Nutritional Strategies to Reduce Nutrient Losses in Intensive Aquaculture

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ABSTRACT

Expansion of aquaculture production is largely the result of intensification of farming activities with a higher degree of feed input and waste output per rearing system. Concerns about the effects of waste output on the quality of receiving water systems have led to efforts to lower levels of enriching nutrients in discharge water. Since the enriching nutrients originate in feed added to the rearing systems, efforts to reduce levels in farm effluents must begin with feed. Phosphorus, nitrogen and fecal solids in farm effluent water are the "pollutants" of concern. Strategies to lower their concentrations in farm effluent water focus on decreasing the amount of uneaten feed, and on increasing the retention of dietary phosphorus and nitrogen by the fish. Modifying feeding levels and methods, plus increasing pellet water stability, are the approaches used to reduce waste from uneaten feed. Reducing phosphorus levels in farm effluents requires effort in three areas, e.g., information on the bioavailability of phosphorus in feeds and feed ingredients, accurate estimations of requirements for phosphorus at various life-history stages, and formulation of feeds to match as closely as is practical the available phosphorus level in the feed to the requirement of the fish or shrimp. Reducing nitrogen levels in farm effluents involves the development of feeds that support high percentages of nitrogen (protein) retention by the farmed fish or shrimp. Another nutritional strategy useful in lowering nutrient levels in farm discharge waters is phase feeding, involving the use of diets containing sub-optimal levels of phosphorus or protein during the final grow-out phase. Using appropriate nutritional strategies, levels of enriching nutrients in farm effluents can be significantly reduced without lowering fish performance or farm profitability.

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INTRODUCTION

Nutrient levels in farm effluents have been under regulatory control since the late 1980s in Denmark (Kossman, 1990), where surface water is diverted into trout farms, returned to the river, and diverted again downstream by other farms. The collective effects of multiple diversions in this type of flow-through aquaculture system are substantial, and water quality in the rivers suffers enrichment and subsequent eutrophication, at least in summer months. In Denmark and eventually in all Scandinavian countries, restrictions were imposed on feed composition and the total amount of feed each farm could purchase (Table 1).

Table 1. Danish regulations on feed composition and performance (from Kossman, 1989). Specification

Feed conversion ratio[1]	1:1.1 (1990)	1:1.0 (1992)
Gross energy	5700 kcal/kg (1990)	6000 kcal/kg (1992)
Nitrogen	9% maximum (1990)[2]	8% maximum (1992)[2]
Total phosphorus	1.1% maximum (1990)	1.0% maximum (1992)

[1] Feed used (kg)/fish weigh gain (kg)
[2] Equivalent to 56% protein (1990) and 50% protein (1992).

These restrictions lowered total phosphorus in feeds and required feeds to achieve relatively low feed conversion ratios (FCRs). The effects of the restrictions were to eliminate an array of useful feed ingredients due to phosphorus, fiber or starch level, and to push feed formulations toward energy-dense feeds having higher lipid levels that were previously used.

Regulatory agencies in several US states, notably Idaho where 70% of rainbow trout food fish production is located, have imposed restrictions on individual farms and hatcheries with respect to levels of enriching nutrients in effluent water discharged into public waters. The Clean Water Act mandates such restrictions when effluent nutrient loads are demonstrated to have an adverse effect on receiving waters. Idaho trout farmers rely on springs to supply water, and the water flows by gravity through the farms and into the Snake River, in a flow-through arrangement. In Idaho, in contrast to Denmark, regulations have been imposed on the rainbow trout industry in the form of Total Maximum Daily Loads (TMDSs). All rainbow trout farms along a specific stretch of the Snake River have been allocated collectively a TMDL of 973 kg phosphorus, and this TMDL is divided among farms based upon their water flow, proportionate to the total water flow of all farms in the affected area. Thus, if one particular farm has a total water flow of 5 m^3/sec, and the total water flow of all farms included in the TMDL is 60 m^3/sec, then this farm is allocated 8.33% of the total industry TMDL, or, in this example, 81 kg of phosphorus per day. Farm management decides how the individual farm chooses to meet this restriction. They can lower production, feed less feed, or use a low-pollution feed. It does not matter how they meet their individual TMDL, as long as they do so.

