Developing extruded cuttlefish feeds

Pea starch
New processing technology
Protein sparing in Tilapia
Organic minerals boost Omega-3 uptake
Physical properties & functionality of Corn Protein Concentrate
Probiotics: a natural approach to fish health & nutrition

Photo: SEPIATECH - Developing extruded diets for the common cuttlefish, Sepia officinalis
Inside this issue:

SEPIATECH — Developing extruded diets for the common cuttlefish, Sepia officinalis 2

PROBIOTICS: A natural approach to fish health and nutrition 10

Pea starch 15

Protein sparing in Tilapia through the supplementation of a digestive/metabolic enhancer 20

Physical properties and functionality of Corn Protein Concentrate in animal feeds 27

Organic minerals boost omega-3 uptake 33

Product update 38

Calendar of Events 43

Advertisers

32  Andritz
36  Aquafeed Horizons
9   Buhler
19  Empyreal 75
37  Famsun
26  Geelen Counterflow
11  Idaho
42  Inve Aquaculture
22  Sai-Pac Fish Farm
31  SWCSNFF 2015
14  Wenger
18  Zheng Chang
Cuttlefish Culture Potential

CCMar started the development of cuttlefish aquaculture technology in 1999 as part of strategy of aquaculture species diversification. We believe that cephalopods have potential for generating a revolution in aquaculture. In fact, the potential of some species, such as the common cuttlefish, has already been recognized by Barnabé (1996) for several reasons, including its nutritional content. According to Kreuzer (1984) and Okuzumi and Fujii (2000), most cephalopods, the cuttlefish in particular, are rich in protein and essential amino acids and have low carbohydrate and lipid (mostly HUFA and HDL cholesterol) levels. In addition, cuttlefish potential as aquaculture product spreads beyond its production for human consumption. In fact, applications include the use of the whole animal, with a recycling of its by-products for feeds and natural products, such as viscera for the fish feed industry (Le Bihan et al. 2006); ink for human food industry (sepia spaghetti) or as a promoter of immune function in vertebrates (Liu et al. 2011, Sundaram 2009); and cuttlebone for the medicinal and pharmacological industry (Rocha et al. 2005, Cadman et al. 2012, Kannan et al. 2007, Kim et al. 2012). On the other hand, cephalopods are used as biological models in neuroscience (Williamson and Chrachri 2004, Sio 2011, Fiorito et al. 2014), mechatronics (Laschi et al. 2012), behavior (Hanlon and Messenger 1996, William...
Tricarico et al. 2011, Gherardi et al. 2012), evolution (Budelmann 1995, Strugnell et al. 2011) and climate changes (Pörtner and Farrell 2008, Melzner et al. 2009). Cuttlefish is also among some of the most charismatic marine animals that seduce the general public in aquaria, due to its intelligence. Therefore, the market application for produced animals is vast.

Developing a prepared diet for cuttlefish

**Pellet development before CCMar**

To have a pellet diet for cuttlefish, or any cephalopod, has long been a goal which has proved difficult to achieve.

The main challenging aspect is that we are dealing with one of the most intelligent marine invertebrates that displays different assessment and behavior depending on which prey it is hunting (Hanlon and Messenger 1996). Therefore, the first trials tried to determine if cuttlefish would accept and ingest a prepared feed. Castro (1991) determined that it was possible to feed cuttlefish on a moist pellet (76% of water) consisting of 20% of prawn (*Palaemon serratus*) powder and 4% alginate as binding agent, which were 2.5cm long and weighed ≈1.2g. In that same year, Lee (1991) tested a surimi diet (a moist prepared feed), and pellets of *penaeid* shrimp, *mysid* shrimp and chicken. Surimi was rejected and never ingested, while the remaining promoted cannibalism after 30-40 days in group-reared animals, which suggested nutritional deficiencies or imbalances of the non-natural diets.

Castro joined Lee, at the National Resource Centre for Cephalopods (NRCC) in the following Project and Partners

**Project SEPIATECH – Development of cuttlefish, *Sepia officinalis*, aquaculture production technology - feed and husbandry refinements – is a PROMAR (National EU Fisheries Fund) funded project which started in 2011 at CCMar, located at the University of the Algarve (Portugal).**

Among several objectives, the main goal is to develop a first extruded diet that will serve as basis for later refinement through traditional feed nutritional optimization. The project is based on a partnership (Fig. 1) of the research center with SPAROS, Lda., which is a SME dedicated to I&D and Nutrition in Aquaculture.

For more information contact: António V. Sykes, Invited Researcher, CCMar – Centre of Marine Sciences.
Relying on the use of premium ingredients of the highest freshness and nutritional value, together with SPAROS Lda, we have now evolved into the manufacture of extruded diets.

*ictalurus*) and a shrimp-based pelleted diet (based on shrimp purée of *Palaemon setiferus*). Surimi was accepted and ingested for 30 days, but survival and growth was poor with either diet (67.5% and 22.5%; 0.33% and 0.54% BW/day, respectively). Castro and Lee (1994) then tested different surimi formulations (fish myofibrillar protein concentrate), varying its content with the use or not of egg albumin, casein, whole egg, menhaden oil, cholesterol and lecithin. All these studies shared the common result of feed acceptance but also of high mortality and very small growth (<1 %BW/day).

**How to fool an intelligent animal? A CCMar approach**

An overview of cuttlefish culture performed at the Ramalhete experimental aquaculture laboratory may be found here and a revision of the most recent technological protocols may be consulted in a dedicated species chapter, which is part of the recently published *Cephalopod Culture book*. We are also working on improving cuttlefish reproduction in captivity. Other videos of juveniles and adults, recorded underwater, may be watched here and here.

We picked up from those studies and started by using the grass shrimp, *Palaemonetes varians*, as a nutritional model species since this prey was able to sustain the culture of cuttlefish for six consecutive captive generations (Sykes et al. 2006). In addition, we determined the egg nutritional content and its use during cuttlefish embryonic development. Moreover, during project SEPIAMETA, we investigated the eventual use of protein, lipids and carbohydrates by cuttlefish. Based on that data and other published, we established the hypothesis that geographical temperature and available prey will have an impact on cuttlefish metabolism (Navarro et al. 2014). Furthermore, we considered the fact that cuttlefish might have problems related to the digestion of heated protein (Domingues et al. 2009). That became the basis for feed raw material choice and preparation.

The 2011 and 2012 experiments were performed with a semi-moist diet (35% of water) and different raw materials on similar proportions of protein, lipids, carbohydrates and ash to grass shrimp. Experiments were performed in groups of 30 juveniles. Since we were not understanding if the observed growth was only due cannibalism, we chose to change the protocol to use a lower amount of individuals (3) and to film at food deliverance and during 3h (Fig. 2 and 3).
We also tested the individualization effect and realized that acceptance was better in groups. Since we had observed a fast disaggregation of the feed, in 2013, we started using gelatine as a binder for the semi-moist feed and, afterwards, we added a source of carbohydrate as raw material to meet the metabolic hypothesis. Not all the cuttlefish ate the diet at time of feeding and there was high disaggregation in a few hours. Therefore, we chose to cold-extrude the same formulation and test it against the semi-moist pellet in early 2014. No differences were found between both formulations in terms of growth and survival but acceptance was still not optimal in either. We thought that this might have
relation with confinement, as the available space for cuttlefish was low. So we changed from baskets to 250L fiberglass round tanks, where we placed 15 cuttlefish (Fig. 4).

