PROJECT COMPLETION REPORT

95-4 "Enhanced Digestibility of Fish Feeds to Reduce Waste Nitrogen, Phosphorus and Solids"

Termination Report Period: March 19, 1996 - April 30, 1998

NRAC Total Funding: $141,840 (May 1, 1996 through April 30, 1997)
(No-Cost Ext'n through Oct. 30, 1997; 2nd through Apr. 30, 1998)

Principal Investigator: H. G. Ketola, Cornell University and U.S. Geological survey at Tunison Laboratory

Participating Investigators/ Cooperative Agencies:

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Scott Lindell	AquaFuture, Inc.	Massachusetts
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Reason For Termination:

Research successfully completed. Scientific and extension reports in preparation.

Project Objectives:

Study 1 Determine true digestibility of amino acids (the major sources of nitrogen) by salmon and striped bass fed fish meal and several plant proteins to formulate efficient diets to reduce nitrogen waste.

Study 2 Determine the minimum amount of phytase needed to digest undigestible phytin phosphorus in a practical-type diet fed to trout and striped bass to formulate efficient diets to reduce waste phosphorus.

Study 3 Determine the effect of nutrient dense diets and phytase on growth, feed efficiency, body composition, and retention and discharge of nitrogen and phosphorus by trout and striped bass.

Study 4 Determine the effect of different methods of pelleting practical diets on growth, feed efficiency, carcass composition, and digestibility of amino acids, nitrogen, and phosphorus by rainbow trout and striped bass.

Study 5 Determine the effect of a new diet (using knowledge gained from previous studies) on growth, feed efficiency, organoleptic characteristics, carcass composition, retention and discharge of nitrogen (N) and phosphorus (P) by rainbow trout and striped bass reared under commercial conditions. No-Cost Extension -- Determine the effect of a new diet containing 16 % corn gluten meal on growth and pigmentation of fish flesh. (*in lieu* of commercial study with trout not completed).

Anticipated Benefits:

Study 1 Four commercially important protein sources will be characterized in terms of their amino acid availability to Atlantic salmon and striped bass. These data will be important for formulating feeds for the salmon, trout and striped bass.

Study 2 Experiments will determine the influence of supplemental phytase on apparent digestibilities of phosphorus (P) in practical diets fed to Atlantic salmon, rainbow trout and striped bass. In salmon and trout the influence of phytase on apparent digestibility of protein and retention of P and protein will be also determined. In striped bass, the increase in apparent digestibilities of calcium, copper, iron, magnesium, and zinc will be determined.

Study 3 These experiments will evaluate effects of high-energy (fat) content or nutrient density in diets on growth, feed efficiency and nutrient retention by Atlantic salmon and striped bass.

Study 4 Experiments will determine the influence on rainbow trout and striped bass of commercially manufactured floating or sinking feeds pelleted by conventional steam processing, expansion, or extrusion. Effects on apparent digestibility of phosphorus, protein, amino acids and dry matter as well as retention of phosphorus and protein will be determined.

Study 5 Experiments will determine the influence on rainbow trout and hybrid striped bass of a commercially manufactured low-phosphorus diet fed under commercial conditions. Effects on growth, feed conversion, nutrient retention, body composition, taste and will be determined.

[The trout study was not completed; therefore, and an alternative study was conducted with Atlantic salmon to determine the impacts of diet on flesh coloration.]

Principal Accomplishments:

Study 1 (amino acid digestibility) was conducted with Atlantic salmon and striped bass.

Atlantic salmon:

1. That corn gluten meal and soybean meal (with optimum toasting) has digestibilities of crude protein and essential amino acids as good or better than that for herring meal.
2. When amino acids were balanced, growth of salmon fed corn gluten meal, soybean meal, or peanut meal to replace 38% fish meal was only slightly or modestly reduced (by 0-9%).
3. Soybean (with adequate toasting) and corn gluten meals appear to be well suited for increased use in trout and salmon feeds with increased efficiency of retention of dietary nitrogen and reduced waste discharges.
4. Amino acids were not optimally balanced unless done according to the profile of amino acids in the eggs of rainbow trout (Syuama and Ogino 1958). Balancing amino acids according to the National Research Council's minimum requirements of rainbow trout resulted in a significantly improved growth but not to as great an extent as with balancing by the trout egg.

Striped Bass

1. Average amino acid digestibility was very similar for herring meal, soybean meal (48%) and corn gluten meal at 89.2, 92.6 and 90.9, respectively.
2. Dry matter digestibilities follow a similar pattern to amino acid digestibility as was 68.3, 72.2, 70.8 and 60.3% respectively for herring meal, soybean meal, corn gluten meal and peanut meal.

Study 2 (phytase) was conducted with Atlantic salmon, rainbow trout, and striped bass fed commercially manufactured diets, according to the proposal.

Atlantic salmon

1. Supplemental phytase (1,000 units/kg feed) significantly increased bone ash but not weight gain or feed efficiency.
2. Phytase (2,000 units/kg) increased retention and reduced discharges of phosphorus.
3. Supplemental phytase (4000 units/kg feed) significantly increased feed efficiency.

Rainbow trout

1. Supplemental phytase (1,000-4000 units/kg feed) significantly increased digestion of phosphorus, but not nitrogen.
2. Phytase (1,000 units/kg) increased weight gain and feed efficiency and bone ash.

Striped bass

1. Supplemental phytase (1,000 units/kg feed) significantly increased bone ash and digestion of phosphorus but not dry matter.
2. Phytase increased feed efficiency (500 units/kg) and weight gains (1,000 units/kg).
3. Supplemental phytase (2,000 units/kg feed) significantly increased digestion of calcium, iron and zinc, but not magnesium or copper.
4. Phytase supplement (1,000 units/kg) in a high phytate diet was shown to increase P utilization by striped bass.

Study 3 (High Nutrient –Density Diets)

Low, medium and high density diets and the high density diet with supplemental phytase were fed to Atlantic salmon and striped bass. The low, medium and high diets contained 44.6, 47.6 and 51.5 % protein, and 11.5, 16.4 and 21.5% fat, and 3451, 3714 and 3981 kcal metabolizable energy per kilogram. The diets were manufactured by Zeigler Brothers.

