

Use of Soybean Meals in Diets of Salmon and Trout

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Introduction

Atlantic salmon, Pacific salmon and rainbow trout, collectively known as salmonids, are the most farmed carnivorous fish species in the world, mainly because they are prized food fish and relatively easy to culture. Salmon and trout can survive in a variety of environmental conditions, such as water temperatures from 0°C to as high as 28°C for some trout strains. They spawn successfully in water temperatures from 2°C to 15°C, and grow at temperatures from 6°C to 25°C. Depending upon their diet, salmon and trout can have pigmented (red) or non-pigmented (white) flesh. Salmon and trout are sometimes thought of as freshwater fish and other times as marine fish, but they are very adaptable to a variety of saline conditions.

Atlantic salmon, *Salmo salar*, are the most widely farmed salmonid species, with global production of 884,200 metric tons (mt) in 2000 (Table 1) (Johnson, 2002). Norway, Chile, Scotland (United Kingdom) and Canada are the top producing countries of Atlantic salmon (Table 2). Pacific salmon, e.g., chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*), are also farmed, mainly in Chile (coho), and Canada and New Zealand (chinook). Total global production of Pacific salmon is less than 100,000 mt. Total production of rainbow trout was 448,000 mt in 2000, making it the second largest production segment of the salmonids, behind Atlantic salmon (Johnson, 2002).

Until 1994, global production of rainbow trout exceeded that of other salmonid species. Top trout producing countries are France, Chile, Denmark and Italy, which account for 48% of global production (Table 3). Production of rainbow trout in marine cages was approximately 150,000 mt in 2000, about one-third of global trout production. The United States accounts for slightly less than 6% of global

trout production, producing 25,863 mt, 70% in the state of Idaho. In North America, Britain, Denmark, France and Italy, most trout farming occurs in freshwater, using flow-through water supply systems. In Chile and Scandinavian countries, rainbow trout are initially grown in freshwater farms followed by grow-out to harvest in marine cages. Trout can be acclimated to sea water once they reach about 100 grams in weight.

Global feed production for salmon and trout was approximately 1,636,000 mt in 2000 and is predicted to reach 2,000,000 mt before 2010. This amount represented 12.5% of total world production of feeds

Table 1: Global Production of Farmed Salmonids (2000)

<u>Salmonid species</u>	<u>Production (mt)</u>
Atlantic salmon	884,200
Rainbow trout	448,000
Coho salmon	66,090
Chinook salmon	16,000

Table 2: Production of Atlantic Salmon by Country (2001)

<u>Country</u>	<u>Production (mt)</u>
Norway	430,000
Chile	220,000
United Kingdom	142,000
Canada	94,000
Faroes	40,000
United States	23,600
Ireland	17,650
Others	92,000

for all fish and crustaceans, mainly shrimp, of 13,106,000 mt (Table 4). Worldwide, dietary fish meal constitutes 25%-35% of rainbow trout feed formulations, but this percentage varies with fish meal price, type of farming (freshwater or marine), and location. Rainbow trout feeds currently utilize about 176,000 mt of fish meal, about 3% of annual fish meal production (Table 5). However, this amount constitutes about 8% of the fish meal used in all aquafeeds, placing the trout sector in fifth place behind salmon, marine fish, shrimp and carp in fish meal consumption by aquatic species groups.

In terms of quantity, it is likely that trout feeds will remain at current levels of fish meal use in the next few years, despite predicted growth in global trout production of approximately 5% per year. This will be accomplished by replacing a portion of fish meal used in diet formulations with alternative protein sources. Salmon are the largest aquatic animal segment users of fish meal for diet production, using 454,000 mt in 2000. This quantity was 21.5% of all fish meal used in feeds for aquatic animals, and about 7% of average annual production of 6.2 million metric tons (mmt). Salmon diets are likewise expected to contain lower percentages of fish meal in the future.

Global fish meal production has been relatively constant in the past 15 years, but production declines as much as 15% when El Nino effects are severe (Hardy and Tacon, 2002). El Nino causes ocean warming off the coasts of Peru and Northern Chile, causing anchovies to move elsewhere, thus reducing the availability of anchovies that supports the world's largest fishery for fish meal production. Although Peru and Chile account for less than one-third of global fish meal production, they produce a large proportion of fish meal traded throughout the world, more than 60%. Other large fish meal-producing countries, such as Norway, utilize nearly all of their production domestically.

El Nino events have a large impact on global fish meal supplies and cost. Production often rebounds in the year after El Nino events, pushing global production over 7 mmt. During severe El Nino years, the price of fair and average quality (FAQ) fish meal can double, from a low of approximately \$330 United States Dollars (USD) per mt to more than \$600 USD per mt (prices free on board Peru). When this happens, salmon and trout feed manufacturers turn to alternatives, including

rendered products (where allowed) and oilseed proteins, mainly soybean meal.