Phosphorus and nitrogen are the two excretory products of farmed fish and shrimp of concern (Wiesmann *et a.,* 1988; Cowey & Cho, 1991; Dosdat *et al*., 1995). Dietary phosphorus not absorbed and deposited in body tissues of farmed fish is excreted into rearing water either in feces (indigestible phosphorus) or in urine (phosphorus absorbed in excess of need for growth). Dietary protein that is not digested is excreted in feces, and this protein contains nitrogen that can be released when the fecal material decomposes, or as ammonia excreted from the gills that originates from protein that is metabolized for energy rather than used to build tissue protein results in increased nitrogen excretion, primarily in the form of ammonia. Fish also produce organic wastes from undigested components of the diet, which contribute to the biochemical oxygen demand of aquaculture systems (Johnsen *et al*., 1993; Kelly & Karpinski, 1994) as well as settle able solids in effluent waters (Pillay ,1992; Midlen & Redding, 1998).

As mentioned above, materials classified as pollutants in fish farm effluents originate from the diet, and are basically materials that are either not digested by the fish, or metabolic excretions. Nutritional strategies for reducing the amounts of these materials are discussed individually for each category or nutrient below.

PHOSPHORUS

Phosphorus is an essential mineral for all forms of life, including fish. Fish can obtain a substantial number of required minerals directly from their rearing water, but phosphorus is one essential mineral that must be supplied by the diet (Lall, 2002). Phosphorus is a structural component of hard tissues such as bone, teeth and scales, as well as a constituent of various coenzymes, phospholipids and nucleic acids. Phosphorus is also involved in energy metabolism by virtue of its role in high-energy bonds of ATP. Dietary requirements for phosphorus have been reported to range rather widely between 0.3 and 0.9% of diet for various fish species (Lall, 2002) and between 0.3 and 2% of diet for crustaceans such as penaeid shrimp (Gatlin, 2000). There is some evidence that fish having scales have a higher dietary phosphorus requirement than do scale-less fish, such as channel catfish *Ictalurus punctatus*.

The first step in using nutritional strategies to lower phosphorus excretion by fish is to obtain accurate data on the bioavailability of phosphorus in common feed ingredients. This step is important because the easiest step in reducing phosphorus excretion is to reduce the proportion lost in feces when indigestible phosphorus levels in the diet are too high. In general, feed ingredients made from animal or fish by-products, such as meat and bone meal, poultry by-product meal, and fish meal, contain relatively high levels of phosphorus coming from the bone component of these ingredients (Table 2).

Table 2. Phosphorus levels and apparent digestibility coefficients (ADCs) for rainbow trout in selected feed ingredients[1].

Feed ingredient	Total phosphorus (%)	ADC phosphorus
Herring meal	2.2	45-52%
Menhaden meal	3.5	36%
Poultry byproduct meal	2.2	48-62%
Meat and bone meal	5.6	27%
Blood meal	0.7	>95%
Feather meal	0.75-1.26	62-79%
Wheat gluten	0.2	75%
Wheat middlings	1.3	55%
Corn gluten	0.5	8.5%
Soy protein concentrate	0.8	<30%

[1] source: Sugiura & Hardy, 2000.

