



Examining Phosphorus in Effluents from Rainbow Trout (*Oncorhynchus mykiss*) Aquaculture

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Excessive phosphorus (P) levels in aquaculture effluents pose a serious threat to the freshwater environment. Thus, the effluent P levels are subjected to environmental protection mandated limits by the U.S. Environmental Protection Agency (EPA). Most of the P in aquaculture effluent originates from P in the feed. Dietary P ingested by fish either becomes incorporated into the fish body or is excreted into the environment (Fig. 1). Increasing P incorporation into the fish body decreases P excretion. In rainbow trout (*Oncorhynchus mykiss*) culture, two research areas can contribute to reducing the P concentrations in effluent waters: improving the retention of dietary P in the fish and increasing our understanding of how that portion of dietary P not utilized by

the fish is excreted in the effluent water (Fig. 2). Studies on the first problem area of dietary retention have shown how P in the food is digested and absorbed from the intestine into the blood and how P is reabsorbed from the kidney tubules back into the blood.

However, the second problem area has not been sufficiently studied on a well controlled experimental scale. P in aquaculture effluents appears as a dissolved, settleable and particulate form. Recent research has shown that the amount of each component is directly affected by time after feeding, concentration of dietary P, digestibility of dietary P and other physical and chemical variables.

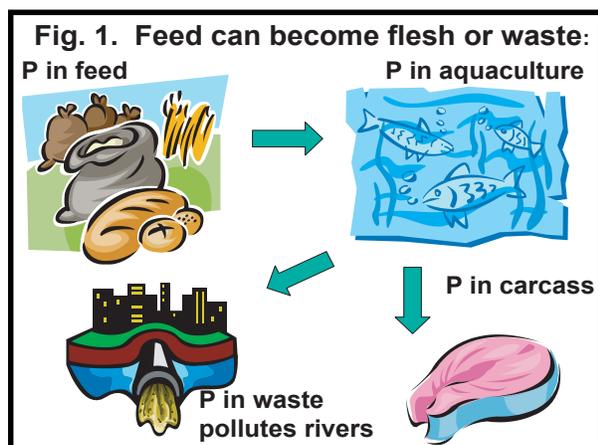


Fig. 1. There are only two outcomes of P fed to fish. Dietary P either becomes incorporated into the carcass of fish or is eliminated into the effluent.

Introduction

The P requirement for juvenile rainbow trout is approximately 0.6 % in the diet. Commercial fish feeds typically contain surplus P because not all P in the feed is available (digestible and absorbable) to the fish. Many common feed ingredients such as fish meal and soybean meal contain non-absorbable forms of P. Dietary P that is consumed by fish, but not digested and absorbed, will appear in the waste as fecal P. Absorbed P above the amount utilized or required by the fish will appear in the waste as urinary P. Effluent water from commercial fish culture may therefore contain different forms of excretory P products at levels potentially high enough to provoke calls by environmentalists for increased regulation.



Fig. 2. Effluent P can be reduced by vacuuming settled feces in ponds and/or by improving feeds.

There is a consensus, however, even among environmentalists that aquaculture is an industry destined for rapid expansion in the near future. The Johannesburg Earth Summit 2002 suggested that aquaculture is the only way that the world can meet the rapidly growing demand for fish because production from freshwater and marine capture fisheries has not adequately increased in the past 15 years. In the same amount of time, aquaculture production quadrupled and now supplies one-third of the world's total edible seafood supply.

An obvious solution to the effluent P problem is to produce diets with 100% available and 0% unavailable P so that all P can be digested and absorbed by the fish. There are many studies on methods to enhance availability of P, particularly those that focus on enhancing digestibility by microbial phytase supplementation of fish feeds containing plant P, thereby reducing fecal P. Phytase is an enzyme that breaks down plant P which is mainly phytate. Phytate is not available to the fish. Another critical consideration is to feed only enough available P to meet but not exceed the requirement for P, thereby reducing P in urine. Hence, some studies focus on estimating P requirements. Understanding the partitioning or division of P in the effluent from aquaculture is also critical because

environmental guidelines must reflect the characteristics of P generation in effluents from trout aquaculture. Along with studies that determine or enhance dietary P availability and dietary P requirement of the fish, P partitioning as a function of diet, feeding time, fish size, and physiochemical variables, like temperature and oxygen, have been investigated. Initial work described in this bulletin has determined how levels in different fractions of the effluent vary with diet and time of day.

