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ALTERNATIVE DIETARY PROTEIN AND LIPID SOURCES FOR SUSTAINABLE PRODUCTION OF SALMONIDS

By (in alphabetical order):
Dr. Ronald W. Hardy, Dr. Dave A. Higgs,
Dr. Santosh P. Lall, Dr. Albert G.J. Tacon



HAVFORSKNINGSINSTITUT
INSTITUTE OF MARINE RESEARCH

Forord

Dersom verdens havbruksproduksjon av fisk og reker fortsetter å vokse vil vi innen få år få mangel på marine oljer og noe lengre fram ti tid også få mangel på fiskemel av god kvalitet.

Norges forskningsråd har utarbeidet rapporten ”Fôr og fôrmidler – den største utfordringen for norsk havbruk”(Waagbø, Torrissen og Austreng, 2001).

Fôrmidler til norsk havbruksnæring er internasjonale handelsvarer der norsk havbruksnæring konkurrerer med annen havbruks- og dyreproduksjon. Havforskningsinstituttet ønsket å få en internasjonal vinkling på problemet med å skaffe nok fôrråstoff til verdens havbruksproduksjon som et supplement til Forskningsrådets rapport. Vi ba derfor Dr. Ronald W. Hardy, Dr. Dave A. Higgs, Dr. Santosh P. Lall og Dr. Albert G.J. Tacon om å utarbeide en analyse over behov og tilgang på fôrråstoff for verdens havbruksproduksjon, og også angi mulige alternative, framtidige, fôrråstoff.

Rapporten er i sin helhet utarbeidet av forfatterne, og Havforskningsinstituttet er ikke nødvendigvis enige i alle analyser og konklusjoner. Vi mener imidlertid at rapporten gir et viktig innspill i debatten om hvor vi skal finne nye fôrråstoff. Nye fôrråstoff som all havbruksproduksjon av karnivore og omnivore dyr vil være totalt avhengige av dersom vi fortsatt ønsker vekst..

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12 sept. 2001

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EXECUTIVE SUMMARY

The predicted growth of the salmon farming industry will place increasing demands on global supplies of fishmeal and fish oil, the main constituents of salmon feeds. Alternative sources of protein and lipid exist, but many questions remain to be resolved concerning their acceptable dietary levels for culturing Atlantic salmon. In this review, we consider sustainable conventional sources of protein and lipid as well as those that may be developed in the future. Basic information is needed on dietary essential amino acid and fatty acid requirements of Atlantic salmon, as well as information on protein, amino acid and lipid (fatty acid) digestibility, suitable economical processing methods to enhance the protein concentration and/or reduce the presence of antinutritional factors in some ingredients, and development of feed formulations that maintain economical growth and health of the fish, and also maintain product quality for the consumer.

CONCLUSIONS AND RECOMMENDATIONS

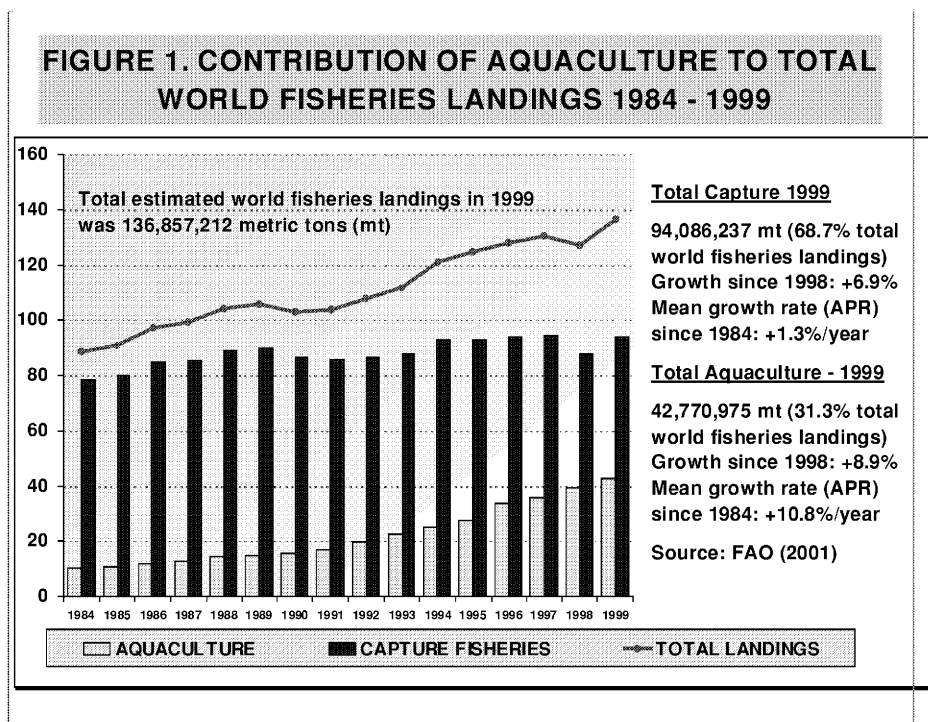
- Predicted supplies of fishmeal and fish oil will be inadequate to meet aquafeed demand
- Prices of fishmeal and fish oil will increase as demand rises
- Supplies of alternative proteins from plant and rendered product sources are adequate to meet the future protein and energy needs for aquafeed production
- Plant proteins will occupy a larger proportion of salmon feeds, as will poultry by-product meals
- The dietary requirements for essential amino acids must be determined for Atlantic salmon
- The digestibility of amino acids in alternate proteins must be determined for Atlantic salmon
- Detailed information on the effects of antinutritional factors in plant proteins and technologies to overcome or inactivate them must be developed.
- Predicted future prices of plant and rendered lipid sources will be less than that of fish oil
- Use of plant and rendered lipid sources in aquafeeds will change the fatty acid composition of salmon flesh
- Research is needed to determine acceptable dietary levels of alternative lipid sources in salmon and other aquafeeds
- Phase-feeding strategies must be developed to ensure that consumer perceptions of the quality of salmon products are maintained
- Issues of food safety are paramount, and before there is introduction of new protein and lipid sources (e.g. genetically modified protein sources or oils) into salmon aquafeeds, thorough risk assessments and transparency of information with respect to the ingredients that are used are required

INTRODUCTION

The new millennium heralds the beginning of a new chapter in aquaculture development. The ever increasing demands of the world's major agricultural food production systems upon a finite quantity of natural resources (i.e. water, nutrients, energy, land) necessitate that farming systems become increasingly more efficient in terms of resource use and have little or no adverse impacts upon society and the environment.

GLOBAL AQUACULTURE PRODUCTION

Total aquaculture production in 1999 (the most recent year for which complete statistical information exists) was reported as 42.8 million metric tons (mmt) and valued at US \$ 53.6 billion; the sector growing at an average percent rate (APR) of 10.8% per year since 1984 compared with an APR of 1.3% per year for capture fisheries (Figure 1). At a species group level, finfish contributed over half of total aquaculture production by weight in 1999 (50.2%), followed by molluscs (23.7%), aquatic plants (22.1%) and crustaceans (3.7%).

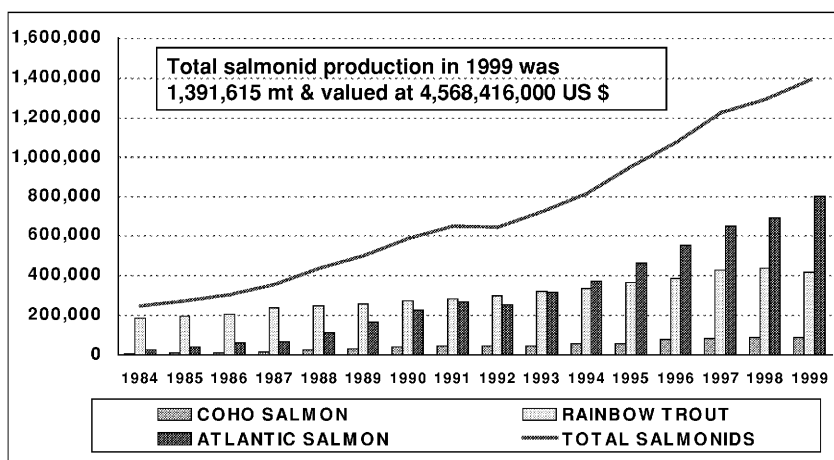


SALMONID AQUACULTURE PRODUCTION

Salmonids represented the third largest aquaculture species group by value in 1999 after freshwater cyprinids and marine shrimp; total salmonid aquaculture production in 1999 amounting to 1,391,615 mt and valued at US \$ 4,568,416,000 (Figure 2). By species the major cultivated salmonid species in 1999 were as follows;

- Atlantic salmon: 797,560 mt, growth since 1998 +15.9%, APR +27.2%/year since 84
- Rainbow trout: 418,654 mt, growth since 1998 -4.2%, APR +6.0%/year since 84;
- Coho salmon: 89,575 mt, growth since 1998 +1.4%, APR +20.7%/year since 84;
- Total salmonids: 1,391,615 mt, growth since 1998 +7.9%, APR +13.1%/year since 84

FIGURE 2. SALMONID AQUACULTURE PRODUCTION 1984-1999
(Total production and main species)



Growth (expressed as % increase since 1998 and APR for 1984-1999): Atlantic salmon 27,404 to 797,560 mt, 15.9% & 27.2%; Rainbow trout 186,167 to 418,654 mt, -4.2% & 6.0%; Coho salmon 6,412 to 89,575 mt, 1.4% & 20.7%; total salmonids 246,894 to 1,391,615 mt, 7.9% & 13.1% (FAO, 2001)

COMPOUND AQUAFEED PRODUCTION

The total production of industrially compounded complete aquatic feeds or 'aquafeeds' was estimated to be about 13.4 mmt in 1999 (Table 1). By far the largest

Table 1. Estimated global aquafeed production and fish meal- fish oil usage in 1999 and projected growth and demand for the year 2000 & 2010

<u>TOTAL GLOBAL ESTIMATES</u>	<u>1999</u>	<u>2000</u>	<u>2010</u>
Total production of major feeding species (mt) <i>IFOMA predicted total finfish/crustacean production (mt)</i>	14,790,069	19,244,000	36,937,000
Total estimated aquafeeds production (mt) <i>IFOMA predicted aquafeed produced (mt)</i>	13,372,583	13,630,000	32,613,000
Total estimated fishmeal used (mt) <i>IFOMA predicted fishmeal used (mt)</i>	2,216,558	2,316,000	3,450,000
Total estimated fish oil used (mt) <i>IFOMA predicted fish oil used (mt)</i>	620,333	716,000	1,209,000

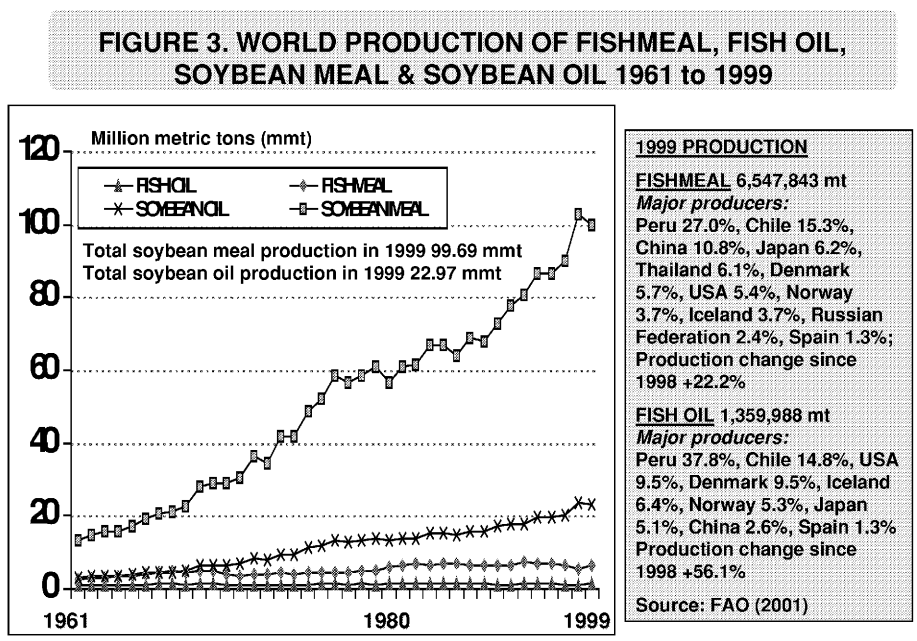
consumers of industrially compounded aquafeeds in 1999 were non-filter feeding carp species (6.68 mmt or 49.8% of total aquafeed production), followed by marine shrimp (1.81 mmt or 13.5%), salmonids (1.79 mmt or 13.3%), marine finfish (0.98 mmt or 7.3%), tilapia (0.86 mmt or 6.4%), catfish (0.65 mmt or 4.8%), eel (0.32 mmt or 2.4%) and milkfish (0.29 mmt or 2.2%).