Salmon and Trout Feeds and Feeding

Salmon and trout are carnivorous fish having relatively short digestive tracts consisting of an acid stomach, a pyloric caeca where digestive enzymes

Table 3: Production of Rainbow Trout by Country (2001)

<u>Country</u>	<u>Production (mt)</u>
France	48,750
Chile	42,656
Denmark	40,864
Italy	40,150
United States	25,863

Table 4: Estimated World Fish Feed Production by Species Groups (2000)

<u>Species Group</u>	<u>mt of Feed</u>	<u>% of Total</u>
Salmon/Trout	1,636,000	12.5%
Shrimp	1,570,000	12%
Catfish	505,000	4%
Tilapia	776,000	6%
Marine finfish	1,049,000	8%
Cyprinids (Carp)	6,991,000	53%
Miscellaneous	579,000	4.5%
Total	13,106,000	

Table 5: Estimated Fish Meal Use in Aquafeeds (2000)

<u>Species Group</u>	<u>mt of Fish Meal</u>	<u>% of Total</u>
Salmon	454,000	21.5%
Marine fish	415,000	19.6%
Shrimp	372,000	17.6%
Carp	350,000	16.5%
Trout	176,000	8.3%
Eels	173,000	8.2%
Flatfish	69,000	3.3%
Other Fish	106,000	5.0%

are released, a small intestine where most nutrient absorption occurs, and a large intestine for water and electrolyte re-absorption. The requirements of trout for essential dietary nutrients have been estimated, and trout are known to require about 40 nutrients, similar to other animals (Table 6) (National Research Council, 1993). They can obtain most minerals directly from water, if mineral levels are high enough, except for phosphorus. Fish meal-based salmon and trout diets contain adequate amounts of most essential minerals to meet their needs directly, regardless of rearing water concentrations. However commercial feeds contain trace mineral supplements. When salmon and trout diets contain high amounts of plant-derived proteins in place of fish meal, additional mineral supplementation is necessary.

Prior to the development of the fish feed manufacturing industry in the late 1950s and 1960s, feeds for salmon and trout were produced on-site by hatchery staff. During the 1920s and 1930s, salmon and trout were fed a variety of feeds based on the local availability of ingredients (Hardy and Barrows, 2002). These ingredients included salmon eggs; fresh, canned or frozen fish; oilseed meals; and brewers yeast. These were combined with beef liver, spleen, horse meat, chicken eggs and cottage cheese. Mixtures such as one-third beef liver, one-third hog liver and one-third salmon viscera were chopped and mixed at the hatchery, 2% salt was added to congeal the mixture, and it was delivered to the fish by a hatchery worker using a spoon or ladle. These feeds contained about 60% moisture with a texture similar to moist sawdust (Hastings and Dickie, 1972). The feed cost per pound of fish ranged from \$0.032 to \$0.57 (Donaldson and Foster, 1937).

In the 1940s, the demand for ingredients used in wet fish feeds increased due to increased hatchery production and to competition from other users. To extend the traditional ingredients, meat-meal mixtures for feeding salmon and trout were developed. These feeds were blends of slaughterhouse byproducts and dry, commercially available feed ingredients. Cortland dry feed mixture No. 6, consisting of 24% each of dry skim milk, cottonseed meal, white fish meal and wheat middlings, with 4% salt added, was a typical dry feed (Phillips et al., 1940). This mixture was blended with an equal weight of beef liver and hog spleen, water was added, and the mixed material was squeezed through a potato ricer to feed small fish in

troughs. Cortland dry feed mixture No. 10 was the same as No. 6, except that soybean meal replaced the cottonseed meal.

Soybean meal was first tested in dry feed mixtures in the early 1940s by the Cortland research team. Feeding trials using brook trout at the Cortland hatchery in New York indicated that growth rates equivalent to those obtained using a meat diet were achieved using the meat-dry meal mixtures. Feed cost per unit of production was reduced by about half (Tunison et al., 1941).

The first dry pellets were reported to increase trout hatchery production by 60% while reducing feed cost by 40% (Grassl, 1956). These formulas did not include a supplemental vitamin premix, so it was necessary to feed beef liver to the trout once every three weeks. Addition of a vitamin mixture to the dry pellet formulation permitted the successful rearing of trout to spawning and subsequent rearing of fry (Phillips et al., 1964). The early formulations of scientists from the Cortland Laboratory and the Abernathy Station provided the basis for the development of current dry pellet formulations produced for salmon and trout throughout the world (Phillips et al., 1964; Fowler and Burrows, 1971). Open formula diets for salmon and trout are modified continually and tested by various governmental agencies to improve fish production efficiency. Recent specifications for the Abernathy salmon diets are shown in Table 7.

Table 6: Required Dietary Nutrients for Salmon and Trout

Protein	10 essential amino acids
Lipids	Omega-3 fatty acids (1% of diet)
Energy	Supplied mainly from lipids and protein
Vitamins	15 essential vitamins
Minerals	10 minerals shown to be essential*
Astaxanthin	Need for viable eggs Pigments

**Note: Other minerals are probably essential, but can be obtained from rearing water.*

Development of Extruded Pellets for Salmon and Trout

Extruded pellets are formed by extrusion of a moist mixture heated from 100°C to 150°C under pressure (20%-24%) followed by drying to reduce the moisture content to 10% or less. Moisture remains in a liquid state in the barrel of the extruder and changes instantaneously to vapor (steam) as the pellets pass through the die and pressure is released. This expands the pellets, lowering density and changing buoyancy. By varying the feed mixture, moisture level and extrusion conditions, pellet density can be altered so that pellets will sink rapidly, sink slowly or float. For the catfish industry, floating pellets are the norm; farmers want to see feeding activity on the surface of ponds. For the salmon industry, pellets that slowly sink in seawater are produced. Given the porous nature of extruded pellets caused by sudden water vaporization, high levels of fish oil (or plant oil) can be added to the pellets to produce high-lipid diets. Since they are dry, extruded pellets can be used in automatic or demand feeders, a desirable characteristic for salmon and trout farming.