Blood meal is an exception to this general statement. In contrast, ingredients produced from grains and oilseeds, e.g., corn, wheat, and soybeans, are relatively low in total phosphorus. However, plants store phosphorus in seeds as phytate-phosphorus, an indigestible compound that passes through the intestinal tract of fish (and other monogastric animals). Several years ago, there was limited information on the bioavailability of phosphorus in common feed ingredients to fish (NRC, 1993). Today, however, there is a wealth of information on this subject that can be used to develop feed formulations for fish that are relatively low in the proportion of indigestible phosphorus (Sugiura & Hardy, 2000). In general, values for the bioavailability of phosphorus in feed ingredients are additive; that is, values for individual ingredients used in a feed formulation can be added together after adjusting for the percentage of each ingredient in the formulation. The sum of the bioavailability values for ingredients in a feed formulation generally yield an accurate estimate of the bioavailability of phosphorus in the feed. Ten years ago, it was impossible to estimate phosphorus bioavailability in a feed formulation, and thus impossible to formulate least-cost feeds on a computer. In certain combinations of feed ingredients, however, the values for individual ingredients are not additive due to antagonistic interactions that can occur when some feed ingredients are combined. An example of this is the effect of fish bone level on apparent availability of phosphorus. At low fish bone content, apparent availability of phosphorus in fish meal is 67%, but phosphorus availability declines as fish bone level in fish meal (or in a feed) increases, reaching a low of about 10% when fish bone level exceeds 16% (Sugiura *et al.,* 2000b). This change is thought to result from the formation of Ca-P complexes that result when food moves from the acid environment of the stomach into the basic environment of the intestine and Ca comes out of solution. Adding citric acid to the diet of rainbow trout inhibits the formation of Ca-P complexes by a combination of acidification, which increases the solubility of calcium phosphates, and through chelation (Sugiura *et al.*, 1998). The observation that the bone content of fish meals changes the bioavailability of phosphorus explains some of variation in reported apparent availability estimates for phosphorus in fish meal for rainbow trout (Richie & Brown, 1996, Nordrum *et al.*, 1997).

The next step in developing feeds to minimize phosphorus excretion involves the formulation of feeds containing available phosphorus levels right at levels required by a species of fish at each stage of production. Surprisingly, the range of estimates of the phosphorus requirement of salmonids, for example, was quite wide, e.g., 0.5% to 0.8% of the diet (NRC, 1993), and this range was too great to accurately formulate low-pollution diets. Research on the dietary requirements for phosphorus has been hampered by the lack of sensitive response variables to identify adequate dietary intake of phosphorus. Early studies based requirements on fish growth and absence of deficiency signs (Ogino *et al.,* 1979). This was followed by studies using total body level of phosphorus as a response variable. However, this approach was not sensitive enough to precisely measure adequate dietary intake because fish can store phosphorus in bones, skin and scales in excess of their immediate needs. When fish are fed a phosphorus deficient diet, they draw upon their body reserves until these reserves are used up (Hardy *et al*., 1993). Measuring phosphorus levels in skin, scales and bones has been shown to be a more sensitive response variable than measuring whole body phosphorus levels (Skonberg *et al*., 1997). Since fish absorb dietary phosphorus in excess of their immediate needs if dietary levels exceed these needs and excrete the excess in urine, measuring urinary phosphorus levels in fish receiving different levels of available phosphorus in their diet is a very sensitive measure of dietary requirements. Using this approach, Sugiura *et al.* (2000a) reported that the requirements of 200g and 400g rainbow trout were 0.66% and 0.55%, respectively. These levels of dietary intake are sufficient to supply the metabolic needs of the fish as they grow.

