New developments in aquatic feed ingredients, and potential of enzyme supplements[1]

Ronald. W. Hardy

Hagerman Fish Culture Experiment Station, University of Idaho, 3059F National Fish Hatchery Road, Hagerman, ID 83332, USA

ABSTRACT: Aquaculture production has expanded at a rate of 15% per year and is predicted to continue to grow at this rate for at least the next decade. Demands on traditional fish feed ingredients, mainly fish meal and oil, which are finite global resources, are increasing. At present, global fishmeal production averages 6.5 mmt per year, of which 23% is utilized in feeds for farmed fish. Global fish oil production averages 1.4 mmt per year, and 25% of this yearly production is utilized in fish feeds. Up to now, 70% of the fish meal and oil used to produce farmed fish has been consumed by salmon, trout and shrimp, despite the fact that these species account for only 30% of global fish feed production and only 7% of global aquaculture production. Clearly, expanded production of carnivorous species requiring high protein, high-energy feeds will further tax global fish meal and oil supplies. Suitable alternative feed ingredients will have to been utilized to provide the essential nutrients and energy needed to fuel the growth of aquaculture production. Rendered products, seafood processing waste, including by-catch, and grain and oilseed by-products are the most likely candidate feed sources to carry aquaculture forward to higher production levels. Worldwide, annual production of rendered products is roughly equivalent to annual fish meal production, with meat and bone meal and poultry by-product meal making up 80% of total production. These products are variable in quality, high in ash content, and fully utilized by other agricultural sectors. They are unlikely to supply a high proportion of the protein needed in fish feeds, but may be valuable as feed components due to their favorable amino acid profiles, which complement plant-derived protein sources. If seafood processing waste and by-catch were converted to fish meal, the quantity would nearly equal annual global fish meal production and potentially provide significant fish protein and oil supplies for aquaculture feeds. However, the high ash content and logistical problems with collection and processing will limit full utilization of this resource. Grain and oilseed by-products are thus the most promising sources of protein and energy for aquaculture feeds of the future. Despite many successful research studies on the use of plant-derived feed ingredients in fish feeds, significant problems remain to be resolved. Innovative collaborative research efforts between geneticists, fish nutritionists and the industrial sectors producing these products are beginning to resolve these technical problems. Use of enzyme supplements is one potential aspect of alternate ingredient utilization that will increase the nutritional value and use of alternate feed ingredients.

KEY WORDS: enzyme supplement, animal by products, grain protein, oilseed protein.

INTRODUCTION

Fish meal production has averaged approximately 6.5 million metric tons (mmt) over the past decade, and prospects for this production to increase are low. The highest annual production of fish meal has been 7.5 mmt, and the lowest production was between 4.5 and 5.0 mmt, during the 1998 El Niño

[1] Hardy, R.W., 2000. New developments in aquatic feed ingredients, and potential of enzyme supplements. In: Cruz -Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A. y Civera-Cerecedo, R., (Eds.). Avances en Nutrición Acuícola V.Memorias del V Simposium Internacional de Nutrición Acuícola. 19-22 Noviembre, 2000. Mérida, Yucatán, Mexico.

period, which lowered production of fish meal from Peru and Chile. Since Peru and Chile have accounted for about 1/3 of global fish meal production, any change in these countries has a major impact annual production. Further, Peru and Chile are major fish meal exporting countries, accounting for up to 2/3 of the amount of fish meal traded throughout the world. Thus, production of fish meal by Peru and Chile greatly influences the supply of fish meal, which in turn affects fish meal price (Fig. 1). The price of fish meal is currently quite low, the result of adequate supplies and relatively low demand, most likely associated with the economic slowdown in Asia. However, this period of low fish meal prices is likely to be short-lived, in that economic recovery in Asia is underway, and production of fish meal in Chile is not expected to recover to pre-El Nino levels in the next few years (Fig. 2). The aquaculture industry must be prepared for higher feed costs, associated with higher fish meal costs, and in addition must seek alternative protein sources to replace a portion of the fish meal in feed formulations to permit expansion of aquaculture production beyond the level at which supplies of fish meal become a factor limiting production of fish feeds, and hence farmed fish.