Feed formulations for salmon and trout have changed greatly since extrusion pelleting has been

introduced. Prior to the late 1980s, diets were produced using compressed (steam) pelleting, a process that produces a dense, hard pellet. Compressed pellets cannot absorb as much added lipid as can extruded pellets, limiting total lipid to about 20%. With extruded pellets, total lipid levels of less than 35% can be realized. Of this amount, no more than 10-12% lipid is present in the feed mixture prior to pelleting; the remainder is added after pelleting (top-dressing). Given this high lipid level, there is not much room in feed formulations for anything other than fish meal and perhaps a small percentage of another protein source, enough starch or ground wheat to hold the pellet together, vitamin/mineral premixes and fish oil.

Salmon feed formulations have changed in the past 20 years, primarily the result of the adoption of high-energy diets. These formulations were embraced in part so that the salmon farming industry could meet European Community regulations governing nutrient losses from farms. Feeds were required to become more efficient (lower feed conversion ratios) and to contain low percentages of phosphorus. As a result, formulations were simplified as described above and shown in Table 8. Similar formulations are used worldwide in the salmon farming industry. Approximate composition

Table 7: Open-Formula Diet Specifications for Dry Salmon Diets

Ingredient	Percent in Diet		
	Starter S8-2	Crumbles (18-2)	Pellets (19-2)
Herring meal	58	55	50
Dried whey	5	5	5
Blood flour (or meal)	10	10	10
Condensed fish solubles <i>or</i> Poultry byproduct meal	3	3	3
Wheat germ meal	1.5	1.5	1.5
Wheat middlings mill-run <i>or</i> shorts	----	5	5
Vitamin premix	Remainder	Remainder	Remainder
Choline chloride (60%)	1.5	1.5	1.5
Ascorbic acid	0.58	0.58	0.58
Trace mineral mixture	0.1	0.1	0.1
Lignon sulfonate pellet binder	0.05	0.1	0.1
Fish oil <i>or</i> Soybean lecithin (max. 2%)	2.0	2.0	2.0
	12	9	9

Abernathy Diets, 1986

of salmon diets has changed also, with total protein decreasing and total lipid (fat) increasing (Figure 1). Digestible protein has not changed as much as total protein because feed manufacturers have eliminated protein sources having lower apparent digestibility coefficients (ADCs) from their formulations, limiting protein sources to those having high ADC protein values.

In contrast, dietary protein levels in trout feeds have increased from 35% to 45% in the last 35 years, and dietary fat levels now exceed 22% in high-energy feeds (Figure 2). In the 1960s, feed conversion ratios (FCR) were about 2.0, but today, the best commercial, high-energy trout feeds yield feed conversion ratios from 1.2:1 to as low as 0.8:1, when their use is combined with good feeding practices). Similar changes have occurred with respect to FCR values for salmon feeds with the adoption of high-energy diet formulations (Figure 3). Feed formulations for rainbow trout utilize fish meal, fish oil, grains and various byproducts derived from production of other food products, e.g. meats and poultry (Table 8).

To lower the percentage of fish meal used in rainbow trout feeds, researchers have substituted other protein sources such as soybean meal, poultry byproduct meal, and small amounts of blood meal and feather meal. In the past decade, the percentage of fish meal used in trout feeds has decreased by about 50% as a result of the use of alternate protein ingredients.

Trends in Salmon and Trout Diet Formulations

Salmon and trout farming has reached a critical period in its evolution, as the foods that it produces are becoming commodities. Similar developments occurred in the poultry and swine industries, and continued production gains depended on genetic selection, changes in husbandry, and improvements in diets and health maintenance. In the case of salmon and trout diet formulations, changes to date have involved: the use of higher quality feed ingredients, particularly protein sources; elimination of high-fiber, plant-derived ingredients; the use of extrusion technology to produce pellets; and the adoption of high-energy, nutrient-dense formulations. These developments will remain, and formulations will advance in terms of refinement, embracing the concept that every ingredient used in

Table 8: Typical Salmon and Trout Diet Formulations

<u>Ingredient</u>	<u>Salmon</u>	<u>Trout</u>
Fish meal	51%	22-25%
Soybean meal	8%	<15%
Animal byproduct meals	0%	~15%
Wheat/wheat byproducts	8%	22%
Corn gluten	0%	<5%
Vitamins/minerals	2.2%	1%
Fish/soy oil	30.8%	7-8%
Crude protein	44%	44%
Crude lipid	>34%	16%

a salmon and trout formulation must be there for a specific reason. Further, it is likely that ingredients will be produced specifically for use in fish feeds. Initially, this will entail the improvement of protein sources derived from fish or animal byproducts to reduce levels of bones and poorly digested fractions, such as skin and connective tissue, and the use of starch fractions from grains rather than ground whole grains.

Superimposed on these expected developments is the issue of limited global supplies of fish meal and fish oil. This issue is becoming critical. The percentage of global fish meal production used in fish feeds has increased from less than 10% prior to 1990, to more than 40% in 2002. In the case of fish oil, the situation is even more critical. Prior to 1990, less than 10% of global fish oil production was consumed annually by the fish feed manufacturing sector, but by 2002, the percentage increased to more than 75%, mainly at the expense of use of fish oil to produce margarine. If current production trends continue upward, fish oil supplies will be inadequate to meet the needs of the fish feed manufacturing industry within a few years. Given the high use of fish oil in salmon and trout feeds, this segment of the aquaculture industry will feel the impact of insufficient supplies most acutely.

