SPAWNING AND GROW-OUT OF *COLOSSOMA MACROPOMUM* AND/OR *PIARACTUS BRACHYPOMUS*

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ABSTRACT

Proximate analysis of broodstock and grow-out feeds for gamitana (*Colossoma macropomum)* and paco (*Piaractus brachypomus*) and their constituent feedstuffs was conducted. Literature values for specific nutrients known to affect fish reproduction were calculated from published sources for the broodstock diet. Fatty acid and amino acid profiles of broodstock eggs were obtained. Analytical information on the feedstuffs and diets currently being used in Iquitos, Peru, together with the egg data and published information on the natural diets of colossomids and broodstock nutrition in other species, was combined to formulate preliminary recommendations for the nutrition and feeding of gamitana and paco broodstock.

INTRODUCTION

Colossomid culture in Iquitos, Peru, may be limited currently by several factors, including the inability to obtain consistent spawning of captive broodstock. Inadequate nutrition of the broodstock may be contributing to this problem. Broodstock nutrition research in fish is rapidly expanding because reliable propagation of captive species is both difficult and vital to the success of most aquaculture industries. Key nutrients such as long-chain highly unsaturated fatty acids, amino acids, and antioxidant vitamins are implicated consistently in the spawning performance and production of quality offspring in many fish species (De Silva and Anderson, 1995). The amino acid profile of fish eggs has been used as an index of the appropriate amino acid balance for broodstock diets (Ketola, 1982), and the fatty acid profile may be used to determine the essential fatty acid composition of the diet (Tocher and Sargent, 1984). Chemical analyses of feedstuffs, prepared broodstock diets, and fish eggs were used in this study, along with published data, to identify nutritional factors that may be hindering reproductive success in captive colossomid broodstock. In addition, preliminary recommendations were made for improvement of broodstock diets.

METHODS AND MATERIALS

Proximate analysis of feedstuffs and broodstock diets were conducted using standard methods (Association of Official Analytical Chemists, 1984). Protein was analyzed using the Kjeldahl method. Total lipid content was analyzed using the Folch method (Folch et al., 1957). The amino acid profiles of fertilized and unfertilized colossomid eggs were determined with an amino acid analyzer. The fatty acid profiles of the eggs were determined with a gas chromatograph equipped with a flame ionization detector. Other data used in this report were obtained from published sources. Small-scale feeding trials were attempted with *Piaractus brachypomus* to bracket the dietary requirements for vitamins C and E. However, most of the fish died during prolonged power outages due to ice storms in Arkansas in December 2000 and January 2001.

RESULTS AND DISCUSSION

The analyzed protein content of the broodstock diet was approximately 32%. The calculated energy:protein (E:P) ratio (kcal of energy per gram of protein) of the current broodstock diet is about 8.7. This is lower than the range of values reported for good growth of colossomid species (10.7 to 13.9) (Castagnolli, 1991). Adult fish require more energy than juveniles simply for maintenance and even more energy for production of gametes. Colossomid eggs are known to be lipid-rich, which appears to be an adaptation to support metabolism rather than growth of newly hatched larvae as they drift downstream for days without feeding (Araujo-Lima, 1994).

The relative abundance of protein compared to energy in the broodstock diet may cause the fish to metabolize a large percentage of protein for basic maintenance requirements, possibly at the expense of gamete production. This imbalance is also not cost-effective, as protein is a more expensive energy source than lipid or carbohydrate. The E:P ratio of the current broodstock diet could be increased by replacing some of the nutrient-poor wheat husks with lipid. Some of the broodstock fish are reportedly "fatty" (Fernando Alcántara, pers. comm.). If this is the case even on a relatively low-energy diet, there may be a lipid transport problem. This problem has multiple potential causes but might be relieved by including dietary lipid in the form of soybean lecithin (1%). Lecithin is a phospholipid which functions in lipid digestion and transport and improves performance in some fish (Hertrampf, 1992). Phospholipid supplementation of broodstock diets also enhances egg quality in some fishes, such as red sea bream (Watanabe et al., 1991), and serves as a source of phosphorus. The present swine vitamin-mineral premix does not contain phosphorus, and fishmeal is the primary source of available phosphorus in the diet. Phospholipid content of the broodstock diet was not addressed in the present study, but will be measured in future studies with colossomid broodstock.