Atlantic salmon

1. When salmon fed increasingly nutrient dense diets at equal levels of energy intake, growth was not significantly improved although feed efficiency was significantly increased, reflecting a simple increase in nutrient content per unit weight of feed.
2. Feeding high density diets did not appear to increase utilization of nutrients except possibly retention of nitrogen.

Striped bass

1. Growth, feed efficiency, and phosphorus balance was not significantly influenced in striped bass fed to satiation diets having increasing nutrient densities.
2. Feeding increasingly nutrient dense diets did not appear to increase utilization of nutrients.

Study 4 (practical formulations and pelleting technology)

The pelleting study involved feeding triplicate lots of rainbow trout and striped bass. A common diet was commercially pelleted by conventional steam (control), expansion and extrusion technology. Extrusion pellets floated. Specific gravity of the various forms of pellets were determined to be approximately 1.17, 1.22, and slightly <1.00 grams/cubic centimeter for the conventional, expanded and extruded pellets, respectively.

Rainbow trout

1. Extrusion pelleting significantly increased growth and feed conversions.
2. Expansion pelleting significantly increased feed conversions, but not growth.
3. Extrusion pelleting significantly increased digestibility of phosphorus and dry matter and decreased digestibility of protein (nitrogen) and amino acids.
4. Expansion pelleting significantly decreased digestibility of phosphorus and had no effect on digestibility of dry matter or protein (nitrogen).
5. Type of pellet had no significant effect on retention or discharge of phosphorus or nitrogen.

Striped bass

1. Type of pellet had no significant effect on growth, feed conversion, or digestibility of dry matter.
2. Expansion pellets significantly decreased digestibility of phosphorus.
3. Steam pellets significantly increased digestibility of protein (nitrogen) and most amino acids.

Study 5 (Commercial diet tests)

Tests with two practical diets were conducted to determine the effect of a new low-phosphorus diet (containing 16% corn gluten meal) on growth, feed efficiency, organoleptic characteristics, carcass composition, retention and discharge of nitrogen (N) and phosphorus (P) by rainbow trout and hybrid striped bass fed a new low-phosphorus diet at Limestone Springs Trout Company and at AquaFuture fish farm. The Limestone Springs test was terminated by the manager due to concerns of off-color flesh observed in a some fish. Therefore a laboratory study was conducted with Atlantic salmon fed a diet to determine the effect of the new low-phosphorus diet containing 16% corn gluten meal on growth and pigmentation of fish flesh. (*in lieu* of commercial study with trout not completed). This diet was made at the University of Maryland according to the same formula used by Zeigler Brothers, Inc. to manufacture the feeds for the practical hatchery tests at Limestone Springs and AquaFuture.

Rainbow trout
(Limestone Springs Trout Co. - preliminary results):

1. Preliminary results feeding the low-P experimental diet and a standard hatchery diet, (Trout Food, Perdue Specialty Feeds). suggested that diet had no significant effect on growth or feed conversion, but may have influenced pigmentation of flesh.
2. A follow-up study was conducted with salmon to more carefully evaluate pigmentation.

Atlantic salmon

1. Atlantic salmon fed the diet containing 16% corn gluten meal and the standard control diet (Perdue Trout Food) for four months revealed no significant influence on pigmentation of edible flesh, either visually or when color was evaluated with a Hunter Color Difference Meter.

Striped bass

1. New low-P diet (0.72% P and 40% protein) tended to reduce weight gain and specific growth rate, but these trends were not significant (P=0.18).
2. Feed conversion (feed/gain) increased in fish fed the experimental feed (P= 0.03).
3. Sensory tests showed no significant effect of diet on taste or smell of fillets.
4. The experimental diet tended to reduce the percentage body fat and reduced increased (P<0.1) lean body mass.

5. Feed intake tended to increase in fish fed the experimental feed (P=0.06), without any significant effect on retention of phosphorus or nitrogen.
6. When diet cost were considered with performance, production costs were similar or slightly improved for the experimental diet as compared to the commercial control
7. Body composition (lean body mass) was improved with the experimental diet.
8. Sensory tests showed that taste, color and general acceptability of fillets was as good for the experimental diet as the commercial control.
9. This study showed that a low-P, high plant protein diet needed to reduce effluent water pollution can be effectively used in commercial hybrid striped bass.
10. AquaFuture chose to further test the new feed on a commercial basis.

Impacts:

Study 1 showed that corn gluten meal and soybean meal (with optimum toasting) have deficiencies of essential amino acids that can be very effectively corrected by supplementation or balancing the amino acids based on the amino acid profile in rainbow trout eggs. Furthermore, the digestibilities of crude protein and essential amino acids in these plant meals were as good as or better than that for herring meal, thereby indicating that they are well suited for increased use in trout and salmon feeds, with increased efficiency of retention of dietary nitrogen and reduced waste discharges. Results suggest that peanut meal was less effective in diets at high levels of supplementation. Since soybean meal (0.6%P) is about half as costly as fish meal (2.9%P), its substitution for much of the dietary fish meal can make significant reduction in the phosphorus content of the diet and potentially reduce pollution of effluent water. These data provide a guide for greater use of plant proteins in place of fish meal in diets of trout and striped bass, thereby reducing excess dietary phosphorus and discharges.

Study 2 shows that phytase supplements increase digestion of otherwise underutilized phosphorus in salmon, trout, and striped bass, resulting in reductions in waste phosphorus discharges. The level of phytase required by trout and salmon (1,000 - 4,000 phytase units/kg feed) appears higher that that for striped bass (500- 1,000 phytase units/kg feed). This finding will enable feed manufactures to further reduce total phosphorus in finished feeds and decrease waste phosphorus discharges.

Study 3 shows that in Atlantic salmon, increased nutrient density of diets increased feed efficiency by about an amount comparable to the reduction in bulk weight of the feed. However, on an equal nutrient basis, diet density had little impact on the growth and utilization of nutrients. Supplemental phytase again significantly increased digestibility of phosphorus but not nitrogen.