Relying on the use of premium ingredients of the highest freshness and nutritional value, together with SPAROS Lda, we have now evolved into the manufacture of extruded diets. SPAROS is specialized in producing pilot-scale batches of customized feeds (Fig 5). Upon a micropulverization of all raw materials (<200 micron), specific vitamin and mineral premixes and a proprietary

Figure 4: 250L fiberglass tanks used for assessment of extruded feeds in 2014.
blend of binders, diets were manufactured by a process of low-shear extrusion under low temperature conditions (Fig 5). Extruded pellets (Fig. 6) showed a high physical stability in water and a controlled sinking speed in the water column. The pellets are shown as mixed in figure 6.

It worked! The first cold-extruded diet was accepted and ingested (VIDEO). The experiment run for 45 days and allowed us to collect information regarding growth, survival, nutrient digestibility with yttrium oxide (still to be confirmed as previous attempts with chromium oxide were useless due to bioaccumulation by cuttlefish).

The good intake and growth performance achieved with a formulated extruded diet for cuttlefish gives us now a powerful tool to generate new knowledge on the nutritional requirements of this species and further optimize growth and reproduction under captivity. The development of this compound feed for cuttlefish is a crucial element for the industrial scale-up of cuttlefish farming.

Figure 5: SPAROS, Lda. facilities (above) and cuttlefish extruded feeds being prepared

Figure 6: Cold-extruded feed tailored for cuttlefish in shape and raw materials.
References


Sykes, A.V. et al. 2006. Effects of using live grass shrimp (Palaemonetes varians) as the only source of food for the culture of cuttlefish, Sepia officinalis (Linnaeus, 1758). Aquacult Int 14:551-568


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The World Health Organization (WHO)’s 2014 report on global surveillance of antimicrobial resistance reveals that antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in the community and hospitals.

One of the stress points is being placed in the sometimes abusive use of antibiotics in animal production. The common trend in all countries is to reduce or even ban the use of these valuable compounds.

As an estimation of the situation nowadays, we can state that in Europe 25,000 deaths per year can be attributed to antimicrobial resistant “superbugs”; 38,000 deaths in Thailand and 23,000 deaths per year in the EEUU. The overall society cost impact is estimated to be US$ 35 billion in the EEUU (sources: ECDC 2007, Pumart et al 2012, US CDC 2013)

New strategies are needed therefore in the fight against the common pathologies suffered by production animals. The supplementation with natural growth promoters (NGPs), also termed as non-antibiotic growth promotors, is one of the alternatives gaining more and more interest. NGPs are feed additives of different categories, such as salts of organic acids, probiotics, prebiotics, phytogenics or yeasts. In addition to the beneficial effects in terms of health status and performance, NGP supplementation does not usually bear any

### PROBIOTICS

A natural approach to fish health and nutrition

By Álvaro Ortiz, Aquaculture Product Manager, NOREL S.A., Spain

Over the last 30 years, no major new types of antibiotics have been developed

Source: [www.who.int](http://www.who.int)

Figure 1.
NGP supplementation does not usually bear any risk regarding bacterial resistance or undesired residues in the edible fish product.

Nutritionists must focus not only on nutritional specifications but also on promoting digestive health. In such tasks probiotics, one of the best documented NGPs, has arisen as a natural solution that results in improved health status, nutrient utilization, and consequently performance.

A probiotic for aquaculture practices can be defined as a live microbial supplement that, administered via feed or directly into the rearing water, provides a benefit to the animal by enhancing nutrient utilization, health status, stress response, disease resistance and performance, and this is in part achieved by optimizing the microbial balance within the animals and water environment (Merrifield et al. 2010).

Numerous studies have demonstrated the beneficial effect of supplementing fish and shrimp diets with Bacillus spp. By re-establishing a beneficial gastrointestinal microbiota composition, this application will exert a major effect within the host, resulting in en-
Enhanced break down of digesta, reduced pathogenic bacteria load, enhanced disease resistance, healthier absorptive surface area, and consequently better nutrient utilization. These host benefits can be explained by different mechanisms of action such as secretion of exo-enzymes, organic acids and other antimicrobial compounds, as well as by competition for nutrient and attachment sites of intestinal cells (Kesarcodi-Watson et al. 2008).

Recent information reveals an immune modulating effect of some *Bacillus* spp. by increasing the activity of phagocytic cells and lysozyme (Yung-Zhang et al. 2010).

*B. amyloliquefaciens* is marketed by Norel as Ecobiol Aqua. Compared to other probionts, the spore form of *Bacillus* spp. offers advantages in use due to its heat-stability, allowing the product to be stored at room temperature. In addition, two other properties make Ecobiol Aqua a highly functional NGP; first, a high survival rate through pelleting, and second, a faster activation and high multiplication rate of the vegetative form when compared to other *Bacillus*.

In a recent study published in the WORLD AQUACULTURE ADELAIDE 2014, NOREL’s probiotic Ecobiol Aqua (*Bacillus amyloliquefaciens* CECT 4950) was tested to evaluate its immune-modulating effects. In order to ascertain this effect, tilapia under a disease challenge using *Aeromonas hydrophila*, was supplemented with the probiotic.

A total of 120 tilapia were stocked in twelve rectangular glass tanks attached to freshwater recirculating aquaculture system (RAS). Fishes were divided into three groups: control (C), treatment 1 (T1) and treatment 2 (T2) of 4 replicates. C, T1 and T2 groups received control feed, feed with 1kg/t and 2kg/t of Ecobiol Aqua supplement respectively. All fish from each replicate of the three study groups were challenged with 1/10 LD50 dose of *Aeromonas hydrophila* by intraperitoneal inoculation. Blood samples were collected from the test groups at days 1, 7, 14, 21 and 28. The samples were analyzed for hematocrit, leukocyte levels, and lysozyme activity. Lysozyme activity was determined based on the
lysis of *Micrococcus lysodeikticus* and correlated to reduction in turbidity measured at A450nm. Both the treatment groups T1 (837.6U/mL) and T2 (1251.3U/mL) had significantly higher lysozyme activity than the control (153.3U/mL). There was a relatively higher WBC level observed among the treatment groups than the control though the differences were not significant. Blood samples analysis results are shown in table 1.

The probiotic induced enhancement in the immunity as evidenced by increase in the lysozyme and leukocyte levels which are important to fight against infectious agents.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>WBC</td>
</tr>
<tr>
<td>Treatment 1</td>
</tr>
<tr>
<td>Treatment 2</td>
</tr>
<tr>
<td>Hematocrit</td>
</tr>
<tr>
<td>Treatment 1</td>
</tr>
<tr>
<td>Treatment 2</td>
</tr>
<tr>
<td>Lysozyme</td>
</tr>
<tr>
<td>Treatment 1</td>
</tr>
<tr>
<td>Treatment 2</td>
</tr>
<tr>
<td>SEM (n=4)</td>
</tr>
<tr>
<td>P</td>
</tr>
</tbody>
</table>

**General conclusion**

In order to meet the world's growing demand for seafood, aquaculture production is increasing significantly. However, current aquaculture practices in many countries are exposed to high risk of disease and reduced performance due to the high stocking densities and use of plant-based diets. In such situation, aquafeed formulators must pay special attention not only to nutritional specifications but also to reduce the potential risk of disease. Therefore, the supplementation with NGPs such as probiotics, capable of exerting immunomodulating effects, might be a natural alternative to develop a successful nutritional program.

For more information contact:
Álvaro Ortiz, Aquaculture Product Manager, NOREL S.A., Spain
TX-3000 RAISES THE BAR ON AQUATIC FEED PRODUCTION

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—Stewart B. Johnson, Dutch Artist

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Starch is a polysaccharide (carbohydrate) consisting of a large number of glucose units that is found in the storage organs of many plants. Its pregelatinization makes starch readily soluble in cold water making it useful for their binding properties in the pelletization and extrusion processes. Also, starch plays an important role in diets used in animal feeding trials for the adjustment of the energy and protein content of the diet.

