Natural Ingredients – Product Opportunities and Process Issues

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Introduction

All over the world we face increasing challenges to support the growth of aquaculture. One of these is the supply of the ingredients that provide our base protein and lipid requirements. The environment we work in becomes increasingly challenging and we are faced with increasing demands for 'natural', 'sustainable', 'ethical' and 'organic' products. These demands in turn are driving us to source naturally derived ingredients with, it is hoped, an optimised delivery of nutritional performance plus added health benefits. It will be increasingly important to source ingredients that deliver the benefits of immune system stimulation, suppress auto-immune response, act as anti-bacterial and anti-viral agents, prevent or reduce cancers, deliver organically linked metals and pigments, and yet be sustainable, ethical and not create manufacturing problems.

Aquaculture Production Trends

From the early 1950's until 2004 global aquacultural production has risen from less than 1 million tonnes to 59.4 million tonnes, with a value of US$70.3 billion. 69.6% of this production in 2004 was in China with 21.9% in the rest of Asia and the Pacific Region. Western Europe contributed 3.5% of this total with 2.1 million tonnes valued at US$5.4 billion. Even in Western Europe with efficient feed conversion ratios (FCR) of 1.2 : 1 this means 2.5 million tonnes of feed were needed in 2004. If feed protein content of 35% average was manufactured using fish meal with 70% protein content this means that in Western Europe 1.25 million tonnes of fish meal would have been needed to supply 3.5% of the global market. The global supply of fish meal was 6.3 million tonnes (Fishmeal Information Network). Supply of fish meal is static with increasing protein demands from aquaculture of about 9% per annum.

Alternative Protein Sources

The required amount of essential amino acids (EAA's) in the feed vary depending upon the protein content of the feed. The following chart demonstrates this for shrimp:

EAA	Dietary Protein Level (%)							Carcass EAA Pattern (%)
	25	30	35	40	45	50	55	
Arg nine	1.36	1.63	1.90	2.17	2.44	2.71	2.98	15.5
Histidine	0.38	0.46	0.54	0.62	0.69	0.77	0.85	4.4
Isoleucine	0.59	0.71	0.83	0.95	1.07	1.19	1.31	6.8
Leucine	1.22	1.47	1.71	1.96	2.20	2.45	2.69	14.0
Lysine	1.29	1.54	1.80	2.06	2.31	2.57	2.83	14.7
Methionine	0.47	0.57	0.66	0.76	0.85	0.95	1.04	5.4
Phenylalanine	0.67	0.81	0.94	1.08	1.21	1.35	1.48	7.7
Threonine	0.84	1.01	1.18	1.34	1.51	1.68	1.85	9.6
Tryptophan	0.24	0.28	0.33	0.38	0.42	0.47	0.52	2.7
Valine	0.74	0.89	1.04	1.19	1.34	1.49	1.64	8.5

Now compare this to the nutrient composition of a range of commonly used ingredients in shrimp formulations.

Ingredient	Protein (%)	Fat (%)	Phosphorus (%)	Available P (%)	Cholesterol (%)	Phospholipid (%)	Linoleic Acid (%)	Arg (%)	Lys (%)	Met+Cys (%)	Thr (%)
Beer Yeast	42.0	0.5	1.40	0.17	0.00	0.14	0.10	2.20	3.30	1.50	2.40
Blood Meal	90.0	1.0	0.24	0.18				3.59	5.40	1.00	4.00
Corn Gluten Meal	60.0	2.0	0.64	0.42				0.91	1.00	1.90	1.70
Feather Meal	82.0	1.0	0.66	0.40				5.35	1.50	0.50	3.80
Fish Meal, Herring	71.0	10.0	1.67	1.08	0.06	2.00	0.35	3.03	3.94	2.07	2.43
Fish Meal (65%)	65.0	7.5	2.50	0.73	0.28	2.14	0.18	2.48	3.30	1.69	1.83
Full Fat Soy	38.0	19.0	0.62	0.07	0.00	1.20	9.00	2.85	2.48	1.12	1.50
Mussel Meal	70.8	8.2	1.36		0.24	1.70	0.15	6.08	6.23	2.97	3.59
Pea Protein Concentrate	70.0	0.0	0.35	0.19				4.00	5.20	0.90	3.90
Polychaete Meal	62.0	9.0	0.95		1.65	2.50	0.10	2.95	2.19	0.82	1.79
Rice Protein Concentrate	60.0	0.1	0.20	0.10				2.80	1.80	0.40	2.90
Shrimp Head Meal	47.0	12.6	1.80	0.58	0.74	2.03	2.00	2.55	2.30	1.41	1.69
Shrimp Meal, with Shells	47.0	5.0	2.00	0.64	0.64	1.02	0.32	2.26	1.40	0.90	1.4
Soya 49%	49.0	1.0	0.64	0.08	0.00	0.06	0.40	3.70	3.20	1.49	2.00
Soy Protein Concentrate	70.0	0.1	0.84	0.60				4.64	3.92	1.70	2.48
Squid Meal	80.0	10.0	0.90	0.58	0.99	3.39	0.45	4.81	4.94	2.35	2.83
Vital Wheat Gluten	80.0	0.1	0.20	0.11				2.94	1.10	1.60	2.20

