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BROODSTOCK DIETS AND SPAWNING OF COLOSSOMA MACROPOMUM AND/OR PIARACTUS BRACHYPOMUS

Tenth Work Plan, Feeds and Fertilizer Research 2 (10FFR2A)
Final Report

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ABSTRACT

The overall objective of this study was to determine the effect of improved broodstock nutrition on maturation and spawning performance of gamitano (*Colossoma macropomum)* and/or paco (*Piaractus brachypomus)*. In October 2002, ovules, semen, and plasma samples from gamitana and paco broodstock fed diets containing 30 or 40% protein were collected for laboratory analysis. The samples were stored frozen at Instituto de Investigaciones de la Amazonia peruana (IIAP) until they were transported to University of Arkansas at Pine Bluff (UAPB) in 2003. Total lipids, lipid class composition and fatty acid composition were analyzed for the tissues from paco and gamitano. However, statistical analysis to determine diet effects could only be performed on the plasma samples because insufficient ovule and semen samples were available. Calculated amino acid and fatty acid composition of the diets based on literature values and unpublished data is presented. The energy:protein (E:P) ratios in the diets for this study were still low, despite the addition of palm oil to both diets. The n-3 fatty acid levels and the n-3:n-6 ratios were also low in both diets relative to published recommendations for reproduction. There was no effect of diet on the total lipid, lipid classes or fatty acids in plasma of either gamitano or paco. Specific fatty acids such as arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) that are important in reproduction in other fish species were present in much higher amounts in the tissues (plasma, ovules, and semen) than in the diets. Because freshwater fish can synthesize some of the long-chain highly unsaturated fatty acids from precursors the identification of lipid sources that will enhance reproduction in characids requires additional research.

INTRODUCTION

Nutrition is known to affect reproductive success in fishes (De Silva and Anderson, 1995), but the effects are not well documented in characids. Past spawning failures of captive characids in Iquitos could be due partly to poor nutrition. The diet used in WP9 to maintain broodstock in Iquitos contained about 32% protein, which is intermediate between the requirements for larval and adult characids (Araujo-Lima and Goulding, 1997). However, the diet appeared to contain a suboptimal amount of total energy compared to diets used in most feeding experiments with characids. Insufficient levels of non-protein dietary energy can cause excessive protein catabolism to meet energy requirements, resulting in loss of essential amino acids for other critical functions such as gamete formation. Therefore, the dietary E:P ratios of the broodstock diets could be a major determinant of spawning success and larval quality in characids.

The objective of the study was to compare the effects of high (40%) and low (30%) protein diets containing similar amounts of total energy on reproductive performance of gamitano (*Colossoma macropomum)* and paco (*Piaractus brachypomus)* broodstock as indicated by biochemical composition of plasma, ovules, and semen. Proteins and lipids as well as essential amino and fatty acids interact metabolically, and analysis of all of these components of the tissues was desired. However, the amount of tissue obtained for analyses in this study was limited. Both diets contained at least 20% fish meal and the diets appeared to have no deficiencies in essential amino acids. Some of the highly unsaturated fatty acids (HUFAs) such as arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) are known to be important for reproduction in other fish species (Izquierdo et al., 2001; Sargent et al., 2002). Tissue stores of these fatty acids can be enhanced through the diet, but diets in the present study had very low levels of HUFAs . The relative proportions of lipid classes can also be manipulated through the diet to variable extents in different tissues, but baseline data is needed before the effects of dietary manipulations can be evaluated. Therefore, the lipid components of plasma, ovules, and semen (total lipid, lipid classes, and fatty acids) were analyzed in this study.

METHODS AND MATERIALS

Two practical diets were formulated for characid broodstock with ingredients commonly used in fish diets in Iquitos. Palm oil (4%) was added to each diet to increase the available energy and alpha-tocopherol levels. Palm oil has been used successfully in *Colossoma* diets previously (Viegas and Guzman, 1998). We determined that pijuayo and corn are similar in proximate composition. Due to the additional beta-carotene content of pijuayo and its availability in Iquitos, part of the corn was replaced by pijuayo. The diets were similar in total calorie content, but differed in total protein content (30 or 40%). The composition of the diets is shown in Table 1. The proportions of feedstuffs in the diets were manipulated to achieve the desired total protein and energy levels. The diets were prepared in Iquitos, Peru, and their proximate fatty acid and amino acid composition were calculated based on values from National Research Council (NRC 1993) and analytical data on pijuayo collected at the University of Arkansas at Pine Bluff. Analyzed amino acid and fatty acid content of pijuayo was not available, so the values for corn were used (a minor

Table 1. Composition (%) of diets for characid broodstock.[a]