The yellow pea (Pisum sativum) is most commonly grown pulse crop in the temperate North America region, and is widely grown as a cool season vegetable crop being one of the most important legume crops. Peas have numerous food (dry seed, vegetable) and feed (seed, fodder) usages. As forage, peas are a high-yielding, short-term crop with a high protein content. Environmentally, peas are highly valued as a rotation crop with cereals because their cultivation breaks cereal disease cycles, facilitates weed control, improves soil condition and fertility, and fixes high amounts of nitrogen in the soil for the following cereal crop to utilize. Its seed peptide fractions have the ability to chelate metals and inhibit linoleic acid, an important fatty acid, oxidation.

Pea starch used for this trial came from the dry milling process by means of mechanical and air separation. Once the seed coat of the pea is peeled, the split pea is hammermilled to a flour, and the starch and protein fractions are separated through air classification. Air classification does not create a 100% pure starch, however the pea starch ingredient is a concentrated carbohydrate source with attractive levels of protein that is also low in fiber. Dry milled pea starch consists of 75% crude carbohydrate, 11% crude protein, 1% crude fiber, and 1% fat. Pea starch is an all natural gluten-free carbohydrate source with high palatability due to the presence of simple sugars. As a raw material it provides many functional characteristics such as high water capacity and low gelatinization temperature, which contributes to excellent pellet/kibble quality and improves processing of the final products. Therefore, pea starch has many possible applications and the capability to be used as a partial or total source of carbohydrate for humans, pet food, and commercial aquaculture.

Various ingredients are used in diet formulations to balance the nutrients profile and physical characteristics of the final product. Although, not all nutrients in feedstuff are available for metabolism; hence, an important consideration is to measure the efficiency of the digestion and absorption of the nutrients present in the diet. The Apparent Digesti-
... pea starch provides superior binding characteristics, is highly digestible, and provides a variety of applications.

The apparent digestibility coefficient (ADC) of the diet components can provide useful information for researchers, nutritionists, feed mills and feed formulators. The digestibility of a particular feed ingredient is species-specific, and in the present case was assessed in rainbow trout (Onchorynchus mykiss) by a team of researchers led by Frederic T. Barrows at the USDA – Agricultural Research Service, in Bozeman, Montana. Wheat flour and pea starch were rapid assessed side-by-side while the other ingredients, soybean meal and corn gluten meal were investigated in other projects.

Due to the scarce information on pea starch, it was assayed along with other plant ingredients and the nutritional value of each ingredient was evaluated by determining the apparent digestibility of nutrients in compounded, extruded diets. Nutrient and energy availability were determined using the methods described by Cho et al. (1982) and Forster (1999) with all diets double labeled using chromic oxide and yttrium oxide as inert markers. A complete reference diet meeting or exceeding all known nutritional requirements for rainbow trout was blended with the test ingredient in a 70:30 ratio (dry matter basis) to form test diets. The reference diet nutritional composition was 48.8% crude protein, 16.5% crude fat, 5,400 Kcal/kg, 0.55% phosphorus, 2.06% lysine, and 0.99% methionine.

All diets were manufactured by cooking extrusion with approximately 18 seconds exposure to an average of 127°C in the six extruder barrel sections. The pressure at the die head was at about 350 psi and pellets of 3-4 mm were produced and dried in a pulse-bed drier to less than 10% moisture, followed by a 20 mins cooling period with forced air at ambient temperature. Diets were top-coated with fish oil using a vacuum coater at 24 mm Hg pressure.

<table>
<thead>
<tr>
<th>Reference Diet</th>
<th>g kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squid Meal</td>
<td>250.0</td>
</tr>
<tr>
<td>Soy Protein Concentrate</td>
<td>171.4</td>
</tr>
<tr>
<td>Corn Gluten Meal</td>
<td>83.4</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>43.0</td>
</tr>
<tr>
<td>Wheat Flour</td>
<td>283.3</td>
</tr>
<tr>
<td>Taurine</td>
<td>5.0</td>
</tr>
<tr>
<td>Menhaden Fish Oil</td>
<td>133.9</td>
</tr>
<tr>
<td>Vitamin Premix (ARS 702)</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>6.0</td>
</tr>
<tr>
<td>Vitamin C (Stay-C 35)</td>
<td>2.0</td>
</tr>
<tr>
<td>Trace Mineral Premix</td>
<td>1.0</td>
</tr>
<tr>
<td>Chromium Oxide</td>
<td>10.0</td>
</tr>
<tr>
<td>Yttrium Oxide</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proximal composition (%)</th>
<th>Soybean meal</th>
<th>Corn gluten meal</th>
<th>Wheat flour</th>
<th>Pea Starch</th>
<th>Reference diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>93.4</td>
<td>91.0</td>
<td>86.9</td>
<td>92.1</td>
<td>99.7</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.1</td>
<td>2.1</td>
<td>4.7</td>
<td>0.6</td>
<td>16.5</td>
</tr>
<tr>
<td>Crude protein</td>
<td>47.6</td>
<td>63.9</td>
<td>11.5</td>
<td>11.3</td>
<td>48.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.83</td>
<td>0.58</td>
<td>0.12</td>
<td>0.15</td>
<td>0.55</td>
</tr>
</tbody>
</table>
Apparent digestibility of each nutrient in the test diet and ingredients were calculated according to the following equations (Kleiber 1961, Forster 1999):

\[
ADCN_{\text{diet}} = 100 - 100\left(\frac{\% \text{ marker in diet}}{\% \text{ marker in feces}} \times \frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in diet}}\right)
\]

\[
ADCN_{\text{ingredient}} = \{((a + b) ADCNt - (A) ADCNr) b^{-1}\}
\]

ADCN diet = apparent digestibility coefficient of the nutrient in the diet;
ADCN ingredient = apparent digestibility coefficient of the nutrient in the test ingredient;
ADCNt = apparent digestibility coefficient of the nutrient in the test diets;
ADCNr = apparent digestibility coefficient of the nutrient in the reference diet;
a = (1-p) x nutrient content of the reference diet;
b = p x nutrient content of the test ingredient;
p = proportion of the test ingredient in the test diet.

The experimental diets were fed to fifteen 250g animals, initial weight, held in each of the 500L tanks in triplicate. Water temperature was maintained at 15°C, lighting on a 13:11 hs diurnal cycle and animals were hand fed to satiation twice daily. Fecal samples were obtained by manual stripping of anesthetized and gently dried fish 16 to 18 hs post-feeding. Fecal samples for each tank were freeze-dried and stored at -20°C until analyses were performed.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Dry Matter</th>
<th>Crude Lipid</th>
<th>Crude Protein</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal, 48%CP</td>
<td>30</td>
<td>76</td>
<td>77</td>
<td>5</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>n/a</td>
<td>89</td>
<td>92</td>
<td>n/a</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>n/a</td>
<td>90</td>
<td>82</td>
<td>n/a</td>
</tr>
<tr>
<td>Pea starch, AGT</td>
<td>40</td>
<td>*</td>
<td>88</td>
<td>57</td>
</tr>
<tr>
<td>Reference diet</td>
<td>76</td>
<td>95</td>
<td>83</td>
<td>47</td>
</tr>
</tbody>
</table>

* trivial amount of fat in ingredient. Digestibility cannot be determined.
n/a nutrient not analyzed

When compared with the other feed ingredients tested, pea starch provides superior binding characteristics, is highly digestible, and provides a variety of applications. Its high viscosity enhances the pellet quality and decreases fines in finished feed by adding a minimal amount of starch, which is slowly digested, providing sustained energy release with a low glycemic index. The protein available in pea starch is highly digestible, which along with the high phosphorus digestibility helps maintain good growth and bone calcification.