Conservative projections for global compound aquafeed production for the year 2000 indicate that production could reach 15 mmt by the year 2000, and about 20 and 25 mmt by 2005 and 2010, respectively (these estimates have been based on the growth assumptions for the individual sectors). These aquafeed projections compare favorably with that made by the International Fishmeal and Fish Oil Manufacturers Association (IFOMA, 2000), who estimated global aquafeed production as increasing from 13.63 mmt in 2000, to 32.613 mmt by 2010, and to 37.561 mmt by 2015 (Table 1).

GLOBAL CHALLENGES TO FEED INGREDIENT SUPPLY

For the past two decades the production of compound aquafeeds, and in particular aquafeeds for carnivorous finfish species and marine shrimp, has been dependent upon the use of fishmeal and fish oil as the major sources of dietary protein and lipid, respectively. These two key ingredient items, together with other

fishery by-product meals usually represent from 25 to 80% of the total aquafeeds for marine shrimp and carnivorous finfish species, including salmonids. For example, Table 1 shows the estimated and predicted utilization of fishmeal and fish oil within compound aquafeeds in 1999 and beyond, for the major species groups. From the data presented, it can be seen that compound aquafeeds consumed an estimated 2,216,558 mt of fishmeal and 620,333 mt of fish oil in 1999, or the equivalent of about 33.8% and 45.6% of reported global production of fishmeal and fish oil in 1999; total fishmeal and fish oil production in 1999 reported as 6,547,843 mt and



1,359,988 mt, respectively (Figure 3).

On the basis of fishmeal consumption alone, and assuming an average conversion ratio for processing pelagics to fishmeal of 5:1, at present all compound aquafeed-fed farming operations for carnivorous diadromous finfish, marine finfish and crustaceans are net consumers of fish rather than net producers. By contrast, the majority of freshwater finfish species are net fish producers, with carps being the most efficient, followed by milkfish, tilapia, and catfish. This is considered in more detail as follows:

Eel (1999): Total farmed eel production 227,704 mt (wet basis), estimated fishmeal use 159,393 mt (dry basis), estimated fish oil use 19,127 mt (dry basis), estimated equivalent use of pelagics 796,965 mt (fishmeal use x 5; wet basis), apparent pelagics: eel conversion efficiency **3.50**. The predicted conversion efficiency for the year 2000 is **4.00**;

Salmon (1999): Total farmed salmon production 901,843 mt (wet weight basis), estimated fishmeal use 492,406 mt (dry basis), estimated fish oil use 257,927 mt (dry basis), estimated equivalent use of pelagics 2,462,030 mt (fishmeal use x 5; wet basis), apparent pelagics: salmon conversion efficiency **2.73**. Predicted conversion efficiencies for the year 2000 range from **2.60** and **2.80**;

Marine finfish (1999): Total farmed marine finfish production 845,275 mt (wet basis), estimated fishmeal use 451,038 mt (dry basis), estimated fish oil use 117,662 mt (dry basis), estimated equivalent use of pelagics 2,255,190 mt (fishmeal use x 5; wet basis), apparent pelagics: marine finfish conversion efficiency **2.67**. Predicted conversion efficiencies for the year 2000 vary between **2.64** and **3.30**;

Trout (1999): Total farmed trout production 474,978 mt (wet basis), estimated fishmeal use 172,892 mt (dry basis), estimated fish oil use 98,795 mt (dry basis), estimated equivalent use of pelagics 864,460 mt (fishmeal use x 5; wet basis), apparent pelagics: trout conversion efficiency **1.82**. Predicted conversion efficiencies for the year 2000 range from **1.56** and **2.10**;

Marine shrimp (1999): Total farmed marine shrimp production 1,130,737 mt (wet basis), estimated fishmeal use 470,386 mt (dry basis), estimated fish oil use 36,184 mt (dry basis), estimated equivalent use of pelagics 2,351,930 mt (fishmeal use x 5; wet basis), apparent pelagics: marine shrimp conversion efficiency **2.08**. Predicted conversion efficiencies for the year 2000 vary between **1.80** and **1.97**;

Catfish (1999): Total farmed catfish production 455,002 mt (wet basis), estimated fishmeal use 38,985 mt (dry basis), estimated fish oil use 6,497 mt (dry basis),

estimated equivalent use of pelagics 194,925 mt (fishmeal use x 5; wet basis), apparent pelagics: catfish conversion efficiency **0.43**. Predicted conversion efficiencies for the year 2000 vary between **0.12** and **0.39**;

Milkfish (1999): Total farmed milkfish production 381,930 mt (wet basis), estimated fishmeal use 29,027 mt (dry basis), estimated fish oil use 8,798 mt (dry basis), estimated equivalent use of pelagics 145,135 mt (fishmeal use x 5; wet basis), apparent pelagics: milkfish conversion efficiency **0.38**. Predicted conversion efficiencies for the year 2000 range from **0.40** to **0.47**;

Tilapia (1999): Total farmed tilapia production 1,099,175 mt (wet basis), estimated fishmeal use 68,588 mt (dry basis), estimated fish oil use 8,574 mt (dry basis), estimated equivalent use of pelagics 342,940 mt (fishmeal use x 5; wet basis), apparent pelagics: eel conversion efficiency **0.31**. Predicted conversion efficiencies for the year 2000 range from **0.25** to **0.28**;

Carp (1999): Total farmed carp production 9,273,425 mt (wet basis), estimated fishmeal use 333,843 mt (dry basis), estimated fish oil use 66,769 mt (dry basis), estimated equivalent use of pelagics 1,669,215 mt (fishmeal use x 5; wet basis), apparent pelagics: carp conversion efficiency **0.18**. Predicted conversion efficiencies for the year 2000 vary between **0.12** and **0.15**;

Global (1999): Total farmed major fish and shrimp feeding species 14,790,069 mt (wet basis), estimated fishmeal use 2,216,558 mt (dry basis), estimated fish oil use 620,333 mt (dry basis), estimated equivalent use of pelagics 11,082,790 mt (fishmeal use x 5; wet basis), apparent pelagics: feeding fish/shrimp conversion efficiency **0.75**. Predicted conversion efficiencies for the year 2000 vary between **0.60** and **0.69**.

Despite the generally optimistic projections concerning the future availability and use of fishmeal and fish oil in aquafeeds (Pike, 2000, Tacon and Forster, 2000; Table 1), there are real concerns about the current dependence of intensive farming systems for high value species (i.e. salmonids, eels, marine finfish, and shrimp)

upon fishmeal and fish oil (Anon, 2000; Hardy, 2000; Tacon, 1998). Apart from the uncertain market availability and cost of these finite and valuable aquatic resources, there are also growing social and environmental concerns regarding the long term sustainability and ethics of catching and processing low-value (in marketing terms) potentially food-grade fishery resources (in the case of whole processed pelagic fish species such as anchovy, sardine and mackerel) and feeding them back to high-value farmed aquatic species (Naylor *et al.*, 2000), rather than using them directly as an affordable source of much needed high quality animal protein and essential nutrients for human consumption; malnutrition currently being the number one killer and cause of ill health on Earth (Tacon and Barg 2001).

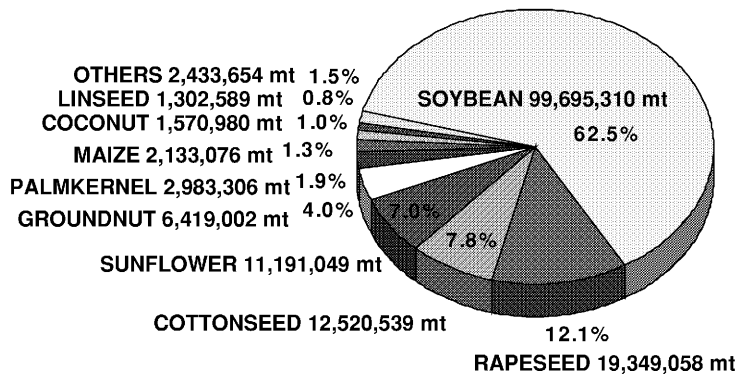
Figure 3 shows the modest global production of fishmeal and fish oil compared with that of soybean meal and soybean oil over the period 1961 to 1999. From the data presented, it can be seen that the global production of soybean meal and soybean oil has grown by a healthy 678% and 685% by weight since 1961, with production increasing from 13.19 to 102.62 mmt (soybean meal) and 2.99 to 23.46 mmt (soybean oil) from 1961 to 1999 at an average compound growth rate of 5.9% per year. By contrast, fishmeal and fish oil production have grown by 160.9% and 29.5% since 1961, with production increasing from 2.51 to 6.55 mmt (fishmeal) and from 1.05 to 1.36 mmt (fish oil) from 1961 to 1999, at a modest rate of 2.6% and 0.7% per year, respectively. In 1999, the global production of plant oilseed meals and cakes, plant pulses, and plant oils and fats in 1999 was estimated to be 163.75 mmt (Figure 4), 59.27 mmt (Figure 5), and 102.23 mmt (Figure 6), respectively.

According to IFOMA (2000), fishmeal and fish oil use within aquafeeds is expected to increase from 2.32 mmt and 0.72 mmt in 2000, to 3.45 mmt and 1.21 mmt by 2010, to 3.70 mmt and 1.26 mmt by 2015, respectively (Table 1). Whilst in the short term efforts should be focused on the use of non-food grade fishery by-products (i.e. fishery by-catch and discards, and fishmeals produced from fish processing plants and industrial non-food fishes), clearly in the long-term, efforts must also be placed on the utilization of by-products arising from the much larger and faster growing terrestrial agricultural production sector, including rendered

animal by-products, grains, oilseeds, and single-cell proteins (Hardy, 2000; Tacon, 1998) to provide proteins and oils for use in aquafeeds.

