NITROGEN AND PHOSPHORUS WASTE IN FISH FARMING

Rafael LAZZARI [1] and Bernardo BALDISSEROTTO [2]

ABSTRACT

The current concerns about the amount of residue generated from fish rearing suggests that this will be a decisive factor in the sustainability of fish farming in the coming years. Due to the great intensification of fish farming, the amount of residue deposited into the rearing tanks has increased significantly. Nitrogen (N) and phosphorus (P) are the main end-products of fish loading, and can affect not only the rearing water, but also the environment as a whole. The output of N and P metabolic wastes by fish was determined by numerous endogenous and exogenous factors such as genetics, life stage, size, rearing system, and diet. Ammonia is predominant type of N excreted, and high levels of ammonia excretion may be due to high protein intake or inadequately formulated diets which provide unbalanced protein synthesis. Phosphorus excretion, usually 69-86% of dietary P, is associated with the sources of origin, which are used in different ways by different species. The use of phytase in fish feeds is a good alternative which can help to reduce P waste. The diet balance should be standardized and the N and P excretion rates in several rearing systems (mainly the intensive farms) should be measured since a two- to three-fold decrease in the excretion of those pollutants in the fish culture systems could be attained.

Key words: excretion, water quality, ammonia, pollution.

EXCREÇÃO DE NITROGÊNIO E FÓSFORO EM PISCICULTURAS

RESUMO

A preocupação com a quantidade de resíduos gerados a partir da criação de peixes é um fator decisivo na sustentabilidade da piscicultura para os próximos anos. Com a grande intensificação dos sistemas de criação de peixes, a carga de resíduos lançados à água de cultivo aumentou significativamente. Os compostos nitrogenados e fosforados são os principais produtos de excreção dos peixes, e ambos podem afetar não só a água de cultivo como o ambiente como um todo. A produção de resíduos metabólicos de N e P pelos peixes é determinada por diversos fatores endógenos e exógenos como genética, idade, tamanho, ambiente de criação e dieta. Em relação ao N, a excreção na forma de amônia é predominante, e quando em altos níveis pode ser devido a uma alta ingestão protéica ou a dietas inadequadamente formuladas, as quais causam desequilíbrio na síntese protéica. A excreção de P, usualmente 69-86% P da dieta, está associada às fontes utilizadas, as quais são aproveitadas de forma distinta nas diferentes espécies. O uso da fitase nos alimentos para peixes é uma boa alternativa para a redução da excreção do P. A padronização da formulação de dietas e a mensuração de taxas reais de excreção de N e P nos diversos sistemas de criação (principalmente os intensivos) deverão ser mais bem estudados, uma vez que ao menos uma diminuição de 2 a 3 vezes da taxa de excreção desses poluentes nas pisciculturas pode ser alcançada.

Palavras-chave: excreção, qualidade da água, amônia, poluição.

Artigo de revisão: Recebido em 08/05/2007 - Aprovado em 04/08/2008

[1] *Animal Husbandry Post-graduation Program, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil. E-mail:rafaellazzari@yahoo.com.br*

[2] *Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil - CEP 97105-900. E-mail: bernardo@smail.ufsm.br (author for correspondence).*

INTRODUCTION

World fish production has increased significantly with the intense use of manufactured diets (CHO, 1993). Nowadays, the main objectives in fish farming are improvement of the foods used and the reduction of nutrients excreted in the water (FOURNIER *et al.*, 2003). Nitrogen (N) and phosphorous (P) in metabolic waste produced by fish are the origin of most dissolved N and P waste resulting from intensive aquaculture operations. The excess of these two elements in the effluents of aquaculture systems leads to eutrophication and a consequent change in the aquatic ecosystem (JAHAN *et al.,* 2003a). Levels of N and P in fish food and the efficiency with which they are used influences the amounts of these nutrients that are excreted into the environment (RODEHUTSCORD *et al.*, 1994). Reducing the outputs of these dissolved wastes is considered to be a key element for the long-term sustainability of aquaculture around the world (CHO and BUREAU, 1997), and appropriate balanced diets allow the amount of these compounds in the water to be significantly decreased (HASAN, 2001). The amount of alimentary residue depends on the rearing size, fish species, rearing practices, alimentary handling, and food characteristics (MALLEKH *et al.*, 1999).

An important advancement in fish nutrition was the use of extruded diets. These diets possess higher stability and digestibility, providing a significant reduction in the amount of nutrients excreted into the rearing water (JOHNSEN *et al.*, 1993). Fish have higher protein requirements than other animal species consumed by humans. Due to the increase in aquaculture production and the implications of poor protein use on N emitted into the effluents, there is an increasing need to optimize the supply of protein and indispensable amino acids (FOURNIER *et al.*, 2002). The concentration of ammonia is often the limiting water quality parameter in intensive aquaculture production systems (THOMAS and PIEDRAHITA, 1998).

Therefore, this review aimed to discuss some important aspects of N and P waste and their relationship with water quality in several fish farming systems.

NITROGEN

Fish are able to utilize protein very efficiently, despite the fact that they use a significant portion of digestible protein for energetic purposes, and produce large amounts of nitrogenous metabolites (DOSDAT *et al.*, 1996). It is well known that feeding of an excess of amino acids will result in amino acid catabolism with associated ammonia excretion and a loss of energy. The balance between digestible protein and digestible energy in the diet is also important in this respect.