Results with striped bass were similar to those with salmon in that nutrient density had no significant effect on growth, whereas feed efficiency was increased. Retention of phosphorus was not improved nor was cost of production. The popular belief that high fat diets are necessary for efficient production of fish is not supported by this study. These data suggest that high fat diets do not induce any extra growth response other than that predicted by the nutrient and energy content of the diet.

Study 4 shows that weight gain and feed efficiency in rainbow trout was improved by feeding extruded pellets, whereas only feed conversion was improved by expanded pellets. Growth and feed conversions were excellent for all pellets fed in this study. Apparent digestibilities of phosphorus and dry matter were significantly increased by extruded pellets, while apparent digestibility of nitrogen and amino acids were significantly decreased. Expanded pellets significantly decreased apparent digestibility of phosphorus for both trout and bass. Retention and discharges of nitrogen by trout showed no consistent tends or significant effects of pelleting. For trout, steam and expansion pellets resulted in higher digestibility of essential and non-essential amino acids than did extrusion pellets. For striped bass, steam pellets generally resulted in higher digestibility of essential and non-essential amino acids than did extrusion or expansion pellets. In bass, the type of pellet resulted in no consistent differences in growth or feed conversion. Striped bass farm managers should therefore buy feed based primarily on formulation, quality and price and not pellet type. Rainbow trout farm managers may realize an improvement in growth and feed conversion when feeding extruded pellets while experiencing no benefit in retention or discharge of phosphorus or nitrogen.

Dr. Subramanyam (Zeigler Bros.) estimated that the commercial cost of conventional pelleting, expansion and extrusion are approximately $30, $40 and $100-200/ton of feed, respectively. For a typical trout feed having an ingredient cost of $408/ton, conventional pelleting adds only about 7% to the cost of feed, expansion adds about 10%, whereas, extrusion adds between 22 and 55% to the cost (including extra vitamins and other costs unique to the process).

Study 5 shows that feeding a new low-P diet had no significant effect on weight gain or feed efficiency in farm-reared rainbow trout when compared with a standard trout feed for one month. No further evaluation was made because the manager observed off-colored flesh in some of the trout. Flesh samples were evaluated from fillets above lateral line near dorsal fin. Feeding diets having the same formulations to Atlantic salmon for 4 months resulted in no significant effect on flesh coloration as determined both visually and by use of a Hunter Color Difference Meter.

Feeding farm-reared striped bass the low-P diet supported acceptable growth of striped bass. When diet costs were considered with performance, production costs were similar or slightly better for the experimental diet as compared to the commercial control. Body composition (lean body mass) was improved with the experimental diet. Sensory tests showed that taste, color and general acceptability of fillets were as good for the experimental diet as they were for the commercial control. These studies show that low-P diets needed to reduce effluent water pollution and heavily dependent on plant proteins can be effectively used in commercial finfish aquaculture.

Publications, Manuscripts, Or Papers Presented:

Publications:

Keene, J. C., R. E. Austic and H. G. Ketola. 1995. Use of plant meals as primary protein sources in diets for juvenile Atlantic salmon (*Salmo salar*). 1995 Cornell Nutrition Conference for Feed Manufacturers (Oct. 24-26). Rochester Marriott Thruway Hotel, Rochester, NY, pp. 179-185.

Keene, J. C., H.G. Ketola and R. E. Austic. 1996. Apparent and true digestibilities of crude protein and indispensable amino acids in dietary herring meal, corn gluten meal, soybean meal and peanut meal for juvenile Atlantic salmon (*Salmo salar*). Proceedings of the first Symposium of the Comparative Nutrition Society, No. 1 1996, Aug. 2-6, Leesburg, VA, pp. 72-74.

Soares, J. H. and K. P. Hughes. 1995. Efficacy of phytase on phosphorus utilization. Maryland Nutrition Conference for Feed Manufacturers.

Dougall, D. S., L. C. Woods, III, L. W. Douglass and J. H. Soares. 1996. Dietary phosphorus requirement of juvenile striped bass *Morone saxatilis*. Journal of the World Aquaculture Society 27(1):82.

Hughes, K. H. and J. H. Soares. 1998. Efficacy of phytase on phosphorus utilization in practical diets by the striped bass *Morone saxatilis*. Aquaculture Nutrition 1998 4:133-140.

Small, B. C., R. E. Austic and J. H. Soares, Jr., 1998. Amino acid availabilities if four practical feed ingredients fed to striped bass, *Morone saxatilis*. Journal of World Aquaculture Society 29: (In press).

Presentations:

Keene, J. C., R. E. Austic and H. G. Ketola. 1995. Use of plant meals as primary sources in diets for juvenile Atlantic salmon (*Salmo salar*). 1995 Cornell Nutrition Conference for Feed Manufacturers (Oct. 24-26). Rochester Marriott Thruway Hotel, Rochester, NY.

Keene, J. C., H.G. Ketola and R. E. Austic. 1996. Apparent and true digestibilities of crude protein and indispensable amino acids in dietary herring meal, corn gluten meal, soybean meal and peanut meal for juvenile Atlantic salmon (*Salmo salar*). Symposium of the Comparative Nutrition Society, No. 1 1996, August 2-6, Leesburg, VA.

Hughes, K. P. and J. H. Soares. 1996. Efficacy of Dietary Phytase on Phosphorus Utilization by the Striped Bass *Morone saxatilis*. World Aquaculture Society, Division U.S. National Aquaculture Society, Expo 96. TX, p. 46.

Soares, J. H. and K. P. Hughes. Improved dietary phosphorus utilization by striped bass fed phytase. 1996. 2nd World Fisheries Congress 1:105.

Small, B. C. and J. H. Soares. 1996. Apparent digested ideal protein requirement of the striped bass (*Morone saxatilis*). (To be presented at World Aquaculture '97, Seattle, Washington.)

Papatryphon, E. and J. H. Soares. 1996. The use of phytase to increase phosphorus utilization in practical diet for the striped bass *Morone saxatilis*. (Presented at World Aquaculture '97, Seattle WA.)

Theses:

Keene, Julie Catherine. 1996. Protein nutrition of Atlantic salmon: The use of plant meals to replace fish meals as primary sources of dietary protein. Master of Science Thesis. Cornell University, Ithaca, NY. January. 133 pp.