**FIGURE 4. GLOBAL PRODUCTION OF PLANT OILSEED
CAKES & MEALS IN 1999**

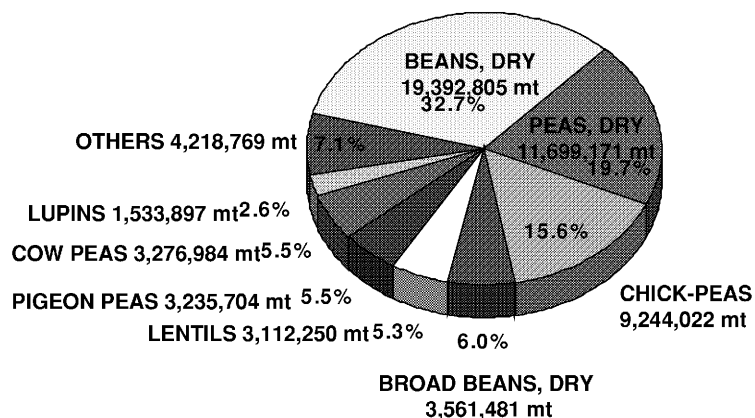
(Source: FAOSTAT Agriculture Database, April 2000)



Total production of plant oil cake & meal in 1999 – 163,749,367 mt

FIGURE 5. GLOBAL PRODUCTION OF PLANT PULSES IN 1999

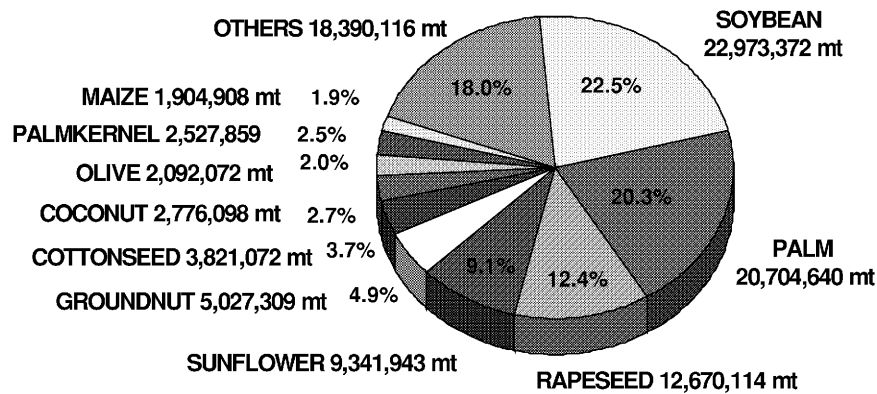
(Source: FAOSTAT Agriculture Database, April 2000)



Total production of plant pulses in 1999 - 59,275,083 mt

FIGURE 6. GLOBAL PRODUCTION OF PLANT OILS & FATS IN 1999

(Source: FAOSTAT Agriculture Database, April 2000)



Total production of vegetable oils and fats in 1999 – 102,229,503 mt

FISH MEAL AND ALTERNATIVE PROTEIN SOURCES

INTRODUCTION

Feed accounts for 35% - 60% of the cost of salmon farming (Forster, 1995; Higgs *et al.*, 1995a). Therefore, feed by far represents the largest operational expense. The protein sources, in turn, presently account for 51% of the cost of high energy extruded grower diets (40% protein and 30-35% lipid) for Atlantic salmon. Much of the high cost of the protein fraction is due to extensive use of mainly South American premium quality fish meals (may supply $\geq 90\%$ of the dietary protein) to meet the dietary protein requirements of salmon in seawater. This cost is not only generally high but is forecast to rise in the future to prices at or above those observed during major El Niño events (e.g. refer to 1998 in Figure 7 below). The other major variables that contribute to the diet cost include marine fish oil (25%) and astaxanthin (20%, when the dosage is 50 ppm), a carotenoid pigment, whereas

the costs of the binders (4%) and vitamin and mineral supplements (3%) are low by comparison (Higgs, 1997).

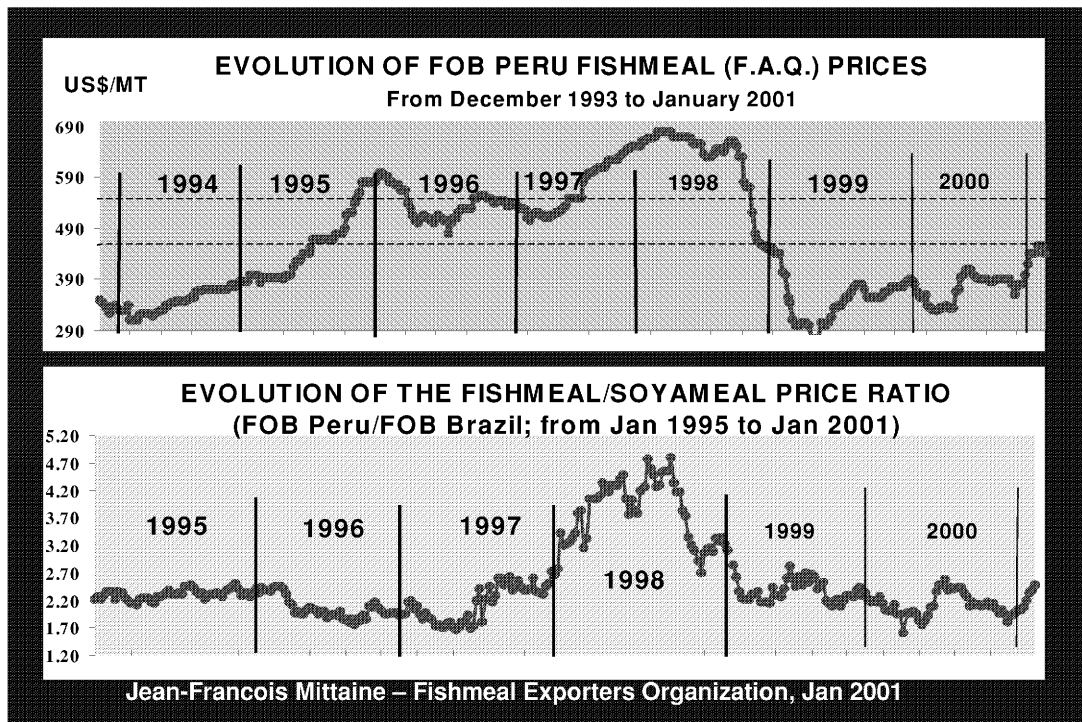


Figure 7. Temporal variations in the prices of Peruvian fishmeal and of the price ratios of fishmeal to soybean meal

Protein provides amino acids, the building blocks of tissue proteins, plus protein is catabolized for metabolic energy. Salmon are piscivores, meaning that they are carnivorous fish that mainly consume other fish in the wild. Thus, it is unlikely that feeds for salmon will ever contain less than 38% crude protein, no matter what advances in feed technology, ingredient selection, or genetic improvement may occur.

Proteins contain about 20 amino acids, of which 10 are essential dietary constituents. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Non-essential amino acids must also be provided in the diet for optimum protein retention.

PROTEIN SOURCES FOR AQUAFEEDS

Many feed ingredients are commonly used to supply protein in fish feeds (reviewed by Li *et al.*, 2000). In recent years several reviews have been also published on the nutritional value of fishmeal (Pike *et al.*, 1990), soybean products (Storebakken *et al.*, 2000) and rapeseed/canola protein products (Higgs *et al.*, 1995a; Higgs *et al.*, 1996) for salmonid fishes.

Animal Proteins

Marine Products or By-Products

Fish Meal

Fishmeal is prepared from dried, ground tissues of whole marine fish, such as menhaden, anchovy, and capelin, or from fish-processing waste. Fishmeal contains 55 to 75% protein, depending on the species of fish used. Fishmeal protein is of excellent quality, both in terms of amino acid profile and apparent digestibility, and is highly palatable to most fish. It contains 5 to 10% oil, making it rich in energy and essential fatty acids, and it also contains bones and other sources of essential minerals. Due to their high ash content, fish meals made from fish-processing waste and residues of canning plants are of lower quality than fishmeals prepared from whole fish. High levels of fishmeal are used in starter diets for most cultured fish and in growout diets for carnivorous species, such as eels, salmon, and trout.

Fish Solubles

Condensed or Dried condensed fish solubles, which contain a minimum of 30% crude protein, are semisolid (50% solids) by-products obtained by evaporating water from "press water" produced during the processing of cooked fish in the manufacture of fishmeal. Dried fish solubles are composed of the same material as condensed fish solubles, only then are dried to powder, and contain about 60% crude protein. Fish solubles are a highly palatable protein feedstuff for use in fish diets.

Shrimp Meal and Crab Meal

Shrimp meal is produced from the waste of shrimp processing and includes the head, shell, and/or whole shrimp. The exoskeleton is primary chitin and has limited nutritional value. Chitin may account for 10 to 15% of the total nitrogen in the meal. Shrimp meal contains approximately 32% protein and 18% ash and is a good source of n-3 fatty acids, cholesterol (essential for crustaceans), and astaxanthin. Crab meal is the by-product of the crab-processing industry and includes the shell, viscera, and flesh. It contains about 30% crude protein and 31% ash. Its high ash content limits its use in fish diets.

Fish Silage

Fish silage is prepared by grinding whole fish or fish-processing waste and then adding an acid, usually formic acid or a combination of sulfuric acid and formic acid, to prevent microbial spoilage. Well-prepared fish silage can be stored for years without spoilage. Good quality silage made from fresh fish contains about 18% crude protein and 74% moisture. Because of their high content of free amino acids and short-chain peptides, which are absorbed and metabolized too quickly following a meal, fish silages do not appear to be as effective as whole-fish meals.

Rendered By-Products

Poultry By-Product Meal

Poultry by-product meal is made of ground, rendered, or clean parts of the carcass of slaughtered poultry. It contains heads, feet, underdeveloped eggs, and visceral organs, but does not contain feathers. The product contains approximately 58% crude protein and 16% ash.

Poultry Feather Meal

Hydrolyzed feather meal is prepared by the high-pressure treatment of clean, un-decomposed feathers from slaughtered poultry. At least 75% of the protein should be digestible, as measured by pepsin digestion. It is high in protein (85%), but the quality of the protein is not as good as that of other animal protein feedstuffs.

Meat and Bone Meal

Meat and bone meal is the rendered product from beef or pork tissues and should not contain blood, hair, hoof, horn, hide trimmings, manure, or stomach and rumen contents, except in amounts as may be unavoidable during processing. Meat and bone meal contains approximately 45 to 50% crude protein, the quality of which is inferior to that of whole-fish meal, because meat and bone meal contains less lysine. In addition, protein quality may vary considerably among products. Meat and bone meal is a good source of minerals, but high ash content limits its use in fish diets, because of the possibility that a mineral imbalance may occur in the diet and because its phosphorus content is high, making it difficult to include in diets designed to have limited environmental impact.

Meat Meal

Meat meal is similar to meat and bone meal, except that there is no added bone. It contains approximately 50 to 55% crude protein, and its ash content is lower than that of meat and bone meal.

Blood Meal

Blood meal is prepared from clean fresh animal blood, excluding hair, stomach belching, and urine, except in trace quantities that are unavoidable. Blood meal contains about 80 to 85% crude protein and is an excellent source of lysine, but is deficient in methionine.

Plant Proteins

Oilseed Meals

Soybean Meal

Soybean meal is prepared by grinding the flakes that result after removal of the oil from soybeans by solvent extraction or by the expeller process. There are three types of soybean meal that can be used in fish diets: dehulled and solvent extracted, solvent extracted, and expeller processed. These types of soybean meal contain 48, 44, and 42% protein and 1, 0.5, and 3.5% oil, respectively. Soybean meal is the major protein feedstuff used in aquaculture diets.

Full-Fat Soybean Meal

Heated, full-fat soybean meal is prepared by grinding heated, full-fat soybeans. The meal contains 39% protein and 18% fat. It is rarely used in channel catfish diets, because of its high fat content, but a limited amount can be used as long as the total fat level in the diet does not exceed 6%.

Cottonseed Meal

Cottonseed meal is obtained by grinding the cake remaining after the oil has been removed from cottonseeds, either hydraulically, by screw-press extraction, prepress solvent extraction, direct solvent extraction, or expander solvent extraction. The products generally contain 41% protein, but must not contain less than 36% protein.

Peanut Meal

Peanut meal is obtained by shelling peanuts, removing the oil, either mechanically or by solvent extraction, and then grinding the peanuts. Solvent extracted peanut meal contains 48% protein, and the mechanically extracted product contains 45% protein. Peanut meal is highly palatable to fish and contains no known antinutritional factors; however, it is deficient' in lysine.

Sunflower Meal

Sunflower meal is prepared by grinding the residue remaining after mechanical or solvent extraction of the oil from sunflower seeds. Dehulled sunflower meal is prepared from sunflower seeds after the hulls are removed. Solvent-extracted, dehulled sunflower meal contains about 44% protein. As the hulls are not easily removed, the meal contains around 13% fiber. In fact, higher levels of fiber are found in meals that are not dehulled. Consequently, its low lysine content and high level of fiber limit its use in fish diets.