The main end-product of protein metabolism in teleost fish is ammonia, but a significant proportion of nitrogenous waste may also be excreted as urea in some species (WOOD, 1993). There are other N waste products, such as creatine, creatinine, trimethylamine (TMA), trimethylamine oxide (TMAO), and uric acid, which have been investigated occasionally in fish studies (KAJIMURA *et al.*, 2004). Mainly excreted through the gills, ammonia production by fish is primarily dependent on the protein intake and metabolic efficiency of the fish, which is species-specific and is affected by waterborne ammonia levels (DOSDAT *et al.*, 2003). Most fish eat protein-rich diets and ammonia is, metabolically, the least expensive means of removing the N produced by deamination of amino acids (CARTER and BRAFIELD, 1992). According to these authors, differences in the amount of excreted N were explained by the differing N content of the diets. Freshwater species tend to excrete more total ammonia nitrogen (TAN) than marine species (JOBLING, 1995). Usually, ammonia is toxic and can affect fish growth. However chronic exposure of rainbow trout (*Oncorhynchus mykiss)* to very low non-ionized ammonia levels (0.01 mg/L at pH 7.6 and 0.002 mg/L at pH 6.3) for 70 days stimulated growth and protein production without increasing food consumption; i.e., ammonia was used to produce amino acids (WOOD, 2004).

The quantification of ammonia and urea excretion of fish species in relation to their nutrition is important for intensive fish culture operations because protein metabolism partly defines the success of a particular nutritional regimen (GÉLINEAU *et al.*, 1998; ENGIN and CARTER, 2001). The capacity of protein use in fishes differs among species and life stages, and there is a strong relationship between protein levels in the food and the production of ammonia N (BEGUM *et al.*, 1994). Proteins are generally considered to contain 16% N (NRC, 1993). Ammonia production is influenced by the relationship of protein or energy and the balance of dietary amino acids (KAUSHIK, 1998). Diets formulated with protein sources with poorer amino acid profile will generally result in higher ammonia excretion.

For grass carp (*Ctenopharyngodon idella*), linear relationships were found between daily rates of ammonia excretion, total nitrogen intake, energy loss and daily rates of food intake (CARTER and BRAFIELD, 1992). Tilapia juveniles fed for 28 days on diets with protein levels varying from 32 to 55% CP/kg food presented a linear increase in N excretion with the increase of dietary protein levels (BRUNTY *et al.*, 1997). High dietary protein levels (35-45%) also increased ammonia excretion in red drum (*Sciaenops ocellatus*), but did not affect weight gain (WEBB Jr. and GATLIN III, 2003).

In different fish culture systems, about 25% of nitrogen added as feed or other nutrient input is recovered by the target organism (HARGREAVES, 1998). For Brazilian species, there is no information about N cycles in fish farming (Table 1). Nitrogen excretion rates in the form of ammonia can be easily measured in closed conditions. However, in ponds, several additional factors interfere in this process because ammonia under these rearing conditions can be produced or consumed in biological processes that do not usually occur in the laboratory (THOMAS and PIEDRAHITA, 1998). In aquaculture ponds, little information regarding the effects of N inputs and outputs on N dynamics is available (GROSS *et al.*, 2000). A more complete understanding of the factors regulating ammonia and nitrite concentrations and the exchange of nitrogenous compounds between sediment and water in aquaculture ponds is needed (HARGREAVES, 1998).

Table 1. Estimates of the range of the percentage of nitrogen recovered by fish and released to the environment in various aquaculture production systems[1]

Fish species	Production system[2]	Recovered fish	Released			References
			Total	Dissolved	Solid	
Oreochromis niloticus	P	18-21	81			GREEN and BOYD, 1995
Ictalurus punctatus	P	27	73			BOYD, 1985
Sparus aurata	T	27		66	7	NEORI and KROM, 1991
Salmo salar	C	25		65	10	GOWEN and BRADBURY, 1987
Salmo salar	C	25		62	13	FOLKE and KAUTSKY, 1989
Clarias macrocephalus	C	24	76			LIN *et al.*, 1993
Ictalurus punctatus	R	14	86			WORSHAM, 1975
Oncorhynchus mykiss	R	19		74	7	FOY and ROSELL, 1991, a, b

[1] Extracted from Hargreaves (1998); [2] Production systems: P=earthen pond; T=tank; C=cages; R=raceway.

Protein sources such as fish meal and soybean meal may improve the efficiency of N assimilation and utilization (HARGREAVES, 1998). The use of vegetable protein sources in fish diets has different effects on fish growth and N wastes. This kind of feed has a poorer amino acids balance, reducing N retention, and consequently, increasing N excretion. Dietary supplementation with synthetic amino acids and including a higher proportion of vegetable protein sources is an important mechanism of decreasing protein levels in the food and reducing N excretion (CHENG *et al.*, 2003). Rodehutscord *et al.* (1994) supplemented diets for rainbow trout with 1.4% lysine and 5.6% other essential amino acids, reducing the protein level in the food from 46 to 30% and consequently reducing the amount of excreted N by up to 43% without affecting growth.