The previous information indicates the possible opportunities for blending various ingredients to achieve required nutrient delivery. Economic pressures will steer the formulator away from some of these ingredients for grow-out feeds, but for speciality feeds such as starter feeds and maturation feeds there are performance challenges that will force the use of the more expensive ingredients. The figures for lipid content illustrate the extra challenges faced when using higher levels of ‘land-based’ proteins, the two main issues being the need to supplement the feed with added cholesterol (especially important for hatchery feeds), phospholipids, HUFA’s and some essential amino acids. Performance of the feed for speciality products is also impaired if the feed is subject to high temperature processing. This leads to the added challenge that some of the speciality ingredients should be dried under very gentle conditions then the feed processed at lower temperatures.

Essential Amino Acids, formulation and processing

It is possible to blend some of the 'protein' ingredients in a way that helps to balance the essential amino acids, but this usually doesn't usually supply the required amino acid content of the feed, making it necessary to add amino acids to the formulation. Some fish species and shrimp do not utilise added crystalline amino acids as efficiently as the same amino acids that are organically bound.

Processing can reduce nutritional availability of amino acids by oxidation, for example the conversion of methionine to its sulphoxide or sulphone. Severe processing can also reduce availability of amino acids if they interact with any reducing sugars in the Maillard Reaction, the end point of which produces extremely stable cyclic compounds. This reaction is a multi-stage reaction, the first stage producing a condensation product known as a Schiff's Base. This unstable sugar-amine is still nutritionally available but the reaction quickly cascades, depending on temperature and availability of reducing sugars, to form the highly stable and unavailable cyclic compounds. In many industries the maillard reaction is limited by the addition of chemicals such as sodium metabisulphite (SMS), which prevents the formation of the highly stable cyclic compounds and the browning effect that accompanies this. The reaction is not completely stopped by SMS, the early condensation reactions still occurring. In feed applications this presents an opportunity to partially stabilise the amino acids with respect to speed of absorption in the intestine so that crystalline amino acids hit the target tissues at the same time as amino acids present in proteins from other feed ingredients. This effect can be encouraged by the deliberate addition of reducing sugars to the formulation with the added amino acids. Some feed compounders have used molasses as a carrier for 'micro' ingredients and this system can be used to facilitate the controlled maillard reaction, but the molasses system can be very difficult to handle if too much crystalline material is suspended in the mix. It may be more convenient to blend the powdered micro ingredients separately, even adding the reducing sugar as a powdered ingredient.

Anti-Nutritional Factors

Another issue to be considered when adding 'land based' proteins is the presence of compounds that can impair growth or cause physiological abnormalities. Even fishmeal can contain levels of histamine, nitrosamine, putrescine and oxidised lipids that are deleterious to palatability, growth and health, depending on the handling of the fish prior to and during processing of the fishmeal. The 'land based' protein sources present a range of other challenges, though.