Predictions of fish meal use in various sectors of the aquaculture industry call for lower use levels in feeds for most species groups (Table 9). The percentage of fish meal use in salmon and trout feed formulations is predicted to decrease by 10% and 5%, respectively, by 2010 (Barlow, 2000). This is expected to lower the actual use of fish meal by

77,000 mt and 29,000 mt for salmon and trout, respectively. This change will require higher use levels of alternative protein sources to replace the fish meal protein removed from diets. Even greater reductions in fish oil use will be needed, perhaps as much as 50% of current use levels. Plant oils will be needed to replace this fish oil.

Environmental Considerations in Salmon and Trout Farming

Salmon and trout farming does not consume water, but it does enrich water with nutrients. These nutrients reduce water quality in lakes and rivers into which farm effluents are discharged by increasing algae and aquatic plant growth. To control this, farms in Europe and the United States are subject to regulations limiting the levels of solids and nutrients in farm effluents. Phosphorus is the main nutrient of concern in effluents. Unassimilated nutrients originate in uneaten feed, feed dust, feces

and metabolic excretions (urine and gill wastes). Many farms have quiescent zones at the ends of raceways where fish are excluded and particles settle without being disturbed. Settled material is regularly removed and this practice significantly lowers the amount of solids and phosphorus in farm effluents. Phosphorus in hatchery effluents is present in two forms: solid particles (e.g., bones and other insoluble forms), and soluble phosphorus excreted by fish in urine. Although particulate phosphorus can be collected and removed, soluble phosphorus cannot be removed economically because it is present in very low concentrations in very large quantities of water. Thus, limiting the amount of digestible phosphorus in feeds to the amount needed by the fish is the approach used to produce low-polluting feeds. Using this approach, the amount of soluble phosphorus excreted by fish has been reduced to very low levels.

Figure 1: Changes in Protein and Fat Levels in Atlantic Salmon Feeds Over the Past Two Decades

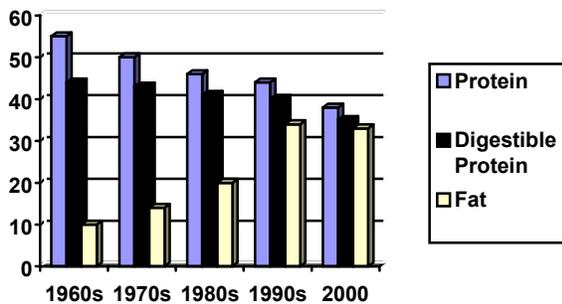


Figure 2: Changes in Protein and Fat Levels in Trout Feeds Over the Past Two Decades

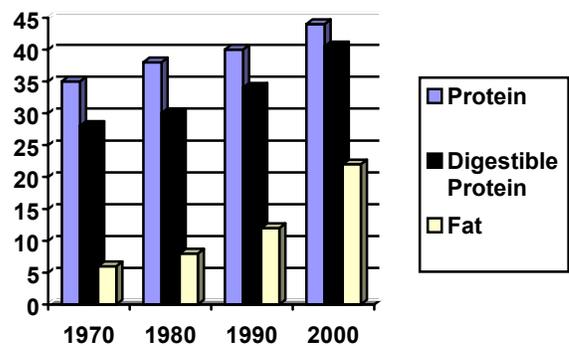
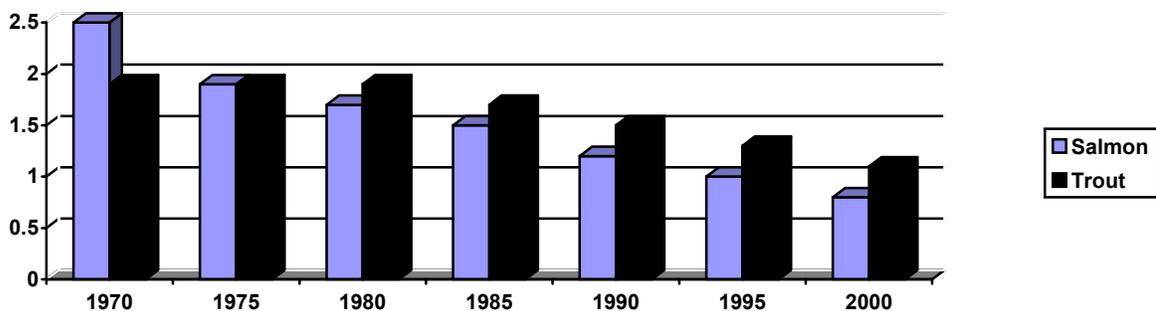


Figure 3: Changes in Feed Conversion Ratios in Salmon and Trout Feeds Over the Past Two Decades



Reducing the amount of insoluble phosphorus in salmon and trout feeds requires two approaches: (1) using low-phosphorus feed ingredients; and (2) increasing the bioavailability of phosphorus in feed ingredients by adding phytase, for example (Cain and Garling, 1995). High-ash feed ingredients, such as fish meal, meat and bone meal, and poultry byproduct meal, contain high amounts of phosphorus associated with bones (Sugiura et al., 2000). Plant protein ingredients, such as soybean meal, are low phosphorus ingredients. Replacing fish meal with soybean meal lowers the total phosphorus content of the diet. However, about 75% of the phosphorus found in soybean meal is bound to phytic acid (Hardy, 2002). This form of phosphorus, called phytate phosphorus, is indigestible to monogastric animals, like fish. As mentioned above, phosphorus can be released from phytate by the enzyme phytase, a natural constituent of seeds. Phytase is available as a feed supplement, but the enzymatic activity of phytase is destroyed by the heat associated with pelleting. Therefore, at present, phytase must be added to pellets by top-dressing.