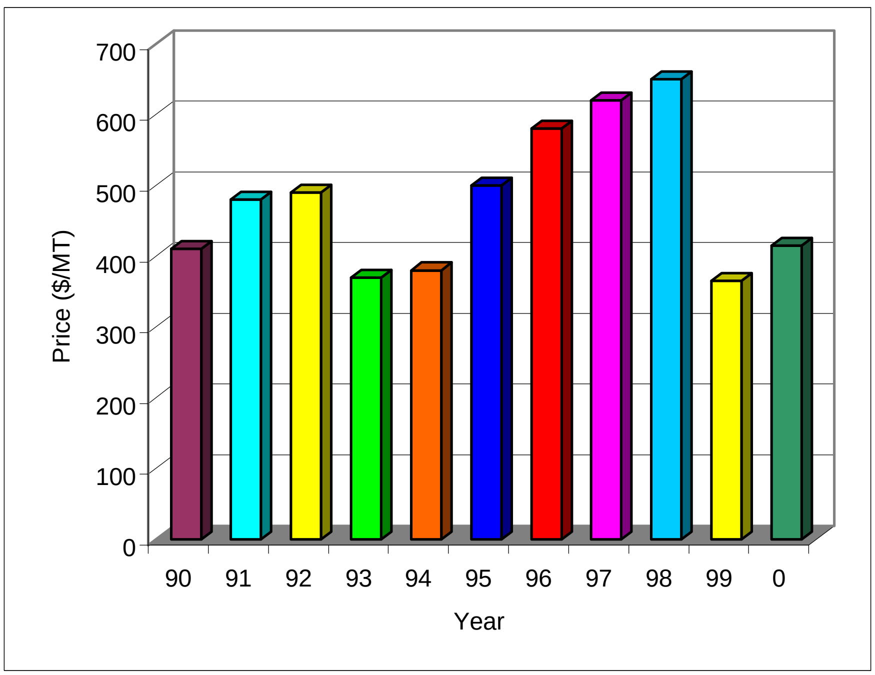

Figure 1.- Average fish meal price from 1990 to 1996

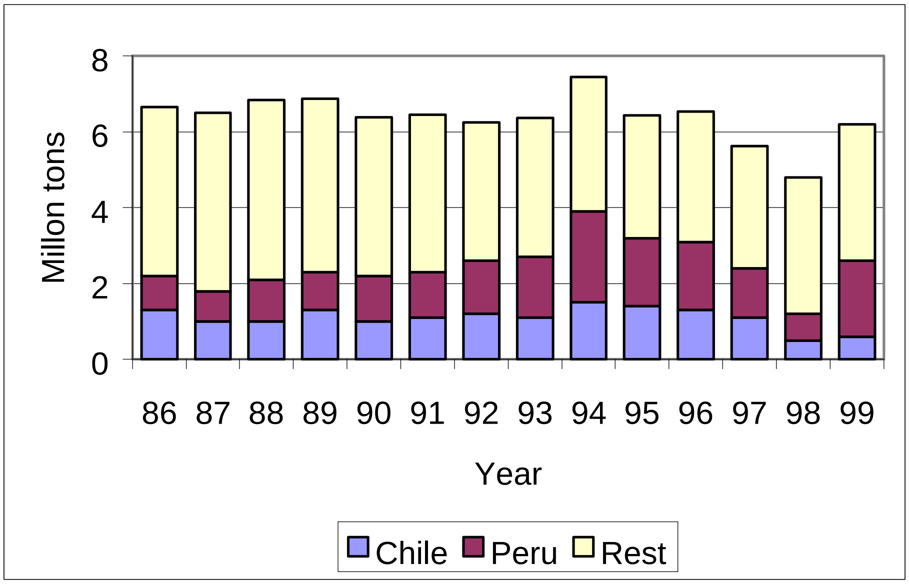

Figure 2.- Production of fish meal from Chile and Peru between 1986 to 1995

Fish meal use in aquaculture feeds

The proportion of global fish meal production that is utilized in fish feeds has increased substantially over the past 10 years. In 1989, aquaculture was a minor consumer of fish meal, using approximately 10% of annual production (Barlow, 1989). In 2000, fish meal consumption by the aquaculture industry will be an estimated 35% of total global fish meal production, an increase 3.5 times in fifteen years. Growth of the Atlantic salmon and shrimp farming industries has been responsible for most of the increase in fish meal use by the aquaculture industry over this period, but the explosive growth of the marine fish farming industry has caused much of the increase in fish meal use by aquaculture in the last five years (Fig. 3). Feeds for Atlantic salmon over the past 15 years have contained more than 50% fish meal, and shrimp feeds 35% fish meal (Barlow, 2000). In the past five years, these percentages have decreased somewhat, but nevertheless, feeds for salmon, marine fish, and eels still contain about 40% fish meal (Barlow, 2000). Predictions of fish meal needs for aquaculture feeds in 2010 are 2.83 mmt, approximately 44% of the ten-year average annual global fish meal production of 6.5 mmt. This represents an increase of 716,000 mt over estimates of fish meal use in 2000. Fish meal use