PHOSPHORUS

Phosphorus is an important mineral in nucleic acids and cellular membranes, the main representative of the structural components of the skeletal tissues, and it is directly involved in energy processes (NRC, 1993). Fish can absorb this mineral from the water, but due to the low waterborne P concentration, dietary supplementation is necessary. In rainbow trout, dietary inorganic P uptake occurs in the intestine (10%) and pyloric caeca (90%). In the pyloric caeca, diffusive uptake represents around 92% of the total inorganic P uptake if the diet contains appropriate P levels, and there is consequently almost no regulation of this uptake. However, in a P-poor diet, the Na^{+}/P transporter becomes essential (SUGIURA and FERRARIS, 2004). The excess of this mineral in fish diets provides higher levels of excreted P, with this being the main cause of eutrophization in the aquatic environment, impairing water quality (KIM *et al.*, 1998b). When fish feeds which produce less P pollution are formulated, the adequacy of available P should be considered, so as to support growth (JAHAN *et al.*, 2003b). With the global concern of reducing water pollution, the reduction of P excretion by fish is becoming imperative for fish food industries (RODEHUTSCORD *et al.*, 2000). According to the same authors, studies on this subject should have two basic objectives: 1. identification of the demands of available P for the different species; and 2. quantification of the proportion of dietary P available and the amount used by the fish.

There are different chemical forms of P in the diet. Very significant differences are observed in the digestibility of the various forms of P (bone, phytin or organic P). Other factors, such as particle size and feed processing techniques, are also known to affect P digestibility (AZEVEDO *et al.*, 1998). Food quality improvement involving ways of retaining dietary P is one of the main strategies of reducing the environmental impact of aquaculture (SATOH *et al.*, 2003). The degree of non-retained P is largely affected by its bioavailability and dietary content, depending on the food type (BUYUKATES *et al.*, 2000). Generally, fish diets that depend on fish meal to provide their main protein source contain a total P level that surpasses the minimum requirements needed to obtain optimum growth (SATOH *et al.*, 2003). P in fish meal is in the form of tricalcium phosphate, which remains almost inaccessible to many cultivated species (SUGIURA *et al.*, 2000a). Phosphorus is found in all plant and animal feed ingredients used in formulate diets. The availability of P varies greatly depending on the source (Table 2).

Table 2. Percent of phosphorus available in common food types used in fish diets (Adapted from NRC, 1993).

Ingredient	Salmonid	*Ictalurus punctatus*	Carp
Blood meal	81		
Brewer's yeast	79-91		93
Feather meal	77		
Poultry meal	81		
Anchovy meal		40	
Menhaden meal	87	39	
Rice bran	19		25
Wheat germ	58		57
Ground corn		25	
Dehulled soybean meal	36	29-54	

The need to formulate diets which minimize fish P excretion and consequent eutrophization of the water requires the replacement of fish meal with low-P protein sources (LALL, 1991). The use of high protein ingredients that have a high percentage of digestible P may help to reduce the unavailable P concentration of the feed (CHO *et al.*, 1994). The suitability of soybean products as a partial replacement for fish meal has been assessed for cost-effective, sustainable and low-P fish feed formulations (NRC, 1993). In general, vegetable sources possess a large amount of P in the phytate form, which is unavailable for the fish because they do not possess the enzyme phytase (NRC, 1993). Higher phytase levels in the feed increase P bioavailability and utilization (BAKER *et al.*, 2001). On the other hand, P contained in animal protein sources, as in the fish meal, is not in the phytate form, but it can be affected by the technique or chemical treatment used in the ingredient production (RODEHUTSCORD *et al.*, 1994).

Fish meal is the main component of fish diets, and has P levels as high as other animal protein sources, such as meat and bone meals. Partial replacement of fish meal by vegetable sources tends to reduce P (KETOLA and HARLAND, 1993) and N excretion (as ammonia) by reducing protein levels (CHENG *et al.*, 2003). Maximum P absorption in rainbow trout is 5.2 g/kg of dry matter, and higher dietary levels only increase the amount of excreted P (RODEHUTSCORD *et al.*, 2000). Usually, the mixture of various protein sources provides lower P excretion without affecting fish performance. Rainbow trout fed fish meal based-diets showed higher P retention than fish fed soy protein concentrate (KIM *et al.*, 1998a). Usually, the availability of phytic P found in vegetable protein sources such as soybean is very low in fish (NRC, 1993). For seabass (*Dicentrarchus labrax*), the supplementation of soybean meal diets with phytase increases P retention (OLIVA-TELES *et al.*, 1998). Supplemental phytase tended to increase bone ash of soy-fed fish, thus indirectly indicating successful gastrointestinal hydrolysis of phytate in the soybean diets, even without effects on growth compared with fish meal based diets (VIELMA *et al.*, 2000). Other studies reported a positive effect of phytase addition in channel catfish (*Ictalurus punctatus*) (JACKSON *et al.*, 1996), rainbow trout (VIELMA *et al.*, 2000), Nile tilapia (PORTZ and LIEBERT, 2004) and common carp (*Cyprinus carpio*) (SCHAEFER, 1995). It is important to emphasize that the form and the phytase levels are dependent of the several factors such as species, age, food type, rearing systems and other factors. For salmonids, replacement of fish meal with high amounts of soybean without phytase treatment or supplementation with calcium phosphate is not recommended to reduce P excretion, as the fish were unable to maintain homeostatic regulation of divalent ions (STOREBAKKEN *et al.*, 2000).