Current Use Levels of Soy Products in Salmon and Trout Diets

As discussed earlier, soybean meal has been used in diets for trout for nearly 60 years, but only in diets for post-juvenile fish and only in limited amounts. Other soy products evaluated or used in salmon and trout diets are soy protein concentrate, soy isolate, full-fat soybean meal and soy oil. Solvent extracted, dehulled soybean meal (48% crude protein) is the meal most commonly used in salmon and trout diets.

Current use levels in trout diets are no more than 20% soybean meal in post-juvenile diets (grow-out diets). Given an estimated global production of 503,000 mt of trout feed, and assuming that 75% of this total is fed to post-juvenile fish, an estimate of maximum soybean use in trout diets worldwide is 75,428 mt. Similar calculations can be applied to salmon diets, although other factors suggest that a lower estimate is more reasonable, as discussed below. This leads to a maximum estimate of soybean meal use in diets of grow-out salmon of 151,000 mt. In Chile, soybean meal is subject to import duties, although product originating in Bolivia, Paraguay or Brazil is exempt from the high duties imposed upon North American product. As a result, soybean meal is used at levels between 5 to 10%, depending on the price of fish meal. Chile is the second largest

producer of farmed salmon in the world, so these factors have a significant impact on global soybean meal use in salmon diets. The situation is different in Norway and Scotland, where soybean meal use in salmon diets can be as high as 25% when economics so dictate. The best estimate of global soybean meal use in salmon diets, taking these factors into account, is between 100,000 mt and 110,000 mt.

Use of other soy protein sources is minimal in salmon and trout diets. Soy protein concentrates and soy isolates are sometimes used in diets for fry, or for specialty diets custom made for growers willing to pay higher feed prices to obtain low fish meal diets that do not contain rendered products. Full-fat soybean meal is not widely used, despite positive research reports concerning its use in salmon and trout diets (described below). Soy oil is a leading candidate to replace a portion of fish oil in salmon diets, especially in the United Kingdom. However, canola oil is a leading competitor, and price dictates use patterns. It is impossible to estimate use levels at present because feed manufacturers vary in their ability to handle both fish and vegetable oils at plant sites. This requires duplicating storage and in-plant conveying of oils unless oils are combined. The industry is currently in the process of evaluating various options for contending with the prospects of insufficient fish oil supplies, and no clear consensus regarding use strategies has yet emerged.

Estimates of future protein requirements for aquaculture feeds depend on future production from various segments of the aquaculture industry and the supplies of fish meal. Barlow (2000) predicted that aquaculture feed production would increase from 13,098,000 mt in 2000 to 37,226,000 mt in 2010 (Table 10). At today's fish meal use levels in formulations for various industry segments, the amount of fish meal needed to produce 37,226,000 mt of fish and shrimp feed would be 4,081,000 mt in 2010, up from 2,115,000 mt in 2000. This slightly exceeds the amount of fish meal traded worldwide in non-El Nino years.

Barlow predicts that the percentage of fish meal in fish feed formulations will decrease and that total fish meal use by the aquaculture industry will be 2,831,000 mt in 2010 (Table 9). The difference of 1,250,000 mt (between 4,081,000 mt and 2,831,000 mt) will be supplied by other protein sources. If fish meal used in fish feeds contains 70% crude protein, 875,000 mt of protein from sources other than fish

meal will be needed annually in fish feeds by 2010. If soybean meal (48% crude protein) were used to supply this protein, the increase in total use in all aquaculture feeds would be 1,822,917 mt. If soy protein concentrate were used, the total would be less, approximately 1,115,000 mt, because of its higher protein content. Salmon and trout feed production currently accounts for 12.5% of global fish feed production. It is likely that this percentage will drop by 2010, as other species sectors expand. If salmon and trout feed production is 7% of global fish feed production by 2010, then 2,606,000 mt of feed will be produced.

At today's use levels of soybean meal in salmon and trout grow-out feeds (approximately 20%), by 2010 391,000 mt will be used annually in salmon and trout diets. If soy protein concentrate were to replace 40% of fish meal in salmon and trout diets by 2010, the potential market would be approximately 780,000 mt. This level of replacement has been demonstrated to be feasible (Stickney et al., 1996; Storebakken et al., 1998; Mambrini et al., 1999).

Full-fat soybean meal has been evaluated in diets for trout, Atlantic salmon and chinook salmon (Smith, 1977; Reinitz et al., 1978; Tacon et al., 1983; Wilson, 1992; Bjerkgeng et al., 1997; Davies et al., 1997). In most studies, full-fat soybean meal was acceptable at levels equal to or exceeding those found suitable when soybean meal is used, perhaps because full-fat soybean meal is often extruded prior to its incorporation into diet mixtures. The additional heat associated with extrusion of full-fat soybean meal lowers the level of trypsin inhibitor activity

(Wilson, 1992) and likely lowers the levels of other heat-sensitive anti-nutritional factors. Full-fat soybean meal has the advantage of adding both protein and lipid to the diet, an important consideration with respect to global fish oil supplies. Providing that the full-fat soybeans are properly extruded to lower levels of anti-nutritional factors (described below) they may see increasing use in the future. At present, however, their use in commercial salmon and trout feeds is minimal.