Rapeseed Meal and Canola Meal

Rapeseed meal is prepared by removing the oil from rapeseeds, using the solvent extraction method, and then grinding the remaining residue. Rapeseed meal

contains glucosinolates (antithyroid factor) which may be detrimental to fish growth. Canola meal is prepared from selected varieties of rapeseed that are low in glucosinolates and erucic acid (another antinutritional factor present in the oil) by solvent extraction to remove the oil. Canola meal contains about 38% protein and is relatively low in lysine as compared with soybean meal, but is higher in sulfur amino acid content relative to soybean meal.

Peas and Lupin

Field beans or peas, particularly low tannin varieties of *Pisum sativum*, are used as a source of protein and starch for animal feeds and they also show potential for aquafeeds. Several anti-nutritional factors including trypsin inhibitors and tannins in feed-grade peas have been substantially reduced or eliminated through selective plant breeding. Dehulled peas contain about 25 % protein and are relatively high in lysine but low in sulfur amino acids and tryptophan.

Lupin meal, especially when dehulled and extruded, has been shown to have some potential for inclusion in Atlantic salmon feeds. The protein content of lupin seed meal is relatively high (35-43 %). Improved varieties of lupin contain low amounts of anti-nutritional factors such as alkaloids. They also contain α -galactosides (7-15 % of dry matter).

Distillers' Dried Grains with Solubles

Distillers' dried grains with solubles are the primary residues from the fermentation of yeast in cereal grains and after the removal, by distillation, of the alcohol in the grains. The product contains approximately 27% protein and is highly palatable to fish.

Brewers' Dried Grains

Brewers dried grains are residues obtained during the brewing of beers and ales after the removal of the starches and sugars of the grains, such as malted barley, corn, rice grit, and hops. This product contains about 28% crude protein and 12% fiber. It can be used in fish diets, but is usually deficient in lysine.

Corn Gluten Meal

Corn gluten meal is the dried residue from corn after the removal of most of the starch and germ and after the separation of the bran by the process of wet milling of corn starch and corn syrup, or by enzymatic treatment of endosperm. There are two types of corn gluten meal, which contain 41% and 60% protein, and 4% and 2.5% fiber, respectively. Corn gluten meal is a good source of methionine, but may contain high levels of yellow carotenoid pigments. The latter may impart an undesirable yellow color in the flesh of salmonids. Corn meal can be produced from white corn, resulting in white corn gluten meal. This product does not contain xanthophyll pigments.

Wheat Gluten Meal

Wheat gluten meal is a protein concentrate resulting from the removal of starch from ground wheat. It is highly digestible and highly palatable to salmonids. It contains approximately 75% protein, but is also relatively high in phytate.

Plant Protein Concentrates

Plant protein concentrates are prepared by various methods that concentrate protein from plant feedstuffs. The concentrates contain high levels of protein (55-80%), and generally lower levels of antinutritional factors than their parent seed or meal. Soybean protein concentrate (SPC) is commercially available, albeit at a relatively high cost compared with that of fish meal. SPC protein is highly digestible (95%) and highly palatable to salmonids. Other promising concentrates include rapeseed/canola protein concentrate, sunflower protein concentrate, cowpea protein concentrate, and other such products from beans, peas and hemp.

Single-cell proteins

Single cell proteins may include bacteria, yeast, and algae. Protein levels can range from 40% to 80% depending upon the species and substrate on which it is grown. Single cell proteins are generally deficient in sulfur-containing amino acids.

QUALITY OF PROTEIN SOURCES

The quality of protein sources is based on the amino acid composition of the protein, and it particularly refers to how well the concentrations and balance of available essential amino acids conform to the essential amino acid requirements of salmon. Table 2 shows the amino acid compositions of commonly used protein sources in salmon feeds. It is noteworthy that the dietary requirements for most essential amino acids have not been determined for Atlantic salmon. One of the shortcomings of the alternate protein sources listed above is that for the most part, protein and amino acid digestibility values are not available. Thus, efforts to simply replace fish meal in salmon feeds with the alternate proteins on an equivalent available essential amino acid basis cannot be done at this time without risking a deficiency or imbalance of dietary amino acid supply. Nevertheless, some information on the apparent protein digestibility coefficients of protein sources for Atlantic salmon is available, and there is also some additional information in this regard for chinook and coho salmon (Table 3).

SUMMARY

Although many protein sources have the potential to be used, relatively few of them are used in commercial diets for salmon, essentially for three reasons. First, not many alternate protein sources are available at a reasonable cost. Second, not many alternate protein sources contain the essential nutrients sufficient for optimum fish growth. Finally, information on the biologically important antinutritional factors is absent. Generally, proteins of animal origin are of higher quality, but more expensive than those of plant origin. Soybean meal has been the major alternate protein source used in aquaculture diets worldwide, mainly because it has one of the best amino acid profile among alternate protein sources. A substantial body of scientific literature exists on the use of alternate proteins in rainbow trout feeds. This information should be examined as a starting point for investigations into their use in feeds for Atlantic salmon.

Table 2. Protein and amino acid composition (%) of ingredients commonly used in fish feeds (as-fed basis)

Essential amino acids	Norse-LT94 ^a	Fish meal, anchovy	Fish Meal, herring ^b	Meat meal ^b	Poultry by-product meal ^b	Blood meal ^b	Corn gluten meal ^b	Soybean meal de-hulled ^b	Soybean protein concentrate ^c	Canola meal ^b	Wheat gluten meal ^d	Yeast, brewers, dehydrated ^b	Lupin, Extruded ^e
Protein	72.1	65.5	72.0	55.6	59.7	89.2	60.7	50.0	65.5	38.0	81.0	42.6	40.3
Arginine	4.67	3.85	4.54	3.6	4.06	3.75	2.02	3.67	4.30	2.32	3.6	2.25	4.76
Histidine	1.73	1.61	1.65	0.89	1.09	5.14	1.31	1.22	1.77	1.07	1.9	1.09	1.25
Isoleucine	3.41	3.17	3.13	1.64	2.30	0.97	2.54	2.14	2.95	1.51	3.5	1.98	1.53
Leucine	5.71	5.05	5.19	2.85	4.11	10.82	10.2	3.63	5.37	2.65	7.0	2.85	2.90
Lysine	6.00	5.04	5.57	2.93	3.06	7.45	1.11	3.08	4.32	2.27	1.5	2.97	1.69
Methionine	2.02	1.99	2.08	0.66	1.10	1.08	1.63	0.68	0.92	0.70	1.6	0.67	0.32
Cystine	0.76	0.60	0.74	0.59	0.84	1.24	1.20	0.75	0.98	0.47	2.2	0.49	0.64
Phenylalanine	3.07	2.78	2.71	1.72	2.10	5.92	3.96	2.44	3.34	1.52	5.0	1.62	1.41
Tyrosine	2.40	2.24	2.20	1.17	1.87	2.55	3.32	1.76	2.42	0.93	3.2	1.5	1.97
Threonine	3.29	2.82	2.90	1.64	0.94	3.76	2.07	1.89	2.82	1.71	3.7	2.04	1.53
Tryptophan	0.53	0.75	0.77	0.34	0.46	1.04	0.43	0.69	0.92	0.44	0.9	0.52	0.40
Valine	3.81	3.50	4.30	2.52	2.86	7.48	3.09	2.55	3.34	1.94	5.0	2.36	1.69

^a Anderson *et al.* (1997)

^b NRC (1993)

^c Profine, Analysis by Central Soya Company, Inc.

^d Storebakken *et al.* (2000)

^e Burel *et al.* (2000)

Table 3. Apparent protein digestibility coefficients of protein sources for salmon.

Feedstuff	International Feed Number	Atlantic salmon	Chinook salmon	Coho salmon
Blood meal, Canada	5-00-381	-	29.4	-
Corn gluten. meal	5-04-900	-	-	91.9
Canola meal	-	-	84.5	-
Canola meal, glucosinolate-free	-	74.1-86.6	87.9	-
Fish, anchovy meal	5-01-985	94-96.5	91.7	91.4
Fish, herring meal	5-02-000	91.5-94.0	90.5	94.7
Fish, menhaden meal	5-02-009	91.7	83.1	87.7
Fish, LT herring/capelin	5-01-977	97.6	93.6	-
Meat and bone meal	5-00-388	-	-	-
Poultry by-product meal, Kansas	5-03-798	-	84.9	94.2
Poultry by-product meal, Canada	5-03-798	-	74.4	-
Poultry feather meal	5-03-795	-	70.8	79.7
Rapeseed/canola protein concentrate	-	97.7	95.6	-
Soybean protein isolate	-	-	86.3	-
Soybean meal (48%)	5-04-612	-	77.0	93.0
Wheat gluten	-	-	-	99.6

Note: Values for Atlantic salmon are from two sources, differing widely in values associated with the use of different fecal collection techniques.

Source: Hajen *et al.*, (1993); Higgs *et al.* (1996); Sugiura *et al.* (1998); Anderson *et al.* (1997).

ANTINUTRITIONAL FACTORS

Plants have developed a number of survival mechanisms to reduce the chances of their seeds being eaten by insects, animals or birds, or, if the plants are eaten, to reduce the chances of their being digested (Dong *et al.*, 2000). One class of defense mechanisms is the production of compounds called antinutrients or antinutritional factors (ANFs) that are toxic to animals or inhibit digestion of seeds. Other compounds present in seeds are not produced for protection, but nevertheless lower the bioavailability of nutrients to animals or otherwise affect their health. Although research with mammalian models in the past 10-15 years has revealed some beneficial effects of certain ANFs such as reduction of blood lipids and reduction in the incidence of cancer (Shahidi, 1997), any health benefits to fish have not been reported. Therefore, since many feed ingredients used in animal and fish feeds are produced from grains, legumes and oilseeds, antinutritional factors are an important consideration in animal and fish nutrition.

There are two main approaches to eliminating ANFs in feed ingredients. Some antinutritional factors can be destroyed or removed by processing conditions, e.g., high- temperature extrusion processing of soybean meal or canola meal, and/or solvent extraction of whole oilseeds themselves and/or their protein- rich by-products following oil extraction. The concentrations of others have been lowered to acceptable levels by selective breeding of some oilseeds and this has led to the development of new plant cultivars that have improved nutritive value for finfish species and other animals relative to the meals derived from their progenitor seeds. Nevertheless, antinutritional factors in feed ingredients of plant origin still remain a source of concern in animal feeds, and especially in those for fish, where much less is known about their sensitivity to these compounds.

GRAINS

Grains are the foundation of most animal and some fish feeds. Ground, whole cereal grains (e.g., corn, wheat, rice, barley, and sorghum) constitute 50% or more of feeds for many other omnivorous fish, but feeds for salmonids contain 10-20% ground, whole grains, and often include products made from grains, e.g., gluten meals (typically wheat and corn) or milling by-products. The antinutritional factors present in grains are protease inhibitor, hemagglutinins, cyanogen, phytic acid, tannin, estrogenic factors, anti-vitamin B-1, amylase inhibitor, invertase inhibitor, dihydroxyphenylalanine. Of these, phytic acid and hemagglutinin are of principle concern to fish feed producers.

Phytic acid, or phytate, is the hexaphosphate of myoinositol and is the storage form of phosphorus in seeds. Phosphorus in this form is unavailable to monogastric animals, including fish because they lack intestinal phytase. Spinelli *et al.* (1983) reported that adding phytates to rainbow trout feeds reduced protein bioavailability, presumably through the formation of protein-phytic acid complexes. Researchers have demonstrated that phytic acid can lower the bioavailability of especially zinc, but also some other divalent ions as well, making it necessary to fortify fish diets with supplemental zinc when ingredients containing phytic acid are included in feeds. Richardson *et al.* (1985) showed that the addition of phytate to chinook salmon feeds that concurrently contained high levels of calcium and phosphorus in relation to an

adequate dietary zinc level (if phytate were absent) induced zinc deficiency. This, in turn, led to bilateral lens cataracts and anomalies in the structure of the pyloric caecal region of the intestine as well as reduced growth, feed efficiency and thyroid function. Certainly, it is desirable to reduce the level of phytate in the ingredients comprising fish feed for the reasons mentioned above and to decrease excretion of unavailable phytate phosphorus into the environment.