Hughes, K. 1995. Efficacy of phytase on phosphorus utilization by striped bass, *Morone saxatilis*. Masters Thesis, University of Maryland, College Park, MD.

TECHNICAL ANALYSIS AND SUMMARY:

Study 1 (amino acid digestibility) was conducted with Atlantic salmon and striped bass.

Atlantic salmon

For experimental methods, data and discussion see attached publication by J.C. Keene, R. E. Austic and H. G. Ketola, 1995, Use of plant meals as primary sources in diets for juvenile Atlantic salmon (Salmo salar). Proceedings of the 1995 Cornell Nutrition Conference for Feed Manufacturers (October 24-26, Rochester Marriott Thruway Hotel, Rochester, NY.) pp. 179-185.

The degree of heat-treatment of soybean meal was heat treated and evaluated by the cresol red dye binding method of Olomucki and Bornstein (1960) according to previous studies by Ketola (1982) and Ketola and Baltusis (1993). After heat-treatment of soybean meal, it had a dye binding value of 4.06 mg/g meal which was shown adequate for growth of salmon (Ketola and Baltusis, 1993). Results (Table 1) showed that iso-nitrogenous substitution of soybean meal (cresol red dye binding value 4.06 mg/gram), corn gluten meal or peanut meal for all the herring meal (38%) in an Atlantic salmon diet (without amino acid supplementation) depressed 14-week growth significantly. Without supplements, growth was depressed least with soybean meal (27%) and more by corn gluten meal (88%) and peanut meal (77%). When these diets were supplemented with amino acids to meet standards of the National Research Council's (NRC, 1993) minimum requirements for the trout or the amino acid content of the trout egg (see appendix 1a), growth of salmon was significantly and markedly increased, and even more so for the egg standard. When amino acids were supplemented to meet the NRC requirements, growth was improved to 74 to 90% of that for salmon fed herring meal. When amino acid were supplemented to meet the rainbow trout egg composition standard, growth was markedly improved to 91 to 100% of that for salmon fed herring meal.

Apparent digestibilities of crude protein in herring, soybean, corn gluten, and peanut meals were 72, 81, 90, and 65%, respectively, with that for corn gluten being significantly ($P<0.05$) greater than that for soybean, and that for soybean being significantly greater than that for herring or peanut meal. Apparent and true digestibilities of essential amino acids in soybean and corn gluten meals were as great or greater than those for herring. Apparent and true digestibilities of essential amino acids in peanut meal were as generally lower than those for herring meal (Tables 2 & 3).

Results demonstrated that plant proteins (soybean, peanut and corn gluten meals) could be fed to Atlantic salmon at very high levels (38-51%) in feed replacing all fish meal without appreciable loss of growth while permitting a marked reduction in phosphorus levels in feed with and discharges in water. This was accomplished only when amino acids were balanced according to a profile of amino acids in the eggs of rainbow trout (Suyama and Ogino 1958). Balancing amino acids according to the 1993 National Research Council's minimum requirements of rainbow trout resulted in a significantly improved growth but less than that with balancing by the trout egg.

Striped bass:

For details on experimental methods, data and discussion see attachment manuscript by Small, B.C., R.E. Austic and J.H. Soares, Jr., 1998, Amino acid availabilities of four practical feed ingredients fed to stripped bass, *Morone saxatilis*, pp. 1-17.

Differences in digestibility of dry matter in these plant proteins were not significant (Table 4). Amino acid digestibility data (Table 5) show that soybean meal, corn gluten meal and fish meal had similar digestibilities of amino acids. Peanut meal, however, had the lowest percentage digestibility of amino acids. Results suggest that both soybean and corn gluten meals have digestibilities equal to or better than herring fish meal, while all three appear to be somewhat superior to peanut meal.

Study 2 (phytase) was conducted with Atlantic salmon and striped bass fed commercially manufactured diets according to the proposal.

Atlantic salmon

Methods:

Fingerling Atlantic salmon (Salmo salar) or rainbow trout (Oncorhynchus mykiss) were acclimated for 1-2 weeks in plexi-glass culture jars and fed a commercially prepared salmon diet. Each jar had a capacity of 6.5 or 20 liters and was supplied with fresh, aerated, well water (9^{o}C) at a rate of about 3 L/min. The phosphorus content of the water was 0.08 mg/L. Fourteen hours (0545 to 1945 hours) of incandescent light was provided each day.

Experimental diets were assigned at random to triplicate jars of fish in experiments 1, 2 and 3. In experiment 1, 30 salmon (initial mean weight, 30 g) were reared in 6-L jars until 14 weeks then transferred to 20-L jars are fed until 16 weeks. In experiment 2, each jar had 75 trout (initial mean weight, 4.7 g). In experiment 3, each jar (20-liter) had 10 trout (initial mean weight, 188 g).

The composition of the basal diet is shown in Appendix 2. Diets were manufactured by Zeigler Brothers, Inc. Commercial phytase (3-phytase; myo-inositol hexakisphosphate 3-phospho-hydrolase; EC 3.1.3.8) was added as a top dressing in liquid form directly to diet. One unit (PU) of phytase enzyme activity is defined as the amount of enzyme required to liberate 1 micromole of inorganic phosphate/min. The amounts of daily feed were calculated by the formula of Buterbaugh and Willoughby (1967) by using a hatchery constant of 7.5 for salmon and 8.5 for trout. Average body weights for fish in each jar were determined at the beginning of each experiment and every 2 weeks thereafter. The durations of experiments 1, 2 and 3 were 14, 12, and 4 weeks, respectively. Fish were euthanized by an overdose of MS222 (tricaine methanesulfonate) until dead. At the end of experiments, bone ash values were determined for vertebrae (with ribs) excised from 10 fish/jar after they were cooked in a microwave oven. Excised vertebrae were extracted for 16 h in a Soxhlet apparatus with a mixture of chloroform and methanol (2:1 v/v), dried at 100^{o}C for 2 h and ashed for 16 h at 600^{o}C. Bone ash values were expressed as percent of fat-free dry bone. Percentage ash content was determined for whole carcasses of 10 fish/jar (fasted for 90 h). Carcasses were minced, ground, dried, and ashed at 600 ^{o}C for 16 h.