Phosphorus retention is also directly affected by growth rate, and higher values were obtained when growth performances were good (JAHAN *et al.*, 2002). In fish, a certain amount of non-fecal P excretion is unavoidable and occurs even at zero intake of P. Consequently, the non-fecal P excretion has been found to be unaffected by P intake up to the level required by the experimental animal (RODEHUTSCORD *et al.*, 2000).

Dietary composition affects P retention and excretion. Diets with high lipid levels and lower P content improved P retention (GREEN *et al.*, 2002). The relationship Ca^{2+}/P also interferes in P use and metabolism. Consequently, an optimum dietary P level would be useless if dietary Ca^{2+}, for example, is low. In addition, KETOLA and HARLAND (1993) and BALLESTRAZZI *et al.* (1994) showed that the reduction of the dietary P levels may increase P absorption if there is an adequate dietary nutrient balance. In common carp, the total P loading calculated based on P retention in whole body was 13.6 kg P/t produced (WATANABE *et al.*, 1999). This value is higher than those from other fish species like salmons, which discharge less than 3 kg P/t produced (CHO and BUREAU, 1997).

IMPACTS IN THE REARING WATER QUALITY

Containment and collection of wastes, both solid and dissolved, is very difficult and costly in aquaculture, as the wastes are rapidly dispersed into the surrounding waters (CHO and BUREAU, 2001). Metabolic waste concentrations may reach high levels in tanks, thereby limiting fish survival and growth, as well as harming the environment by discharging the enriched water from tanks (LEMARIÉ *et al.*, 1998). Quantification of fish waste production is required to monitor such risks and to develop integrated high-density culture systems using recirculated water (LEMARIÉ *et al.*, 1998; PORRELLO *et al.*, 2003). As it is difficult for the producer to measure P and N waste, diet manipulation is the main means by which producers can minimize the discharge of soluble nutrients in the water. In salmonid culture, 2 kg P and 40 kg soluble N were excreted for each 1,000 kg of fish produced (CHO *et al.,* 1991). The production of 1,000 kg fish of marine caged-cultured carnivorous fed with discarded fish yielded a higher amount of P (3.4-10.9 kg P) and a similar amount of N waste (34.4-67.2 kg N) (XU *et al.*, 2007).

In the context of aquaculture, the release of dissolved and suspended N and P can be significantly reduced through precise knowledge of the requirements of the fish and the supply to and retention by the fish (KAUSHIK, 1998). One of the most effective ways of improving aquaculture effluent water quality is through modifications of the diets fed to culture fish (CHO and BUREAU, 1997). Digestibility of the ingredients and nutrient composition of the diet are the main factors that affect waste outputs by fish. However, data of the apparent digestibility coefficients (ADC) of protein and other nutrients are highly variable. According to CHO and BUREAU (2001), this variability results from four main factors: (1) differences in the fecal material collection method used; (2) experimental errors; (3) differences in the manufacturing and chemical composition of the ingredients; and (4) biological and environmental differences (fish species, fish size, water temperature).

The use of the extruded diets may provide a significant decrease in the nitrogen discharge in waste waters from fish farms (BALLESTRAZZI *et al.*, 1998). However, these authors did not observe any difference in P excretion between sea bass fed with extruded or pelleted diets.

The relationships among ingested, kept and excreted nutrients are fundamental to estimating the amount of excreted residues (KAUSHIK, 1998). The mean daily ammonia production rates have been found to increase linearly with increasing protein intake (BALLESTRAZZI *et al.*, 1994; FORSBERG, 1996). For similar feed types, N and P waste production depends on species, mean weight and rearing temperature (LEMARIÉ *et al.*, 1998). For example, in gilthead sea bream rearing, fish fed with food containing 47%CP and a feed conversion ratio of 1.8:1 produce 1000 kg fish, 180 kg solids, 13 kg P (10.8 kg in the feces and 2.2 kg in the water) and 105.4 kg N (24.4 kg in the feces and 81kg in the water) (ALVARADO, 1997). In gilthead sea bream culture (fish produced up to 400 g), from the total N and P intake (132 kg N; 25 kg P), the fish retention was 22% and 29%, respectively (LUPATSCH and KISSIL, 1998). These authors suggest that fish size, digestibility and water temperature are important in predicting N and P waste.

Measuring ammonia production rates in white sturgeon (0.09-3.8 kg) maintained in ponds and fed with diets containing 40%CP, THOMAS and PIEDRAHITA (1998) showed that they produced 36.2-662.4 mg total ammonia/kg.day. Tambaqui (*Colossoma macropomum*) kept in aquaria (25-27 ºC) showed a peak ammonia excretion four hours after feeding, where the amount of excreted ammonia was influenced directly by temperature and inversely by fish weight according to the following equation (ISMIÑO-ORBE *et al.*, 2003):

$$\ln(\text{daily total ammonia excretion}) = 31.03(2.96) + \ln W \times 0.79(0.06) - t \times 1.32(0.64),$$

where total ammonia is expressed in mg/L, W = weight (g), t = temperature (ºC), and the number between the parentheses is the standard error.