Phytic Acid is a compound that reduces the intestinal absorption of divalent cations, specifically calcium, magnesium, zinc and iron. Phytic acid may exist as the acid, or more commonly its salt, known as phytate, and is the principle source of phosphate from plant sources. It is highly stable and in animals is normally only broken down by micro-organisms in the rumen. Even in human beings a brown bread diet with no dietary supplementation of calcium has produced rickets. This is why bread in many countries is still supplemented with calcium, usually added in the form of powdered chalk. The phytate can pass through the intestine of aquatic species, carrying with it in chelated form the metals named previously. The double problem thus caused is nutritional deficiency of the metals and increased excretion of phosphorus into the water. The addition of the enzyme phytase to the feed has been shown to greatly improve growth rates in many different species. There is a practical issue here, in that phytase is rendered inactive by thermal processing. For feeds made on pellet mills the enzyme can be added in powdered form and so needs to be added to the feed pellets after drying. For extruded pellets the phytase is added in liquid form in the pellet coating system. This can then partially leach into the water when feeding, as can other heat sensitive ingredients such as astaxanthin in salmonid feeds. Enzymes need carefully controlled conditions of pH, temperature, time and mixing to guarantee performance. It is far better, if possible, to pre-treat key ingredients in a warm wet mixing system with the enzyme before manufacture of the finished feed. Note that enzymes are proteins that denature under conditions of high heat and shear, which is why phytase needs to be added as a topical coating after the pellet dryer.

Anti-nutritional factors occur in abundance in some of the alternate protein sources, particularly legumes. These include trypsin inhibitors, lectins, and haemagglutenins. Many of these can be partially or fully inactivated by using severe heat and shear during processing of the individual ingredient. Processing to concentrate the proteins significantly reduces these factors, and also reduces the oligossacharide content.

Handling Problems

Most of the ‘land based’ protein ingredients are manufactured as fine powders. This creates handling and storage problems for the feed miller, as listed below:

- Dust emission creating losses and environmental/health & safety problems

- Reduced conveying capacities in bucket elevators and mechanical conveyors

- Hygroscopic characteristics create clumping, easy wetting, mould growth

- Low bulk densities reduce storage capacities and reduce carrying capacity of haulage

- Fine dust increases explosion hazards

- Can easily fluidise, creating losses, process control problems and waste

- Powders need to normally be handled from trucks with air conveying systems, which have high capital and energy costs

- If fine powders are mixed and conveyed with coarser materials there can be problems with segregation of ingredients within the mix

If it is possible to buy these ingredients as pelleted products this greatly reduces the handling problems, as long as the pelleting process does not increase cost of ingredients too much.

Lipids

Four fatty acids are considered essential for shrimp; listed below:

Linoleic acid - 18:2 (9,12)
Linolenic acid - (18:2 (9,12,15)
Eicosapentaenoic acid - 20:5 (5,8,11,14,17) – (EPA)
Decosahexaenoic acid - 22:6 (4,7,1013,16,19) – (DHA)

Vegetable Oils are high in linolenic and linolenic acid, whereas marine oils are high in eicosapentaenoic acid and decosahexaenoic acid.

Phospholipids containing choline (phosphatidylcholine) and inositol (phosphatidylinositol) and cholesterol are also considered essential for growth and survival in shrimp. Cholesterol requirement has been proposed at a level of 0.2-2.0% of the diet.

Cholesterol content of various materials

Ingredient	Cholesterol (% of fat)	Ingredient	Cholesterol (% of fat)
Squid Mantle	18.0	Pollack Meal	7.1
Squid Viscera	3.0	Cod Meal	6.2
Shrimp, Whole	9.9	Tuna Meal	1.4
Shrimp, Head	10.0	Herring Meal	0.6
Crab	7.8	Mackerel Meal	0.6
Clam	5.2	Cod Liver Oil	0.6
Mussel	2.9	Menhaden Oil	0.5
Squid	9.9	Salmon Oil	0.5
Herring Oil	0.8	Polychaete worm meal	18.0

The above figures can be used to calculate required admix levels of the ingredients to achieve the required levels of cholesterol in the diet. It can be seen that very high levels need to be added to achieve cholesterol levels of 2% of the diet, and explains why some of the above ingredients are preferred in nutrient critical diets such as maturation feeds and starter feeds. It is now possible to use purified forms of cholesterol as single ingredients, which gives much more freedom in formulation development.