A few factors affect the ability of plant ingredients to be included in animal diets, such as, the protein, fat, starch and fiber levels, amino acid and fatty acids balance, the type and level of anti-nutritional factors, palatability, and cost. Several plant ingredients have been
successfully utilized in trout diets despite concerns with respect to palatability, fiber, glucosinolates and phytate content. Peas, although lower in protein than oilseed meals, have been successfully fed to trout, sea bass, tilapia, white legged shrimp, etc. without negative effects. Simple procedures such as dehulling, to decrease fiber levels, to more advanced treatments involving fine grinding, air classification, and thermal treatment are beneficial in reducing heat-labile anti-nutritional factor levels and in increasing nutrient availability. Peas differ from soybeans and canola in that the energy fraction of the seed is comprised of starch rather than oil and it can be a direct replacement for wheat due to its excellent binding characteristics, relatively protein levels and low fiber. The higher digestibility can be attributed to fiber removal, as well as gelatinization of the starch and anti-nutrient destruction during pelletization.

Acknowledgements
The authors would like to thank Cargill Inc. and Alliance Grain Traders for the financial support and for providing pea starch for the investigation. Also, a special thanks to Dr. Frederic Barrows and his team for conducting the research and sharing the data.

More information
Eric Bartsch, Alliance Grain Traders. Bismarck, ND, 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.

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Tilapia has drawn much attention lately and been recognized by FAO as the most potent culture fish species in supplying human protein source of the Century. Production of tilapia has been increasing throughout the world and a future increase in production has been projected. As the industry expands and technology development continues, traditional extensive culture of tilapia is being replaced by semi-intensive and intensive production systems. As stocking rates increase, the contribution of natural food decreases and more nutritionally complete diets are needed. In semi-intensive and intensive culture systems, diet is the most expensive cost item, often ranging from 30% to 60% of the total variable expenses, depending on the intensity of the culture operation. Thus, the use of least-cost, nutritionally balanced diets and good feeding management are two most important requisites for successful fish production.

Traditionally, dietary non-protein energy sources, such as carbohydrate or lipid have been demonstrated to spare the protein for tilapia (Shiau, 2001). However, these common carbohydrate sources also show low digestibility due to the high fiber content, whereas high dietary fat levels cause fatty fish. Therefore, feed additives that can improve nutrient utilization have a potential to promote protein sparing in tilapia and reduce the feed cost per kg of fish and/or filet produced. 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 (Ceulemans et al., 2009) as well as field conditions (Sampaio Gonçalves et al., 2012). The present...
study was conducted to investigate the use of a feed additive with digestive/metabolic enhancing action to reduce dietary protein level in Tilapia without affecting performance and final product quality. During this trial, a wide range of parameters were evaluated, including fish performance, filleting yield, metabolic indicators, lipid utilization and intestinal morphology.

**Growth trial**

Experimental diets (formulation and composition are shown in Table 1) were designed by National Pingtung University of Science and Technology (NPUST) based on a common feed formulation for tilapia in Taiwan. The diets were extruded by Tungkang Biotech Research Center (Pingtung, Taiwan). The feed additive with digestive/metabolic enhancing properties consisted of Aquagest® OMF (Nutriad International, Belgium). Experimental diets consisted of a control diet (28% protein and 7% lipid without Aquagest OMF, coded as CONTROL 28/7) and a test diet with 2% less protein and the feed additive added (26% protein and 7% lipid, 0.3% Aquagest OMF; coded as LOPRO 26/7+AG).

Male hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) were supplied from the local farm in Tainan, Taiwan. All the fish were reared at the “88 platform” test farm in Changjhih, Pingtung. Upon arrival, they were acclimated to farm conditions for 2 month in a cement pond [5 m (w) × 5 m (l) × 0.75 m (h)] and fed commercial tilapia diet (Hanaqua Tech Inc., Taiwan). The conditions during the acclimation period were similar to those at the initiation of the experiment. Three cement ponds were used for the feeding trial. Each pond was divided into two parts by a nylon net. Forty five tilapia with average initial weight of approximately 175 g were randomly selected into each pond. Fish were reared in a flow-through system with underground freshwater. Around 80% of the water in the system was changed every three weeks.

Fish were fed with 2-2.5% of their body weight per day. This amount was close to the maximal daily ration for tilapia. The daily ration was divided into two equal meals (08:00 and 15:00 h) and hand-fed to the fish. Fish were weighed once every 3 weeks by students from NPUST, to monitor growth performance and adjust feeding rations. Fish were fed the test diets during 18-weeks from July 28 to November 30, 2013. Water temperature was recorded every day. Other water quality parameters, including ammonium and nitrite concentrates were determined eve-

| Table 1. Diet formulation and proximate composition (%) |
|---------------------------------|-----------------|-----------------|
|                                | CONTROL 28/7    | LOPRO 26/7+AG   |
| Local fish meal                | 10.88           | 10.85           |
| Meat and bone meal             | 2.18            | 2.17            |
| Soybean meal                   | 13.06           | 8.77            |
| Rapeseed meal                  | 8.71            | 8.68            |
| Wheat meal                     | 10.88           | 10.85           |
| Wheat flour                    | 10.88           | 10.85           |
| Coconut meal                   | 8.71            | 8.68            |
| Whole fat rice bran            | 10.88           | 10.85           |
| Corn DDGS                      | 19.59           | 19.53           |
| Salt                           | 0.70            | 0.69            |
| Local fish oil                 | 2.61            | 2.60            |
| DCP                            | 0.44            | 0.43            |
| Premix                         | 0.44            | 0.43            |
| Choline                        | 0.04            | 0.04            |
| Oyster shell powder            | -               | 4.34            |
| Aquagest OMF                   | -               | 0.30            |

**Proximate composition**

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL 28/7</td>
<td>8.7</td>
<td>27.8</td>
<td>7.4</td>
<td>8.2</td>
</tr>
<tr>
<td>LOPRO 26/7+AG</td>
<td>8.3</td>
<td>26.1</td>
<td>7.4</td>
<td>10.4</td>
</tr>
</tbody>
</table>
At the end of the feeding trial, body length and weight of tilapia were recorded. Weight gain (WG, as measured by the percentage of body weight gain), feed efficiency (FE), protein efficiency ratio (PER), protein retention, condition factor and hepatosomatic index were calculated. Five fish were sampled randomly to be sacrificed. Liver, blood and muscle were collected and stored in -20°C until analysis. The midgut samples were analyzed by histological assay. Blood was examined on the hematological parameters, including red blood cell count, hematocrit and hemoglobin concentration, and plasma triglyceride concentrations. Proximate composition of muscle was also measured. Thirty fish were transferred to Hung-Yi Frozen Food Factory (Pingtung, Taiwan, Fig. 1) to determine the fillet yield (%) and visceral fat (%).

Multiple effects from supplementing the feed additive to a low protein feed

Growth performances of tilapia fed different diets were shown in Table 2. Fish fed LOPRO 26/7+AG, grew faster (daily weight gain 5.2% higher) than control fish, although these differences were not significantly different. FCR, pro-

Fig. 1. Collect the fillet and visceral fat in Hung-Yi Frozen Food Factory (Pingtung, Taiwan)
tein efficiency ratio (PER) and protein retention were significantly better (P<0.05) in tilapia fed the LO-PRO 26/7+AG-diet compared with the control (showing -7.1%, +14.8% and +7.3%, respectively, change versus control). These results clearly indicated that the supplementation of the feed additive showed positive effects on protein utilization and growth for tilapia, resulting in an effective protein sparing effect.