Juveniles of brown trout, turbot and sea bass of 10 g also produced proportionally more ammonia and urea than fishes that were 100 g in weight (DOSDAT et al., 1996). California halibut (*Paralichthys californicus*) 4-20 g in weight that were fed 43-45% CP in a marine water recirculation system presented peaks of ammonia excretion 4-6 h after feeding, and the daily excretion was 91-113 mg total ammonia/kg.day (MERINO *et al.* 2007). Studies of rainbow trout (*Oncorhynchus mykiss*) showed that water temperatures from 6 to 15ºC had no effect on digestible N retention efficiency (AZEVEDO *et al.*, 1998).

Retention of dietary P by fish used to be about 20% in typical commercial aquaculture feed, and most dietary P (69–86%) is excreted in the effluent. Recent experiments presented new foods with similar compositions and approximately 40-60% P retention. Rainbow trout kept in raceways and fed on diet with 1% P showed similar growth rates to specimens fed on a diet with 1.4 %P, but production costs were higher in fish fed on the low P diet (SUGIURA *et al.*, 2006). The total P calculation is based on the P retention rate and is considered to be a more accurate estimation as it includes both fecal and non-fecal excretion (JAHAN *et al.*, 2002). The values based on this calculation supported those found in previous studies of fish, and were higher than the apparent values, suggesting that post-absorption output through the gills and urine cannot be ignored (SUGIURA *et al.*, 2000b). The total values of P excreted by carp were reduced by the lower inclusion of fish meal, while N waste was increased. Carp fed on food with 10% fish meal excreted 5.9 kg P/1000 kg carp production, and those fed on food with 30% fish meal excreted 9.6 kg P/100 kg (JAHAN *et al.*, 2000). In turbot rearing, Mallekh *et al.* (1999) found that for production of 1000 kg of turbot, 51 kg N and 8.7 kg P were excreted. According to these authors, N and P retention were 35.7 and 42.1%, respectively, in turbot rearing in ponds. For rainbow trout, the values of N and P excretion ranged from 47 to 87 kg and 4.8-18.7 kg per 1000kg of fish produced, respectively (AXLER *et al.*, 1997). Apparently, rates of N and P excretion are more dependent on feed content than on the feeding habits of the species.

Results from a variety of culture systems indicate that, on average, about 25% (with a range from 11 to 36%) of N added as in feed or other nutrient inputs was recovered by the target organism (HARGREAVES, 1998). The inherent efficiency of nutrient utilization by fish implies that N loading of fish ponds may be limited by the capacity of the ponds to assimilate nitrogenous excreta (HARGREAVES, 1998; PASPATIS *et al.*, 2000). Fish species and time after food intake also influenced excretion (LUPATSCH and KISSIL, 1998).

FUTURE DIRECTIONS

The relative proportions of ammonia, urea and other nitrogenous compounds comprising total nitrogenous excretion per unit of N intake need further investigation in Brazilian species. The primary concern is related to the values of N and P waste of Brazilian species in different rearing systems, and therefore the qualitative and quantitative protein requirements in different growth stages should be determined. The standardization of methodologies assessing digestibility is the next step for comparing results of different fish studies. In addition, improvement of technologies for increasing the digestibility of feed is also necessary.

REFERENCES

ALVARADO, J.L. 1997 Aquafeeds and the environment. In: A. TACON and B. BASURCO, Eds. *Feeding tomorrow´s fish.* p.275-289. Proceedings of the workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM). Mazzarron, Spain, 24-26 June 1996 CIHEAM, Apodo, Spain.

AXLER, R.P.; TIKKANEN, C.; HENNECK, J.; SCHULDT, J.; McDONALD, M.E. 1997 Characteristics of effluent and sludge from two commercial rainbow trout farms in Minnesota. *Progressive Fish-Culturist,* 59:161-172.

AZEVEDO, P.A.; CHO, C.Y.; BUREAU, D.P. 1998 Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources,* Paris, 11(4):227-238.

BAKER, R.T.; SMITH-LEMMON, L.L.; COUSINS, B. 2001 Phytase unlocks plant potential in aquafeeds. *Global Aquaculture Advocate*, 4(2).

BALLESTRAZZI, R.; LANARI, D.; D'AGARO, E.; MION, A. 1994 The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax,* L.). *Aquaculture*, Amsterdam, 127:197-206.

BALLESTRAZZI, R.; LANARI, D.; D'AGARO, E. 1998 Performance, nutrient retention efficiency, total ammonia and reactive phosphorus excretion of growing European sea-bass (*Dicentrarchus labrax,* L.) as affected by diet processing and feeding level. *Aquaculture*, Amsterdam, 161:55-65.

BEGUM, N.N.; CHAKRABORTY, S.C.; ZAHER, M. 1994 Replacement of fish meal by low-cost animal protein as a quality fish feed ingredient for Indian major carp, *Labeo rohita*, fingerlings. *Science Food Agriculture,* London, 64:191-197.

BOYD, C.E. 1985 Chemical budgets for channel catfish ponds. *Transactions of the American Fisheries Society,* 114:291-298.

BRUNTY, J.I; BUCKLIN, R.A; DAVIS, J.; BAIRD, C.D.; NORDSTEDT, R.A. 1997 The influence of feed protein intake on tilapia ammonia production. *Aquaculture Engineering,* 16:161-166.

BUREAU, D.P.; CHO, C.Y. 1999 Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): estimation of dissolved phosphorus waste output. *Aquaculture*, Amsterdam, 179:127-140.

BUYUKATES, Y.; RAWLES, S.D.; GATLIN III, D.M. 2000 Phosphorus fractions of various feedstuffs and apparent phosphorus availability to channel catfish. *North American Journal Aquaculture*, 62:184-188.