No supplemental lipid was added to the current broodstock diet, but it contains 7% lipid. Most of the lipid comes from fishmeal (2.9%), wheat husks (1%), soybean meal (0.7%), and corn flour (0.7%). The lipid content of the broodstock diet strongly influences the fatty acid profile of fish eggs (Watanabe et al., 1978, 1984). The lipid content and fatty acid profile of fertilized and unfertilized colossomid eggs from captive broodstock in Iquitos is shown in Table 1. The total lipid content of fertilized and unfertilized eggs ranged from 41.5 to 42.5%. This lipid level is considerably lower than the value (60%) reported for unfertilized colossomid eggs by Heming and Buddington (1988). The apparent lipid deficit in colossomid eggs in this study might have resulted directly from the low available energy content of the current broodstock diet. This problem can be resolved by supplementing lipid in the diet, as recommended earlier. The total lipid content of unfertilized eggs was significantly higher than that in fertilized eggs, probably signifying the use of lipid to fuel ontogenetic changes following fertilization (Table 1). There were no differences in the relative amounts of individual fatty acids between fertilized and unfertilized eggs.

Lipids from the fish meal supplied several of the long-chain (≥ 20 carbons) fatty acids known to be important for reproduction in fish: eicosapentaenoic (EPA, 20:5n-3), eicosatetraenoic (arachidonic, 20:4n-6), and docosahexaenic (DHA, 22:6n-3). The 20-carbon fatty acids are precursors of prostaglandins, which regulate ovulation in females (Goetz, 1983) and synchronization of reproductive behavior in males and females (Kobayashi et al., 1986a, 1986b). The DHA plays prominent roles in the development of the brain and other parts of the nervous system, especially the eye (Mourente and Tocher, 1998). Because the n-3 and n-6 fatty acids are antagonistic, a ratio of 1:1 of n-3 to n-6 fatty acids has been suggested as optimal for most fish functions, including reproduction (Tacon, 1987). Colossomid eggs in this study appeared to have about twice as much total n-3 fatty acids as n-6 fatty acids (Table 1). Eggs of wild colossomids were not available during this study, but comparison of fatty acid patterns from wild and cultured eggs should indicate whether or not the n-3:n-6 ratio of approximately 2:1 represents an imbalance in the eggs from cultured fish. A deficiency or imbalance of the n-3 and n-6, 20- to 22-carbon fatty acids in broodstock diets has been identified or implicated in impaired spawning or reduced gamete and larval quality or both in rainbow trout (Fremont et al., 1984), fathead minnows (Cole and Smith, 1987), goldfish (Wade et al., 1994), milkfish (Ako et al., 1994), and many marine fishes (Izquierdo et al., 2001). Because the main source of these fatty acids in the present colossomid broodstock diet is the lipid associated with the fish meal, any change in diet composition that results in a lower level of fish meal should be accompanied by an increase in supplementation of marine fish oil. There are very few other practical lipid sources that contain highly unsaturated fatty acids of both the n-3 and n-6 families.

The combination of fish meal (25%) and soybean meal (30%) meets the essential amino acid requirements of most warm-water fish for most functions (National Research Council, 1993). The amino acid profile of fertilized and unfertilized colossomid eggs is shown in Table 2. Leucine was the predominant indispensable amino acid in both fertilized and unfertilized eggs. Histidine was present in lower amounts than all other indispensable amino acids in both fertilized and unfertilized eggs. The high levels of leucine, glutamic acid, and alanine indicate that the broodstock diet is providing sufficient quantities of the amino acids needed to synthesize vitellogenin, the primary precursor of yolk proteins in teleosts. Although omnivorous and vegetarian fishes need little or no fish meal for optimal growth, *Oreochromis niloticus* broodstock fed diets with isonitrogenous amounts of either fish meal or legume meal had better ovarian growth and produced larger eggs when fed the fish meal diet (Cumaranatunga and Thabrew, 1990). The authors concluded that fish meal is richer in