Analyses of phosphorus in diets and fish carcasses were determined either by the method of Kitson and Mellon (1944) as reported by Ketola et al. (1991) and commercially by use of an inductively coupled argon plasma atomic emission spectrophotometer at the analytical service laboratory at Cornell University, Ithaca, NY.

The amount of waste phosphorus discharged was determined by calculation from the amount of phosphorus fed during duration of the study less the amount retained in fish. The amount of dietary phosphorus fed to trout was determined (for each lot of fish) from analyses of P in feed fed. The amount of dietary phosphorus retained in trout was determined (for each lot of fish) from analyses of samples of fasted trout at the start and at the end of the study. The amount of dietary phosphorus discharged as wastes was determined by difference between the amount of phosphorus fed and the amount retained in the carcass during the study. Analyses of diets for rancidity were performed by Woodson-Tenent Laboratories, Memphis, TN. Data were subjected to analyses of variance followed by Duncan's New Multiple Range Test when appropriate (Steel and Torrie 1960).

Results:

Atlantic Salmon

At 6 weeks in the experiment with salmon, the growth appeared to be slow. Rancidity of feed was the suspected cause of slow growth. Therefore, samples of diets were sent to Woodson-Tenent Laboratories for analyses of rancidity (thiobarbituric acid and peroxide values). Analyses revealed high oxidative rancidity in the basal diet (peroxide value, initial was 96-100 meq/kg oil and the thiobarbituric acid result was 92.4 - 99.8 mg/kg), whereas the practical trout feed was found acceptable (peroxide value, initial was 4.2 meq/kg oil; thiobarbituric acid was 3.3 mg/kg). Zeigler Brothers agreed to remake the diets with fresh ingredients to determine digestibility by both salmon and bass.

Although growth of salmon was suppressed at 6 weeks, it was significantly increased (17%) by supplementation with phosphate. Supplementation with phytase tended to increase growth, but the response was not significant. Salmon were fed for 16 weeks. Further, two of these diets were fed to rainbow trout in order to tests effects of rancidity in that species.

The salmon study was completed and showed that growth of salmon fed the experimental diets was not significantly increased by supplements of phytase enzymes (Table 6 & 7). Growth was significantly increased by supplemental P (0.2%). In contrast feed efficiency (gain x 100/feed) was significantly increased by the P supplement and the highest level of phytase (4,000 units/kg). Bone ash measurements were more sensitive measurements of P deficiency and responses to supplements of at least 1,000 units of phytase or P. This effect on bone ash was previously demonstrated by Ketola (1994). All measurements of growth, feed efficiency and bone ash were reduced for experimental diets (with or without supplements) relative to those measurements for the practical control diets. Some reduction, however, was expected because the experimental diets were designed to be sufficiently deficient in order to detect responses to phytase or P supplements. Further reduction in growth may be due to rancid ingredients as mentioned above. Bone ash measurements did not appear to be influenced by rancid ingredients.

The concentrations of P in salmon carcasses tended to increase with supplements of phytase enzyme, but they were not significant ($P > 0.05$). The concentration was significantly increased by supplemental P. The retention of P in carcasses tended to increase with supplements of phytase but was not significant except at the highest levels of phytase (2,000 and 4,000 phytase units/kg) or with supplemental P. Discharges of phosphorus in effluents tended to decrease with 500 and 1,000 units of phytase/kg feed and were significantly reduced at the highest levels of phytase (2,000 and 4,000 units/kg) but not with supplemental P (Table 7).

Rainbow trout (phytase):

An experiment was conducted for 12 weeks with triplicate lots of 75 fingerling rainbow trout fed three rancid diets (the basal alone, and with supplements of phytase or P) in order to further examine the effects of these diets. The experimental results (Tables 8-9) showed that supplemental phytase (1,000 phytase units/kg) or phosphorus significantly increased growth, feed efficiency and bone ash. Supplemental phytase (1,000 phytase units/kg) or phosphorus significantly increased retention of phosphorus in carcasses and significantly reduced discharges of phosphorus. The reductions in P discharges ranged from 13 to 19% depending on whether the discharges were expressed as grams of P discharges per kilogram of weight gain or feed fed.

Measurements of digestibility of phosphorus, nitrogen and dry matter in the rancid diets and remanufactured fresh diets fed to rainbow trout are shown in Table 10. Phytase significantly increased digestibility of phosphorus but not nitrogen or dry matter (DM). Rancidity did not significantly influence digestibility of phosphorus. Maximum digestibility was achieved with 4,000 phytase units/kg feed.

Striped bass

For experimental methods, data and discussion see attached Publication by K.H. Hughes and J. H. Soares. 1998. Efficacy of phytase on phosphorus utilization in practical diets by the striped bass *Morone saxatilis*. Aquaculture Nutrition 1998 4:133-140, and manuscript by Papatryphon, E. R.A. Howell and J. H. Soares. 1998. The use of phytase to increase mineral absorption in a high-phytate diet for striped bass, *Morone saxatilis*.

<u>Summary of results</u>:

This study was completed and showed significant improvements ($P<0.05$) were found in mean weight gain in the 1,000 PU/kg diet (53g), 2,000 PU/kg diet (49g), and positive control (47g) groups when compared to the 0 PU (34g) supplemented group. There were no differences observed between the two highest phytase treatments and the positive control. Significant differences in mean feed conversions were also found between the 0 PU treatment (1.57) and the 1,000 PU, 2,000 PU/kg groups (1.32 and 1.36 respectively, $P<0.05$). Bone ash concentrations (mg/kg DM) at the end of the experimental period were significantly greater in the 2,000 PU (34), positive control (33) ($P<0.05$), and 1,000 PU/kg (32) ($P<0.06$) treatments, when compared to the 0 PU (25) treatment (pooled SEM=19.7). In addition, apparent P digestibility was significantly greater in the 1,000 PU/kg (67) and 2,000 PU/kg (78) treatments, when compared to the unsupplemented group (42), (pooled SEM=6.2). Incidence of scoliosis was significantly greater in the unsupplemented (0 PU) treatment when compared to all other treatments.