Ten years ago, feed formulators were mainly concerned with ensuring that there was enough phosphorus in feeds to prevent deficiency signs, so emphasis was on formulating feeds to contain more than the minimum required level of phosphorus with little regard to upper limits. It was common to see levels of total phosphorus in salmonid feeds that exceeded 2.2%, well above the known required level. Today, levels of phosphorus in rainbow trout feeds range from 0.9% to 1.1%, a significant drop from levels in feeds of the recent past. However, if the dietary requirement is in the range of 0.55-0.66%, why do feeds contain nearly twice as much total phosphorus? The answer is that not all of the phosphorus in feed ingredients is available to fish, and therefore, feeds are formulated to contain the required amount of available phosphorus, and at the same time the minimum amount of total phosphorus. One of the complicating factors in the use of feed ingredients of plant origin is that plants store phosphorus in seeds as phytate phosphorus, and, as mentioned above, phosphorus in this form is indigestible to all animals except ruminants. Approximately two-thirds of the phosphorus in plant ingredients is present at phytate-phosphorus, with the remaining portion present as inorganic salts or other compounds that are bioavailable to fish. Phosphorus is released in germinating seeds by the enzyme phytase, and supplementing feeds with microbial phytase increases the availability of phosphorus in plant ingredients to fish (Cain & Garling 1995; Eya & Lovell, 1997; Li & Robinson, 1997; Sugiura *et al*., 2001). Phytase activity, like the activity of other enzymes, is destroyed by exposure to temperatures over approximately 65^0C, so the heat of extrusion processing of fish feeds destroys phytase activity. If phytase is applied to pellets after extrusion, activity can be retained. Another promising development with respect to phytate phosphorus is the development of single-gene mutant varieties of grains in which the proportion of phosphorus stored as phytate phosphorus is reduced by 50-75%. Studies with rainbow trout have demonstrated that low-phytate barley cultivars have higher apparent digestibility coefficients for phosphorus than regular barley (Sugiura *et al.,*1999). Low-phytate corn and soybean varieties will also soon be available. All of these approaches to increasing the availability of phosphorus in diets are useful in the formulation of low-pollution feeds because they can be used to reduce the amount of total phosphorus in feeds while at the same time ensuring that the level of available phosphorus is at required levels.

A final opportunity to employ nutritional strategies to lower phosphorus excretion and subsequent pollution involves phase feeding, defined as changing feed formulations for specific segments of the production cycle. As mentioned above, salmonids maintain a reserve of phosphorus in hard tissues that can be depleted over short periods to compensate for inadequate dietary intake (Hardy *et al.,* 1993). No signs of clinical deficiency or reduced fish performance are evident until body phosphorus reserves are reduced to critical levels. In fish for which there are well-defined production cycles, such as rainbow trout *Oncorhynchus mykiss*, dietary phosphorus levels can be purposefully reduced below required levels during the last stages of production before harvest without reducing fish growth or efficiency, or lowering product quality (Lellis *et al.,* 2002). Fish draw upon their tissue phosphorus reserves to meet metabolic needs. As long as fish do not reach critical body phosphorus levels over the period of depletion, there are no negative effects on production. This strategy has been demonstrated to reduce the total amount of excreted phosphorus during a production cycle by over 35%, but care must be taken to avoid excessive tissue phosphorus depletion.

NITROGEN

Nitrogen from dietary protein is another enriching nutrient of concern in aquaculture, particularly marine aquaculture. Nitrogenous wastes are dietary in origin with estimates of up to 52-95% of feed nitrogen being excreted as waste, depending on the species of fish and the diet (Wu, 1995). Adequate dietary intake of high-quality protein is required to support rapid growth of fish and crustaceans, but fish do not have a dietary requirement for protein, per se, but rather for amino acids. Fish are metabolically adept at utilizing protein for energy, and many early estimates of dietary protein requirements failed to take this fact into consideration, resulting in a wide range of reported protein requirements for aquatic species (NRC, 1993). Fish species vary in their ability to use dietary carbohydrate for energy, and this complicates the picture. For example, salmonids are fed diets containing 40% to 50% protein whereas diets for channel catfish typically contain 28 to 36% protein. Catfish are much more able to utilize cooked starch for energy than are salmonids, suggesting that part of the apparent protein need of salmonids is associated with supplying metabolic energy. A general relationship between natural feeding habits and dietary protein requirements has been observed among aquatic species such that the carnivorous species have higher protein requirements than omnivorous and herbivorous species (NRC, 1993). The reason that this is important is that amino acids used for metabolic energy, rather than for tissue synthesis, are catabolized, and nitrogen from the amino acids is excreted via the gills, contributing to environmental degradation.