Nutritional Considerations Affecting Soy Product Use in Salmon and Trout Diets

Fish are similar to other animals in that they require 40 or more nutrients in their diets, including the same 10 essential amino acids required by mammals and birds. Fish meal has been the protein source of choice in salmon and trout diets because of its relatively high protein content (65-72%, depending on the species from which it is produced), and the fact that the amino acid profile of fish meal is similar to the amino acid requirements of salmonids. The protein content of dehulled soybean meal is 48% and the amino acid profile, while not as close to the dietary requirements of salmon and trout as fish meal, is sufficient in most essential amino acids, except for methionine. Further, residual oil in fish meal contributes essential fatty acids, e.g., long-chain omega-3 fatty acids, especially eicosapentanoic acid (EPA, C20:5 n-3), and docosahexanoic acid (DHA, C22:6, n-3). Soybean oil contains about 8% linolenic acid (C18:3, n-3) that can be lengthened and desaturated to long-chain omega-3 fatty acids by salmon and trout. Soybean

Table 9: Fish Meal Use by Species Group (% in diet and 000mt), Showing an Increase of 38% in Fish Meal Use by 2010

Species Group	2000	2010*	2000	2010*
Catfish	3%	0%	15 mt	0 mt
Salmon	40%	30%	454 mt	377 mt
Trout	30%	25%	176 mt	147 mt
Marine Fish	45%	40%	415 mt	688 mt
Flatfish	55%	45%	69 mt	263 mt
Shrimp	20%	20%	372 mt	485 mt
Carp	5%	2.5%	350 mt	675 mt

Source: Barlow (2000)

meal contains less than 2% residual oil and negligible amounts of linolenic acid.

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 (Storebakken et al., 2000). These vary in practical significance, and there are a number of uncertainties with respect to the effects of various anti-nutritional factors on fish health or performance (Dong et al., 2000). Soy products are heated during processing, and the degree of heat exposure influences the level or activity of several anti-nutritional factors present in soybeans. The principal compounds of concern in soybean meal are trypsin inhibitors, which reduce protein digestibility by binding with the digestive enzyme trypsin in the intestine of the animal (Krogdahl et al., 1994).

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 levels, 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 (Krogdahl et al., 1994; Rumsey et al., 1995). However, overheating 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) per milligram 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 mg to 3.5 mg trypsin inhibited per gram sample (Tacon et al., 1983). Further heating occurs during feed pelleting, especially during cooking-extrusion pelleting, 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 trypsin inhibitor levels (Arndt et al., 1999; Mambrini et al., 1999).

Given the importance of heat treatment on trypsin inhibitor levels in soybean meal, tests to determine the adequacy of heat treatment of soybean meal are critically important. 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 (Vohra and Kratzer, 1991). Chemical tests to detect underheated soybean meal are determined by urease activity, trypsin activity and protein solubility. Urease, an enzyme naturally present in soybeans, 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. Urease activity in commercial soybean meal ranges from 0.02 to 0.1 increase in pH (Vohra and Kratzer, 1991). Values that have more than a 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

Table 10: Predicted Use of Fish Meal in Aquafeeds in 2010

Compared to amount needed if percentage of fish meal in feeds remains the same as 2000

	Feed (000 mt)	Fish meal (000 mt)
2000 (est.)	13,098	2,115
2010 (today's use levels)	37,226	4,081
2010*		2,831

Difference for 2010 at today's use levels and Barlow's prediction - 1,250,000 mt

Barlow (2000)

a urease activity of more than 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 (Vohra and Kratzer, 1991). The method has been slightly modified by extracting the sample in 0.2% potassium hydroxide (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 to 1.0 (Araba and Dale, 1990).

Soy products contain compounds that influence feed intake, gut histology and immunological function (Rumsey et al., 1995; Storebakken et al., 2000). Complete replacement of fish meal with soybean meal in trout feed lowers growth, primarily by lowering feed intake, but soybean meal levels of 20% or less have little or no effect on trout performance. Replacement of fish meal with soybean meal at levels between 20% and 40%, e.g., 29% soybean meal and 42% fish meal in the diet, have had variable effects on feed intake or growth, depending on the study (Rumsey et al., 1995; Medale et al., 1998; Refstie et al., 2000). This variance is perhaps associated with the source of the soybean meal, feed formulation or experimental design. Thus, studies involving partial or total replacement of fish meal with soybean meal in diets for rainbow trout are difficult to compare.

Early studies used diets that were low in total lipid compared to today's diets (Reinitz, 1980; Dabrowski and Wojno, 1977; Tacon et al., 1983; Alexis, 1990). This is significant because fish oil likely contributes to feed palatability. Given the increase in lipid content associated with fish oil addition to high-energy feeds, the negative effects on feed intake associated with the addition of soybean meal to salmon and trout diets may be somewhat reduced with today's feeds. In some studies, experimental diets differed in protein content, thus confounding interpretation of results (Pongmaneerat and Watanabe, 1992; Pongmaneerat and Watanabe, 1993; Watanabe and Pongmaneerat, 1993). In carefully-conducted studies designed to evaluate the effects of soybean meal inclusion in diets for rainbow trout, no differences have been observed up

to 40-50% replacement (Refstie et al., 2000). Medale et al. (1998) showed that trout feed intake decreased stepwise as the level of soybean meal in the diet increased. However, trout are reported to adapt to diets containing soybean meal, increasing feed intake after a period of adaptation from pre-study diets that nearly always contain fish meal. This suggests that the effects of soybean meal on feed intake may be temporary, at least in some feed formulations.