Recently, plant geneticists have developed varieties of corn and barley that are lower than common varieties in phytate content. These varieties contain the typical amount of phosphorus, but much less of the phosphorus is bound to phytic acid. The bioavailability of phosphorus to rainbow trout from low-phytate corn and low-phytate barley was significantly higher than in common corn and barley (Sugiura *et al.*, 1999). Most likely similar results will be found in other fish species. High temperature extrusion promising also appears to be a practical way to partially reduce the levels of phytate in some plant protein products before their dietary incorporation with concomitant beneficial effects on fish performance (Sato *et al.*, 1998).

Hemagglutinins, also known as lectins, are proteins that cause agglutination of erythrocytes (red blood cells), at least *in vitro*. Luckily, hemagglutinins are destroyed by the acid conditions in the stomach, suggesting that species of fish possessing acid stomachs will not be affected by them. For carp and other agastric fish species, the possibility exists that hemagglutinins could be a problem, although heat treatment inactivates them. Thus, the heat involved in pelletizing fish feeds likely eliminates these compounds as practical problems in fish farming.

Estrogenic factors in plants, also called phytoestrogens, are compounds found in several grains (e.g., barley, oats, rice, and wheat) that have weak estrogenic activity. The compounds having estrogenic activity in grains are isoflavones, coumestans, and resorcylic acid lactones (Hendricks and Bailey, 1989). The amounts present in grains are low, and it is unlikely that they have any significant effect to fish at the levels present in fish feeds, although studies to confirm this have not been done.

Amylase is an intestinal enzyme used to digest carbohydrates. Amylase inhibitors are found in wheat and beans and have been reported to be heat sensitive (e.g., 95-100°C for 15 min) and digested by pepsin (Whittaker and Feeney, 1973). Carnivorous fish (e.g., salmon and trout) have low levels of amylase in their intestine (Hendricks and Bailey, 1989). In trout, wheat flour inhibits about 80% of the amylase activity of intestinal fluid. Ground wheat is less inhibitory than wheat flour. Use of wheat flour in fish feeds likely results in reduced amylase activity and thus reduced rates of carbohydrate digestion, especially in fish with little endogenous amylase.

OILSEEDS

A number of feed ingredients produced from oilseeds are important in fish feeds, including soybean meal, cottonseed meal, canola (rapeseed) meal and sunflower meal. To a lesser extent other oilseed meals, including peanut meal, and sesame seed meal, are included in fish feeds in areas where availability and economics support their use. The antinutritional compounds found in oilseeds are trypsin inhibitors, glucosinolates, phytic acid, saponin, tannin, phytoestrogens, gossypol, anti-vitamin E, A, D, B-12, arginase inhibitor, and cyclopropenoic acid.

Soybean products

Soybean meal contains anti-nutritional compounds that must be removed or inactivated by processing before the meal can be used successfully in animal or fish feeds (reviewed by Storebakken *et al.*, 2000). The principal compounds of concern in soybean meal are trypsin inhibitors that reduce protein digestibility by binding with the digestive enzyme trypsin in the intestine of the animal. Trypsin inhibitors are sensitive to heat, and ordinary processing after oil is extracted from raw soybeans lowers the level of trypsin inhibitors in the dried meal to levels that do not affect the growth of most domestic animals and some species of fish. Salmon and trout are more sensitive to trypsin inhibitor level, and more extensive heat treatment is necessary to reduce residual trypsin inhibitor levels below the levels affecting protein digestibility and growth performance, which is 5 mg/g (Rumsey, 1995). However, over-heating soybean meal may reduce protein quality by fostering reactions between amino acid residues and

portions of the carbohydrate fraction in soybeans. Trypsin inhibitor levels were rapidly lowered in unheated soy flakes from 181 trypsin units inhibited (TUI)/mg sample to 1.8 TUI after 20 minutes of heat treatment (120°C, 25 psi) (Arndt *et al.*, 1999). Protein solubility was reduced from 98% to 70% by this treatment, but further heating to 40 minutes or more reduced protein solubility to below 33%, an indication of over-heating (Araba and Dale, 1990). Protein digestibility, measured *in vivo* using rainbow trout, was increased from 74% to 91% by 20 minutes of heat treatment; this difference was presumably the result of heat inactivation of trypsin inhibitors.

Regular solvent-extracted soybean meal, the most commonly used soybean product in feeds, is heat treated to some extent during its manufacture, resulting in values of about 3.0 to 3.5 mg trypsin inhibited/g sample (Tacon *et al.*, 1983). Further heating occurs during feed pelleting, especially during cooking-extrusion pelleting (see entry on feed manufacturing), presumably lowering trypsin inhibitor activity further. Full-fat soybeans (toasted whole soybeans) containing 46.5 mg TUI had TUI values of 7.6 and 8.5 after being extruded (Wilson, 1992), illustrating the effects of cooking-extrusion on trypsin inhibitor levels.

Soy protein concentrates have low levels of trypsin inhibitor levels (Arndt *et al.*, 1999; Olli *et al.*, 1989), but contain levels of phytic acid that are at least as high as those in soybean meal (Arndt *et al.*, 1999). The enzyme phytase releases phosphorus from phytic acid, and the addition of phytase to feeds significantly improves phosphorus availability in soybean meal-based feeds.

Chemical tests for detecting underheated soybean meal

The various chemical tests used to determine the adequacy of heat treatment of soybean meal can be divided into two groups: those that detect underheated soybean meal; and those that detect overheated meal (Vorha and Kratzer, 1991). Chemical tests to detect underheated soybean meal are determination of urease activity, trypsin activity, and protein solubility. Urease is an enzyme naturally present in soybeans that does not have any substantial nutritional relevance except that it is heat-sensitive and

its activity correlates well with residual trypsin activity in dried soybean meal. It is also relatively easy to measure (AOAC, 1990). Urease activity in commercial soybean meal range from 0.02-0.1 increase in pH (Vohra and Kratzer, 1991). Values over 0.5 increase in pH indicate insufficient heat treatment of the soybean meal. If no increase in pH is detected with the urease test, this may mean that the soybean meal has been overheated, so some residual urease activity in the meal is preferred, at least for soybean meal intended for use in poultry feeds. Unheated soybean meal has a urease activity of >2.25 pH rise (Waldroup *et al.*, 1985).

Another method for measuring the extent of heat treatment of soybean meal is the water solubility test, which involves measuring Kjeldahl nitrogen levels in the soybean meal and in a water extract of the soybean meal (Vorha and Kratzer, 1991). The method has been slightly modified by extracting the sample in 0.2% KOH (Araba and Dale, 1990). Heating decreases the percentage of 0.2% KOH-extractable proteins, from about 99% in raw soybean meal to about 72% after 20 minutes of autoclaving, corresponding to a decrease in trypsin inhibitor units from 21.1-1.0 (Araba and Dale, 1990).

Soy products contain compounds that influence feed intake, gut histology, and immunological function (Rumsey 1995). Complete replacement of fish meal with soybean meal in trout feeds lowered growth, primarily by lowering feed intake, but partial replacement of fishmeal with soybean meal, e.g., 29% soybean meal and 42% fishmeal in the diet, had no effect on trout feed intake or growth (Rumsey, 1995). The tolerance of trout to dietary soybean meal appeared to be higher at higher water temperatures and in larger fish. The intestinal mucosa of trout fed soybean meal-containing diets was blunted or flattened, thus decreasing the absorptive surface of the proximal and distal intestine, but it is not known if these changes were responsible for differences in growth associated with feeding diets containing high levels of soybean meal (Rumsey, 1995). Antigens present in soybean products stimulate the nonspecific defense mechanisms of trout, but it is unknown if such stimulation of the immune system results in higher resistance to infectious disease (Rumsey, 1995).

Other oilseed products

The other major antinutritional factors associated with other oilseed meals are: glucosinolates, erucic acid, and phenolic compounds (e.g., tannins and sinapine) in rapeseed/canola meals; and gossypol and cyclopropenoic acid in cottonseed meal. Glucosinolates interfere with the function of the thyroid gland in fish, posing problems during metamorphosis, smoltification, and maturation (NRC, 1993). Glucosinolates themselves are not harmful compounds, but when they are hydrolyzed by the enzyme myrosinase in poorly processed meals or by intestinal microorganisms, an array of possible products can result such as thiocyanate ions, isothiocyanates, nitriles and goitrin, depending upon the conditions of hydrolysis. Isothiocyanates and nitriles are precursors to thiocyanate, which inhibits uptake of iodine by the thyroid gland. Extra supplementation of the diet with iodine can overcome this. Goitrin actually inhibits the ability of the thyroid to bind iodine. Consequently, the provision of supplemental iodine cannot overcome this problem. Typically, the major consequences of impairment of thyroid function include thyroid hypertrophy and hyperplasia, and depressed thyroid hormone synthesis and plasma thyroid hormone titres. These effects lead to reductions in growth, feed intake, and feed utilization. Fortunately, varieties of rapeseed have been developed through genetical selection (called canola) that contain very low levels of glucosinolates, and residual levels of these compounds can be decreased further by solvent extraction and other means. Hence, glucosinolates present in some canola protein products, e.g., concentrates and isolates, are very low in concentration and do not pose a problem.

Erucic acid is a 22-carbon monounsaturated fatty acid, and may constitute 20-55% of rapeseed oil (NRC, 1993). This compound causes lipid accumulation and necrosis of heart tissue (Slinger 1977), and is toxic to coho salmon when fed at 3-6% of the diet (Hendricks and Bailey, 1989). However, no erucic acid problems have been reported when rapeseed or canola meals are included in fish feeds, presumably because nearly all of the oil has been removed from these meals.

Tannins are known to compromise protein and dry matter digestibility. Sinapine is bitter and may decrease the palatability of rapeseed/canola meals and it is noteworthy that

treatment of canola products with various solvents can dramatically reduce the levels of sinapine and other phenolic compounds.

Varieties of canola have been developed that contain lower levels of glucosinolates and erucic acid than earlier varieties. The use of rapeseed/canola protein products in fish feeds has recently been thoroughly reviewed (Higgs *et al.*, 1995a and 1996).

The presence of fiber in oilseed meals like commercial canola meal singly and perhaps in combination with phytate appear to have the greatest adverse effects on their digestibility in salmonids like the rainbow trout (Mwachireya *et al.*, 1999). Indeed, the soluble fibers in the meal may depress the gut transit time and the absorption of amino acids and peptides whereas the soluble fibers could inhibit digestive enzymes and also restrict diffusion of hydrolysis products (reviewed by Mwachireya, 1995).

Gossypol causes a number of problems in fish, including anorexia and increased lipid deposition in the liver. Gossypol is contained in the pigment glands of cotton; glandless cotton is gossypol-free. Fish species differ in their sensitivity to gossypol, with trout being the most sensitive, channel catfish more sensitive, and blue tilapia the most sensitive of the species for which information is available (NRC, 1993). Growth depression occurred in trout fed more than 290 mg gossypol/kg diet, with more than 900 mg/kg for channel catfish and 1800 mg/kg for tilapia needed to reduce growth. Solvent-extracted cottonseed meal samples from the USA contained between 400 and 800 mg gossypol/kg (Robinson and Li, 1995). Thus, catfish feeds containing no more than 20% cottonseed meal would not deliver enough gossypol to affect the fish. The use of cottonseed meal in fish feeds has also been thoroughly reviewed recently (Robinson and Li, 1995). Cottonseed meal also contains cyclopropenoic fatty acids (sterculic and malvalic acids). These fatty acids are quite toxic in their own right, and powerful carcinogens when fed to rainbow trout or salmon in combination with aflatoxins, fairly common toxins produced by *Aspergillus flavus*, a common mold typically found on grains (Hendricks and Bailey, 1989). Cottonseed meal contains residual levels of cyclopropenoic fatty acids, even after oil extraction; thus it should not be used in feeds for trout or salmon.