in feeds for carp is predicted to increase by 325,000 mt, and use for marine fish by 447,000 mt, while use in feeds for eels, salmon, trout, milkfish, and catfish is predicted to decrease (Table 1). Use of fish meal in shrimp feeds is predicted to increase from 372,000 mt to 485,000 mt between 2000 and 2010. The percentage of fish meal in feeds for all species groups is predicted to decrease (Table 2). If the percentage of fish meal use in fish feeds was to remain the same as today, and aquaculture production increased to predicted levels in 2010, fish meal needs would be 4086 mmt, or 63% of the average amount produced over the past decade. The difference between the predicted need for fish meal by the aquaculture feed industry in 2010 (2.83 mmt), and the amount that would be needed if the percentage of fish meal in fish feeds did not decrease (4.086 mmt) is 1,255,000 mt. This is the amount of fish meal-equivalent protein sources that will be needed to replace the 'missing' fish meal in fish feeds by the year 2010.

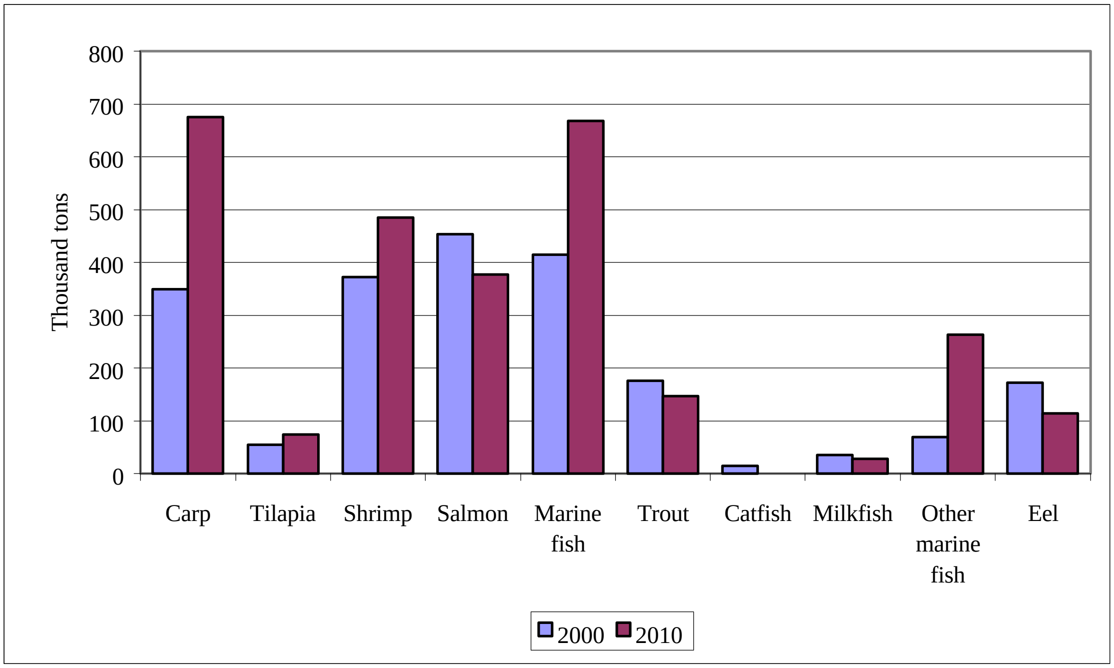

Figure 3.- predicted fish meal use in 2000 and 2010 by sector

Table 1. Amount of fish meal (1000 mt) used in fish feeds in 2000 and estimated for 2010.

Species group	2000	2010	Change
Carp	350	675	+93%
Tilapia	55	74	+35%
Shrimp	372	485	+30%
Salmon	454	377	-17%
Marine fish	415	668	+61%
Trout	176	147	-16%
Catfish	15	0	
Milkfish	36	28	-22%
Marine flatfish	69	263	+281%
Eel	173	114	-34%
Total	2115	2831	+34%

Table 2. Percent fish meal used in fish feeds in 2000 and estimated for 2010.