Table 1. Total lipid (%) and fatty acid (% of total fatty acids) content of freeze-dried fertilized and unfertilized eggs from *Piaractus brachypomus* in Iquitos, Peru.[1, 2]

Lipid or Fatty Acid Name	Fatty Acid Formula[3]	Fertilized Eggs	Unfertilized Eggs
Total Lipid	NA	41.48 ± 0.72*	42.50 ± 1.36*
Myristic	14:0	2.77 ± 0.10	2.74 ± 0.13
Palmitic	16:0	27.45 ± 1.44	27.10 ± 1.33
Palmitoleic	16:1	4.03 ± 0.52	3.82 ± 0.57
Stearic	18:0	13.45 ± 1.53	13.24 ± 1.53
Oleic	18:1	31.08 ± 1.66	30.64 ± 2.05
Linoleic	18:2n-6	4.10 ± 0.16	4.12 ± 0.08
Eicosatrienoic	20:3n-3	1.03 ± 0.02	1.02 ± 0.03
Eicosatetraenoic	20:4n-6[4]	1.39 ± 0.09	1.37 ± 0.06
Eicosapentaenoic	20:5n-3[4]	1.10 ± 0.07	1.11 ± 0.05
Docosahexaenoic	22:6n-3[4]	8.68 ± 0.44	8.88 ± 0.37

1 Values are means of three replicates ± SD. Fatty acids present at less than 1% of the total are not shown.
2 Means in rows followed by an asterisk (*) are significantly different ($P < 0.10$) using a paired t-test.
3 Fatty acids are designated using the formula xy (n–z), where x = the number of carbons in the chain, y = the number of double bonds, and z = the carbon number where the first double bond from the methyl end (n) of the fatty acid is located.
4 The 20- and 22-carbon fatty acids with 4 or more double bonds are known to be important for reproduction in many fishes.

Table 2. Amino acid content (%) of freeze-dried fertilized and unfertilized eggs from *Piaractus brachypomus* in Iquitos, Peru.[1, 2]

Amino Acid[3,4]	Fertilized Eggs	Unfertilized Eggs
Phenylalanine[3]	2.55 ± 0.01	2.53 ± 0.03
Valine[3]	3.55 ± 0.01	3.48 ± 0.04
Threonine[3]	2.49 ± 0.07	2.53 ± 0.04
Isoleucine[3]	3.28 ± 0.02*	3.18 ± 0.03*
Methionine[3]	1.85 ± 0.05	1.86 ± 0.08
Histidine[3]	1.47 ± 0.01	1.48 ± 0.08
Arginine[3]	4.22 ± 0.08	4.23 ± 0.04
Leucine[3]	5.39 ± 0.03**	5.30 ± 0.05**
Lysine[3]	4.38 ± 0.01	4.32 ± 0.08
Aspartic Acid[4]	4.44 ± 0.03*	4.49 ± 0.05*
Serine[4]	2.70 ± 0.19	2.85 ± 0.07
Glutamic Acid[4]	7.08 ± 0.02	7.00 ± 0.09
Glycine[4]	1.87 ± 0.02	1.86 ± 0.03
Alanine[4]	6.50 ± 0.03*	6.37 ± 0.08*
Cystine[4]	0.64 ± 0.02	0.64 ± 0.04
Tyrosine[4]	1.98 ± 0.07	1.91 ± 0.09

1 Values are means of three replicates ± SD.
2 Means in rows followed by one or two asterisks (*) are significantly different at $P < 0.10$ and $P < 0.05$, respectively, using a paired t-test.
3 Indispensable amino acid. Tryptophan was the only indispensable amino acid excluded from analysis because it could not be analyzed simultaneously with the rest.
4 Dispensable amino acid.

vitellogenic proteins and n-3 fatty acids that enhance reproduction in many fishes. In this study isoleucine, leucine, and alanine were higher in fertilized eggs than in unfertilized eggs, and aspartic acid was higher in unfertilized eggs than in fertilized eggs (Table 2). These differences can be attributed to differences in the relative activities of energy generation and protein synthesis from amino acids in unfertilized and fertilized eggs.