Visceral fat (Table 3) and plasma triglyceride concentrations (Table 4) in fish fed LOPRO 26/7+AG, showed -7.1% and -14.0% reduction compared to the control group. This indicates that the feed additive ingested by tilapia can enhance lipid utilization and decrease the metabolism of protein to energy by the fish. In addition, hepatic glucose-6-phosphate dehydrogenase (G6PDH) activity was higher in the LO-PRO 26/7+AG group compared to control fish (Table 4). This enzyme is involved in the pentose phosphate pathway to generate the coenzyme NADPH for metabolism. High performance of G6PDH generally indicates high activity of fatty acid synthesis derived from carbohydrate. In the present study, enhanced G6PDH activity indicated a higher rate of carbohydrate utilization in fish fed the feed additive.

The improvement of nutrient utilization was also reflected in filleting yield. Tilapia fed LOPRO 26/7+AG showed 1.1% higher filleting yield (Table 3) compared to control fish.

Hematological parameters, including WBC, RBC, Hct and Hb, were not affected by the dietary treatments (Table 4). These results suggest that in the present trial, the general health status of Tilapia was not influenced by the changes in dietary protein levels or the supplementation of the feed additive. The histological evaluation of midgut from all treatments (Fig. 2) showed a little atrophy in fish fed diets without Aquagest OMF compared with fish fed diets with Aquagest OMF. In all experimental diets, many plant ingredients were used, such as soybean meal, rapeseed meal, wheat meal, wheat flour, coconut meal, rice bran and corn DDGS. By our knowledge, anti-nutritional factors in plant ingredients were considered to cause intestinal enteritis for fish. So the damage to the villi was commonly found in fish fed plant-based diet. In the present study, the villi integrity in fish fed diets supplemented Aquagest OMF was better than that in fish fed diets without the feed additive.

### Table 2. Growth performance of tilapia fed different diets for 18 weeks.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL 28/7</th>
<th>LOPRO 26/7+AG</th>
<th>% change vs control</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>93.3±2.2</td>
<td>96.3±2.6</td>
<td>+3.2%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Initial wt (g)</td>
<td>177.3±1.8</td>
<td>174.7±2.1</td>
<td>-1.5%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Final wt (g)</td>
<td>469.6±13.2</td>
<td>482.0±12.1</td>
<td>+2.6%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Daily weight gain (g/d)</td>
<td>2.32±0.11</td>
<td>2.44±0.03</td>
<td>+5.2%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Feed intake (g/fish)</td>
<td>860.5±21.6</td>
<td>840.3±17.0</td>
<td>-2.4%</td>
<td>n.s.</td>
</tr>
<tr>
<td>FCR</td>
<td>2.95±0.20</td>
<td>2.74±0.04</td>
<td>-7.1%</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>PER</td>
<td>1.22±0.08</td>
<td>1.40±0.02</td>
<td>+14.8%</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Protein retention (%)</td>
<td>27.70±1.73</td>
<td>29.72±0.04</td>
<td>+7.3%</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Different superscripts in the row indicate significant (P<0.05) difference between different dietary treatments. Values are means ± SD from three groups of fish (n=3) with 45 fish per group. n.s.: non significant difference (P<0.05).

FCR: food conversion ratio; PER: protein efficiency ratio.
Table 3. Hepatosomatic index (HSI), condition factor (CF), visceral fat content and filleting yield of tilapia fed different diets for 18 weeks.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL 28/7</th>
<th>LOPRO 26/7+AG</th>
<th>% change vs control</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSI (%)</td>
<td>3.30±0.74</td>
<td>3.09±0.26</td>
<td>-6.4%</td>
<td>n.s.</td>
</tr>
<tr>
<td>CF</td>
<td>1.88±0.14</td>
<td>2.02±0.04</td>
<td>+7.4%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Visceral fat (%)</td>
<td>6.31±1.81</td>
<td>5.83±1.62</td>
<td>-7.6%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Filleting yield (with skin)</td>
<td>41.46±0.17</td>
<td>41.91±0.40</td>
<td>+1.1%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Filleting yield (without skin)</td>
<td>34.82±0.14</td>
<td>35.20±0.34</td>
<td>+1.1%</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Different superscripts in the row indicate significant (P<0.05) difference between different dietary treatments. n.s.: non significant difference (P>0.05). Values are means ± SD from three groups of fish (n=3) with 5 fish per group.
Table 4. Hematological parameters of tilapia fed different diets for 18 weeks.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL 28/7</th>
<th>LOPRO 26/7+AG</th>
<th>% change vs control</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell (10^3/μl)</td>
<td>235.23±10.83</td>
<td>236.46±12.24</td>
<td>+0.5%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Red blood cell (10^6/μl)</td>
<td>2.55±0.08</td>
<td>2.50±0.24</td>
<td>-2.0%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33.09±2.29</td>
<td>31.61±3.02</td>
<td>-4.5%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hemoglobin (mg/dl)</td>
<td>10.12±0.56</td>
<td>10.64±0.82</td>
<td>+5.1%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hepatic G6PDH</td>
<td>163±40^a</td>
<td>267±29^b</td>
<td>+63%</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Plasma triglyceride (mg/dl)</td>
<td>15.73±0.40^a</td>
<td>13.53±0.80^b</td>
<td>-14.0%</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Different superscripts in the row indicate significant (P<0.05) difference between different dietary treatments. n.s.: non significant difference (P>0.05). Values are means ± SD from three groups of fish (n=3) with 3 fish per group.

Conclusion
The present study demonstrated that the dietary protein level can be reduced in tilapia by supplementing a feed additive capable of enhancing nutrient utilization. Tilapia fed 2% less protein and supplemented with a digestive/metabolic enhancer, showed even better performance in terms of growth, FCR, PER, protein retention and fileting yield compared to control fish. Furthermore, fish fed the enhanced low protein diet showed lower levels of visceral fat and plasma triglycerides, but enhanced G6PDH activity, which indicated effects on lipid and carbohydrate metabolism. The metabolic effects can explain the release of non-protein energy, which in turn results in protein sparing and more effective utilization of protein for muscle growth. Increased nutrient utilization efficiency is key to achieve more cost-efficient feeds.

Acknowledgement
The authors would like to acknowledge the practical support of Taiwanese industrial partners in this research project, i.e. Mr. Peter Chiang, founder of Hanaqua Tech Inc. produced the extruded feeds and Mr. Neil Shih, farm manager responsible for the test farm facilities “88 platform”.

References
Sanitary Design Cooler
For highest food safety standards
Physical properties and functionality of Corn Protein Concentrate in animal feeds

Fabio Soller, PhD, Nutritionist, Cargill, Inc.

Physical Properties

Empyreal® 75 (E75), a corn protein concentrate (CPC) is a natural protein golden in color with low levels of ash. Not only does it complement animal proteins in formulation but it also gives the option of lowering the production costs by using fewer grades of animal protein meals, resulting in lower formulation cost. Its high methionine-to-lysine ratio serves as a good source of essential amino acids at a lower cost than other vegetable or animal proteins of comparable purity, consistency and crude protein level. Since animal proteins and other plant ingredients are naturally high in lysine, the addition of CPC will result in a more balanced formulation. Its granular material possess low water solubility but highly soluble in aqueous concentrations of ethanol (Table 1) and it has good oil binding properties.