Species group	2000	2010
Carp	5	2.5
Tilapia	7	3.5
Shrimp	25	20
Salmon	40	30
Marine fish	45	40
Trout	30	25
Catfish	3	0
Milkfish	12	5
Marine flatfish	55	45
Eel	50	40

Alternative Protein Sources; Availability and Quantity

Seafood Processing Waste and By-Catch

Seafood processing waste and fishery by-catch together exceed in tonnage the global landings of fish for fish meal production (New, 1996). If half of the fishery by-catch discarded by the fishing industry each year could be converted into fish meal, this quantity (2,600,000 mt) could supply the expected needs of the aquaculture feed industry for the next 15 years or more. Seafood processing waste, which is mainly the carcass of fish after fillets are removed, contains too much bone to be producing suitable fish meal for fish feeds. Therefore, the bone content of the processing waste must be reduced, either before it is made into fish meal by mechanical de-boning, or after it is made into fish meal by screening (Babbitt *et al*. 1994). Fish processing waste contains ca. 25% ash on a dry weight basis, but fish meals made from de-boned fish filleting waste can be as low as 7% ash, half the level of ash in fish meals used in feeds. This is particularly valuable for the production of low-pollution fish feeds. Expanded production of low-ash fish meals produced from seafood processing waste is likely, as is further refinement of the production process to ensure that the nutritional value of these fish meals remains high.

Rendered Products

Rendered products are meat & bone meal (annual US production 2,819,322 mt) and blood meal (annual US production 101,300 mt). By-products of poultry processing include feed grade poultry by-product meal (annual US production 265,910 mt), pet food grade poultry by-product meal (annual US production 177,270 mt), low-ash pet food grade (annual US production 24,000 mt), and feather meal (annual US production 363,640 mt). Together, annual US production of all rendered products plus poultry processing products totals 3,751,442 mt, or about 50-60% of average annual world fish meal production. These products are fully utilized in poultry feeds, pet foods, and other animal feeds. At present their prices are very low in comparison to 10-year average prices, both on a weight basis and on a protein-unit basis (Table 3).

Table 3. Current prices (October, 2000) and production levels of protein sources used in fish feeds.

Ingredient	Percent Protein	US Annual Production (mmt)	Price per mt (US$)	Price per mt protein (US$)
Anchovy Meal	65-68	--	450	692
Corn Gluten	60	1.2	300	500
Wheat Gluten	70-80	0.085	1760	2200
Soybean Meal	48	30.6	210	438
Soy Protein Concentrate	65-76	0.085	990	1414
Canola Meal	38	1.74 (Canadá)	140	368
Rapeseed Protein Concentrate	61	0	427 (estimated)	700 (estimated)
Brewers Yeast	54	.064	770	1426
Meat and Bone Meal	51	2.819	228	447
Blood Meal	89	0.101	426	479
Poultry By-product Meal	60	0.266	282	470
Feather Meal	83	0.364	254	306

Rendered products have not been extensively studied as replacements for fish meal in feeds for carnivorous fish. Dong *et al.* ((Dong *et al*. 1993) reported that poultry by-product meal varied considerably in quality among suppliers, as measured by apparent protein digestibility. Feeding trials involving poultry by-product meals have demonstrated that up to 40% of fish meal could be replaced with pet-food grade poultry by-product without lowering trout growth, but that higher replacement levels resulted in reduced growth. Protein digestibility of poultry by-product meal, measured in trout, is 94-95%, equivalent to herring meal (Sugiura *et al*. 1998a) but recent research results suggests that the availability of certain amino acids in poultry meal is lower than average protein digestibility. Meat and bone meal and feather meal were considered to be unsuitable for use in salmonid feeds because early data showed less than 70% protein digestibility (Cho and Slinger, 1979). Recent re-evaluation of several of these ingredients has shown that earlier work underestimated the protein digestibility of meat & bone meal and blood meal, with more recent values showing apparent digestibility coefficients ranging from 87% to 92% (Hajen *et al*. 1993; Sugira *et al*. 1998b, Bureau *et al*. 2000). As is the case with poultry by-product meal, individual amino acid digestibility coefficients are higher and lower for specific essential amino acids than the average protein digestibility value for meat and bone meal (unpublished data, Sugiura and Hardy, 1998). Initial studies suggest that up to 25% of fish meal protein can be replaced with meat and bone meal without compromising growth, but that higher levels of replacement significantly reduce growth (Schelling and Hardy, unpublished data, 2000). The nutritional value of rendered products varies among producers, and even among manufacturing plants owned by the same company. The most important determinate of nutritional value is the source and freshness of the raw material used to produce the meals. At present, rendered products are sold at commodity prices, but efforts are being made within the rendering industry to range products and establish grades corresponding to nutritional value.

