is still limited (Deng et al. 2010 & 2011). Previous studies have shown that dietary lipid at a level of 10-14% mainly provided by fish oil supports good growth of this fish. Therefore, the quality of fish oil will be very important for the growth and health of Pacific threadfin. With this study, we intended to evaluate the nutritional quality of fish oil previously stored under different conditions based on their effects on growth performance and proximate composition of fish.

MATERIALS AND METHODS

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<td>6 months, -80°C</td>
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Newly farmed aquatic species, changing raw material availabilities, and even controversial ecological issues have created serious needs for advancements in aquafeed processing. As a long-time leader in extrusion, Wenger is addressing these and other challenges with ground-breaking approaches. Consider these recent Wenger innovations: Oblique Tube Die and Diverging Cone Screw result in small diameter feeds at rates three to five times those of previous technology; Thermal Twin Screw Extruder permits high percentages of fish slurry, oil and high moisture ingredients; HIP pre conditioner, with adjustable mixing intensity, addresses recipe challenges - especially those with varying content of starch, fiber and oils. And the list goes on.

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What will tomorrow bring
Pink salmon oil supplemented with ethoxyquin at a level of 1,000 mg /Kg oil was obtained from the Kodiak Fish Meal Company in Alaska. The fish oil was stored following the conditions described in Table 1.

Five test diets were formulated with the same formulation except fish oil stored at different conditions was used (Table 1). Test diets were manufactured according to similar protocols used in industry and pelted using a California Pellet Mill. The pellets were air-dried overnight at room temperature and packaged and stored at 4 °C until used. A commercial diet containing 50% crude protein and 14% crude lipid (Skretting Marine Grower, Vancouver, Canada) was used as a reference diet.

**FISH MAINTENANCE**

Juvenile Pacific threadfin were acclimated to laboratory conditions for 7 days by feeding the commercial diet before the start of experiment. The growth trial was conducted indoors in 150-L flow-through aquaria (115-L water) with 31% seawater at 4L/min. Water temperature was maintained at 26.7 ± 0.1°C.

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There were three replications per dietary treatment with 20 fish per replication. Fish were batch-weighed at two week intervals. Feeding rates were adjusted according to body weight. The initial body weight of fish was 5.2±0.03g (mean ± SEM). Fish were hand fed with the assigned diet three times a day (08:30, 11:30 and 14:30 h) with equal portions at each feeding. The daily feeding rates were 5-7% of body weight. The growth trial lasted for 8 weeks. Animal care, maintenance, handling, and tissue sampling of the fish followed the protocols approved by the Oceanic Institute Animal Care and Use Committee.

SAMPLE COLLECTION AND ANALYSIS

At the end of the 8-week feeding trial, total weights were recorded for each tank. Individual body weight, body length and liver weights were recorded to calculate condition factor and hepatosomatic index. Five fish from each tank were used to measure liver and carcass weight (gut removed). Five fish were collected from each tank as whole body and stored at -80°C until use for proximate composition analysis. Proximate composition of the test diets and fish samples was analyzed following AOAC (2000) methods. Fatty acids (FA) were analyzed according to the method described by Ju et al (2009).

Calculation and statistics

- Weight Gain (%) = 100* (final body weight (g)-initial body weight (g))/initial body weight (g)
- Specific Growth Rate (SGR) (% day⁻¹) = 100×Ln (Final body weight (g)/initial body weight (g))/ feeding period (day)
- Feed Conversion Ratio (FCR) = Feed weight as dry (g)/weight gain (g)
- Protein Efficiency Ratio (PER) = Fish weight gain (g)/protein fed (g)
- Hepatosomatic index (HSI, %) = 100*liver weight (g)/body weight (g)
- Carcass Index (CSI, %) = 100* carcass weight (g)/body weight (g)
All data obtained for the test diets were subjected to one-way ANOVA to determine if there was significant effect due to dietary lipid. Differences among means were determined by Turkey HSD test and were considered significantly different when P-values were <0.05.