The quantity and quality of dietary protein are factors that influence nitrogen excretion. Protein quality is largely determined by a feedstuff's amino acid composition and digestibility. Dietary protein that cannot be digested into constituent amino acids and absorbed in the gastrointestinal tract is excreted in the feces, contributing to fecal nitrogen losses. However, the major (75-90%) nitrogenous waste produced in fish is ammonia, mostly excreted via the gills (Kaushik & Cowey, 1991). Elevated ammonia production by fish has been readily observed when the concentration of dietary protein is excessive relative to non-protein energy, such that a portion of dietary protein not used for protein accretion is broken down and used for energy. For example, Ballestrazzi *et al.* (1994) found that ammonia excretion of European sea bass *Dicentrarchus labrax* increased linearly with protein level of the diet. Médale *et al.* (1995) also found that higher digestible protein relative to digestible energy resulted in increased ammonia excretion in rainbow trout. Thus, increasing digestible energy content in the diet relative to the digestible protein content is the one of the nutritional strategies used to lower nitrogen excretion (Kaushik & Cowey, 1991). The positive results from this approach have been demonstrated for several fish including rainbow trout (Kaushik & de Oliva Teles, 1985), carp species (Steffens, 1996), Atlantic salmon *Salmo salar* (Johnsen *et al.,* 1993), turbot *Scopthalmus maximus* (Andersen & Alsted, 1993), lake trout *Salvelinus namaycush* (Jayaram & Beamish, 1992), gilthead sea bream *Sparus aurata* (Vergara *et al.,* 1996) and red drum *Sciaenops ocellatus* (McGoogan & Gatlin, 1999, 2000). Including higher dietary lipid levels relative to protein generally increases protein sparing but with a concomitant increase in body lipid levels. One of the best examples of increasing energy density of diets by lipid supplementation to enhance growth and protein retention has been seen in the production of Atlantic salmon (Hardy, 1999a). Diet formulations for this species have evolved in recent years to include up to 35% lipid; they contained <20% lipid in the late 1980s (Figure 1). Protein retention values over this period have increased from 20-25% to 45% or more as a result of higher dietary lipid levels and the use of higher quality protein sources. Increases in protein retention lower nitrogenous losses.

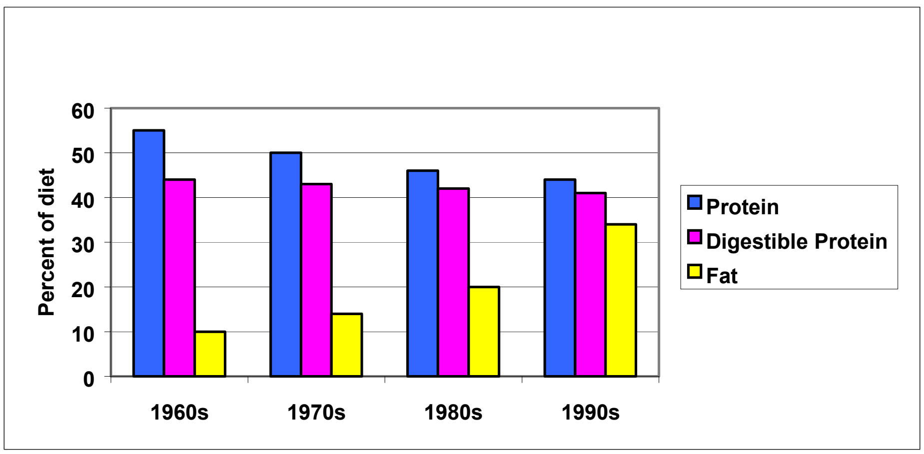

Figure 1. Changes in protein and fat content of salmon feed

Another nutritional strategy to lower nitrogen excretion is to replace feed ingredients having low protein digestibility with those having high protein digestibility coefficients. This approach depends upon using the results of studies of the digestibility and nutritional value of various feed ingredients (Hardy, 1999b). However, some highly digestible protein sources, e.g. fish hydrolysates, do not result in higher protein retention due to asynchronous absorption of amino acids from the hydrolysates and intact proteins in diets that leads to higher rates of amino acid catabolism (Stone & Hardy, 1989). Cho *et al.* (1994) explained that the most beneficial approach to increasing nutrient density of diets generally involves excluding ingredients with low protein and energy contents. This strategy was evaluated with red drum and observed to have positive effects on water quality of a closed recirculating system (Jirsa *et al.,* 1997).