Since animal proteins and other plant ingredients are naturally high in lysine, the addition of CPC will result in a more balanced formulation.

Table 1  Corn protein concentrate solubility index (SI).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Deionized water</th>
<th>Water: Ethanol</th>
<th>Ethanol</th>
<th>2</th>
<th>4</th>
<th>5.5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility (%)</td>
<td>2.01</td>
<td>17.70</td>
<td>12.87</td>
<td>0.250</td>
<td>0.150</td>
<td>0.112</td>
<td>1.193</td>
<td>0.612</td>
<td>1.325</td>
</tr>
</tbody>
</table>

Obs: Protein analyses were done on the suspensions using the Bio-Rad Protein Assay. Protein solubility indices were calculated as follows:

\[ SI = \frac{[\text{protein content of supernatant}]}{[\text{protein content of suspension}]} \times 100 \]

E75 is dried with a flash dryer, which results in a consistent low moisture product with a fine granular appearance (Table 2), with minimal damage to its proteinaceous and vitaminic fractions.
Functionality

The manufacturing process creates a new ingredient functionality for greater: elasticity, binding and co-mingling with other ingredients, coating, and fat absorption. CPC has superior extrusion functionality over other high protein corn ingredients with finer and more uniform cell structure at comparable extruder setup and specific mechanical energy (SME). Better incorporation and intermixing with other ingredients leads to less fines loss due to non-incorporated particles.

The following examples show, in dog and cat food, the dough-like properties of the extrudate provides a smoother kibble surface both on the die and knife surfaces (Figures 1 and 3). The extrusion properties of CPC provide a new tool to control expansion with an economical source of protein and lessen the reliance on starch for structure and expansion properties (Figure 2), also requiring less steam during the process.

Shelf life

CPC is rich in nutrients and possesses a high concentration of natural antioxidants. This benefits the overall health of the animal, while also extending the product's shelf life which should be shelf-stable for up to twelve months.

<table>
<thead>
<tr>
<th>Retained on US Standard Sieve</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>35</th>
<th>60</th>
<th>80</th>
<th>170 throughs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sieve Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical % Overs</td>
<td>0.9</td>
<td>2.6</td>
<td>5.1</td>
<td>13.0</td>
<td>23.6</td>
<td>15.7</td>
<td>26.6</td>
</tr>
</tbody>
</table>

Table 2  Typical particle size distribution

Figure 1: internal cross section of cat kibble with CGM included (left), kibble surface of cat kibble with CPC included (right). 15x magnification.
Figure 2: Internal matrix of extruded samples of CGM (a.), CGM plus 15% fat (b.), CPC (c.), and CPC plus 15% fat (d.). Twin extrusion system used. 15x magnification.

Figure 3: Physical appearance of cat kibbles. Internal matrix of fractured (a.) and knife surface (b.) with CGM or CPC, fractured (c.) and knife surface (d.). 15x magnification.
Water Absorption and Solubility Indexes

Water absorption index (WAI) and water solubility index was determined as outlined in American Association of Cereal Chemists (AACC) Official Methods and calculated as:

\[
\%WAI = \frac{\text{net weight of wetted sample}}{\text{original weight sample (dry basis)}} \times 100
\]

Water solubility index (WSI) was calculated as the percent solids of the original material (db) present in the supernatant. Additionally, the % solubilized protein was calculated as the calculated percent of the original samples protein content that was measured in the supernatant after centrifuging as measured by CRA Official Method G-66 and a protein conversion factor of 6.25.

Oil Binding and Adsorption Index

The oil binding index (OBI) and oil adsorption indexes (OAI) were calculated as follows:

- Oil Binding Index = weight of oil lost/weight of material (db)*100.
- Oil Adsorption Index = Weight increase of pellet (db)/weight of protein material (db) *100.
- Increase of dry basis weight of the protein material was assumed to be oil adsorbed during treatment.

<table>
<thead>
<tr>
<th>WAI (%)</th>
<th>WSI (%)</th>
<th>% Solubilized Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>280</td>
<td>5.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Water absorption index and water solubility index of CPC was also determined in either deionized water (DI H2O) or in 0.5% sodium chloride (NaCl) solution and incubated at 22, 50, or 99°C for 1hr.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Solvent</th>
<th>WAI (%)</th>
<th>% Solubilized Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>DI H2O</td>
<td>228</td>
<td>3.6</td>
</tr>
<tr>
<td>22</td>
<td>NaCl</td>
<td>227</td>
<td>3.5</td>
</tr>
<tr>
<td>50</td>
<td>DI H2O</td>
<td>211</td>
<td>3.5</td>
</tr>
<tr>
<td>50</td>
<td>NaCl</td>
<td>219</td>
<td>3.6</td>
</tr>
<tr>
<td>99</td>
<td>DI H2O</td>
<td>210</td>
<td>4.2</td>
</tr>
<tr>
<td>99</td>
<td>NaCl</td>
<td>209</td>
<td>4.4</td>
</tr>
</tbody>
</table>
The physical attributes of CPC allow good extrusion or other mechanical processing characteristics with minimal water requirements for functionality with, also, increased water stability of the finalized diet, an essential requirement of any aquaculture feed. Its high oil binding and adsorption indexes make it a very desirable ingredient for any diet in which high amounts of fat are required (e.g. marine species).

The cost-benefit of this alternative protein source during manufacturing, as well as the increase in pellet quality, gives the manufacturer and the final consumer total satisfaction with economical savings.

Table 5 – Percentage of corn oil bound (OBI) and adsorbed (OAI) by the CPC.

<table>
<thead>
<tr>
<th></th>
<th>OBI (%)</th>
<th>OAI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>236</td>
<td>103</td>
</tr>
</tbody>
</table>

More information
Fabio Soller, PhD, Nutritionist, Cargill, Inc.
ANDRITZ is one of the world's leading suppliers of technologies, systems, and services relating to advanced industrial equipment for the aqua feed industry. With an in-depth knowledge of each key process, we can supply a compatible and homogeneous solution from raw material intake to finished feed bagging.

We have been delivering products and plant solutions for the feed industry since the 1930s and have thereby gained extensive knowledge and insight into the increasing demands of the specific feed markets. We put this knowledge into all our efforts to ensure that we have satisfied customers.
Organic forms of trace mineral have been demonstrated to have higher bioavailability and tissue storage than their inorganic counterparts. This is of primary importance when it comes to their nutritional performance in aquafeeds. Their increased bioavailability and effectiveness promotes performance, reduces mineral loading in diets and decreases waste discharge.

Organic mineral forms can greatly enhance the absorption of an element through their structural integrity. Minerals chelated to organic molecules interact less with each other in the digestive tract and have also been shown to be less sensitive to the inhibitory action of other compounds because of their reduced solubility in water. Amino acid chelates of cobalt, manganese and zinc are more available than their inorganic forms while the organic chelate zinc methionine has been estimated to be three times more potent than the inorganic sulphate.

Salmon producers are acutely aware of the environmental and social challenges that the aquaculture industry faces. The responsible sourcing of marine and vegetable raw materials for salmon feed is an increasingly important factor in production and a vital component of a sustainable industry.

In the last 10 years changes in diet formulation have seen the content of fish feed change from 70% marine raw materials to 70% vegetable and 30% marine raw materials in 2013. This trend to decreasing marine raw materials is continuing and this shift has increased the complexity of feed formulation with more functional ingredients and additives being utilized to satisfy the nutrient requirement of the species being cultured. This provides balanced nutrition promoting good health, growth and performance.