LEGUMES

Legumes include peas, beans, alfalfa and ipil ipil, and many contain trypsin inhibitors, hemagglutinin, cyanogens, phytic acid, saponin, anti-vitamin factors, and mimosins. Most of these antinutritional factors have been discussed above, with the exception of saponin and mimosin. Saponin is a non-protein constituent of soybeans making up about 0.5% of the weight of the soybean, extractable from soybean globulins with alcohol. Isolated saponins do not harm chicks, rats or mice when fed up to three times the amount found in soybean meal, but a crude saponin extract of soybean meal has been reported to lower feed intake of chinook salmon fingerlings and to reduce growth of rainbow trout (Bureau *et al.*, 1998).

OTHER ANTINUTRITIONAL FACTORS FOUND IN FEED INGREDIENTS

Thiaminase

Many species of fish, mainly freshwater species, contain thiaminase, an enzyme that hydrolyzes thiamin in feed preparations (NRC, 1993). Thiaminase is destroyed by heat treatment, and also by acidification used to produce fish silage. Thiaminase is a relatively slow-acting enzyme; therefore feeding raw fish combined with dry mash within hours of preparation is not a high risk. An alternative strategy is to feed raw fish and dry mash containing thiamin separately.

Histamine and gizzerosine

Histamine, 4-(2-aminoethyl) imidazol, is a primary amine arising from the decarboxylation of the amino acid, L-histidine. The toxic effects of histamine in the food supply of humans has been positively associated with scombroid-fish poisoning (Morrow *et al.*, 1991), an allergic reaction resulting from the ingestion of spoiled fish, usually of the families Scombridae and Scomberesocidae. The U.S. Food and Drug Administration has established a hazard action level of 50 mg histamine/100 g canned

tuna (U.S.F.D.A., 1982), and for fresh and frozen fish, the level is 20 mg histamine/100 g fish (U.S.F.D.A., personal communication as cited in U.C.S.G.E.P., 1990)

Histamine production in fish muscle can be controlled by low storage temperatures, which often help to reduce enzymatic and most microbial activities. However, if onboard refrigeration is inadequate, much of the fish captured could undergo significant deterioration prior to processing into fishmeal. During the processing of fishmeal (capture, transport, storage), microbial and enzymatic degradation can take place rapidly at warm temperatures to produce high concentrations of histamine. If a significant portion of the catch is delivered to processing plants in a partially decomposed state, then further degradation can occur after the fish have been unloaded, especially if the raw material is stored in non-refrigerated concrete pits prior to processing.

Gizzard erosion and the resultant black vomit disease of chickens has long been associated with feeding thermally abused fish meal, but it was not until 1983 that an active substance from fishmeal was isolated (Okazaki *et al.*, 1983). The active compound named gizzerosine was presumably formed via condensation of histamine with the epsilon amino group of lysine during high temperature processing of raw fish into fishmeal. Mori *et al.* (1983, 1985) synthesized several forms of the compound and established the L-form as a potent inducer of gizzard erosion in chickens. In one study, Mori *et al.* (1983) observed severe gizzard erosion in chickens fed less than 50 µg gizzerosine/day (about 2.2 mg/kg diet) for one week. Considerable experimental evidence indicates that gizzerosine induces gizzard erosion in chickens by hyperstimulating gastric acid secreting cells of the proventriculus.

Putrescine and cadaverine, as well tyramine, β-phenylethylamine, and tryptamine, have been shown to potentiate histamine toxicity *in vivo* by inhibition of the histamine-metabolizing enzymes diamine oxidase and histamine-N-methyltransferase (Hui and Taylor, 1985). These data strongly suggest that fish meal toxicity to chickens is most likely related to complex interactions among many chemical compounds found in degraded fish products.

In a study by Fairgrieve *et al.*, (1994), growth, feed intake, and development of gastric abnormalities were assessed in juvenile rainbow trout fed diets containing fish meal acutely toxic to chickens, or fed casein or fish meal diets supplemented with histamine and two suspected potentiators of histamine toxicity, putrescine and cadaverine, and abusively heated. Rainbow trout were less sensitive than chickens to gastric erosion (GE)-positive fishmeal, and there was no correlation between the GE score and the nutritional value of the fishmeal for rainbow trout. Fish fed diets containing GE-positive fishmeal had distended stomachs, but no gastric lesions or cellular abnormalities; similar effects were obtained by feeding diets containing casein or GE-negative fishmeal supplemented with histamine (2,000 mg/kg dry diet). The addition of putrescine and cadaverine (500 mg/kg dry diet each) to the histamine-supplemented diets had no further effect. Feed consumption, feed efficiency, and growth were similar among dietary treatments, indicating that stomach distention did not reduce feed intake or impair gastric function. This study also showed that stomach distention resulting from feeding diets containing GE-positive fishmeal could be duplicated by feeding diets supplemented with 2,000 mg histamine/kg diet.

Phytotoxins (toxins of algal origin)

Many species of marine algae are capable of producing toxins, including paralytic shellfish toxin (PSP), diarrhetic shellfish toxin (DSP) and domoic acid, which causes amnesic shellfish poisoning (ASP) (Bricelj and Shumway, 1998). Shellfish, specifically bivalve mollusks, concentrate these toxins and are the principal vector through which the toxins are transferred to man, and possibly to farmed fish. Very little is known about the direct effects of these toxins on farmed fish, or on accumulation and/or biotransformations that may occur if farmed fish are fed diets containing contaminated shellfish, or other marine product containing toxins. However, the potential human health risk is serious (Bricelj and Shumway, 1998).

Sardines, and presumably other fish utilizing algae as food, consume algae containing domoic acid without apparent signs of toxicity (Work *et al.*, 1993). If these fish are harvested and used to produce fishmeal, domoic acid, which is heat-stable, can be concentrated in the fishmeal by a factor of at least three, reaching 130 µg

domoic acid per g fishmeal (Hardy *et al.*, 1995). Rainbow trout fed diets containing contaminated fishmeal did not exhibit any signs of toxicity or growth retardation, even though they consumed 50 µg domoic acid per kg body weight, much more than is required to cause illness in humans. Domoic acid was present in the GI tract of the trout, but not in edible tissues (Hardy *et al.*, 1995).

MICROBIAL TOXINS

Molds that grow on feed ingredients and on prepared feeds are an important group of toxins that affect fish (Hendricks and Bailey, 1989). In particular, toxins produced by the mold *Aspergillus flavus*, called aflatoxins, cause serious health problems in fish at much lower intake levels than terrestrial animals. Cottonseed meal, peanut meal, and corn products are the most problematic feed ingredients with respect to aflatoxins, and grains including wheat, rice, barley and oats are the next most problematic feed ingredients (Hendricks and Bailey, 1989). Prolonged intake of very low levels of aflatoxin (< 1 ppb) causes liver cancer after one year in rainbow trout, the most sensitive vertebrate to aflatoxin intake. Acute toxicity in rainbow trout is observed when fish are fed diets containing between 0.8 µg and 1.9 µg aflatoxin per g feed, depending upon the type of aflatoxin. Differences in sensitivity exist among strains of rainbow trout, among trout species, among other salmonids, and among other species of fish. Coho salmon and channel catfish, for example, are much more resistant to aflatoxin exposure than are rainbow trout (Hendricks and Bailey, 1989). Other mold toxins of concern in fish feeding include ochratoxin, sometimes found as a contaminant of corn and wheat, vomitoxin, found in cereal grains, and T-2 toxin, also found in cereal grains. Of these, only vomitoxin has been evaluated in fish, with dietary levels of 20 µg toxin/g feed or higher causing feed refusal in trout (Woodward *et al.*, 1983). Obviously, fish should never be fed moldy feed.

SUMMARY

The biological significance of anti-nutritional factors in feed ingredients varies among factors and within factors among fish species. Research to document the relative importance of anti-nutritional factors in fish is extensive for some (e.g.,

glucosinolates), less extensive for others (e.g., phytic acid, trypsin inhibitors, gossypol), and nearly absent for others. If compounds that lower feed palatability are included in the anti-nutritional factor category, the practical significance of anti-nutritional factors increases. As use of feed ingredients from grains and oilseeds in fish feeds increases, the importance of understanding the biological effects of anti-nutritional factors on farmed fish and of developing methods of inactivating anti-nutritional factors or overcoming their effects will become a critical element of the expansion of aquaculture production.

FISH OIL AND ALTERNATIVE LIPID SOURCES

INTRODUCTION

Lipids and their constituent fatty acids, together with their metabolic derivatives, i.e., eicosanoids and other associated compounds, play essential and dynamic roles in the maintenance of optimum growth, feed efficiency, health (immunocompetence and cardiovascular function), kidney and gill function, neural and visual development, reproduction, and flesh quality (market-size) of finfish species (reviewed by Higgs and Dong, 2000). In this review, we provide a brief overview of the general types of lipids and families of fatty acids that are of nutritional significance and of the types of metabolic derivatives that are elaborated from some of the key fatty acids that are especially important from a nutritional standpoint. Also, we consider the dietary lipid and fatty acid requirements of salmonids. Further, we consider the need to partially replace marine lipid sources with alternative lipids of plant and/or animal origin in diets for Atlantic salmon as well as the possible consequences of this on fish growth, health, and flesh quality. A brief summary of research needed to support this shift in dietary lipid sources is also provided.

Lipids, Fatty Acids, and Eicosanoids

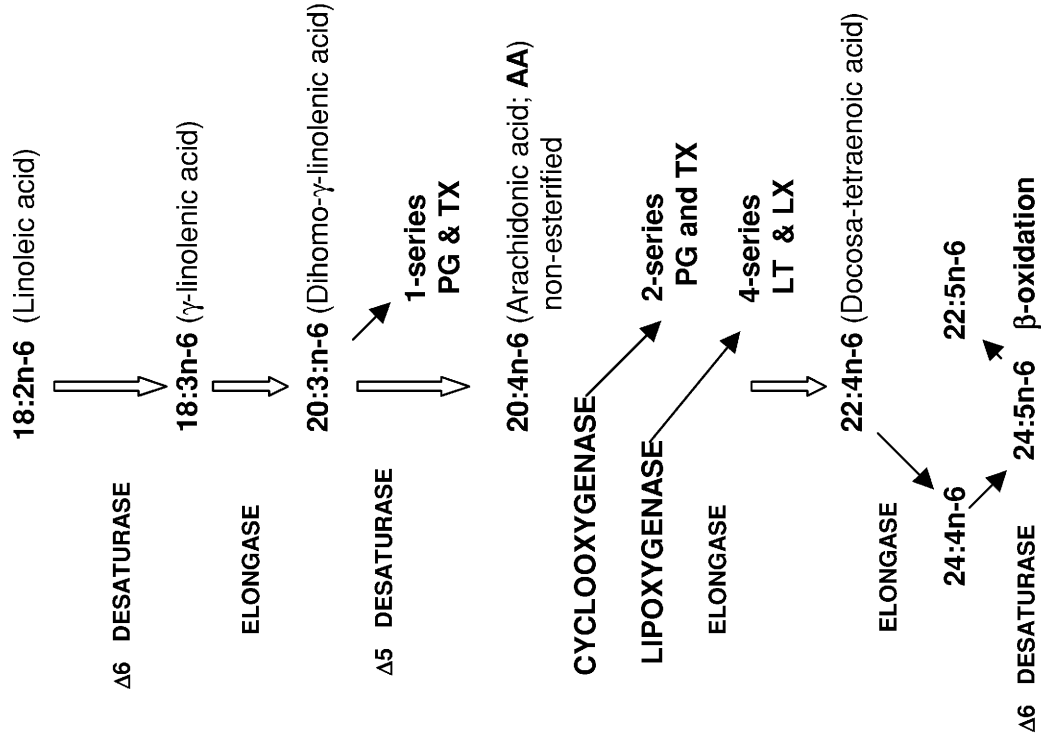
Lipids refer to compounds that are relatively insoluble in water but are soluble in organic solvents such as chloroform, ether, hexane, and benzene. There are many types of lipids, and they are frequently differentiated according to their polarity. In this regard, some lipids such as triacylglycerols, wax esters and sterol esters are insoluble in water and are therefore called nonpolar lipids. This category is generally the storage

form of lipids (energy) in the body. Other lipids such as phosphoglycerides have varying degrees of water solubility and are termed polar lipids. These are essential structural components of biological membranes, and they influence their physical and functional properties. The fatty acids within nonpolar and polar lipids generally contain a single carboxyl group and a straight unbranched carbon chain. The carbon chain may have no double bond, one double bond, or two or more double bonds, in which case the fatty acid is referred to as saturated, monounsaturated, or polyunsaturated (PUFA), respectively. The unsaturated fatty acids present in fish prey, formulated diets, and body lipids can be divided further into three major families or series, namely the oleic (n-9), the linoleic (n-6), and linolenic (n-3). The latter two families of PUFAs have the greatest nutritional significance and are depicted in Figure 8.