CARTER, C.G.; BRAFIELD, A.E. 1992 The bioenergetics of grass carp, *Ctenopharyngodon idella* (Val.): the influence of body weight, ration and dietary composition on nitrogenous excretion. *Journal of Fish Biology*, Leicester, 41:533-543.

CHENG, Z.J.; HARDY, R.W.; USRY, J.L. 2003 Plant protein ingredients with lysine supplementation reduce dietary protein level in rainbow trout (*Oncorhynchus mykiss*) diets, and reduce ammonia nitrogen and soluble phosphorus excretion. *Aquaculture*, Amsterdam, 218:553-565.

CHO, C.Y. 1993 Digestibility of feedstuffs as a major factor in aquaculture waste management. In: KAUSHIK, S.J., LUQUET, P. (Eds.), *Fish nutrition in practice.* Les Colloques n.61, INRA ed., Versailles Cedex, France, 365-374.

CHO, C.Y.; BUREAU, D.P. 1997 Reduction of waste output from salmonid aquaculture through feeds and feeding. *Progressive Fish Culturist,* 59:155-160.

CHO, C.Y.; BUREAU, D.P. 2001 A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture International,* Amsterdam, 32:349-360.

CHO, C.Y.; HYNES, J.D.; WOOD, K.R.; YOSHIDA, H.K. 1991 Quantification of fish culture wastes by biological (nutritional) and chemical (limnological) methods; the development of high nutrient dense (HND) diets. In: *Nutritional Strategies and Aquaculture Waste* (ed. by C.B. Cowey & Cho, C.Y.), pp. 37-50.

CHO, C.Y.; HYNES, J.D.; WOOD, K.R.; YOSHIDA, H.K. 1994 Development of high-nutrient-dense, low-pollution diets and prediction of aquaculture waste using biological approaches. *Aquaculture,* Amsterdam, 124, 293-305.

DOSDAT, A.; RUYET, J.P.; COVÈS, D.; DUTTO, G.;

GASSET, E.; LE ROUX, A.; LEMARIÉ, G. 2003 Effect of chronic exposure to ammonia on growth, food utilization and metabolism of the European sea bass (*Dicentrarchus labrax*). *Aquatic Living Resources,* Paris, 16:509-520.

DOSDAT, A.; SERVAIS, F.; MÉTAILLER, R.; HUELVAN, C.; DESBRUYÉRES, E. 1996 Comparison of nitrogenous losses in five teleost fish species. *Aquaculture*, Amsterdam, 141:107-127.

ENGIN, K.; CARTER, C.G. 2001 Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary protein level. *Aquaculture,* Amsterdam, 194:123-136.

FOLKE, C.; KAUTSKY, N. 1989 The role of ecosystems for a sustainable development of aquaculture. *Ambio*, 18:234–243.

FORSBERG, O.I. 1996 Ammonia excretion rates from post-smolt Atlantic salmon, *Salmo salar* L., in land-based farms. *Aquaculture Research*, Amsterdam, 27:937-944.

FOURNIER, V.; GOUILLOU-COUSTANS, M.F.; MÉTAILLER, R.; VACHOT, C.; GUEDES, M.J.; TULLI, F.; OLIVA-TELES, A.; TIBALDI, E. KAUSHIK, S.J. 2002 Protein and arginine requirements for maintenance and nitrogen gain in four teleosts. *British Journal of Nutrition,* London, 87:459-469.

FOURNIER, V.; GOUILLOU-COUSTANS, M.F.; MÉTAILLER, R.; VACHOT, C.; MORICEAU, J.; LE DELLIOU, H.; HUELVAN, C.; DESBRUYERES, E.; KAUSHIK, S.J. 2003 Excess dietary arginine affects urea excretion but does not improve N utilization in rainbow trout *Oncorhynchus mykiss* and turbot *Psetta maxima*. *Aquaculture,* Amsterdam, 217:559-576.

FOY, R.H.; ROSELL, R. 1991a Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. *Aquaculture*, 96:17–30.

FOY, R.H.; ROSELL, R. 1991b Fractionation of phosphorus and nitrogen loadings from a Northern Ireland fish farm. *Aquaculture*, 96:31–42.

GÉLINEAU, A.; MÉDALE, F.; BOUJARD, T. 1998 Effect of feeding time on postprandial nitrogen excretion and energy expenditure in rainbow trout. *Journal of Fish Biology*, Leicester, 52:655-664.

GOWEN, R.J.; BRADBURY, N.B. 1987 The ecological impact of salmonid farming in coastal waters: a review. *Oceanogr. Mar. Biol. Annual Reviews*, 25:563–575.

GREEN, B.W.; BOYD, C.E. 1995 Chemical budgets for organically-fertilized fish ponds in the ry tropics. *Journal of the World Aquaculture Society,* 26:284-296.

GREEN, J.A.; BRANNON, E.L.; HARDY, R. 2002 Effects of dietary phosphorus and lipid levels on utilization and excretion of phosphorus and nitrogen by rainbow trout (*Oncorhynchus mykiss*). 2. Production-scale study. *Aquaculture Nutrition*, 8:291-298

GROSS, A.; BOYD, C.; WOOD, C.W. 2000 Nitrogen transformations and balance in channel catfish ponds. *Aquacultural Engineering*, 24:1-14.