In a similar way it is interesting to study the phospholipids content of various ingredients:

Phospholipid content of various materials

Ingredient	Phospholipid %
Clam Meal	1.27
Egg Powder	2.14
Fish Meal	2.47

Krill Meal	2.03
Mussel Meal	1.70
Oyster Meal	16.26
Shark Liver Oil	2.50
Shrimp Head Meal	3.82
Shrimp Meal	1.02
Squid Liver Meal	6.36
Squid Liver Oil	1.53
Squid Meal	3.39
Squid Oil	0.76
Tuna Meal	0.50
Yeast, Torula	1.27
Polychaete Worm Meal	2.50
Soy Lecithin	48.00

It can be seen from the above list why soy lecithin is widely used as an ingredient in shrimp mariculture. Note that soy lecithin is not just a pure source of Phosphatidyl choline. It contains a complex mix of lipids, and supplier specification should be studied. A typical liquid soy lecithin will contain 14.5% Phosphatidyl choline and 8.8% phosphatidylinositol, with 69% fat of which 54.8% is linoleic acid and 6.7% linolenic acid.

From a practical perspective it can be seen that the lipid mix added to the process system can be quite complex. The mix can contain marine oils, vegetable oils, soy lecithin, which is quite gummy and difficult to handle, and cholesterol, which is equally difficult to handle. Process design can ensure these products are homogenously mixed, but normally the mixture will need to be held at elevated temperatures of about 40 degrees Celcius. This is to ensure that the cholesterol and any higher melting point lipids are in a liquid and low viscosity state. Care has to be taken about the dangers of oxidation, so mixing and day tanks need to be sized carefully so that time held at the elevated temperatures is minimised, along with ensuring that an appropriate anti-oxidant system is used. Polyunsaturated oils oxidise very rapidly on exposure to the air. It possible to buy specialised oil products that are packaged with an inert gas atmosphere that have extended shelf life at ambient temperature. These must be used very quickly after opening the package, or stored in a system with an inert (nitrogen flushed) atmosphere. Oxidative deterioration will be accelerated by high process temperatures, so products containing high levels of HUFA's should be processed on short-time systems with minimised temperatures, then packaged in nitrogen flushed bags that are impermeable to oxygen, water and ultra-violet light.

Pigments

Crustaceans do not synthesise carotenoid pigments de novo thus these must be supplied in the diet. These pigments are have a potent anti-oxidant effect and have been shown to protect developing offspring against free radical attack, improving survival and health. The pigments act as precursors of Vitamin A, which plays an important role in lipid metabolism and is particularly critical in broodstock feeds. It has been demonstrated that, in shrimp, there was a decrease in nauplius quality in successive hatchings associated with a loss of pigmentation in the ovary of L.

vannamei. Dietary supplementation with paprika at low levels has been shown to result in significant improvement in nauplius quality.

Astaxanthin is widely used in salmonid culture to colour the flesh. This pigment also has strong antioxidant properties, which makes it beneficial as a free radical scavenger. The only problem is that a chemical that is strongly anti-oxidant oxidises very easily. Astaxanthin used to be added to feeds before processing resulting in large losses in the process. Practice now is to add the pigment in oil suspension in the coating system, after the extruder and dryer. This creates a potential problem with leaching of the pigment during storage (in high oil feeds) and during feeding. This may not be particularly significant for farmed salmonids, where the pellets are eaten very quickly. Astaxanthin may be extracted from ‘natural’ materials or chemically manufactured, in either case the ingredient being very expensive. There are now commercial sources of powders made from Phaffia yeast and algae that are used as sources of carotenoid pigments in feed products. Seaweeds also contain significant quantities of these pigments, and the use of selected powdered seaweeds in the diet gives potential advantages in terms of pigment delivery and antimicrobial effects.