Results of this experiment confirm that a phytase supplement in a high phytate diet will increase P utilization by the striped bass, and furthermore indicate that a level of approximately 1,000 PU/kg diet is adequate to maintain proper growth and health comparable to an inorganic P supplemented diet.

<u>Study 3</u> (High Nutrient Density Diets):

Low, medium and high density diets and the high density diet with supplemental phytase were fed to triplicate lots Atlantic salmon and striped bass. The low (#1), medium (#2) and high density (#3) diets contained 44.6, 47.6 and 51.5 % protein, and 11.5, 16.4 and 21.5% fat, and 3,651, 4,057 and 4,448 kcal digestible energy per kilogram. These diets (#1-3) provided a calculated 122, 117, and 116 mg of protein/kcal of digestible energy, respectively. The diets were manufactured by Zeigler Brothers. For composition, see Appendix 3. Feeding levels for salmon and bass differed.

Atlantic salmon

Methods:

Fingerling Atlantic salmon *(Salmo salar)* were acclimated for 2 weeks in 200-liter supplied with fresh, aerated, well water (9^oC) at a rate of about 6.5 L/min. Fourteen hours (0545 to 1945 hours) of incandescent light was provided each day. Experimental diets were assigned at random to triplicate 200-liter tanks of 20 fish each (initial mean weight, 123 g). The amounts of daily feed for the first four weeks were intended to provide equal weights of feed (according to the size of fish) calculated by the formula of Buterbaugh and Willoughby (1967) by using a hatchery constant of 8.5. From week 4 to the end (12 weeks), equal amounts of energy (according to the size of fish) were calculated by the formula of Buterbaugh and Willoughby (1967) by using a hatchery constant of 8.5 high-density diet (#3). In order to feed equal levels of digestible energy, diets 1 and 4 were fed at 120% of the 8.5 hatchery constant level, and diet 2 at 110%.

Average body weights for fish in each jar were determined at the beginning of each experiment and every 2 weeks thereafter for four months. Samples of fish at the beginning and end were euthanized by an exposure of MS222 (tricaine methanesulfonate) until dead and frozen until analyzed. Analyses of phosphorus in diets and fish carcasses were determined by the inductively coupled argon plasma atomic emission spectrophotometer at the analytical service laboratory at Cornell University, Ithaca, New York. Analyses of nitrogen in diets and fish carcasses were determined by the use of a Nitrogen Determinator (Model FP228, Leco Corporation, St. Joseph, MI).

The amounts of waste phosphorus and nitrogen discharged were determined by calculation from the amounts of nutrients fed during duration of the study less the amounts retained in fish. The amounts of dietary nutrients fed to fish were determined for the amounts of feed fed during the entire study. The amounts of dietary nutrients retained in trout were determined (for each lot of fish) from analyses of samples of fasted fish at the start and at the end of the study. The amount of dietary nutrients discharged as wastes were determined by differences between the amount of nutrient fed and the amount retained in the carcass during the study.

At the end of the study, apparent digestibility was determined following the indicator method (Austreng, 1978) by adding 0.5% chromic acid to each diet (ground) and re-pelleting in the laboratory. Indicator diets were feed for 10 days before feces were collected by abdominal pressure to avoid leaching by water. Samples of freeze-dried feed and feces were analyzed for chromium, phosphorus, nitrogen and amino acids. Digestibility was calculated by the formula of Austreng (1978).

Analyses of diets for rancidity were performed by Woodson-Tenent Laboratories, Memphis, TN. Data were subjected to analyses of variance followed by Duncan's New Multiple Range Test when appropriate (Steel and Torrie 1960).

Results:

Growth and feed efficiencies for salmon for the first 4 weeks tended to increase as nutrient density increased generally reflecting energy density; however, only growth at the highest nutrient density was significant ($P<0.05$) (Tables 13 and 14). For weeks 4-12, when salmon were fed at equal levels of energy, growth was not significantly increased as nutrient density (energy) increased. Feed efficiencies for weeks 4-12 significantly increased for medium and high nutrient density diets and the numerical values generally reflect energy density. There was, however, no significant ($P<0.05$) difference between feed efficiencies for the medium and high nutrient density diets.

Weight gain data show that the most significant impacts of diet on growth were during the early part of the study—up to four weeks. After that, the effect on growth diminished. The reason for the diminution is not known. Retention of nitrogen and phosphorus both tended to increase with nutrient density of feed, but the effect was not significantly ($P>0.05$) except for P in fish fed the high density diet (Table 14). Supplemental phytase significantly increased digestibility of phosphorus but not nitrogen or dry matter.

It is not known why the increase in P retention occurred with the high density feed. Unfortunately the first analyses of excreta samples for digestibility were not reliable and there was not enough sample remaining to repeat excreta samples for diets 2 and 3.

With the exception of the apparent increase in retention of dietary phosphorus by salmon fed the high nutrient density diet, the results of this study with Atlantic salmon fed diets having increasing energy density, the differences in growth and feed efficiency appear to be related simply to quantity of nutrients consumed and density of feed without any significant influence on nutritional quality or physiology of salmon.

Striped bass

Growth of striped bass fed to satiation diets having differing nutrient densities was not significantly influenced (Table 15). Feed conversion (feed/gain), however, was significantly ($P<0.05$) impaired by feeding the low density diet (without added phytase) when compared to the other diets. Also, supplemental phytase significantly improved (decreased) feed conversion. Nutrient density of diets did not significantly improve retention of dietary nitrogen or phosphorus, while phytase significantly increased nitrogen retention (Tables 16 & 17).

With the exception of improved feed conversion for diets with increasing nutrient density, the results of this study with striped bass fed diets having increasing nutrient density, there was no significant increase in growth or retention of nitrogen or phosphorus.

<u>Study 4</u> (practical formulations and pelleting technology):

The pelleting study involved feeding triplicate lots of rainbow trout and striped bass. A common diet was commercially pelleted by conventional steam (control), expansion and extrusion technology. Extrusion pellets floated. Specific gravity of the various forms of pellets were determined to be approximately 1.17, 1.22, and about 1.00 grams/cubic centimeter for the conventional, expanded and extruded pellets, respectively.