RESULTS AND DISCUSSIONS

The overall fatty acids profiles were similar among fish oil stored at different conditions. There was however a minor decrease in the levels of long chain polyunsaturated fatty acids such as C20:n-3, C22:5n-3 and C22:6n-3 for the fish oil stored over the 6 to 12 months period under 4°C and 30°C, compared to the fish oil stored at -80°C (Table 2). Oxidation of fatty acid may be the cause of this change.

The growth performance, feed conversion ratio and protein efficiency ratio was similar among fish fed the test diets formulated with fish oil stored under different conditions. The values of condition factors and liver ratio (HSI), as parameters of fish health or fitness, and carcass index (production yield) were also similar among treatments fed the test diets with different fish oil. Body composition of whole fish was

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<td>C20:5n-3</td>
<td>140.47</td>
<td>140.53</td>
<td>135.77</td>
<td>131.93</td>
<td>144.74</td>
</tr>
<tr>
<td>C22:5n-6</td>
<td>4.77</td>
<td>2.66</td>
<td>2.55</td>
<td>2.33</td>
<td>2.77</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>27.83</td>
<td>28.46</td>
<td>27.02</td>
<td>26.83</td>
<td>28.99</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>130.49</td>
<td>132.67</td>
<td>124.81</td>
<td>123.84</td>
<td>136.99</td>
</tr>
<tr>
<td>Identified</td>
<td>846.78</td>
<td>824.36</td>
<td>801.22</td>
<td>782.71</td>
<td>832.91</td>
</tr>
<tr>
<td>Unidentified</td>
<td>153.22</td>
<td>175.64</td>
<td>199.34</td>
<td>217.63</td>
<td>167.09</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>1000.00</td>
<td>1000.11</td>
<td>1000.44</td>
<td>1000.33</td>
<td>1000.00</td>
</tr>
</tbody>
</table>

Table 2. Major Fatty acid profiles of salmon oil stored at different conditions.
not affected by different fish oil (data is not presented). The overall growth performance for fish fed the commercial feed was poorer than the fish fed the test diets (P<0.05). In our previous study, the optimal dietary protein level for Pacific threadfin was shown to be 35-40% and the growth of this fish was not different between diets with 10 or 12% lipid (Deng et al 2011). Therefore, the poor performance of fish fed the commercial diet may be due to the over formulation of protein or lipid for this fish.

The results of this study suggest that fatty acids of the salmon oil containing 0.1% ethoxyquin stored at either 4°C or 30°C up to 12 months is relatively stable although there were some minor changes in a few fatty acids. With 7% of the salmon oil used in the current formulation, no adverse effect was found for both growth performance and the proximate composition of fish under the current testing condition. A high level of this oil or a longer feeding trial may show different effects. This will warrant further investigations. Furthermore, the oxidation state of fish oil measured based on 2-thiobarbituric acid reactive substances (TABARs) and peroxide value will be measured before a better evaluation can be achieved on the quality of the fish oil.