The simultaneous replacement of fish meal with plant protein sources and the reduction of fish oil through vegetable oil substitution impacts the fatty acid composition of the filleted product. This results in high levels of C18 PUFAs and reduced levels of the omega-3 PUFAs.
docosahexanoinc acid (DHA) and eicosapentaenoic acid (EPA).
This is of concern to producers and consumers as salmon is an excellent source of high-quality protein and a rich source of health enhancing long chain omega-3 essential fatty acids good for the heart, brain, eyesight and many other conditions. In order to maintain the health benefits of salmon the FHL (Norwegian Seafood Federation) announced that the minimum levels of EPA+DHA present in diets for salmonids should be no lower than 4% of the total dietary oils.
Recent studies have shown that it is possible to replace fish oil in salmon feed using an algal meal (Alltech Inc.) rich in the marine omega-3 fatty acid DHA and obtain high n-3 fillet levels with improved DHA, EPA and total n-3 ADC while maintaining good fillet technical quality and without affecting the growth or having any negative effect on fish health.
However unpublished new data from a currently running European research project has demonstrated that low fish meal (15%) diets containing higher than commonly used levels of minerals show similar growth with fish fed a control diet with high fish meal (40%) inclusion.
Within the framework of the Alltech- Nofima strategic research alliance, a study on the effect of fish oil replacement by algae on salmon performance and fillet quality, an organic mineral premix was added to one of the experimental diets to study the effects of these on fillet quality.
The diet formulations are shown below and are balanced to contain 4.85% EPA+DHA (% in dietary oil).
Five dietary treatments were prepared:
Diet 1  MFM_0_AA - Medium fish meal diet (15% fish meal)
Diet 2  MFM_2.5_AA - Medium fish meal diet + 2.5% algae
Diet 3  MFM_5_AA - Medium fish meal diet + 5.0% algae
Diet 4  LFM_5_AA   - Low fish meal diet (10% fish meal) + 5.0% algae
Diet 5  LFM_0_AA_BP - Low fish meal diet (10% fish meal) + 5.0% algae with organic minerals

All test diets were formulated to contain equal amounts of crude protein, crude lipid, digestible energy, total saturated fatty acids, EPA+DHA and n-3/n-6 fatty acid ratio using different oil and plant protein mixes. The algae used in this study was SP1 (Alltech Inc) and the organic mineral mix was prepared using Bioplex minerals supplied by Alltech Inc.

Table 1 shows the growth performance and fatty acid fillet composition.

The salmon growth rates were high and the FCR low and similar in all the dietary treatments. The highest fillet levels of DHA were found in the fish in the lowest fish meal inclusion rate fed the organic minerals (LFM_0_AA_BP). The Bioplex mineral supplementation in low fish meal diets resulted in lipid levels similar to that of the medium fish meal diets.

No differences in fillet quality was measured across the 5 dietary treatments when drip loss, Lightness, SalmoFan Cscore or firmness were measured. However the gaping % was nearly eradicated by the supplementation of the Bioplex minerals (Figure 1) indicating that Bioplex minerals play a positive role in fillet quality.
Bioplex minerals are more available than inorganic minerals and may protect the polyunsaturated fatty acids in the diet from oxidative stress and promoting higher PUFA incorporation levels in the fillet tissue of fish.

There is still much to learn about the role of micronutrients as co-enzymes for key LCC-PUFA biosynthesis. Their influence on gene regulatory elements and their interaction with fatty acid β-oxidation may play a significant role in future sustainable feed formulations.

**Figure 1:** Fish fillet gaping (%) in the different dietary treatments

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New and complete range of hammer mills from Dinnissen, with a focus on multitasking

Dinnissen Process Technology specializes in the development and production of process technologies and equipment for the feed and Petfood industry. Dinnissen is now offering a new and extremely wide range of hammer mills that provide an effective solution for any challenge when it comes to grinding and screening even the most difficult ingredients.

The new hammer mills are characterized by a broad range of capacities and product output particle size as well as flexibility and special add-on functionalities, making them real multitasking 'monsters'.

**Wide range of screening panels, perforations, and functionalities**

Dinnissen’s new range of multitasking hammer mills enable the user to screen output particles ranging in size from 3 mm down to 150 microns. Single hammers or double hammers are used depending upon the specific ingredients to be ground down.

A crucial feature of the new product range is the enormous range of screening panels, surfaces and perforations available, as these features determine the capacity, quality and effectiveness of the screening process and make it possible for the company to offer its clients an effective solution for practically any grinding and/or screening challenge. Two striking members of the new product range are the small Easy-to-Clean DINNOX Mill and the Dinnissen D-Topline Hamer Hammer mill with automatic screen changer.

**Small Easy-to-Clean DINNOX Mill**

The Easy-to-Clean DINNOX Mill is a very compact hammer mill with a minimum capacity of 30 kg per hour. The small Easy-to-Clean DINNOX Mill is fitted with exchangeable grinding rotors, providing it with a variety of breaking, cutting, and grinding functionalities, as well as exchangeable screening panels and adjustable RPMs. This allows the user to easily and quickly switch between an almost endless variety of grinding and screening applications, depending upon the specific ingredients process and the desired end result.

In spite of its very compact design, the new Easy-to-Clean DINNOX Mill is also fitted with completely detachable grinding rotors and extra-large inspection hatches, making it easy to access all the components, including the screening panels, and to clean everything quickly and thoroughly.

The Easy-to-Clean DINNOX Mill is available in stainless steel 304, RVS 316L, and polished or electrolytically polished RVS. It can also be fitted with an automatic cleaning system based on compressed air, CIP,
or hot steam/air for extra hygienic applications, and complies with all EHEDG requirements.

**D-Topline Hamex Hammer mill with automatic screen changer**

Dinnissen’s largest and most sophisticated hammer mill is the new D-Topline Hamex Hammer mill with automatic screen changer. It can handle grinding capacities of up to 45,000 kg per hour, and the screen changer has space for four to six different sets of screening panels. The mechatronic screen changing system automatically selects and places the appropriate set of screening panels in the hammer mill. After the production process has been completed, it also removes and stores the screening panels in less than 40 seconds. This makes it possible to change screens extremely quickly and greatly reduces downtime.

The D-Topline Hamex Hammer mill features a new and innovative design that greatly increases the surface area of the grinding and screening panels inside the hammer mill, which in turn provides increased grinding and ringing capacity. The screen storage facility is also fitted with an extra-wide chamber that can be automatically opened. This makes it easier to carry out maintenance and remove damaged screens if necessary, thereby minimizing downtime. The new hammer mill has a maximum speed of 1500 RPM, giving it a longer usable lifetime and in lower energy consumption than its predecessors.

**Extru-Tech releases advanced feature Aseptic Dual Preconditioner**

Extru-Tech, Inc. didn’t take the designation lightly when it released its new Advanced Feature Aseptic Dual Preconditioner (ADP) as a replacement for its Dual Shaft Conditioning Cylinder.

Technically, “aseptic” is defined as sterile, which, in turn, calls for a complete pathogen kill. To that end, Extru-Tech offers a proven scientific validation that the new ADP can sterilize the product prior to its discharge from the preconditioner, and can be cleaned/sanitized through validated SSOPs.

Moreover, studies show that the new Aseptic Dual Preconditioner offers a 2.56 coefficient of variation for greater efficiency than our competitor’s latest preconditioner designs. As a result of the ADP advanced design, the coefficient of variation improves even further as beating speeds increase.

“As formulas become more nutritionally refined through the high ratio meat inclusion and finished product quality increases, preconditioning is becoming a vital part to any production process,” says Norm Schmitt, corporate sales manager for Extru-Tech.