At this point, some explanation of the shorthand abbreviations for fatty acids should be provided. In Figure 8, for example, linolenic acid, the parent acid of the n-3 family, is abbreviated as 18:3n-3. This signifies the number of carbon atoms (18), the number of double bonds (3), and the position of the first double bond counting from the terminal methyl (CH_3) group carbon to the carbon atom of the first double bond. Finfish are similar to other vertebrates and cannot synthesize either linoleic acid (18:n-6), the parent acid of the n-6 family, or linolenic acid. Consequently, these fatty acids or their highly unsaturated metabolic derivatives must be of dietary origin. Also, depending upon the finfish species, the parent acid of the n-3 series or proper levels and proportions of some of the highly unsaturated members of this family alone (i.e., eicosapentaenoic acid and docosahexaenoic acid) or together with counterparts of the n-6 series of fatty acids (i.e., linoleic acid and arachidonic acid) are essential for normal growth, food utilization, health, and reproductive viability (refer to Higgs and Dong, 2000 for information on the lipid and fatty acid requirements of commercially important finfish species). Further, it should be mentioned that the members of each of the families of fatty acids are created from their respective parent acids by a common enzyme system of alternating desaturases and elongases which yield series of fatty acids of increasing unsaturation and length. It is also noteworthy that the members of one family are not interconvertible to those of another. The respective highly unsaturated fatty acids (HUFAs) of the n-9, n-6, and n-3 families of nutritional significance are eicosatrienoic

acid (20:3n-9), dihomog- γ -linolenic acid (20:3n-6), arachidonic acid (20:4n-6; AA), eicosapentaenoic acid (20:5n-3; EPA), and docosahexaenoic acid (22:6n-3; DHA). The latter four fatty acids are progenitors

MOST VEGETABLE OILS;
FRESH WATER PREY
DIET



SOME VEGETABLE OILS (AS 18:3n-3), FRESH WATER PREY
(AS 18:3n-3, EPA & DHA), MARINE PREY (AS EPA & DHA),
DIET

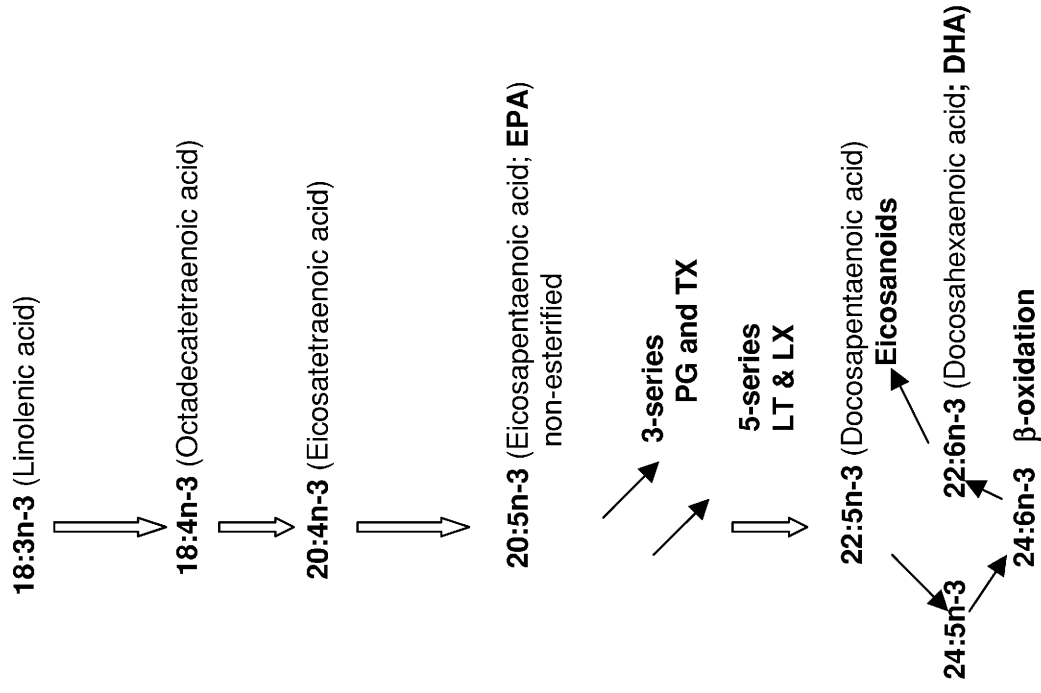


FIGURE 8. Probable pathways involved in the desaturation and elongation of n-6 and n-3 series of fatty acids in freshwater fish. The parent acids of each family together with their respective nutritionally important highly unsaturated fatty acids and series of cyclooxygenase-derived (prostaglandins, PG and thromboxanes, TX) and lipoxygenase-derived (leukotrienes, LT and lipoxins, LX) compounds, collectively termed eicosanoids are indicated (Adapted by Balfry and Higgs, 2001 from Higgs and Dong, 2000). The production of 22:5n-6 and 22:6n-3 is thought to occur as illustrated rather than through Δ 4 desaturation of 22:4n-6 to yield 22:5n-6 and 22:5n-3 to produce 22:6n-3 (refer to Buzzi *et al.* 1997; Calder 1997). There is some evidence for eicosanoid formation from DHA e.g., 14-hydroxydocosahexaenoic acid, through the action of 12-lipoxygenase (German *et al.* 1986).

of a series of compounds collectively called eicosanoids. For instance, non-esterified AA through the action of cyclooxygenase enzymes yields 2-series prostanoids (prostaglandins and thromboxanes) and, through the action of lipoxygenase enzymes, 4-series leukotrienes and lipoxins. Alternatively, the metabolic derivatives stemming from non-esterified EPA are 3-series prostanoids and 5-series leukotrienes and lipoxins. Collectively, these compounds play essential roles in the regulation of many physiological processes, including aspects of the immune response. EPA and DHA together are frequently referred to as n-3 HUFAs, but this term can also include other C20 members of the n-3 family.

LIPID AND FATTY ACID REQUIREMENTS

The known dietary lipid and fatty acid requirements for salmonids (salmon, trout and charr) are provided in Table 4.

The information presented in Table 4 reveals that there are wide differences in dietary lipid requirements within and between salmonid species. Lipid is the preferred dietary non-protein energy source in salmonids owing to their limited ability to utilize digestible carbohydrate as an energy source. This likely stems from the fact that salmonids in the wild derive most of their energy needs from the high levels of protein

Table 4. Recommended dietary levels (g/kg dry weight basis and % of dietary lipid where established) of lipid and fatty acids for maximum growth and feed efficiency as well as reproductive performance of salmonid species. It is assumed that the dietary levels and sources of the other energy-yielding nutrients viz., protein and carbohydrate are optimal and the digestibility of lipid is $\geq 90\%$. In many studies, n-3HUFAs refers to 20:5n-3 (EPA) and 22:6n-3 (DHA) while in others, this term also includes small amounts of 20:4n-3 and 22:5n-3 and sometimes 20:3n-3 (Adapted from Higgs and Dong, 2000).

Species/ life history stage	Lipid (g/kg)	Fatty acid					Source
		18:3n-3 (g/kg) (%)	18:2n-6 (g/kg) (%)	n-3 HUFAs (g/kg) (%)	20:4n-6 (g/kg)	20:3n-3 (%)	
A) Salmonids <i>Oncorhynchus mykiss</i> -Freshwater (FW) (juvenile-adult)	150-230	8.3-16.6 ≥ 20 ^{1/} ≤ 80	<10	20-30	≥ 10 ^{1/} ≤ 40	R? ^{1/}	Watanabe (1982); Cho (1990); Cho (1996); Cho and Cowey (1991)
<i>O. tshawytscha</i> (juveniles in FW) (post-juveniles in sea water (SW), <500g)	>63-200 150-200	R ^{2/} R	≤ 26	R R	R R		Higgs <i>et al.</i> (1995b) Higgs <i>et al.</i> (1995b)
<i>O. kisutch</i> (juvenile in FW) (maturing fish in FW)	160-180	10-25 R ^{2,3/}	≤ 10	R	R	R	Higgs <i>et al.</i> (1995b); Yu and Sinnhuber (1979); Hardy <i>et al.</i> (1989)
<i>O. keta</i> (juvenile in FW) ^{4/} (juvenile in SW) ^{4/}	55-109	10 10	10 10	10 10	10 10	R ^{2,3/} R?	Akiyama <i>et al.</i> (1981); Takeuchi <i>et al.</i> (1979) Takeuchi and Watanabe (1982)
<i>O. masou</i> (juvenile in FW) ^{5/}	10	10	5				Watanabe (1988)

Table 4-continued

Species/ life history stage	Lipid (g/kg)	Fatty acid						Source
		18:3n-3 (g/kg)	(%)	18:2n-6 (g/kg)	(%)	n-3 HUFAs (g/kg)	(%)	
<i>Salmo salar</i> (fry; 4g)		10						Ruyter <i>et al.</i> (2000)
<i>Salmo salar</i> (juvenile in FW; 80g)	240	R				R		Grisdale-Helland and Helland (1997)
(post-juveniles in SW; >200g-adults)	≥330	R				R		Johnsen and Wandsvik (1991); Hillestad <i>et al.</i> (1998)
<i>Salvelinus alpinus</i> (juvenile in FW)	200	10-20	20-40	≤7				Tabachek (1986); Yang and Dick (1993); Yang and Dick (1994); Yang <i>et al.</i> (1994)
<i>Salmo trutta</i> (post-juveniles in SW; 1600g)	290							Arzel <i>et al.</i> (1994)

^{1/} The rainbow trout requires 20% of the dietary lipid content as 18:3n-3 or 10% as n-3 HUFAs; ^{2/} Required; ^{3/} Maturing coho salmon in fresh water need ≥ 10g of n-3 fatty acids/kg diet for optimal reproductive performance. It is unknown whether there is a small requirement for 20:4n-6; ^{4/} *O. keta* require either 1% 18:3n-3 and 1% 18:2n-6 or 10% of dietary lipid as n-3HUFAs; ^{5/} *O. masou* need 1% 18:3n-3 or 0.5% n-3HUFAs in their diet.

and lipid in their prey. Indeed, the needs of wild salmonids for glucose are largely met through the process of gluconeogenesis, using the glucogenic amino acids derived from the digestion of dietary protein or tissue proteolysis (e.g., alanine, serine and glycine) plus lactate and glycerol as the substrates. Salmonids also have other metabolic deficiencies that restrict the utilization of high dietary levels of digestible carbohydrate and it is generally recommended that the dietary level of digestible carbohydrate should not exceed 150g/kg and in some cases should be even lower than this (Higgs *et al.*, 1995b).

The data in Table 4 also suggest that the dietary lipid needs of salmonids may vary in relation to the stage of life history. This is clearly evident in the Atlantic salmon (*Salmo salar*) where it has been found that very high-energy (≥ 330 g lipid/kg) diets support maximum performance (growth and feed efficiency) of post-juvenile salmon (>200g) in sea water whereas the dietary lipid needs of the juvenile salmon in fresh water are lower (240g/kg).