Studies were conducted over 5 years. These initially used purified diets before shifting to practical diets containing ingredients found in commercial trout diets. The research used 12 - 25 kg of trout cultured for 1 - 3 months in 0.7 metric ton tanks. This simulated as much as possible commercial fish density situations before actually performing the study in raceways using commercial-type feeds. The concentrations of soluble, particulate (10 - 200 μm), settleable (> 200 μm) P were tracked in the effluent, as well as the amount of P fed to the fish and the amount of P retained by the fish. From the results, a "balance" was calculated as follows:

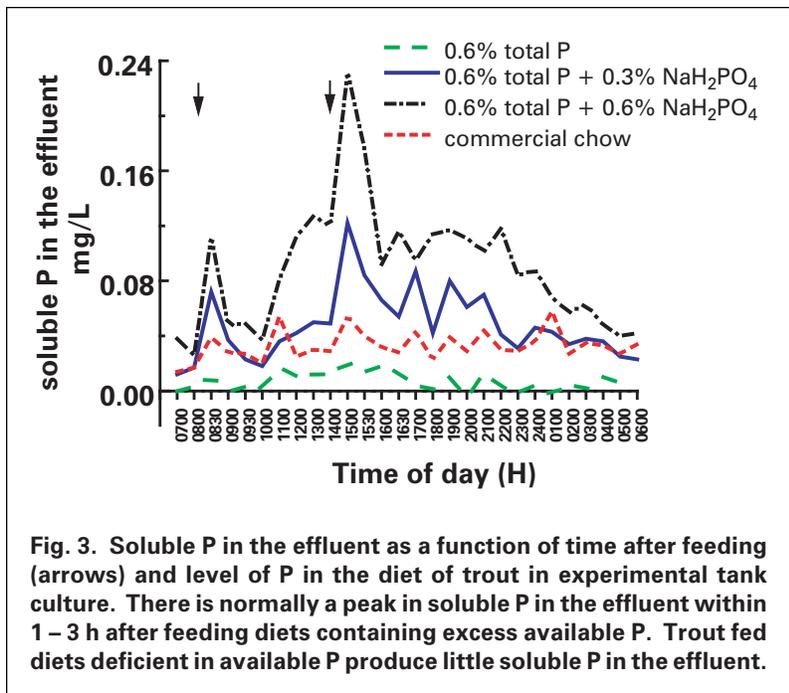
$$\text{Amount of P fed} = \text{P in fish carcass} + \text{soluble P} + \text{particulate P} + \text{settleable P} + \text{residual P (or P not accounted for)}.$$

Soluble and particulate P levels were monitored every hour over a 24 hour period, and settleable P over several days. When the total P in the total trout biomass has been determined, it can then be estimated where the P consumed by the fish went.

Soluble P in the Effluent Usually Peaks Immediately After Feeding

The most troublesome component of the effluent is soluble P. It is not only the P that is readily available to aquatic phytoplankton and algae, but also is the component that is difficult to strip from the effluent before it leaves the hatchery. Effluent P in feces and large particulates can be minimized by the use of settling ponds, but not soluble P.

Production of soluble P by fish is not constant. Several studies, each using tanks with several hundred trout,



indicate a sharp peak of soluble P in the effluent immediately after feeding, while broad peaks show up about 3 – 4 hours after feeding. The intensity of the peak depends on the amount of available P in the diet. In one study, juvenile rainbow trout were fed four different diets. One is marketed commercially and the other three diets were formulated as fish meal-based commercial-type feed low in total P content (0.6%). Two of these three diets were then supplemented with 0.3% or 0.6% readily available P as sodium phosphate (Fig. 3). The fish grew well and gained about 250% of their initial weight after 11 weeks. The Feed Conversion Rate (FCR) was good in all of the treatments. In the trout given the fish meal based 0.6% total P diet, some excretion of soluble P occurred during the day, but none at night. The average soluble P excreted over 24 hours was close to zero. In the fish that consumed the diets supplemented with readily available sodium phosphate or NaH_2PO_4 (0.3% or 0.6% P), considerably more P appeared within an hour after feeding. The increases in soluble P that appeared about 3 hours after feeding were sustained for several hours before gradually decreasing. Addition of 0.3% P as sodium phosphate to the diet increased by 10 times the average soluble P in the effluent to about 0.04 mg/L. Addition of 0.6% P as sodium phosphate increased the average to 0.09 mg/L. Fish fed commercial pellets had an average of 0.03 mg/L of soluble

P. The pattern shown in Fig. 3 was observed again when effluent samples were collected over a 24 h period four weeks later.