“Fortunately, the new Aseptic Dual Preconditioner offers the best of all worlds. Customers benefit from unmatched efficiency, flexible configurations, variable retention times, total product quality control and a long list of new hygienic features.”

The new design also includes Extru-Tech’s Advanced Venting Technology (AVT) system for steam and fines suppression from the conditioner without the need to vent to the atmosphere.

Other sanitary features include a new hygienic frame and man-
ual clean-out valve. In the meantime, a higher-speed shaft configuration and reversible, variable-frequency drives provide greater operator control over mixing speed and conditioning efficiency. Aseptic Dual Preconditioners are available in a variety of sizes to fit the varying applications and needs of Extru-Tech customers worldwide.

Extru-Tech, Inc., headquartered in Sabetha, Kansas, currently produces and markets one of the industry’s most complete lines of extrusion processing systems along with a full line of ancillary equipment and customized equipment solutions for specialized processes.

Merck Animal Health shows benefits of treating salmon sequentially with SLICE® (emamectin benzoate) and Hydrogen Peroxide

Merck Animal Health (known as MSD Animal Health outside the USA and Canada) recently presented results from a study assessing the benefits of treating salmon with SLICE® (emamectin benzoate) followed by hydrogen peroxide to reduce sea lice infestations and re-infection in sea lice populations that have demonstrated resistance to emamectin benzoate. The study found that a sequential treatment approach provided effective clearance of existing sea lice infections and subsequently reduced resettlement of an emamectin benzoate-resistant sea lice strain, more effectively than treatment with hydrogen peroxide alone. Results were presented at the 10th International Sea Lice Conference held August 31 to September 5 in Portland, Maine, U.S.A.

“Sequential treatment with SLICE and hydrogen peroxide has been adopted by a number of veterinarians and producers to improve sea lice control, particularly in sea lice populations that have shown resistance to the treatments,” said Dafydd Morris, Merck Animal Health. “We are pleased to present this important research to help producers optimize their sequential treatment approach, thereby improving the effectiveness of their control strategy and reducing re-infection.”

In the study, mixed-sex Atlantic salmon were challenged with infective sea lice copepods from a population that is resistant to emamectin benzoate. After four weeks, the fish were randomly allocated to 28 identical treatment tanks. On Day 36, the fish were challenged with a second cohort of sea lice copepods to ensure a mixed-stage sea lice population on the fish.

The 28 treatment tanks were randomly assigned to 14 treatment groups, with ten groups receiving treatment with SLICE from days zero to six. Nine of these groups were also treated with hydrogen peroxide at nominal concentrations of 600, 1000, or 1400ppm, administered at either three, seven, or 12 days after the end of the SLICE treatment. The other four groups did not receive a SLICE treatment. Of these, three groups received hydrogen peroxide treatments at 1400ppm and one received no treatments.

The study found that efficacy against sea lice infection was greater when hydrogen peroxide was administered seven or 12 days after SLICE in a concentration of 1000 or 1400ppm. Sequential treatment of hydrogen peroxide seven days after SLICE had 86.1 percent efficacy at 1000ppm and 81.8 percent efficacy at 1400ppm. When hydrogen peroxide was administered 12 days after treatment, efficacy was 83 percent at 1000ppm and 91.2 percent at 1400ppm. Efficacy of hydrogen peroxide at 1400ppm—
without a preceding treatment with SLICE—was similar, at 86 percent when administered on day seven and 86.6 percent when administered on day 12. Treatment groups receiving only SLICE experienced limited reduction of infestation, due to resistance in the sea lice population.

In addition to determining the most efficacious timing and dosing of hydrogen peroxide after treatment with SLICE, the study also sought to determine the residual effect of SLICE and hydrogen peroxide in reducing re-infection by a resistant strain of sea lice. The study found that treatment with hydrogen peroxide alone gave little or no benefit against sea lice re-infection, and that sequential treatment showed similar efficacy to treatment with SLICE alone, suggesting that hydrogen peroxide has no residual efficacy. Treatment with SLICE, however, provided 52.9 percent efficacy against re-infection 39 days following treatment.

Cargill EMS Risk Tool to help shrimp farmers battle disease

Cargill's animal nutrition business has launched a new EMS Risk Tool to help customers determine the best farm management program to manage the risk of Early Mortality Syndrome (EMS) on their shrimp farms. EMS, also known as Acute Hepatopancreatic Necrosis Disease (AHPND), is caused by certain strains of the bacteria vibrio and has led to devastating production losses in China, Vietnam, Malaysia, Thailand and most recently in Mexico.

With this tool, Cargill can identify the main risk factors for individual farms and provide tailored recommendations to help reduce the severity of the disease to promote shrimp survival. Outbreaks of EMS have resulted in mortality rates as high as 100 percent.

"With comprehensive risk assessment tools we are able to develop biosecurity programs tailored to identifying and reducing the risk of EMS losses in our customers' shrimp farms," said Ryan Lane, Cargill's global aquaculture technology director.

The EMS Risk Tool assesses the risk of EMS based on the best available knowledge of the factors associated with the disease, from seed (postlarvae), genetics, sanitary practices, environment and farm management, to shrimp nutrition and health.

Cargill – in partnership with researchers, industry and government – has been investigating strategies to help shrimp producers reduce the risk of EMS outbreaks. Cargill's EMS Risk Tool encompasses feedback and learnings from all of the EMS-affected countries, including a workshop in Mexico in November 2013. This workshop, hosted by Cargill, brought together a wide range of academic researchers, government representatives and industry experts to share experiences and explore strategies to deal with the disease.

"This tool is the next step in our journey to mitigate the effects of EMS," added Lane. "Helping shrimp producers manage risks of diseases like EMS will lead to better farming practices and can help mitigate other diseases. The Cargill technical team continues to focus on finding effective ways to combat EMS and is working closely with our customers to develop solutions."

Beyond implementing strong biosecurity practices, Cargill Animal Nutrition studies have shown that using high quality hatchery and farm feeds are better at delivering nutrients to young fish and shrimp.

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For more information, contact your local INVE Aquaculture representative.
OCTOBER
Oct 6 – 9: GOAL 2014
Ho Chi Minh City
Vietnam
Details

Oct 7 – 9: EurOCEAN 2014
Connecting Science, Policy and People
Rome, Italy
Details

Oct 14 – 17: Aquaculture Europe 2014
San Sebastian, Spain
Details

Oct 14: 3rd European Percid Fish Culture (EPFC) workshop 2014
San Sebastian, Spain
Details

Oct 15 - 17: Vietstock
HCMC, Vietnam
Details

Oct 16-18: World Congress of Aquaculture and Fisheries
Dalian, China
Details

Cascas, Portugal
Details

NOVEMBER
Illinois, USA
Details

Nov 6 - 8: AFIA Equipment Manufacturers Conference
Palm Springs, Ca., USA
Details

Nov 11 -14: EUROTIER
Hanover, Germany
Details

DECEMBER
Dec 1-3: International Algae Congress
Florence, Italy
Details

Dec 9 - 10: AFIA 2014 Import & Export Seminar
Arlington, Va., USA
Details

2015
FEBRUARY
Feb 19 - 22: Aquaculture America 2015
New Orleans, La., USA
Details

MARCH
Mar 16-18: AquaME
Dubai, UAE
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MAY
Jeju Island, South Korea
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JUNE
Jun 9: Aquafeed Horizons Conference
Cologne, Germany
Details

Jun 10: FIAAP International Conference
Cologne, Germany
Details

June 9—11: FIAAP/VICTAM/GRAPAS 2015
Cologne, Germany
Details