Halogenated Compounds and Seaweed

The natural ‘marine’ flavour of wild caught shrimp and fish is said to come from the diet of these creatures, which is rich in bromo-phenols. It has been shown that polychaetes are rich in these compounds and that when polychaetes are part of the diet there is a very positive ‘marine’ flavour development.

Ocean fish eat a range of algae, sea mosses, sandworms and sea salt, all of which are rich sources of bromophenol. Red seaweed is known to synthesis halogenated substances containing bromine and iodine. Green seaweeds and brown seaweeds produce a range of halogenated compounds which impart a range of different flavours. These compounds in part play a role in protecting the seaweeds from herbivorous fish and crustaceans and in part they have potent anti-microbial effects. It is well known that the addition of small levels of dried seaweed such as Ascophyllum nodosum to a feed formulation has a potent antimicrobial effect. In vitro studies with isolated bromophenols have shown them to have a potent antibacterial effect and a cytotoxic effect on cancer cells

These natural, sustainable and readily available materials present a strong case for inclusion in diets, with the added benefit that a ‘wild caught’ marine flavour profile can be developed. It has been shown that the flesh of wild caught saltwater fish contains levels of 6-34 ng/g of total bromophenols whereas the flesh of freshwater fish contains virtually none and the flesh of cultivated atlantic salmon contains less than 2 ng/g. For shrimp it has been shown that total bromophenols content of wild caught shrimp contained up to 1100 ng/g whereas cultivated shrimp contained <1 ng/g. It has even been shown that freshwater species develop a ‘marine’ taste profile when given feeds high in bromophenols. If the type of seaweed used is controlled it is possible to create main line feeds have beneficial anti-microbial properties and finishing feeds to develop final flesh flavour and texture. This gives multiple potential benefits in reduction of ‘chemical’ antimicrobial treatments, claims for natural and sustainable diets, reduced mortalities and improved profitability and the creation of high value products that are not bland in flavour, (although for some markets bland is a requirement as the finished product is simply a vehicle for a sauce flavouring).

Flavonoids

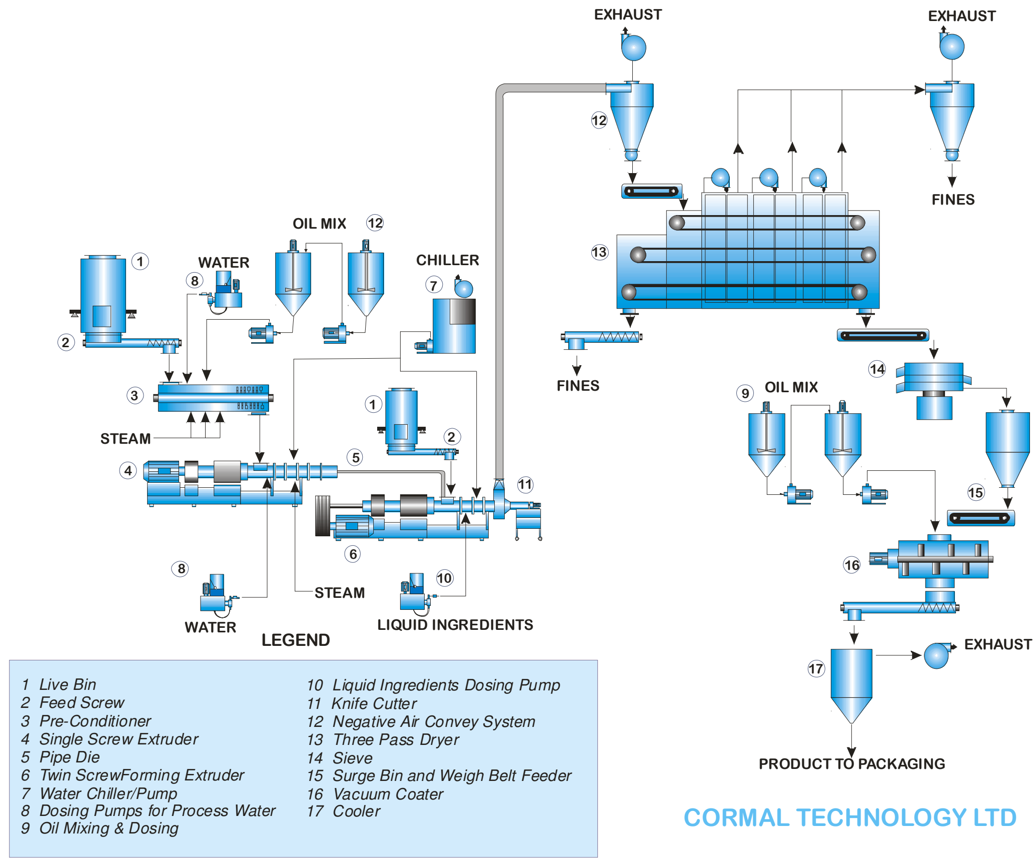
Not many farmed animals would naturally eat citrus fruits, but in recent years citrus flavonoids have found a place in land based agriculture and increasingly in aquaculture. Research has shown suppression of mast cell activity and associated tissue inflammation. On a wider front flavonoids in mammals have been shown to exert anti-bacterial and anti-viral activity, anti-inflammatory, anti- angionic, analgesic, anti-allergic effects etc. The compounds that interact with flavonoids include cytochtome p450, monooxygenases and steroids. In vitro studies have demonstrated suppression of microbial growth at relatively low concentrations. Soy Beans are an important source of flavonoids and claims are made in human nutrition that they have the ability to reduce the incidence of hormone-dependant breast and prostate cancers. In intensive aquaculture the anti-microbial and anti-inflammatory properties of these compounds can be very beneficial. Commercial products containing concentrated flavonoids are made by drying the rind and seeds of citrus fruits, the best sources being those companies that control supply chain from farm to finished products. Citrus flavonoids have a bitter flavour, being part of the natural defence mechanism of the citrus fruits. At the levels normally used in feeds palatability is not normally detrimentally affected, but addition of small levels of naturally extracted masking agents (strong sweeteners) can counteract this effect.