Grain Proteins

Wheat gluten is an excellent protein source, containing 70-80% protein that is highly digestible to rainbow trout, coho salmon and presumably other fish species (Sugiura *et al*. 1998). Up to 25% of fish meal has been replaced with wheat gluten without negative effects on growth or feed conversion ratios (Weede, 1997). Higher replacement levels combined with lysine supplementation are reported to support trout performance equivalent to fish meal-based diets (Rodehutscord *et al*. 1994). The main drawback of wheat gluten is its relatively high price. Wheat gluten is currently produced for human consumption as a high-value, non-meat protein source. If lower quality, cheaper, feed-grade wheat gluten were developed, this ingredient could become an important aquaculture feed ingredient.

Corn gluten is an excellent protein source, containing a minimum of 60% protein (Morales *et al*., 1994) which is 97% digestible to trout (Sugiura *et al*. 1998). Corn gluten can substitute for 25–40 % of fish meal without negative effects on growth or feed conversion ratios in trout (Morales et al., 1994; Weede, 1997). The main disadvantage of corn gluten for commercial trout diets is that it imparts a yellow color to fish flesh when included at a high proportion of the diet (Weede, 1997). Nevertheless, it is a valuable ingredient when included at levels up to 10% in trout diets, and, when trout or salmon are raised with the intention of producing fish with pink colored flesh, corn gluten can be included as up to at least 22.5% of the diet, along with canthaxanthin or astaxanthin, which masks the yellow color in fillets (Skonberg *et al*. 1998). For fish species that do not deposit carotenoid pigments in their flesh, corn gluten can be used at even higher dietary levels. Corn gluten has the advantage of being plentiful and low priced. In 1997, U.S. production alone amounted to 1.178 mmt, and was priced at $380 per mt. Currently, corn gluten produced from white corn is being evaluated as a feed ingredient for salmonids, and initial results appear promising (Hardy, unpublished data, 2000). White corn gluten meal will likely be priced at a premium to regular (yellow) corn gluten meal, but protein levels are at least 10% higher, which justifies the increased cost.

Oilseed Proteins

Soybeans, as other plant-derived protein sources, have several antinutritional factors (ANFs), which can reduce palatability, protein utilization or growth (Hardy, 1996). These can be divided into two categories: heat-labile and heat-stable ANFs (Rumsey *et al.* 1995). *Heat-labile ANFs* include trypsin inhibitors, phytates, lectins, goitrogens and antivitamins. *Heat-stable ANFs include* carbohydrate or soluble fiber, saponins, estrogens, allergins, and lysinoalanine. Heat-labile constituents can be at least partially degraded by heat treatments, so the effects of these antinutritional factors reduced by adjusting the heat treatment used during soybean presscake drying (Vohra and Kratzer, 1991). Trypsin inhibitors decrease the activity of trypsin, a digestive enzyme that breaks down proteins in the intestine. Trypsin inhibitors lower protein digestibility in diets for salmon and trout (Arndt *et al*., 1999). Phytate or phytic acid has been reported to reduce protein digestibility and limit the bioavailability of minerals (Spinelli *et al*. 1983; Riche and Brown, 1996). Much of the phosphorus in plant-derived ingredients is bound in phytate. Supplementing the diet with the enzyme phytase can break down a portion of the phytate, increasing availability of dietary phytate-phosphorus in diets for fish (Rodehutscord and Pfeffer, 1995; Schafer *et al*., 1995; Cain and Garling, 1995). Soybean lectins have been shown *in vitro* to bind to the brush border membrane of Atlantic salmon small intestine (van den Ingh *et al*. 1991), but no studies have been conducted on performance or health effects of lectins on fish. The carbohydrate fraction of a soybean is approximately 30% of its dry weight, and only 33% consists of the soluble fraction (oligosaccharides, raffinose, sucrose, and stachyose), or the fraction available for energy use (Arnesen *et al*. 1989). Arnesen *et al*. (1989) suggested that a large fraction of the potential carbohydrate energy is not available to salmonids because most of the soybean polysaccharides cannot be absorbed. This carbohydrate fraction is unavailable because salmonids only have the enzyme necessary to digest starch and starch makes up less than 1% of soybean meal. A crude saponin extract of soybean meal was found to lower feed intake of chinook salmon fingerlings and to reduce growth of rainbow trout (Bureau *et al*. 1996). Overall, there are several ANFs that could influence the nutritional value of soybean meal and other plant-derived ingredients for fish; additional processing or diet supplementation may be required to realize the full nutritional potential when these ingredients are used in fish feeds.