HARGREAVES, J.A. 1998 Nitrogen biogeochemistry of aquaculture ponds. (Review). *Aquaculture*, Amsterdam, 166:181-212.

HASAN, M.R. 2001 Nutrition and feeding for sustainable aquaculture development in the third millennium. In: *Technical Proceedings of the Conference on Aquaculture in the Third Millennium,* Bangkok, Thailand, 193-219.

ISMIÑO-ORBE, R.A.; ARAÚJO-LIMA, C.A.R.M.; GOMES, L.C. 2003 Excreção de amônia por tambaqui (*Colossoma macropomum*) de acordo com variações na temperatura da água e massa do peixe. *Pesquisa Agropecuária Brasileira,* Brasília, 38(10):1243-1247.

JACKSON, L.; LI, M.; ROBINSON, E. 1996 Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytase phosphorus. *Journal of the World Aquaculture Society,* 27:309-313.

JAHAN, P.; WATANABE, T.; SATOH, S.H.; KIRON, I. 2000 Effect of dietary fish meal levels on environmental phosphorus loading from carp culture. *Fisheries Science*, Tokyo, 66:204-210.

JAHAN, P.; WATANABE, T.; SATOH, S.H.; KIRON, I. 2002 Different combinations of protein ingredients in carp diets for reducing phosphorus loading. *Fisheries Science*, Tokyo, 68:595-602.

JAHAN, P.; WATANABE, T.; KIRON, I.; SATOH, S.H. 2003a Improved carp diets based on plant protein sources reduce environmental phosphorus loading. *Fisheries Science,* Tokyo, 69:219-225.

JAHAN, P.; WATANABE, T.; KIRON, I.; SATOH, S.H. 2003b Balancing protein ingredients in carp feeds to limit discharge of phosphorus and nitrogen into water bodies. *Fisheries Science,* Tokyo, 69:226-233.

JOBLING, M. 1995 *Environmental biology of fishes.* Chapman and Hall, New York, 455p.

JOHNSEN, F.; HILLESTAD, M.; AUSTRENG, E. 1993 High energy diets for Atlantic salmon. Effects on pollution. In: KAUSHIK, S.J., LUQUET, P. (Eds.), *Fish nutrition in practice***.** Les Colloques n.61, INRA ed., Versailles Cedex, France, 391-402.

KAJIMURA, M.; CROKE, S.J.; GLOVER, C.; WOOD, C.M. 2004 Dogmas and controversies in the handling of nitrogenous wastes: the effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. *Journal of Experimental Biology,* 207:1993-2002.

KAUSHIK, S.J. 1998 Nutritional bioenergetics and estimation of waste production in non-salmonids. (Review) *Aquatic Living Resources,* Paris, 11(4):211-217.

KETOLA, H.G.; HARLAND, B.F. 1993 Influence of phosphorus in rainbow trout diets on phosphorus discharges in effluent water. *Transactions of the American Fisheries Society*, 122:1120-1126.

KIM, J.D.; KAUSHIK, S.J.; BREQUE, J. 1998a Nitrogen and phosphorus utilization in rainbow trout (*Oncorhynchus mykiss*) fed diets with or without fish meal. *Aquatic Living Resources*, Paris, 11(4):261-264.

KIM, J.D.; KIM, K.S.; SONG, J.S.; LEE, J.Y.; JEONG, K.S. 1998b Optimum level of dietary monocalcium phosphate based on growth and phosphorus excretion of mirror carp, *Cyprinus carpio. Aquaculture*, Amsterdam, 161:337-344.

LALL, S.P. 1991 Digestibility, metabolism and excretion of dietary phosphorus in fish. In: COWEY, C.B., CHO, C.Y. (Eds.). *Nutritional Strategies and Aquaculture Waste.* Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Ontario, 21-36.

LEMARIÉ, G.; MARTIN, J.M.; DUTTO, G.; GARIDOU, C. 1998 Nitrogenous and phosphorous waste production in a flow-through land-based farm of European seabass (*Dicentrarchus labrax*). *Aquatic Living Resources,* Paris, 11(4):247-254.

LIN, C.K.; JAIJEN, K.; MUTHUWAN, V. 1993 Integration of intensive and semi-intensive aquaculture: concept and example. *CRSP Research Reports*, 93-54.

LUPATSCH, I.; KISSIL, G.W. 1998 Predicting aquaculture waste from gilthead seabream (*Sparus aurata*) culture using a nutritional approach. *Aquatic Living Resources,* Paris, 11(4):265-268.

MALLEKH, R.; BOUJARD, T.; LAGARDÈRE, J.P. 1999 Evaluation of retention and environmental discharge of nitrogen and phosphorus by farmed turbot (*Scophthalmus maximus*). *North American Journal of Aquaculture,* 61:141-145.

MERINO, G.E.; PIEDRAHITA, R.H.; CONKLIN, D.E. 2007 Ammonia and urea excretion rates of California halibut (*Paralichthys californicus*, Ayres) under farm-like conditions. *Aquaculture*, 271: 227-243.

NEORI, A.; KROM, M.D. 1991 Nitrogen and phosphorous budgets in an intensive marine fishpond: the importance of microplankton. In: COWEY, C.B., CHO, C.Y._Eds.., *Nutritional Strategies and Aquaculture Waste*. University of Guelph. Guelph, Ontario, Canada, pp. 223–230.