Table 3. Growth performance of fish fed different test diets for 8 weeks

<table>
<thead>
<tr>
<th></th>
<th>FO6-CT</th>
<th>FO12-CT</th>
<th>FO6-AT</th>
<th>FO12-AT</th>
<th>FO-C</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBW (g)</td>
<td>42.4±0.7b</td>
<td>41.8±0.8b</td>
<td>41.7±2.5b</td>
<td>43.6±0.7b</td>
<td>43.4±0.7b</td>
<td>33.5±1.3a</td>
</tr>
<tr>
<td>WG (%)</td>
<td>742±25b</td>
<td>697±11b</td>
<td>698±40b</td>
<td>733±12b</td>
<td>738±4b</td>
<td>539±30a</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>3.8±0.1b</td>
<td>3.7±0.0b</td>
<td>3.7±0.1b</td>
<td>3.8±0.0b</td>
<td>3.8±0.0b</td>
<td>3.3±0.1a</td>
</tr>
<tr>
<td>FCR</td>
<td>1.2±0.0b</td>
<td>1.2±0.0b</td>
<td>1.2±0.1b</td>
<td>1.2±0.0b</td>
<td>1.2±0.0b</td>
<td>1.5±0.1a</td>
</tr>
<tr>
<td>PER</td>
<td>2.2±0.0b</td>
<td>2.1±0.0b</td>
<td>2.2±0.1b</td>
<td>2.2±0.1b</td>
<td>2.3±0.0b</td>
<td>1.3±0.1a</td>
</tr>
<tr>
<td>CF</td>
<td>0.91±0.01a</td>
<td>0.91±0.01a</td>
<td>0.91±0.01a</td>
<td>0.90±0.00a</td>
<td>0.92±0.01a</td>
<td>0.94±0.02a</td>
</tr>
<tr>
<td>HSI (%)</td>
<td>1.34±0.05b</td>
<td>1.43±0.04b</td>
<td>1.36±0.04b</td>
<td>1.30±0.08b</td>
<td>1.38±0.09b</td>
<td>1.97±0.07a</td>
</tr>
<tr>
<td>CSI (%)</td>
<td>93.1±0.3a</td>
<td>93.0±0.3a</td>
<td>92.9±0.2a</td>
<td>93.0±0.2a</td>
<td>92.9±0.2a</td>
<td>92.6±0.2a</td>
</tr>
</tbody>
</table>

Data was presented as mean ± se (n=3). Different letters indicate the significant difference among treatments tested by Tukey HSD test (P<0.05). FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; CF, condition factor; HSI, hepatosomatic index; CSI, carcass index.

Acknowledgements

Funding for this study, through Grant No 58-5341-4-591 from the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) and through a Specific Cooperative Agreement with the University of Alaska Fairbanks UAF-05-0039, also funded by USDA-ARS, is gratefully acknowledged.
New CSIRO laboratories for Aquafeed Technologies:
Leading the development of Asia-Pacific’s aquaculture feed and production sectors in the 21st century

By Brett Glencross, Katherine Morton and Nick Wade
CSIRO Food Futures Flagship, and CSIRO Marine and Atmospheric Research, PO Box 2583, Brisbane, QLD 4001, Australia

The development and application of aquafeed technologies is an expanding area of national and global research within the broad portfolio of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia’s peak research organization. The aquafeed technologies research portfolio integrates multidisciplinary capabilities from within CSIRO’s 6,000 staff, together with complimentary capabilities from external research and industry partners. In alignment with CSIRO’s mandate, the focus of the aquafeed technologies research group is on developing knowledge and technology to assist industry and facilitating the use of the results of the research.

NEW LABORATORIES

Aquaculture research at CSIRO gained momentum in the 1990s, drawing on established strengths in agricultural and marine research. Since then the foci of research in the aquaculture domain has centred around health, environmental impacts, genetics and nutrition. Initially the CSIRO aquaculture nutrition research was based at the Cleveland Laboratories in on the edge of Moreton Bay, 35 km from Brisbane. In 2011 the old Cleveland labs were closed and the CSIRO aquaculture nutrition research moved to two new purpose built facilities.
The molecular and analytical laboratories are now at the new EcoSciences Precinct (ESP) at the inner-Brisbane suburb of Dutton Park. While the aquaria and feed production laboratories are now co-located with the Queensland State government’s aquaculture research at their Bribie Island Research Centre. There a state-of-the-art laboratory has recently been built that houses multiple laboratories for fish and shrimp work, as well as dedicated feed processing and feed extrusion facilities, cold and freezer rooms and a series of other accessory laboratories.

CLOSE LINKS WITH INDUSTRY

A significant proportion the CSIRO aquafeed technologies R&D is conducted on farm or in feed mills with industry. Today the CSIRO aquafeed technologies group is actively involved in about 20 different projects with both Australian and international companies. The group works with both the feed manufacturing and animal production sectors of the aquaculture industry. Notably, the group has active projects with five different feed companies, three feed additive companies and seven different
shrimp/fish production companies. The close linkage with industry demands that a balance is maintained between IP protection and publication.