Low Temperature Processing to Protect Heat Sensitive Nutrients

The addition of heat sensitive ingredients and prevention of loss during processing, storage and feeding is an issue. Traditional extrusion cooking systems have a single extruder that cooks the majority of the ingredients that are then dried as pellets, following which some liquid ingredients are added in the coating system. Even with vacuum coating there are losses. It would be far better to firmly bind these ingredients in the matrix of the pellets so that distribution of the ingredients within the pellets is more even and leaching losses are minimised. The diagram below shows an extrusion system with two extruders. The first is a cooking extruder that only adds heat and shear to those ingredients that need this type of processing. The cooked ingredients are then passed into the second forming machine, which blends various streams together and forms the feed pellets, all at reduced temperatures. If the dryer is well balanced after this second machine heat damage is minimised. This technology has the added advantage of reduced total energy costs.

The manufacturer using this process can concentrate on hydrating and efficiently cooking starch in the first machine. It was stated earlier that ‘land based’ proteins have high avidity for water. This two pass system can separate these ingredients so that the starch doesn’t have to compete for available water. The fact that most of the energy is now being applied to a small proportion of the total feed also results in significant energy savings.

PROCESS DIAGRAM FOR THE PRODUCTION OF ‘LOTEMP’ EXTRUDED AQUATIC FEED



It is important to follow this with a drying process that is sophisticated enough to avoid further heat damage to the product. Avoiding overheating of the pellet is important within the conveyor drier, since heated air is used to evaporate the moisture in the pellet. A properly designed drier will therefore allow for the use of elevated air temperature in the early stages of drying, while evaporative cooling effectively cools the product temperature. As the pellet dries, temperature gradients are used to optimize the product temperature to the drying curve, while also balancing for the highest energy efficiency.

Conclusion

Aquaculture is an old industry but intensive aquaculture is very new. Many lessons have been learned over the last 50 years as this industry has grown into a globally significant sector. There are ingredient and process challenges as we face the onward pressure to nurture the seas and the soil to provide ourselves with sustainable resources to support the industry. At the same time we need to approach processing issues in an imaginative way to optimise process efficiencies and product delivery.

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