The observed peaks after feeding are consistent and have been observed in four independent experiments. The peaks are not due to leaching of P from diets or feces. Typically, almost all feeds are consumed by fish immediately after feeding so dietary P cannot leach out. Pellets swirled for one minute in control tanks without fish revealed that there were no pellet-derived P after 30 min. Therefore, the sharp peaks in effluent P observed 0.5 to 1 hours after feeding are due mainly to feeding-induced excretion of soluble P.

Other studies using different diets confirmed the findings that feeding-related peaks exist in soluble P excretion. For example, urine P in trout confined in metabolic chambers increases after feeding.

Soluble P in the Effluent Increases with Available Dietary P

It is obvious from Fig. 3 that average soluble P concentrations in the effluent increases as a function of available P in the diet. Using the data averaged from studies conducted over several years, it was estimated that rainbow trout raised in large flow-through tanks produced an average of about 7 mg of soluble P per kg of fish per day when fed diets containing greater than 0.3 – 0.4% available P. This soluble P likely emanates from fish urine. Rainbow trout raised in recirculating tanks urinated about 9 mg of P per kg of fish per day when available P concentration in diet reached about 0.7%. These findings indicate that trout will urinate P when available dietary P goes above a certain level. This has two important implications. From a nutritional perspective, P utilization will decrease after the trout has consumed nutritionally sufficient levels of dietary P because trout will urinate the excess P.

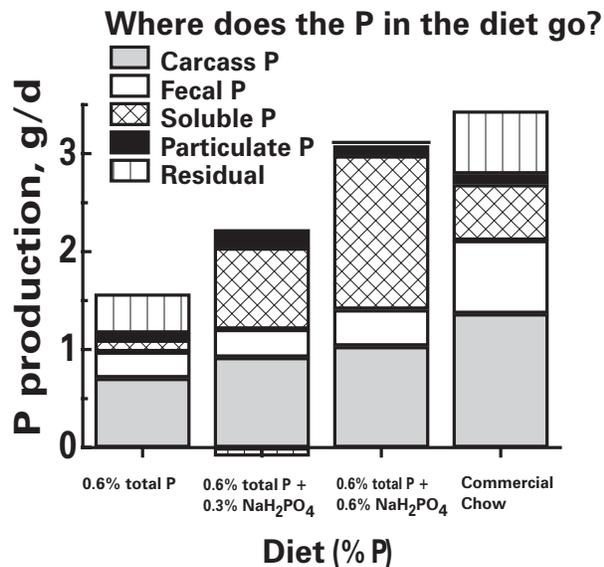


Fig. 4. The total P in the diet and the P concentrations in the carcass of fish, as well as in the soluble, particulate and settleable fractions of the effluent, were determined. The height of each bar represents the total amount of P fed to the trout each day. The subdivisions in each bar represent the outcome of dietary P; residual P is P not accounted for in the balance equation. A positive residual (e.g., the 0.6% total P) means that measurements of P in the carcass and effluent are not sufficient to account for dietary P consumed by fish. In fish fed 0.6% P, almost half of the dietary P was incorporated into the carcass. In fish fed diets supplemented with Sodium Phosphate, fish incorporated more P, but a lesser proportion of the total amount of P consumed. In trout fed commercial feed, there was a large component of settleable P and a large residual.

From an environmental perspective, soluble P in the effluent is produced only if trout are fed dietary P at levels above those required. Therefore, adjusting P concentrations in feeds is a precarious balancing act between production efficiency and environmental concerns.

These results also imply that trout will not produce any soluble P in the effluent if their dietary P is insufficient. In fact, research has consistently observed that rainbow trout deficient in P intake from diet will absorb P from the water. The mechanisms underlying this phenomenon are yet to be understood.