In an interesting study on adaptation to a diet containing soybean meal, two groups of rainbow trout were fed either a fish meal-based diet or a diet containing 60% soybean meal and weight gain was measured for two periods (Refstie et al., 1997). During the first 28 days, gain was 40% higher in the group fed the fish meal-based diet, but in the second 28 day period, weight gain was similar. Refstie et al. (1997) then fed trout a fish meal-based diet or a 40% soybean meal diet for seven days, after which the fish were offered a 1:1 mixture of the two diets. Diets were supplemented with different inert markers, allowing the researchers to estimate feed preference by analyzing the proportion of the inert markers in the feces of the fish. Fish adapted to the fish meal-based diet overwhelmingly preferred that diet, but those adapted to the 40% soybean meal diet consumed it in a 6:4 ratio, eating slightly more of the fish meal diet. The authors suggested that the ratio might have been nearly even had the adaptation period been longer. No similar studies have been conducted with salmon.

Some studies with Atlantic or Pacific salmon suggest that lower dietary soybean meal levels may be warranted in salmon compared to rainbow trout. Pacific salmon fingerlings are very sensitive to even small percentages of soybean meal in the diet and feed intake was severely reduced as dietary soybean meal was increased (Fowler, 1980). Growth of Atlantic salmon smolts was reduced when fish meal was replaced with soybean meal during freshwater rearing, mainly the result of lower nutrient digestibility (Olli et al., 1994). Subsequent studies with post-juvenile Atlantic salmon reared in seawater confirmed that growth was 44% higher in fish fed a diet without soybean meal compared to a diet containing 30% soybean meal (Refstie et al., 2000). Feed intake was not a factor in the differences in weight gain. Digestibility values for fat and energy were 16% and 9% higher for the fish meal diet than for the diet containing soybean meal.

Alcohol-soluble components of soybean meal are reported to lower fat digestibility in Atlantic salmon (Olli and Krogdahl, 1995) and also have been implicated in distal intestine enteritis (van den Ingh et al., 1996). Fish can recover from distal enteritis when fasted (Baeverfjord and Krogdahl, 1996).

Including soybean meal in diets is reported to alter the intestine of salmon and trout. 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 et al., 1995). In Atlantic salmon, an inflammation of the distal intestine (enteritis) when soybean meal is included in the diet has been reported by many researchers (van den Ingh et al., 1991; Refstie et al., 2000; Refstie et al., 2001). This condition is associated with higher moisture levels in feces, suggesting more rapid passage through the gut, with less time available for digestion and absorption to occur. It is not known what component of soybean meal causes intestinal changes, although alcohol-soluble components have been suggested as a factor in the development of distal intestine enteritis (van den Ingh et al., 1996).

Soy protein concentrate production involves extraction steps that remove the component of soybeans that causes distal enteritis (Storebakken et al., 2000). Rainbow trout performance is unaffected by replacing 50% of dietary protein with soy protein concentrate (Stickney et al., 1996; Medale et al., 1998; Mambrini et al., 1999).

Other components of soybean products postulated to affect growth, feed intake or metabolism of salmonids are saponins, isoflavones, oligosaccharides and phytate. Methanol extraction of soy flour improved growth rate of chum salmon fingerlings fed diets in which 77% of the fish meal protein in the diets was replaced, providing that essential amino acids were supplemented, but growth was significantly less than fish fed the fish meal-control diet (Murai et al., 1987). Purified ethanol extracts containing saponins were added to the diets of chinook salmon and rainbow trout and found to significantly lower feed intake (Bureau et al., 1998). The authors postulated that saponins are the component of soybean meal and soy protein concentrates (plus some soy isolates) that lowers

palatability of feeds containing soy products. Isoflavone levels are highly variable in soy products, but to date no definitive research has demonstrated that trout growth is affected by high levels (Mambrini et al., 1999).

The effects of oligosaccharides in soybean meal on feed intake or growth performance of Atlantic salmon or trout are not clear. However, when salmon were fed a diet containing 40% of a soybean meal having lower than normal levels of oligosaccharides, trypsin inhibitors, lectins and soy antigens, feed intake and fish weight gain were similar to those of fish fed a fish meal-control diet, and both groups performed better than fish fed a diet containing regular soybean meal (Refstie et al., 1998). The levels of lectin were 0.15 milligrams per gram (mg g^{-1}) and 0.04 mg g^{-1} in regular and special soybean meal, respectively. Trypsin inhibitor activity was 0.13 mg g^{-1} and 0.09 mg g^{-1} , respectively. Oligosaccharide levels were not reported. The relative importance of these anti-nutritional components of soybean meals remains unknown.