In addition to ingredient composition of diets, manufacturing procedures also may influence nutrient digestibility of diets. For example, the use of extrusion processing in the manufacture of fish diets generally has resulted in increased digestibility of diets and reduced ammonia excretion (NRC, 1993). This is principally because the heat and pressure associated with extrusion processing improves the digestibility of the protein and carbohydrate fractions and thus increases the digestible energy of the diet. In addition, extruded pellets can absorb high amounts of added lipid (oil) compared to compressed pellets, and higher oil levels in diets increase nutritional density. Thus another nutritional strategy for producing low-pollution diets is to use extrusion processing to produce pellets.

Given the fact that fish typically regulate feed intake to meet an energy need (NRC, 1993), the feeding of high-energy diets may reduce intake, which may contribute to a decrease in ammonia production. However, If feed intake is severely restricted, protein intake may be too low to support rapid growth rates. Therefore, increasing the percentage of dietary protein in conjunction with an increase in energy may help to provide amino acids at levels needed for maximum growth even if feed intake is reduced (Cho & Bureau, 1997). Such interactions between diet formulations and feeding practices have been demonstrated with a number of species including salmonids (Cho & Bureau, 1997) and red drum (McGoogan & Gatlin, 2000). For other species such as the channel catfish, there has been a trend of reducing dietary protein concentrations, which does not adversely affect growth as long as feed intake is not limited (Robinson & Li, 1997).

FECAL SOLIDS

Fecal waste is another component of the diet that is typically a concern with regard to waste production in aquaculture. Various organic constituents of the diet besides protein and lipid, such as soluble and fibrous carbohydrates, become fecal waste products when not digested by fish. These organic wastes contribute to the biochemical oxygen demand of aquaculture systems (Johnsen *et al.,* 1993; Kelly & Karpinski, 1994) as well as to the amount of fecal solids in effluent waters (Pillay, 1992; Midlen & Redding, 1998). The primary means of limiting these organic wastes in aquaculture has been to formulate diets using ingredients with high nutrient digestibility. Salmon and trout feeds are much more efficient today than in the past, as reflected by improvements in feed conversion ratios (FCRs), and fecal solids output per unit feed input is much lower than in the past as a result (Figure 2). Another approach with some terrestrial animals had been the supplementation of diets with microbial enzymes such as amylases and proteases to facilitate the digestion of carbohydrate and protein, respectively, and hence improve nutrient utilization (Lobo, 1999). However, at this time such enzyme supplements have been evaluated only to a limited extent with fish.