Particulate P Represents a Phantom Menace

The amount of fecal P is a function of undigestible P in the feed. For any given level of total dietary P, the less digestible or available dietary P, the more P will be found in the feces. In our studies, commercial type diets (typically using fish meal and soybean meal as the major P source) with similar total P levels as semi-purified research diets always had more fecal P.

An overlooked component of the effluent is particulate P. This is the amount of P suspended in the water column (Fig. 4). Particulate P is likely from fecal matter that disintegrated into particulates small enough so that they escape into the settling area. Because they are not soluble, they will escape detection unless captured by filters during sampling. Levels of particulate P in the water column are highly variable, independent of dietary P, and are likely dependent on factors such as fish activity (e.g., feeding when fish stir up the water column), stocking density, feeding rate and water flow rate. Stronger flows prevent larger particles from settling. About 5 – 10% of effluent P is particulate in nature. This is not large, but enough is present to represent a significant fraction of effluent P.

Where does Dietary P Go?

A large fraction (almost 50%) of P fed to the fish actually becomes incorporated into the carcass. This is shown in Fig. 4. The next biggest component, for the commercial chow, is fecal P. The sources of P in this diet are not readily available forms such as sodium phosphate. In contrast, for experimental diets supplemented with sodium phosphate, soluble P was in fact much larger than fecal P and even higher than carcass P. Interestingly, the three experimental diets with fish meal based P had similar fecal P levels. This suggests that the addition of sodium phosphate in two of the diets merely served to increase urinary P.

What about Vitamin D?

In mammals, vitamin D is a precursor for the endocrine system regulating P metabolism. However,

studies in trout have indicated that dietary vitamin D had no significant effect on P utilization and therefore P in the effluent. In fact, supplemental dietary vitamin D had no effect on plasma levels of major vitamin D metabolites in trout.

Summary and Conclusions

Fish feeds need to supply a certain amount of bio-available forms of P in order for fish to grow normally. Reducing dietary P increases the risk of P-deficiency and growth depression in fish. Critical considerations in effluent P reduction must include the following: First, the precise dietary requirement of P in the fish must be known. This differs from diet to diet more than from species to species. Second, the bio-availability (absorption) of P in each diet or ingredient must be known. This is the function of the dietary source of P and is greatly affected by certain dietary components which interact with P within the diet. Third, factors of how effluent P are produced must be known. These include the time course of P appearance in various fractions of effluent, effects of dietary composition and management methods. In this report, the third problem was addressed. This, in turn, helps address the first and the second problems. Effluent fecal P is a factor of undigested P in diet. This fraction can be reduced by selecting highly digestible feed ingredients for P or applying various methods that can increase digestion / absorption of dietary P. Effluent soluble P, however, is a factor of excess available P in diet. This component, therefore, must be reduced by lowering available P, including sodium phosphate, in the diet to the minimum amount required by the fish. The rate of appearance of soluble P in the effluent also varies greatly depending on the time after feeding and the amount of readily available P in the diet. Thus, there can be a significant variation among different diets in the effluent soluble P concentrations a few hours

after feeding; however, such a difference can almost disappear to a baseline low level in all dietary treatments before feeding or while fasting. This will certainly be an important consideration when monitoring effluent soluble P. Effluent particulate P is only slightly influenced by dietary P levels, although reducing the portion of effluent P awaits further research to develop methods of preventing fecal disintegration in ponds.

Acknowledgments

The authors are deeply indebted to Reli Coloso, John Fletcher, Kim King, Wade Jodun, Pat Farrell, Nichole McDaniel, Sandra Basantes, Mary Kang, Jackie Choi, Tom Kehler, Ana Garcia, and Marivi Suarez. They helped maintain the fish, collected and analyzed the samples every summer from 1997 to 2001. Further indebtedness is extended to Mike Hendrix, Pete Weis, Linda Smith-Lemmon, Mark Subramanyam, Vic Mancebo and Kim Harrison for invaluable advice, support and encouragement.

This material is based upon work supported by the Cooperative State Research, Education, and Extension Service (CSREES), U.S. Department of Agriculture, under Agreement No. 95-38500-1423 awarded to the Northeastern Regional Aquaculture Center at the University of Massachusetts, Dartmouth. The laboratory of Dr. Ferraris is also supported by USDA Grants No. 2001-35102-09881 and 2003-35102-13502.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Agriculture, the Northeastern Regional Aquaculture Center or the University of Massachusetts.

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Published: December 2003

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