A FOCUS ON RAW MATERIALS

One of the priority areas of active research of the CSIRO aquafeed technologies group is the development and assessment of raw materials for both fish and shrimp feeds. Substantial progress in this area has been made in Australia over the past 20 years with current Australian commercial diets among the lowest users of fish meals and oils of any aquafeed sector in the world. In particular the work on the use of grain products as both protein and energy sources for fish and shrimp are considered some benchmark works in the field and have led to major reviews on the field (Glencross et al., 2007). In addition to the work on grains, there has also been considerable work done on rendered animal meals and oils, and together both the grain and rendered animal products form the basis of most aquafeeds in Australia (Williams et al., 2003a, b; Glencross et al., 2011). Work in this area continues in order to gain a better understanding of key drivers of variability in raw material quality and to develop and refine rapid assessment technologies that will allow the feed industry to adapt to that variability in real-time.

In addition to the work targeting increasing the raw material scope for aquafeeds, has been the development of some new technology focusing on improving the efficiency of animal performance through the use of natural bioactive substances. This resulted in the granting of international patent rights to a technology now being promoted as Novacq™. This technology has been shown to dramatically stimulate the growth of shrimp and is now

Feed grains have made a significant impact on reducing the reliance on fishmeal in aquafeeds in Australia.
being commercialised with a series of industry partners around the world.

A third tier of research is focusing on why some raw materials are so ‘important’ in terms of identifying how these raw materials, like fishmeal, comprise such relative importance in the diets of some species. Using a materials chemistry approach the different raw materials are being ‘pulled apart’ before being re-fed to key species to identify those important components.

**IMPROVING PERFORMANCE BY UNDERSTANDING ANIMAL FUNCTION**

Although most diet specifications are relatively well established for key aquaculture species now, there is surprising potential for significant gains still to be made. Recent re-evaluations of key nutritional paradigms for some species are shedding new light on how by understanding animal function at a physiological and molecular level that significant gains in performance can be made. Recent use of nutrigenomic technologies has revealed at a molecular level why carnivorous species like barramundi perform so much better when the diets are subtly changed to focus on protein supply (Wade et al., 2012). These findings are also being used to further enhance nutritional models developed by the CSIRO group that are presently being widely used by both the Australian feed and fish production sectors (Glencross, 2009; Glencross and Bermudes, 2012).

Similarly, classical dose-response designs to assess essential nutrient requirements, like those for the essential fatty acid in shrimp diets, have also contributed to not only improving performance but also identifying opportunities for alternative raw materials (Glencross et al., 2002).

As is often the case, work in one area underpins that in another, such is the integrated nature of nutrition.

**References**


For more information about aquaculture research at CSIRO, please contact Dr. Brett Glencross.
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- Minimizing reconfiguration acquired.

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Heat Stable Protease Enzyme Improves Growth and Nutrient Digestibility of Fish Fed Diets Extruded At ~120°C

By M A Kabir Chowdhury, Product Manager - Aquaculture, Jefo, St-Hyacinthe, Quebec, Canada, J2S 7B6

World production of compounded aqua feeds is expected to exceed 50 MMT in the next few years. The need for high quality protein sources to achieve maximum growth potential of farmed aquatic species leaves feed formulators worldwide with a very limited choice of ingredients that are widely available, have relatively less variable nutritional profiles and better digestibility of nutrients. However, these commonly used sources are usually expensive and their price fluctuates considerably depending on the supply-demand situation, speculative forces, and various other factors. Alternative protein sources are continuously being sought and evaluated but the presence of anti-nutritional factors, poor characterization of macro- and micro-nutrients, and the large variations in nutrient composition from source to source and from batch to batch of a common source, limit the inclusion of most of these ingredients to less than 15% in compounded aquafeed. The presence of protease inhibitors or other poorly characterized factors result in poor utilization of crude proteins of these ingredients. The addition of protease enzymes in the diets will increase the digestible nutrient contents resulting in better growth and nutrient utilization. It will also allow formulators to increase the amount of the poorly digestible ingredients in feed.