There is little information on vitamin requirements of colossomid species. The natural diets of these fish are especially rich in vitamins C and E and carotenoids. All of these nutrients are known to affect reproduction in at least some fish species (De Silva and Anderson, 1995). The broodstock diet is currently supplemented with vitamin C at a rate of 500 mg kg^{-1}. This supplement is critical, as the intrinsic vitamin C content of the feedstuffs is very low. A diet with 139 mg ascorbic acid kg^{-1} was optimal for growth of pacu (*P. mesopotamicus*) fingerlings (Martins, 1995). However, the requirement may be higher for larvae due to their more rapid growth rate. The quality of eggs and spermatozoa of rainbow trout was substantially higher when fish were fed diets containing vitamin C at 8 to 10 times the level required for optimal growth (Blom and Dabrowski, 1995). The stability of vitamin C is poor under conditions of high heat and humidity, as in Iquitos. Therefore, the form of C is critical; a stabilized form should be used. If a stable form is not currently used, a different form can be used or other antioxidants (e.g., ethoxyquin or equivalent) can be added to the diet to ensure stability.

The swine vitamin-mineral premix currently used in the broodstock diet supplies about 100 mg vitamin E kg^{-1} diet, and the feedstuffs supply another 20 to 30 mg. One hundred mg kg^{-1} meets or exceeds the vitamin E requirements of most fish species for weight gain and absence of deficiency signs (National Research Council, 1993). However, only alpha-tocopherol has high biological activity. The form of vitamin E in the premix is not specified. The form should be verified since the supplement supplies most of the dietary vitamin E. Also, a stabilized form of vitamin E should be used (e.g., alpha-tocopherol-acetate). Vitamin E is very prone to oxidation under conditions of high heat and humidity. In addition, vitamin E is quickly used up in the presence of unsaturated lipids (as from fish oil) because it is a powerful antioxidant. The amount of vitamin E should be increased proportionately if unsaturated lipids (especially those found in marine fish oil) are increased in the diet. Furthermore, there are studies showing that broodstock diets containing large amounts of vitamin E given to broodstock just prior to spawning have positive effects (Kanazawa, 1988). In carp, vitamin E increases gonadosomatic index, facilitates vitellogenisis, and protects essential fatty acids in the oocytes. The specific amount of vitamin E needed to optimize these activities is not known, but vitamin E nutriture has not been investigated in colossomids.

Of pigments reported to have beneficial effects in fish or other animals, only xanthophylls (from corn flour, with a xanthophyll content of 17 mg kg^{-1}) are present in the broodstock diet (2.2 mg kg^{-1}). Other carotenoids such as beta-carotene are important for egg viability in some fish and are prevalent in the natural diets of colossomid species. Therefore, carotenoid supplementation of the broodstock diet may be beneficial for spawning success of colossomids. Further research is necessary to identify inexpensive, available sources of carotenoids (such as fruits, vegetables, or flowers) that could be used in Iquitos. Finally, most studies indicate that there is little or no benefit in adding fish meal to grow-out diets of vegetarian or omnivorous colossomid species. However, the nutritional requirements for growth are not always the same as for those for reproduction. If reduction of the fish meal in the current broodstock diet is considered for environmental or cost reasons, there are multiple nutritional factors to consider as well, as outlined in this report. The chemical composition of the colossomid eggs from the present study and other studies should serve as a guideline for formulation of broodstock diets in the future.

ANTICIPATED BENEFITS

Improving the nutritional status of colossomid broodstock should increase spawning success and possibly the quality of resulting fry. These changes would enhance the economic viability of commercial colossomid farming in Peru.

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