Soybean products are generally high in protein, ranging from about 45% protein for soybean meal to over 70% protein for soy protein concentrate. Soybeans are the most plentiful of oilseed crops, with a worldwide production of 132.53 mmt in 1996. US production of soybean meal in 1996 was 30.6 mmt, and the price was $289 per mt. Soy protein concentrate is produced in smaller quantities, with US annual production of 85,000 mt, and is priced at about $990 per mt. Early studies with soybean meal in trout feeds showed that trout tolerated relatively high levels of soybean meal in their feeds, especially if the meal was heat-treated to inactivate trypsin inhibitor levels (Cho *et al*. 1974; Reinitz, 1980). Studies with Pacific salmon fingerlings, however, are less promising, with some studies showing that feed intake was reduced even at 5% soybean meal in the diet (Higgs *et al.* 1979; Fowler, 1980). Recent studies with post-juvenile Pacific salmon have been more encouraging, suggesting that larger fish are more tolerant of soybean meal in their feed than are fry and fingerlings. Wilson (1992) found that full-fat soybean meal, heat-treated by double extrusion to lower trypsin inhibitor levels, could be used in diets for post-juvenile chinook salmon at levels up to 15% of the diet without reducing growth rates or feed efficiency ratios, but that diets containing more than 15% full-fat soybean meal resulted in reduced feed intake and growth. Further research is necessary to determine whether higher levels of soybean meal can be included in diets for salmon when appetite stimulants are included in the diet, and to determine the relative importance of various antinutritional factors in soybean meal for fish. In contrast to salmon and trout feeds, catfish feeds depend heavily on soybean meal to provide dietary protein. Current catfish feed formulations in the US contain 45-50% soybean meal, with less than 10% fish meal (Wilson, 1991). Similarly, tilapia and carp feeds generally contain less than 15% fish meal, with soybean meal or other alternate protein sources providing the bulk of dietary protein (Luquet, 1991) (Satoh, 1991).

Other By-Product Proteins

By-products of the brewing and distilling industries are widely available and underutilized in feeds for fish. Rumsey *et al*. (1991) found that the protein quality of *Saccharomyces* yeast (brewers or bakers yeast) in diets for rainbow trout is improved by a treatment to disrupt the cell walls, thereby making the protein more available. When yeast cell walls are disrupted, 50% of the protein in rainbow trout diets can be supplied by bakers yeast with equivalent growth and feed conversion ratio to a control diet with protein supplied by casein and gelatin. Although single cell proteins are potentially good protein sources, limited availability or high cost has so far limited their use in fish diets. This situation may change in the near future, however, as new processes are developed to recover single cell proteins from brewery waste, and upgrade its quality by air-classification to lower fiber content.

Role of Enzyme Supplements

As fish meal is increasingly replaced in fish feeds with non-traditional protein sources, the opportunity to improve the nutritional value of these protein sources by enzyme supplementation will increase. Phytase is already used in swine and poultry feeds to increase phosphorus availability in grains and oilseeds by dephosphorylation of myo-inositol hexakisphosphate (phytate)) (Cromwell *et al*. 1993). Studies with catfish (Jackson *et al*. 1996; Eya and Lovell, 1997; Li and Robinson, 1997) and trout (Cain and Garling, 1995; Rodehutscord and Pfeffer, 1995; Vielma *et al*. 2000) demonstrate the effectiveness of phytase at increasing phosphorus availability in fish, although these studies also demonstrate the significance of rearing water temperature on effectiveness and optimum dietary phytase level. Li and Robinson (1997) found that the cost of adding phytase to catfish feeds was nearly equal to the savings associated with eliminating dietary supplementation with inorganic phosphorus.

Other enzyme supplements are not widely used, but may be added to future fish feeds to increase nutritional value when alternate ingredients are included. For example, mixtures of proteases may be used increase the digestibility of protein in rendered products. Such products would contain enzymes that hydrolyze connective tissue and skin, two components of rendered products that are difficult for fish to digest. Another category of enzyme supplements is those that break down fiber and certain carbohydrates found in protein sources from grains and oilseeds. One such product, designed specifically for use in high-wheat feeds for poultry, contains endo-xylanase, which breaks down pentose sugars. A similar product breaks down glucans found in wheat, barley, triticale and rye, releasing glucose. To date, these products have been only used in poultry and swine diets, but it is likely that they will be effective in diets for tilapia, catfish, and perhaps shrimp.