The dietary essential fatty acid needs of salmonids shown in Table 4 largely reflect the lipid compositions of their respective natural prey. *Oncorhynchus* species in fresh water, for example, ingest prey that contain substantial amounts of n-3 and n-6 fatty acids mostly in the form of the parent acids and highly unsaturated members of each series (i.e., AA, EPA and DHA, with EPA often greater than DHA). The levels of n-3 series fatty acids in the fresh water prey items always exceed those of the n-6 fatty acids. In the marine prey of these species, the levels of n-3 and n-6 fatty acids are respectively much higher and lower than those found in the fresh water prey. Also, the fatty acids of the n-3 series are largely represented by EPA and DHA and frequently the level of DHA is equivalent to or greater than that of EPA (Higgs *et al.*, 1995b). Accordingly, the essential fatty acid needs of salmonids in fresh water are mostly satisfied by 18:3n-3 alone and in one instance (chum salmon, *Oncorhynchus keta*), by a combination of 18:3n-3 and 18:2n-6 or by n-3 HUFAs alone (≥ 10 % of the dietary lipid level). All of the species in fresh water appear to have good ability to convert 18:3n-3 to n-3HUFAs, and depending upon the speed of the bioconversion in each species,

n-3HUFAs may have greater essential fatty acid activity than 18:3n-3 (e.g., in rainbow trout) or equivalent essential fatty acid activity (e.g., in coho salmon, *Oncorhynchus kisutch*). The essential fatty acid needs of salmonid species in sea water have only been studied in chum salmon, and they did not differ from those established for this species in fresh water (i.e., 10% of the dietary lipid level as n-3HUFAs). It is assumed that this is also the case for the essential fatty acid requirement of the other salmonid species in the marine environment.

Lipid Sources

MARINE AND NON-MARINE LIPID SOURCES IN FEEDS FOR FINFISH

Fish oil is the traditional source of lipid for fish feeds because it is a rich source of the dietary essential fatty acids needed by fish and a by-product of fishmeal production. However, the challenge of finding environmentally and economically sustainable sources of fish feed ingredients raises questions about the future suitability and availability of fish oil. As the demand for fish oil increases relative to supply, the price increases, making other lipid sources economically competitive. In addition, there has been a trend to increase the percentage of lipid in feeds for some species, such as salmon and trout and it is clear that lipid ingredients are a major part and major expense of fish feeds. The trend of increasing demand for fish oil in a market of static or dwindling supply (see below) further supports the need to investigate the suitability of non-fish sources of lipid ingredients. In general, when the price of fish oil exceeds that of soybean oil, the use of plant oils offers an advantage. Animal fats are favorably priced relative to fish oil most of the time.

While marine lipid sources such as menhaden, herring, anchovy, capelin, and sardine oils, as well as the oil that originates from fish silages, fish processing waste, and squid, have traditionally been used in aquafeeds, other lipid sources are already being included in feeds for some aquatic species. For instance, increased attention has been given to studying the nutritional value of other more sustainable animal lipid resources and processing by-products such as rendered fat from hogs (lard), chickens (yellow grease), and cattle and sheep (tallow), plus possibly oils recovered from fisheries by-catch or extracted from novel marine resources, such as krill, amphipods, and so on. Sustainable vegetable sources have also been studied and these include

soybean oil, canola oil, rapeseed oil, corn oil, safflower oil, linseed oil, and the lipid from white lupin. World production of plant oils in 1999 exceeded 100 mmt (Figure 6), whereas that of fish oil ranges from 1.2-1.4 mmt, and in El Nino years may be as low as 800,000 mt. Thus, supplies of plant oils and fats are more than sufficient to supply the needs of aquafeed production over the next century. Of the plant oils, soybean, corn, sunflower, rapeseed, and palm oils are the most available, whereas canola, lupin, other legume oils, olive, and linseed are less available. Transgenic oils are commercially unavailable at present, and may not be viable candidates for use in aquafeeds (for certain markets) due to consumer concerns. Prices for common oils and fats vary depending upon supply and demand, and when fish oil prices are low, fish oil is less expensive than plant oils. However, when prices for fish oil are high, plant oils are relatively competitively priced (Table 5). In the future, fish oil prices will generally exceed those of plant oils. Also, other lipids such as those from hemp, algae, single-cell sources and transgenic plants may also be considered in the future. Whether or not each of these oils or a combination thereof constitutes a suitable replacement for fish oils depends on the feed and lipid ingredients meeting the essential fatty acid requirements of the fish, the oxidative stability of the lipids, the extent of breakdown

Table 5. Range of prices of common oil and fat sources from 1989 to 1999 (USD\$/mt, Rotterdam)

Lipid source	Low	High
Soybean	335	642
Corn	461	638
Sunflower	413	730
Rapeseed	359	637
Palm	271	601
Fish oil	350	650

before and after incorporation into feed, the cost of the ingredient, and the effect, if any, of the lipid source(s) on the fatty acid composition of the fillet and on the lipid deposition

pattern in the whole fish. Fish health concerns (immunocompetence) also need to be considered as well as issues related to contaminants within the lipid sources.

LIMITATIONS OF ALTERNATIVE LIPID SOURCES

The main limitation of alternative lipid sources relates to their fatty acid composition relative to fish oils (Tables 6 and 7). Fish oils generally contain 20-30% n-3 fatty acids, whereas with the exception of linseed oil (52-55% n-3) and hemp, plant oils rarely contain more than 10% n-3 fatty acids. Further, the n-3 fatty acids of fish oils are mostly in the form of n-3 HUFAs, while plant oils do not contain HUFAs. Moreover, plant oils are rich sources of n-6 fatty acids, whereas fish oils contain low concentrations of these fatty acids. This difference has important implications for the fatty acid profiles of farmed fish, which in turn has important implications for human health and consumer perceptions (market value) of farmed fish products. Similar concerns exist for rendered fat products. These are also not rich in n-3 fatty acids and further are rich in saturated fatty acids, and may also be rich in n-6 fatty acids. Of these sources, poultry fat appears to have the most favorable fatty acid composition because it is a relatively rich source of monounsaturated fatty acids, which are important for human health. The fatty acid profile of fish fillets largely reflects the dietary lipid composition which is, in turn, is influenced by the fatty acid compositions of the dietary lipid sources. Thus, diet can potentially affect fillet storage quality, with fillets having high levels of highly unsaturated fatty acids being more susceptible to oxidation than those containing increased proportions of monounsaturated fatty acids. The fatty acid profile in the fillet can also affect the sensory properties of the fillet both in the raw and/or cooked form.

Table 6. Fatty acid composition of common marine fish oils and animal fats

Fatty acid	Anchovy	Capelin	Herrings, Atlantic	Menhaden	Redfish	Cod liver	Squid	Poultry	Beef tallow	Pork fat
16:0	17.4	11.1	12.7	19.0	13.2	13.5	14.0	21.6	24.9	23.8
16:1	10.5	11.1	8.8	9.0	13.2	9.8	7.0	5.7	4.2	2.7
18:0	4.0	1.0	0.9	4.2	2.2	2.7	8.0	6.0	18.9	13.5
18:1 (n-9)	11.6	17.0	12.7	13.2	13.3	23.7	12.3	37.3	36.0	41.2
18:2 (n-6)	1.2	1.7	1.1	1.3	0.9	1.4	2.7	19.5	3.1	10.2
18:3 (n-3)	0.8	0.4	0.6	0.3	0.5	0.6	0.7	1.0	0.6	1.0
20:1	1.6	18.9	14.1	2.0	17.2	7.4	9.0	0.1	0.3	1.0
20:4 (n-6)	0.1	0.1	0.3	0.2	0.3	1.6				
20:5 (n-3)	17.0	4.6	8.4	11.0	8.0	11.2	11.3			
22:1	1.2	14.7	20.8	0.6	18.9	5.1				
22:5 (n-3)	1.6	0.3	0.8	1.9	0.6	1.7	2.3			
22:6 (n-3)	1.8	3.0	4.9	9.1	8.9	12.6	16			
Total n-6	1.3	1.8	1.4	1.5	1.2	3.0		19.6	3.1	10.2
Total n-3	31.2	12.2	17.8	25.1	19.1	27.0		1.0	0.6	1.0

Source, NRC (1993); Gunstone *et al.* (1995).

Table 7. Fatty acid composition of common vegetable oils and *Sinapsis alba*

Fatty acid	Soya	Canola	Rapeseed	Corn	Sunflower	Safflower	Hemp	Linseed	Sesame	Palm	Olive	<i>Sinapsis alba</i> ^{1/}	Lupin
16:0	10.3	3.1	3.9	10.9	5.9	6.2	5.7	5.3	11.2	43.5	13.8	3.7	9.7
18:0	3.8	1.	2	1.8	4.5	2.2	4.2	4.1	5.8	4.3	2.2	1.9	1.5
18:1(n-9)	22.8	60.0	19.4	24.2	19.5	11.7	7.7	20.2	40.1	36.6	64.5	69.2	56.3
18:2(n-6)	51.0	20.2	14.3	58.0	63.7	74.1	56.4	12.7	44.7	9.1	16.2	10.5	15.1
18:3(n-3)	6.8	11.7	8.8	0.7	0.1	0.4	19.0	53.3		0.2		12.0	104
20:1	-	1.3	13.2				1.8					1.1	3.4
22:1	-	1.0	39.8										0.9
Total (n-6)	51.0	20.2	14.3	58.0	65.7	74.1	60.6	12.7	44.7	9.1	16.2	10.5	15.3
Total (n-3)	6.8	11.7	8.8	0.7	0.0	0.4	19.1	53.3	0.3	0.2	0.6	12.0	10.4

^{1/} Line T093-0876

Source, NRC (1993); Gunstone *et al.* (1995); Raney *et al.* (1999); Higgs *et al.* (2001, unpublished data)

Another strategy to supply lipids to aquafeeds is through the use of lipid-rich ingredients, such as full-fat soybean meal, poultry by-product meal, and possibly blended marine oil analogs that contain plant oils and/or rendered fats, plus a supplement of n-3 fatty acids.

PRACTICAL CONSIDERATIONS

There is less metabolic turnover of lipid stores within the body of post-juvenile salmon. Thus, once lipids are deposited in triacylglycerides, the fatty acid profiles of these lipid stores are more or less fixed. Given the need for harvested salmon to have a fatty acid profile that maintains the healthful image of salmon to the consumer, alternative oils cannot be fed exclusively throughout the production cycle. Rather, alternative oils will likely have to be restricted to the earlier stages of the salmon growth cycle, with fish oils or alternative oils containing high levels of n-3 fatty acids being used in the last stages of the salmon growth cycle to ensure that the fatty acid content of the edible product meets consumer expectations. Thus, research will be needed to determine suitable substitution levels of alternative lipids in salmon diets for growth and feed efficiency as well as fish health and reproduction. Further, there will be a need to establish phase-feeding programs that lead to maximum substitution levels yet maintain desirable fatty acid profiles in the final product. Research conducted to date on the effects of substituting alternative lipids in salmon diets suggests that using lipid sources that are rich in monoenes, and preferably concurrently rich in linolenic acid, rather than those rich in linoleic acid, facilitates enrichment of tissue lipids with n-3 HUFAs when finishing diets are fed during the final stages of fish culture before harvest (Dosanjh *et al.*, 1996).

CONCLUSIONS AND RECOMMENDATIONS

- Predicted supplies of fish oil will be inadequate to meet aquafeed demand
- Prices of fish oils will increase as demand rises

- Supplies of plant and rendered lipid sources are adequate to meet the future energy needs for aquafeed production
- Predicted future prices of plant and rendered lipid sources will be less than that of fish oil
- Use of plant and rendered lipid sources in aquafeeds will change the fatty acid composition of products
- Research is needed to determine acceptable dietary levels of alternative lipid sources in salmon and other aquafeeds
- Phase-feeding strategies must be developed to ensure consumer perceptions of the quality of salmon products are maintained
- Issues of food safety are paramount, and before there is introduction of new lipid sources (e.g. genetically modified oils) into salmon aquafeeds, thorough risk assessments and transparency of information/ingredients used are required

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