NRC. 1993 National Research Council. *Nutrients requirements of fish.* National Academy Press. 114p.

OLIVA-TELES, A.; PEREIRA, J.P.; GOUVEIA, A.; GOMES, E. 1998 Utilization of diets supplemented with microbial phytase by seabass (*Dicentrarchus labrax*) juveniles. *Aquatic Living Resources,* Paris, 11(4):255-259.

PASPATIS, M.; BOUJARD, T.; MARAGOUDAKI, D.; KENTOURI, M. 2000 European sea bas growth and N and P loss under different feeding practices. *Aquaculture,* Amsterdam, 184:77-88.

PORRELLO, S.; LENZI, M.; PERSIA, E.; TOMASSETTI, P.; FINOIA, M.G. 2003 Reduction of aquaculture wastewater eutrophication by phytotreatment ponds system I. Dissolved and particulate nitrogen and phosphorus. *Aquaculture,* Amsterdam, 219:515-529.

PORTZ, L.; LIEBERT, F. 2004 Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fed plant-based diets with graded levels of microbial phytase. *Journal Animal Physiology and Animal Nutrition,* 88:311-320.

RODEHUTSCORD; M., GREGUS, Z.; PFEFFER, E. 2000 Effect of phosphorus intake on faecal and non-faecal phosphorus excretion in rainbow trout (*Oncorhynchus mykiss*) and the consequences for comparative phosphorus availability studies. *Aquaculture*, Amsterdam, 188:383-398.

RODEHUTSCORD, M.; MANDEL, S.; PFEFFER, E. 1994 Reduced protein content and use of wheat gluten in diets for rainbow trout: effects on water loading N and P. *Journal Applied Ichthyology,* 10:271-273.

SATOH, S.; HERNÁNDEZ, A.; TOKORO, T.; MORISHITA, Y.; KIRON, V.; WATANABE, T. 2003 Comparison of phosphorus retention efficiency between rainbow trout (*Oncorhynchus mykiss*) fed a commercial diet and a low fish meal based diet. *Aquaculture*, Amsterdam, 224:271-282.

SCHAEFER, A. 1995 Effects of microbial phytase on the utilization of native phosphorus by carp in a diet based on soybean meal. *Water Science Technology*, 31:149-155.

STOREBAKKEN, T.; SHEARER, K.D.; ROEM, A.J. 2000 Growth, uptake and retention of nitrogen and phosphorus, and absorption of other minerals in Atlantic salmon *Salmo salar* fed diets with fish meal and soy-protein concentrate as the main sources of protein. *Aquaculture Nutrition*, 6:103-108.

SUGIURA, S.H.; BABBITT, J.K.; DONG, F.M.; HARDY, R.W. 2000a Utilization of fish and animal by-product meals in low-pollution feeds for rainbow trout *Oncorhynchus mykiss*. *Aquaculture Research*, 31:585-593.

SUGIURA, S.H.; DONG, F.M.; HARDY, R.W. 2000b A new approach to estimating the minimum dietary requirement of phosphorus for large rainbow trout based on nonfecal excretions of phosphorus and nitrogen. *Journal of Nutrition*, 130:865-872.

SUGIURA, S.H.; FERRARIS, R.P. 2004 Contributions of different NaPi cotransporter isoforms to dietary regulation of P transport in the pyloric caeca and intestine of rainbow trout. *Journal of Experimental Biology*, 207:2055-2064.

SUGIURA, S.H.; MARCHANT, D.D.; KELSEY, K.; WIGGINS, T.; FERRARIS, R.P. 2006 Effluent profile of commercially used low-phosphorus fish feeds. *Environmental Pollution*, 140: 95-101.

THOMAS, S.L.; PIEDRAHITA, R.H. 1998 Apparent ammonia-nitrogen production rates of white sturgeon (*Acipenser transmontanus*) in commercial aquaculture systems. *Aquacultural Engineering,* 17:45-55.

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*, Amsterdam, 183:349-362.

XU, Z.N.; LIN, X.T.; LIN, Q.; YANG, Y.F.; WANG, Y.X. 2007 Nitrogen, phosphorus, and energy waste outputs of four marine cage-cultured fish fed with trash fish. *Aquaculture*, 263: 130-141.

WATANABE, T.; JAHAN, P.; SATOH, S.; KIRON, V. 1999 Total phosphorus loading on to the water environment from common carp fed commercial diets. *Fisheries Science*, Tokyo, 65:712-716.

WEBB Jr., K.A.; GATLIN III, D.M. 2003 Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum *Sciaenops ocellatus*. *Aquaculture*, Amsterdam, 225:17-26.

WOOD, C.M., 1993 Ammonia and urea metabolism and excretion. In: EVANS, D.H. (Ed.), *The physiology of fishes*, 1st, ed. CRC Press, Boca Raton, 379-425.

WOOD, C.M. 2004 Dogmas and controversies in the handling of nitrogenous wastes: is exogenous ammonia a growth stimulant in fish? *Journal of Experimental Biology,* 207:2043-2054.

WORSHAM, R.L. 1975 Nitrogen and phosphorus levels in water associated with a channel catfish *Ictalurus punctatus* feeding operation. *Transactions of the American Fisheries Society,* 104:811–815.