<u>Methods</u>:

Fingerling rainbow trout were acclimated for 2 weeks in 200-liter tanks supplied with fresh, aerated, well water (9.3^{o}C) at a rate of about 7.8 L/min. Fourteen hours (0545 to 1945 hours) of incandescent light was provided each day. Each experimental diet was assigned at random to triplicate 200-liter tanks of 30 fish each (initial mean weight, 58 g). The composition of the basal diet is shown in Appendix 2. The amounts of daily feed were calculated by the formula of Buterbaugh and Willoughby (1967) by using a hatchery constant of 12.5. Average body weights for fish in each tank were determined at the beginning of each experiment and every 2 weeks thereafter for four months.

At the end of the study, apparent digestibility was determined following the indicator method (Austreng, 1978). Indicator (chromic acid, 0.5% of diet) was added to re-ground diet which were re-pelleted in the laboratory. Indicator diets were feed for 10 days before feces were collected by abdominal pressure to avoid leaching by water. Samples of freeze-dried feed and feces were analyzed for chromium, phosphorus, nitrogen and amino acids. Digestibility was calculated by the formula of Austreng (1978).

Samples of fish were euthanized by an overdose of MS222 (tricaine methanesulfonate) until dead and frozen until analyzed. Analyses of chromium and phosphorus in diets and fish carcasses were determined by the inductively coupled argon plasma atomic emission spectrophotometer at the analytical service laboratory at Cornell University, Ithaca, New York. Analyses of nitrogen in diets and fish carcasses were determined by the use of a Nitrogen Determinator (Model FP228, Leco Corporation, St. Joseph, MI). Amino acids were measured by the method of Davis and Austic (1994).

The amounts of waste phosphorus and nitrogen discharged was determined by calculation from the amounts of nutrients fed during duration of the study less the amounts retained in fish. The amount of dietary nutrient fed to fish was determined for the amount of feed fed during the entire study. The amount of dietary nutrient retained in trout was determined (for each lot of fish) from analyses of samples of fasted fish at the start and at the end of the study. The amount of dietary nutrient discharged as wastes was determined by difference between the amount of nutrient fed and the amount retained in the carcass during the study. Analyses of diets for rancidity were performed by Woodson-Tenent Laboratories, Memphis, TN.

Data were subjected to analyses of variance followed by Duncan's New Multiple Range Test when appropriate (Steel and Torrie 1960).

<u>Results</u>:

Rainbow trout

Observations at 6 and 12 weeks showed a significant improvement ($P<0.05$) in weight gain and feed efficiency when rainbow trout were fed extruded pellets, whereas feed conversion was improved by expanded and extruded pellets (Table 18). Growth and feed conversions were excellent in this study. Apparent digestibility of phosphorus, nitrogen, amino acids and dry matter were significantly influenced by pelleting methods. Although retention and discharges of phosphorus tended to be improved by expansion and extrusion pelleting, these changes were not significant. Retention and discharges of nitrogen showed no consistent tends or significant effects of pelleting.

Compared with steam pelleting, digestibility of phosphorus was significantly reduced by expansion pelleting but increased by extrusion pelleting (Table 18). Compared with steam pelleting, digestibility of nitrogen (protein) was not significantly influenced by expansion pelleting but decreased by extrusion pelleting. Digestibility of dry matter (DM) was not significantly increased by expansion pelleting ($P>0.05$), but was significantly increased by extrusion pelleting ($P<0.05$).

Compared with steam pelleting, digestibility of all essential and non-essential amino acids was significantly reduced by extrusion pelleting but was not significantly influenced by expansion pelleting (Table 19).

In spite of the fact that extrusion pelleting significantly reduced digestibility of amino acids and total protein, it apparently had no measurable negative effect on weight gain, feed utilization, nitrogen retention, or nitrogen. While there was a positive correlation between increased digestibility of phosphorus and growth of fish fed extruded feed, the correlation was negative for fish fed expanded feeds, suggesting that the effects of pelleting on growth and feed utilization was not related to digestibility of phosphorus. The increase in growth and feed utilization observed in trout fed extruded feed was about 9 to 11% when compared with conventional steam pelleting. The increase feed utilization by trout fed expanded feed was only about 5%. The importance relative costs of feeds will, in large part, determine the potential economic benefit of extrusion feeds.

Striped bass

Results with striped bass differ from those with trout in that neither growth nor feed conversion were significantly influenced by pellet processing (Table 20). Compared with steam pelleting, digestibility of phosphorus was significantly reduced by expansion pelleting but not by extrusion pelleting (Table 20). Digestibility of nitrogen (protein) was significantly reduced by expansion and extrusion pelleting. Extrusion significantly reduced digestibility of all amino acids, whereas expansion significantly reduced digestibility of most of the amino acids tested (Table 21). Digestibilities of the practically important essential amino acids lysine and methionine were significantly reduced by both extrusion and expansion. Digestibility of dry matter was not significantly influenced by pellet processing.

For rearing striped bass, extrusion and expansion processing of pellets gave no clear significant benefit over conventional steam pelleting. Dr. M. Subramanyam (Zeigler Bros. Inc.) estimated that the commercial cost of conventional pelleting, expansion and extrusion are approximately \$30, \$40 and \$100-200/ton of feed, respectively. For a typical trout feed having an ingredient cost of \$408/ton, conventional pelleting adds only about 7% to the cost of feed, expansion adds about 10%, whereas, extrusion adds between 22 and 55% to the cost (including extra vitamins and other costs unique to the process).