Specific enzyme supplements are needed to overcome various components of the carbohydrate fraction of oilseeds. As mentioned above, soybean non-starch polysaccharides are suspected of being one of the problems that limits soybean meal nutritional value for some species of fish. Studies in poultry show that supplementing feeds with a glycanase increases the performance of the birds when their diet contained low metabolizable energy wheat (Choct, Hughes, *et al.* 1995 #20771). Supplementation with the enzyme significantly increased solubilization of non-starch polysaccharides in the intestine of the birds. Enzymes that break down non-starch polysaccharides must be tested in fish to determine if nutritional value, specifically energy availability, is increased in soybean meal-containing diets when enzyme supplements are used.

SUMMARY

Expanded aquaculture production will require more fish feed, which will in turn require higher quantities of alternate protein sources to substitute for fish meal. An estimated 1.5 mmt of alternate proteins will be needed just in the next decade to supply global needs. If fish meal supplies decrease, higher amounts will be needed. Most likely, these proteins will be supplied from a variety of sources, most of which requiring special processing or enzyme supplementation to realize their full nutritional value. The aquaculture industry should look to blends of protein sources from plant sources and from animal or fish sources. Such blends would more closely approximate the excellent amino acid profile of fish meal that any single protein source, with the exception of fish meal produced from seafood processing waste.

REFERENCES

Arndt, R.E., Hardy, R.W., Sugiura, S.H., Dong, F.M., 1999. Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour for coho salmon, *Oncorhynchus kisutch*. Aquaculture, 180, 129-145.

Arnesen, P., Brattås, L.E., Olli, J., Krogdahl, Å., 1989. Soybean Carbohydrates Appear To Restrict the Utilization of Nutrients by Atlantic Salmon. Proc. Third Int. Symp. on Feeding and Nutr. in Fish, 273-280.

Babbitt, J.K., Hardy, R.W., Reppond, K.D., Scott, T.M., 1994. Processes for improving the quality of whitefish meal. J. Aquat. Food Product Tech, 3, 59-68.

Barlow, S., 1989. Fishmeal - world outlook to the year 2,000. Fish Farmer, 40-41, 43.

Barlow, S., 2000. Fishmeal and fish oil. The Advocate, 3, 85-88.

Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A., Cho, C.Y., 2000. Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. Aquaculture, 181, 281-291.

Bureau, D.P., Harris, A.M., Cho, C.Y., 1996. The effects of a saponin extract from soybean meal on feed intake and growth of chinook salmon and rainbow trout. Proc. VI. Int. Symp. on Feeding and Nutrition in Fish, (Abstract)

Cain, K.D., Garling, D.L, 1995. Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. Prog. Fish-Cult., 57, 114-119.

Cho, C.Y., Bayley, H.S., Slinger, S.J., 1974. Partial replacement of herring meal with soybean meal and other changes in diets for rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can., 31, 1523-1528.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurements in feedstuffs for rainbow trout. Proc. World Sym. Finfish Nutrition and Fishfeed Technology, 2, 239-247.

Cromwell, G.L., Stahly, T.S., Coffey, R.D., Monegue, H.J., Randolph, J.H., 1993. Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybeam meal diets for pigs. J. Anim. Sci., 71, 1831-1840.

Dong, F.M., Hardy, R.W., Haard, N.F., Barrows, F.T.B., Rasco, B.A., Fairgrieve, W.T., Forster, I.P. 1993. Chemical composition and protein digestibility of poultry by-product meals for salmonid diets. Aquaculture, 116, 149-158.

Eya, J.C., Lovell, R.T., 1997. Net absorption of dietary phosphorus from various inorganic sources and effect of fungal phytase on net absorption of plant phosphorus by channel catfish. J. World Aqua. Soc., 28, 386-391.

Fowler, L.G., 1980. Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and coho salmon. Prog. Fish-Cult., 42, 87-91.

Hajen, W.E., Higgs, D.A., Beames, R.M., Dosanjh, B.S., 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (Oncorhynchus tshawytscha) in seawater. 2. Measurement of digestibility. Aquaculture, 112, 333-348.

Hardy, R.W. (1996). Alternate protein sources for salmon and trout diets. Animal Feed Science Technology, 59, 71-80.