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 et al., 1995). Further research is needed to determine if low-antigen soy products are advantageous with respect to immune function in salmonids. Susceptibility to furunculosis, caused by *Aeromonas salmonicida* ssp. *salmonicida*, is higher in Atlantic salmon fed diets containing soybean meal compared to fish fed diets containing fish meal or soy protein concentrate, presumably because the soybean meal diet caused distal enteritis, and the pathogen is presumed to infect fish via the gut (Krogdahl et al., 2000). Immune function may also be affected when diets containing soybean meal are fed (Bakke-McKellep et al., 2000).

Soybean meal and soy protein concentrate contain approximately 8.0 mg and 8.2 mg phosphorus kg^{-1} , of which about 75% is present as phytic acid. The processes used to produce soy protein concentrates increase the level of phytic acid to 1.3% to 2.2% (Storebakken et al., 1998). The enzyme phytase releases phosphorus from phytic acid, and the addition of phytase significantly increases phosphorus availability in soybean products from less than 40% to more than 94% (Hardy, 2002).

To summarize the anti-nutritional factors of interest in salmon and trout nutrition, trypsin inhibitors, saponins and phytate are the factors appearing at present to have the potential to lower fish performance when soy products are included in salmon and trout diets. Heat treatment affects several, but further processing, e.g., extraction or enzyme treatment, are required to deal with the others (Table 11).

Effect of Diet on Product Quality

Studies designed to examine the effects of soybean products on sensory attributes of salmon and trout are rare. No differences in taste or flavor of rainbow trout fillets was found when fish were fed diets containing protein sources primarily of animal (including fish) protein sources or plant protein sources (Smith et al., 1988). Differences in sensory and physical characteristics of trout fillets from fish fed 33% or 100% replacement levels of soy protein concentrate or 50% soybean meal for fish meal have been reported, but the differences were not dramatic or described in detail (Kaushik et al., 1995). Substituting full-fat soybean meal for 10% of the fish meal in a diet for Atlantic salmon resulted in no significant differences compared to salmon fed the control (no soy) diet (Bjerkeng et al., 1997).

The omega-3 fatty acid content of trout fillets depends upon the omega-3 content of their feed, but averages 22% of fillet lipids when fish are fed diets containing fish oil. The current trend is toward replacing up to 50% of added fish oil with plant oil in grow-out feeds for trout and salmon. The effects of such replacement on fatty acid composition of fillets are likely to be an elevation of linoleic acid (C18:2, n-6) if soybean oil is used, as has been reported in Atlantic salmon (Hardy et al., 1987). Sensory attributes of salmon and trout fed diets

containing plant oils are reported to be altered, with panelists preferring samples from fish fed diets containing plant oils over those receiving all fish-oil diets (Skonberg et al., 1993).

Future Outlook

Aquaculture is the fastest growing segment of livestock production worldwide. It is expected to exceed cattle farming in terms of total production by 2010. Aquaculture is growing in large part because production is switching to more intensive, higher-input systems. The main input is feed, and as mentioned earlier, feed production for aquatic animals is expected to nearly triple by the end of the decade. This growth absolutely dictates greater use of protein sources other than fish meal. Soy products are the leading candidates to supply this protein (and possibly oil).

Further research is needed to determine the importance of various anti-nutritional factors in soy products on fish performance, and to either develop cultivars to minimize the presence of these factors, such as the development of low-phytate cultivars, or to develop processing methods to lower their levels in final products. Further research is also needed to identify the components of soy responsible for reducing feed intake and for causing distal enteritis in salmon and trout.

Feed processing, e.g., extrusion, requires further study as well, given the prospect that the relatively high temperatures and pressures of extrusion could increase or decrease nutritional value, depending upon operating conditions (Cheng and Hardy, 2002). Most attention has thus far been given to soybean meal, but extruded full-fat soybeans may be increasingly useful in salmon and trout diets because they contribute both protein and lipid to the diet.

Table 11: Anti-Nutritional Factors in Soy Products and Processing Steps to Remove or Inactivate Them

<u>Anti-Nutrient</u>	<u>Heat Sensitive</u>	<u>Extractable</u>	<u>Other Treatment</u>
Trypsin inhibitors	yes	no	no
Hemagglutinin	yes	no	no
Phytic acid	no	no	phytase
Saponin	no	yes	no
Phytoestrogen	no	yes	no
Anti-vitamin	yes	?	no

Overall, however, the prospects are very encouraging for soy products as future components of salmon and trout diets.

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Managed Aquaculture Program

This technical review paper was created through the Managed Aquaculture Marketing and Research Program (AquaSoy Initiative), funded through the United Soybean Board and American Soybean Association. The AquaSoy Initiative is designed to remove the barriers to soybean meal use in diets fed to aquaculture species. The program has been divided into two components, one focused on awareness, the other on research.

The awareness program initially focuses on Southeast Asia and India, where there are significant opportunities to intensify production within established aquaculture industries with the use of soybean meal-based diets.

The focus of the research component is salmonids, specifically rainbow trout and Atlantic salmon, and commercial crustaceans, all of which are large industries currently underutilizing soybean meal. The highly integrated and collaborative nature of this initial series of projects should result in expansion of soybean meal into new rapidly growing existing markets in North America, Europe and Asia.

This paper is one of a series of four technical review papers prepared by aquaculture specialists that summarize soy product use and potential in the diets for key aquaculture species groups. The technical reviews address the following species groups: 1) freshwater omnivorous fish; 2) marine fish; 3) marine shrimp; and 4) salmonids. All of these papers can be viewed at www.soymeal.org.



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