Study 5 (Commercial diet tests):

Practical diet tests were conducted to determine the effect of a new low-phosphorus diet (containing 16% corn gluten meal) on growth, feed efficiency, organoleptic characteristics, carcass composition, retention and discharge of nitrogen (N) and phosphorus (P) by rainbow trout and hybrid striped bass. Tests were conducted at Limestone Springs Trout Company and AquaFuture fish farm. The trout test was terminated by the manager due to concerns of off-color flesh observed in some fish. Therefore a laboratory study was conducted with Atlantic salmon fed a diet to determine the effect of the new low-phosphorus diet containing 16% corn gluten meal on growth and pigmentation of fish flesh (*in lieu* of commercial study with trout not completed). This diet was made at the University of Maryland according to the same formula used by Zeigler Brothers, Inc. to manufacture the feeds for the practical hatchery tests at Limestone Springs and AquaFuture. Preliminary results of a test with rainbow trout at Limestone Springs Trout (Pennsylvania) Company are summarized.

Rainbow trout

(Limestone Springs Trout Co. - preliminary results)

Triplicate lots of rainbow trout (initial mean weight, 229 g; initial mean number of trout/lot, 6438) were fed for one month an experimental diet containing 16% corn gluten meal to determine the effect of the new low-phosphorus diet or a standard hatchery diet, Trout Food (Perdue Specialty Feeds). The remaining planned results were not obtained due to early termination.

Results (Table 22) showed that diet had no significant effect on growth or feed conversion.

Atlantic salmon

Triplicate lots of Atlantic salmon (initial mean weight, 40.1 g, 30 fish/lot) were fed an experimental diet containing 16% corn gluten meal to determine the effect of the new low-phosphorus diet on growth and pigmentation of fish flesh. The main purpose of the study was to determine the effect of the diet containing 16% corn gluten meal for four months on pigmentation of edible flesh and to examine the effect of feeding a depletion diet for the last two months. This diet and a standard control diet (Perdue Trout Food) fed at Limestone Springs Trout Company was done to better evaluate the changes of yellow pigmentation imparted by a natural and harmless pigment (xanthophyll) found in corn gluten. The control diet was manufactured by Perdue Specialty Feeds in Catawissa, Pennsylvania, and it contained no corn gluten meal.

In this experiment, there were three treatment groups: Treatment 1 involved feeding the experimental diet containing corn gluten meal for 4 months. Treatment 2 involved feeding the experimental diet containing corn gluten meal for 2 months followed by feeding the control feed for the last 2 months. Treatment 3 involved feeding the control diet containing no corn gluten meal for 4 months. In this experiment, salmon were reared in 200-L tanks for four months. Each tank of fish was fed 4 times per day, 5 days per week for four months. Each tank of fish was weighed every two weeks.

Flesh color was evaluated visually and by using a Hunter Color Difference Meter (Hunter Associates Laboratory, Reston, VA) at the beginning and throughout the study until the end. The meter was calibrated using against a standard tile (L=67.8, a=22.6, and b=10.4) from Hunter Associates Laboratory. Hunter color measurements of back muscle were made three times on each of five fish per replicate. "L" values measure lightness and ranged from 0 (black) to 100 for perfect white. The "a" value indicates redness for positive values and greenness for negative values. The "b" value indicates yellowness for positive values and blueness for negative values. Initial back muscle Color Difference values were L=34.0 (SD=9.0), a= -1.30 (SD=0), and b = 2.60 (SD=17.6).

Table 23 summarizes the results of this study. Growth in salmon fed the experimental CGM diet was slower that expected; therefore, samples were sent to Woodson-Tenent Laboratories to be analyzed for lipid oxidation. Analyses revealed oxidation of lipids in the experimental feed (thiobarbituric acid rancidity, TBA= 81 mg/kg; peroxide value, initial, PV=43 meq/kg) but not in the control diet (TBA= 7.9; PV=17). This result, unfortunately, compromised the growth aspects of the study. Because of the duration of the of extension, there was not enough time to remake feeds and start over. However, the effect of diet oxidation was not expected to influence the primary objectives concerning pigmentation.

The experimental diet resulted in significantly depressed growth and feed efficiency apparently due to oxidized lipids, probably not the formula of feed. The Hunter color difference values showed no significant influence of diet on flesh color in salmon fed for four months. The observation of "L" values of back flesh showed that lightness of the fish fed the diets was fairly consistent. The negative "a" values showed a tendency for "greenness" though by the visual examination it was not perceptible. The positive "b" values showed a tendency for "yellowness" though by the visual examination it was not perceptible. However, the ability of the color meter to detect small differences that are not perceived by the human eye has been previously recognized (Regenstein, 1998, personal communication).

This study showed that feeding salmon the diet for four months (containing 16 % corn gluten meal) as fed to rainbow trout at Limestone Springs Trout Company and hybrid striped bass at AquaFuture, had no significant influence on flesh color. Unfortunately, growth potential of the diet was not suitably evaluated in this test because of the presence of oxidized lipids.

Hybrid striped bass

A practical diet formulated to have relatively low but adequate in P (0.72%) and protein (40%) and utilizing a number of alternative protein supplements (corn gluten meal, soybean meal, feather meal and blood flour) was fed under practical conditions to triplicate lots of hybrid bass (40 fish/lot) at a commercial farm. A commercial standard fish feed (Moore & Clark) was fed as a control. We measured fish growth, feed efficiency, body composition and sensory acceptance of fillets. The experimental low-phosphate diet was formulated to support rapid growth of striped bass.

Tables 23 and 24 summarize the findings in this commercial study. Although the experimental feed tended to reduce weight gain and specific growth rate, these trends were not significant (P=0.18). Feed conversion increased in fish fed the experimental feed (P= 0.03). Sensory tests showed no significant effect of diet on taste or smell of fillets. The experimental diet tended to reduce the percentage body fat and increase (P<0.1) lean body mass (Table 24). Feed intake tended to increase in fish fed the experimental feed (P=0.06), whereas intake of phosphorus was reduced with no significant effect on retention. Diet did not significantly influence intake of nitrogen or nitrogen retention.

When diet cost were considered with performance, production costs were similar or slightly better for the experimental diet as compared to the commercial control. Body composition (lean body mass) was improved with the experimental diet. Sensory tests showed that taste, color and general acceptability of fillets was as good for the experimental diet as the commercial control. This study showed that a low-P, high plant protein diet needed to reduce effluent water pollution can be effectively used in commercial hybrid striped bass.

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The Full Report with all the data and tables is available at the NRAC office upon request.