Higgs, D. A., Markert, J. R., MacQuarrie, D. W., McBride, J. R., Dosanjh, B. S., Nichols, C., Hoskins, G., (1979). Development of practical dry diets for coho salmon *Oncorhynchus kisutch*, using poultry by-product meal, feather meal, soybean meal, and rapeseed meal as major protein sources. J.E.Halver and Tiews, K. W. Finfish Nutrition and Fishfeed Technology. 2, 191-218. 79. Berlin, Heenemann.

Jackson, L.S., Li, M.H., Robinson, E.H., 1996. Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytate phosphorus. J. World. Aqua. Soc., 27, 297-302.

Li, M.H. Robinson, E.H., 1997. Microbial phytase can replace inorganic phosphorus supplements in channel catfish *Inctalurus punctatus* diets. J. World Aqua. Soc., 28, 402-406.

Luquet, P., 1991. Tilapia, *Oreochromis* spp. In R.P. Wilson (Ed.), Handbook of Nutrient Requirements of Finfish (pp. 169-180). Boca Raton: CRC Press.

Morales, A.E., Cardenete, G., De la Higuera, M., Sanz, A., 1994. Effects of dietary protein source on growth , feed conversion and energy utilization in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 124, 117-126.

New, M.B., 1996. Responsible use of aquaculture feeds. Aquaculture Asia, 1,

Reinitz, G., 1980. Soybean Meal as a Substitute for Herring Meal in Practical Diets for Rainbow Trout. Prog. Fish-Cult., 42, 103-106.

Riche, M., Brown, P.B., 1996. Availability of phosphorus from feedstuffs fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 142, 269-282.

Rodehutscord, M., Mandel, S., Pfeffer, E., 1994. Reduced protein content and use of wheat gluten in diets for rainbow trout: effects on water loading with N and P. J. Applied Ichthyology, 10, 271-373.

Rodehutscord, M., Pfeffer, E., 1995. Effects of supplemental microbial phyatse on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). Water Sci. Technol., 31, 143-147.

Rumsey, G.L., Endres, J.G., Bowser, P.R., Earnest-Koons, K.A., Anderson, D.P., Siwicki, A.K., 1995. Soy protein in diets of rainbow trout: Effects on growth, protein absorption, gastrointestinal histology and nonspecific serologic and immune response. In C.E. Lim & D.J. Sessa (Eds.), Nutrition and Utilization Technology in Aquaculture (pp. 166-188). Champaign, IL: AOCS Press.

Rumsey, G.L., Hughes, S.G., Smith, R.R., Kinsella, J.E., Shetty, K.J., 1991. Digestibility and energy values of intact, disrupted, and extracts from brewers dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). Anim. Feed. Sci. Tech., 33, 185-193.

Satoh, S., 1991. Common Carp, *Cyprinus carpio*. In R.P.Wilson (Ed.), Handbook of Nutrient Requirements of Finfish (pp. 55-68). Boca Raton: CRC Press.

Schafer, A., Koppe, W.M., Neyer-Burgdorff, K.H., Gunther, K.D., 1995. Effects of microbial phytase on utilization on native phosphorus by carp in diets based on soybean meal. Water Sci. Tech., 31, 149-155.

Skonberg, D.I., Hardy, R.W., Barrows, F.T., Dong, F.M., 1998. Color and flavor analysis of fillets from farm-raised rainbow trout (*Oncorhynchus mykiss*) fed low-phosphorus feeds containing corn or wheat gluten. Aquaculture, 166, 269-277.

Spinelli, J., Houle, C.R., Wekell, J.C., 1983. The effects of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture, 30, 71-83.

Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998a. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonids . Aquaculture, 159, 177-200.

Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998b. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture, 159, 177-202.

van den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J.M., Koninkx, J.G.J.F., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon: a morphological study. Aquaculture, 94, 297-305.

Vielma, J., Makinen, T., Ekholm, P., Koskela, J., 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture, 183, 349-362.

Vohra, P., Kratzer, F.H., 1991. Evaluation of soybean meal determines adequacy of heat treatment. Feedstuffs, 23-28.

Weede, N., 1997. Low phosphorus plant protein ingredients in finishing diets for rainbow trout (*Oncorhynchus mykiss*). 97. Seattle, WA, University of Washington. 97.

Wilson, R.P., 1991. Channel Catfish, *Ictalurus punctatus*. In R.P.Wilson (Ed.), Handbook of Nutrient Requirements of Finfish (pp. 35-54). Boca Raton: CRC Press.

Wilson, T.R., 1992. Full-fat soybean meal-an acceptable, economical ingredient in chinook salmon grower feeds. Ph.D. Dissertation, Seattle, WA: University of Washington.