A major concern for the inclusion of enzymes in aquafeeds has been their thermal stability. Most modern aquaculture feeds are extruded and produced through high temperature extrusion process (90-120°C) to improve apparent digestibility of carbohydrates, destroy some anti-nutritional factors, and improve texture and water stability of the feed. Exposure to high temperature and pressure during cooking and extrusion processes of aquaculture feeds may significantly reduce the efficacy of any exogenous enzymes and nullifies their uses in extruded feed. At high temperature, major degradation mechanisms of a prote-
Enzyme are deamidation of asparagine and glutamine, and succinamide formation at aspartate and glutamate leading to peptide bond hydrolysis.

Jefo, a Canadian company, is a world pioneer in protease enzyme production. The enzyme has been successfully applied in poultry and aquaculture feeds. The enzyme is produced from a unique bacterial strain and has been tested through a comprehensive research program covering carps, salmonids, shrimps, tilapia and other aquatic species. Findings from some of these studies related to high temperature extrusion process are presented in this article.

**IMPROVED GROWTH AND FEED CONVERSION**

A trial was conducted on juvenile hybrid tilapia, *Oreochromis aureus* × *O. niloticus* (2 g IBW) at the Shanghai Fisheries University, China, to compare the effects Jefo protease has on growth and nutrient digestibility of fish fed pelleted (50-60°C) and extruded (120°C for ~1 min) diets, for 30 days. Two diets containing 3% and 9% fishmeal were prepared either by pelleting or extrusion. The growth of fish fed both 3% fishmeal diets with Jefo protease was significantly better than those fed without the enzyme (Figure 1) or those containing 9% fishmeal. Feed conversion ratio (FCR) was also improved by 7% and 5% in fish fed 3% fishmeal pelleted and extruded diets with the enzyme, respectively compared to those fed diets without the enzyme.

![Figure 1: Weight gain (BWG - g) of tilapia fed pelleted (60°C) or extruded (120°C) diets with or without the Jefo protease (PEC)](image)

In another trial, rainbow trout, *Oncorhynchus mykiss* (~395 g IBW) were fed a commercial feed (extruded at 120°C for 30 sec) with or without the Jefo protease for 12 weeks. Two levels of enzymes, 175 mg kg⁻¹ and 250 mg kg⁻¹ were added to the diets. Growth rate and FCR of the fish fed the diets with added en-
zymes were significantly better (TGC = 0.294, FCR = 1.35) than those fed the control diets (without enzyme). However, there were no differences in either growth rate or FCR between the two treatments with added enzyme.

**IMPROVED INTESTINAL PROTEASE ACTIVITY AND DIGESTIBILITY OF NUTRIENTS**

In the same trial with tilapia, protease activity in the proximate intestine and apparent digestibility (ADC) of crude protein were evaluated. Protease activities showed a marked increase in the fish fed low fishmeal (3%) diets with added enzyme in both extruded and pelleted feed treatments. However, ADC of crude protein improved only in fish fed low fishmeal pelleted diets (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protease Activity U mg⁻¹ protein min⁻¹</th>
<th>ADC Crude Protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.9a</td>
<td>81.9a</td>
</tr>
<tr>
<td>Jefo Protease</td>
<td>21.7b</td>
<td>83.9b</td>
</tr>
<tr>
<td>Extruded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23.2a</td>
<td>84.5a</td>
</tr>
<tr>
<td>Jefo Protease</td>
<td>28.1b</td>
<td>84.7b</td>
</tr>
</tbody>
</table>

Note: Different superscripts in a column for either pelleted or extruded feed indicate significant differences (P<0.05)

In a recent trial with three salmonids species, coho salmon (*Oncorhynchus kisutch*), Atlantic salmon (*Salmo salar*) and rainbow trout (*O. mykiss*), apparent digestibility of nutrients and gross energy of the test diets with or without the protease were evaluated. Two diets of about 45% CP were prepared with 15% fishmeal, poultry by-product meal (12%), soybean meal (15%) and corn gluten meal (12%) as protein source. Both diets were extruded at 120°C for ~30 sec. Fish fed diets with the Jefo protease showed significantly higher ADC of crude protein (Figure 2), total carbohydrates and gross energy than those fed the control diets.