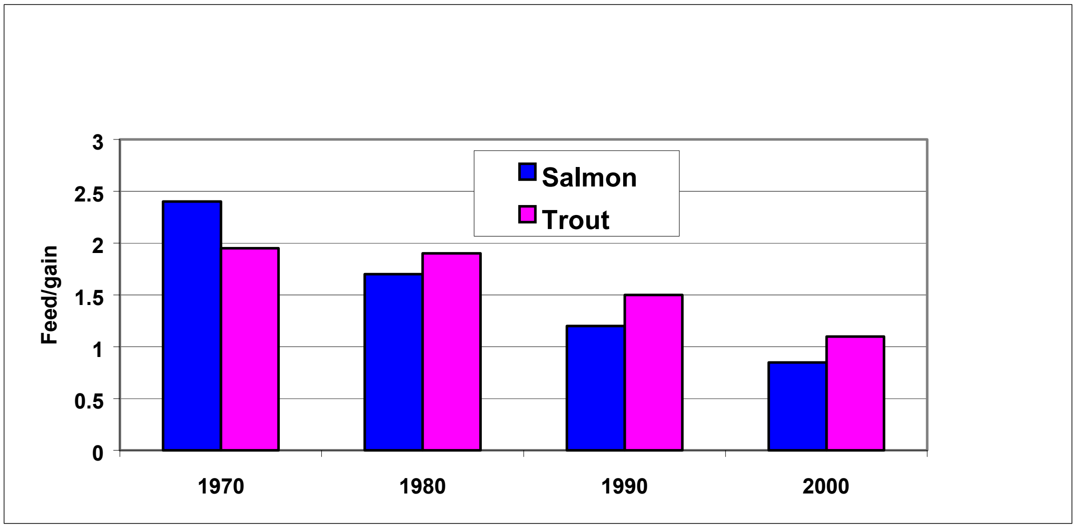

Figure 2. Changes in feed conversion ratios for grow-out salmon and trout

ACCOMPLISHMENTS TO DATE AND OUTLOOK FOR THE FUTURE

Impressive reductions in the amount of phosphorus excreted by fish have been achieved over the past decade by reducing the level of total phosphorus in the diet, and by formulating the diet so that the amount of available phosphorus closely matches the metabolic requirements of the fish. For example, trout diets used in the USA five years ago typically contained 2.2% total phosphorus, of which about 65% was available (1.4% available phosphorus). The emphasis in trout feed formulation was to ensure that sufficient levels of available phosphorus were present in the diet. Diets contained high levels of available phosphorus because the dietary requirement was not precisely understood, available phosphorus levels in feed ingredients were not known, and there was no information or even awareness of antagonistic interactions among feed ingredients in diets. It is now known that trout require between 0.55 and 0.7% available phosphorus, depending upon their size. Five years ago, trout diets typically contained twice as much available phosphorus than the fish actually required. Most of this excess available phosphorus was absorbed and excreted via the urine as soluble phosphates, which are virtually impossible to remove except by plants. Fecal phosphorus excretion by trout accounted for the balance (about 0.8% of total dietary phosphorus). Today, trout feeds are formulated to contain 1.1-1.2% total phosphorus, of which 0.7-0.9% is available. Hence, urinary losses have been reduced by 70%, and fecal losses by 50%. Further reduction of phosphorus excretion must be achieved by increasing the availability of dietary phosphorus and simultaneously reducing the total phosphorus levels in diets. The trend toward higher use of plant protein sources in fish diets to replace fish meal will increase the level of phytate in diets, making it necessary to utilize phytase, or possibly use low-phytate grains (Sugiura *et al.,*1999).

Reducing nitrogen losses from aquaculture is more problematic than reducing phosphorus because fish, like all animals, have an upper limit on the amount of dietary proteins (amino acids) that are incorporated into tissue proteins, rather than catabolized. Tissue proteins are in a constant state of flux, being continually degraded and re-synthesized. Nitrogen is excreted as a result of this protein turnover. Anabolic hormones lower the rate of turnover, thus lowering the rate of nitrogen excretion, suggesting that it is possible to achieve approximately 70% protein retention, but administration of anabolic hormones for this purpose is not anticipated in aquacultural production of food fish. Given the fact that protein retention has doubled in Atlantic salmon farming (from ca. 22% to 45%), it is likely that protein retention values can be increased in other species of farmed fish. Strategies for increasing protein retention include balancing dietary amino acid levels, perhaps in a factorial manner that changes with life history stage or size of fish, optimizing dietary protein (and amino acids) and energy levels, and using enzyme supplements to facilitate digestion of less available nutrients in various feedstuffs.

Reducing organic material excreted by fish basically revolves around increasing the digestibility of feed ingredients, especially those of plant origin. As mentioned earlier, feed processing technology will be a key in this effort, as will the use of enzyme supplements to facilitate digestion of starches and cellulose components of plant-derived feed ingredients.

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