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CONCLUSION

It can be concluded from the findings that heat stable proteases can significantly improve growth or apparent digestibility of nutrients in fish. A number of potential beneficial effects such as an increase in endogenous protease production, reducing the requirement for supplemental amino acids and energy yielding nutrients, and improved digestibility of dietary proteins, carbohydrates and gross energy are associated with exogenous protease application. A protease enzyme may also hydrolyze protein-based anti nutritive factors such as lectins and trypsin-inhibitors improving the efficiency of amino acid utilization and reducing protein turnover. The benefits of a protease enzyme can be maximized when low-digestible proteins such as cottonseed meal are used in the feed matrix.

For more information, please contact M A Kabir Chowdhury, Product Manager — Aquaculture, Jefo, St-Hyacinthe, Quebec, Canada, J2S 7B6
WEIGHING & DOSING

Three conditions for fast and accurate dosing

For accurate dosing not only is a good weigher necessary, but also a capable dosing instrument and an adequate controller. When these three conditions are met, it’s possible to dose fast and precisely with guaranteed accuracy.

Fast and precise dosage requires a controlled inflow, a good weigher and an adequate controller. The quality of the weigher is determined by mechanical as well as electronic elements. From a mechanical perspective, it is essential that the structure of the weigher is rigid. Even the smallest deflection, for example in an oblong weigher, causes a measuring deviation. In addition, the weigher should not be too heavy in relation to the products or raw materials to be weighed. It’s clear that for an increase in weight of 100 grams, greater accuracy can be determined on a weigher of 5 kg than on a weigher of 100 kg. This also demonstrates the weak point of a ‘loss in weight’-weighing system: after all, this weighing strategy measures a comparatively minimal decrease in a large weight.

WEIGHER DESIGN

In practice it is evident that the design of a weigher needs attention. It often happens that not all product ends up on the weigher, but partly on a funnel to the weigher: the product should be dosed directly on the weigher, so that there will be no ‘leverage effect’. For example, leverage will occur when the product lands on the extreme side of the weigher, causing the weigher to exert a torque on a load cell. And of course, a weigher should have sufficiently weight before the product is weighed. That sounds obvious, but in practice it is sometimes forgotten.

The most common error, without a doubt, is insufficient ventilation. The air which will be moved by the product being weighed, must be able to escape without disturbing the weighing. A flexible sleeve of filter cloth, which is often nearly closed, is absolutely insufficient for this venting. By an influx of, for example, 50 kg/s wheat, 75 liters/s = 270 m3/hour of air must be removed. Another important design point is the emptying of the weigher, which
should be smooth and complete, without leaving residue.

**ELECTRONICS**

The quality of a weigher from an electronic aspect depends on the quality of the weigh cell (load cell or force transducer) and inverter (digitizer or indicator). In both cases you need to ensure there is a sufficiently broad weight range (taking into account some overload) and distinctiveness. This distinctiveness determines the smallest possible weighing unit. At a distinctive character of for example 3000 steps - taking into account 20% overload - 2400 steps still remain for the actual weighing range. For a 100 kg weigher a weighing unit of 42 gram is a feet. In practice, in this case, they will often choose to go down to 2000 steps, which creates a better workable weighing unit of 50 grams. A large number of weighing units (slabs) aren’t able to give all the information and therefore it’s possible to receive false accuracy. It’s the combination of the mechanical and electronic properties of the weigher that determines the actual accuracy.

**SIGNAL LATENCY**

Signal delay (latency), the time that elapses between the signal from the weighing unit and it’s processing by the controller, is a problem that is misunderstood in dosing weighers. The signal latency arises at electronic filtering and averaging to improve the stability of the signal. But also the delay through the network between weigher and controller should not be underestimated. A process control will calculate with outdated data because of signal latency. Therefore it is more appropriate to speak of ‘backlash’ instead of ‘for lash’. The weight should constantly be long enough for the final determination. The pitfall here is an electronic

---

**Fig. 2** The ALFRA FCD doses raw materials, like dry powders, granulates and pellets, using an innovative ‘weigher-in-weigher’ system for extremely accurate dosing of quantities ranging from 20 g to 100 kg. It uses ALFRA Dosing Slides and the ALFRA dose&weigh controller.

**Fig. 3** The ALFRA MCD and KCD are fast, accurate and extremely robust machines for dosing powders, granules or pellets in quantities ranging from 20 g to 30 kg (MCD) and 200 g to 100 kg (KCD). They are equipped with a moveable weighing hopper and ALFRA Dosing Slides.
created stability which doesn’t match with reality.

EXTERNAL INFLUENCES

A well-designed weigher doesn’t guarantee proper weighing. There are also external factors that can affect the weighing result. It is obvious that the weighers should be free from interference due to connections with stabilizers and flexible sleeves. Yet it happens that even when this is correct, that perhaps a stepladder is placed against the weigher or spilled product disturbs the weigher. Other external influences should be avoided whenever possible. Think of vibration, buckling floors or supports, compressed air leaks and wind, but also to over- or under pressure due to aspiration, pneumatic conveying or product movements in connected silos.

QUICKLY AND ACCURATELY

A good weigher alone does not guarantee correct dosing; the weigher just establishes how much is dosed. All systems of the triangle ‘Weigher-Controller-Dosing Tool’ are in the business of proper dosing, in which these systems are optimally matched. The controller uses the information from the weigher to control the dosing tool. The dosing tool is able to work with a fixed or a variable dosing speed. With a variable speed it’s possible to realize more accurate and faster dosing. The exit point (trail) is standard corrected so that the final standard weight usually is within the tolerance. A variable dosing speed is only fully utilized as the ‘settings’ (turning points of dosing speeds) are constant optimized. This is a labor-intensive activity, which mostly results in disappointing results. Modern software makes it possible to automate this optimization, whereby the quality of dosing greatly improves.

DOsing TOOL

The dosing tool is the tool that delivers the product to the weigher; for example a (grid) slide, a screw conveyor or a vibrating chute. The highest achiev-

---

**Fig. 4 (Left)** Screen of the self-learning ALFRA dose&weigh control software

**Fig. 5 (Right)** The ALFRA GCD doses powders and granulates very accurate, ranging from 100 g to 1.000 kg using an innovative ‘weigher-in-weigher’ system. It uses ALFRA Dosing Slides and the ALFRA dose&weigh controller.
able dosing accuracy, contrary to popular belief, usually does not depend on the weigher. In practice, it appears that the dosing tool is the weakest and therefore the limiting factor. The accuracy depends on the smallest controllable product flow. If the dosing tool continues dropping a quantity of 100 grams in the weigher, than the guaranteed dosing accuracy never becomes better than 50 gram. After all, an extra quantity won’t make the deviation ever better than 50 grams. A smaller deviation is just coincidence and not a guarantee. The smaller the minimum controllable flow, the more accurate the dosing will be. For the combination of fast and accurate dosing, it is necessary that the dosing tool has a wide range in the dosing speed (flow). The ideal dosing tool must not only be fast, but it must also be able to dose less and precisely. The final consideration is the power supply of the dosing tool. A dosing tool which doesn’t completely and constantly feed itself is not able to provide the weigher with the ideal product flow. Therefore it is recommended to choose a dosing tool which influences the product flow from a parent silo or container.

ONE STEP GUARANTEE IS TWO STEPS DOSING

To weigh the smallest quantity of the product requires twice the smallest weight unit. This can be clarified by the example of a weigher with weight units of 100 gram. When there is 49 grams in this weigher, the weigher-readout will indicate 0 grams because of the rounding. When 1 gram is added, there will be 50 gram in the weigher and the weigher-readout will indicate 100 gram. This means we have to count a minimum of two weighing units for a guaranteed addition of 100 grams. Although it won’t be known whether 101 or 299 grams (from 49 to 150 = 101 grams or from -50 to 249 = 299 grams) are added, it is guaranteed that in all cases at least 100 gram is added. In short: one step guarantee is two steps dosing.

For more information, please contact Marijke Vreugdenhil, KSE, the Netherlands