Ingredient	40% Protein [b]	30% Protein [b]
Fish Meal	26.00	20.00
Soybean Meal	46.00	30.00
Wheat Husks	16.00	28.00
Corn Flour and/or Pijuayo [c]	6.00	16.00
Vitamin/Mineral Premix	1.95	1.95
Vitamin C	0.05	0.05
Palm Oil	4.00	4.00

a. The calculated energy to protein ratios of the diets with 40 and 30% protein are 7.3 and 8.5 kcal/gram dietary protein, respectively, based on digestible energy data for paco reported in Fernandes et al. The values are 8.2 and 10.1 kcal/gram dietary protein, respectively, based on digestible energy values for channel catfish or physiological fuel values (NRC 1993).
b. Total dietary protein levels calculated using analyzed protein content of the individual ingredients.
c. The diet with 40% pijuayo had 6% pijuayo and the diet with 30% protein had 10% pijuayo and 6% corn.

Table 2. Calculated lipid and fatty acid composition (%) of diets for characid broodstock.

Fatty Acid [a]	40% Protein [a]	30% Protein [a]
14:0	0.22	0.18
16:0	2.37	2.36
16:1	0.35	0.30
18:0	0.30	0.28
18:1	1.95	2.10
18:2n-6	1.06	1.44
18:3n-3	0.14	0.16
18:4n-3	0.07	0.05
20:4n-6	0.01	0.04
20:5n-3	0.28	0.21
22:5n-3	0.05	0.04
22:6n-3	0.23	0.18
Ratio of n-3:n-6 Fatty Acids	0.73	0.43

a. The calculated total lipid of diets with 40% and 30% protein was 7.8 and 8.0 %, respectively.

contributor to both the amino acid and fatty acid profiles). Diet samples were not available for analysis so calculated values could not be verified analytically.

Plasma, semen, and ovule samples from broodstock were collected by personnel at IIAP in November and December of 2002 and stored frozen until they were transported to UAPB. The plasma samples were received in February, 2003 and the ovule and semen samples were received in April, 2003. Total lipid of the plasma was determined by the method of Folch et al. (1957). Lipid classes of the plasma, ovules and semen were determined using an iatroscan as described in Lochmann et al. (1995). Fatty acid analysis was also conducted on the samples by a commercial lab (Woodson-Tenent Laboratories, Inc., Des Moines, Iowa).

Table 3. Calculated essential amino acid composition (%) of diets for characid broodstock.

Amino Acid	40% Protein	30% Protein
Phenylalanine + Tyrosine [a]	3.08	2.42
Valine	1.91	1.53
Threonine	1.57	1.23
Tryptophan	0.51	0.41
Isoleucine	1.72	1.34
Methionine + Cystine [a]	1.32	1.07
Histidine	1.02	0.81
Arginine	2.76	2.16
Leucine	2.30	2.40
Lysine	2.65	2.01

a. The total amount of these amino acids is given due to the ability of the non-essential amino acid in each pair (listed second) to partly fill the requirement for the essential amino acid.

Table 4. Analyzed total lipid (% of tissue), lipid classes (g/100g lipid), and selected fatty acids (g/100g lipid) of plasma of *Colossoma macropomum* (gamitano) broodstock fed diets differing in protein content.[a]

Lipid or Lipid Class	40% Protein	30% Protein
Total Lipid	2.93 ± 0.59	1.75 ± 0.56
Sterols	6.07 ± 0.59	6.75 ± 0.83
Sterol Esters	6.21 ± 2.05	6.41 ± 1.96
Triglycerides	53.81 ± 7.27	43.38 ± 8.37
Phospholipids	29.08 ± 5.29	38.34 ± 8.13
Free Fatty Acids	3.11 ± 0.48	2.81 ± 0.69
16:0	26.08 ± 0.50	25.35 ± 1.06
18:0	9.50 ± 0.22	10.02 ± 0.53
18:1	25.00 ± 1.36	28.51 ± 1.28
18:2n-6	9.66 ± 0.42	8.41 ± 0.79
18:3n-3	0.84 ± 0.05	0.63 ± 0.08
20:4n-6	1.67 ± 0.31	1.56 ± 0.24
20:5n-3	2.03 ± 0.15	1.76 ± 0.17
22:5n-3	1.00 ± 0.06	0.81 ± 0.06
20:6n-3	12.28 ± 1.80	11.99 ± 1.29
n-3/n-6 ratio	1.15 ± 0.11	1.23 ± 0.11

a. Values are means + SE of 4 individual fish per diet. There were no significant differences between treatments as determined by ANOVA ($P>0.05$).

Only the plasma data was complete enough to analyze statistically to determine diet effects. Analysis of covariance was used to detect differences in lipid and lipid classes due to diet or gender, and weight was used as a covariate. Because there were no significant gender effects ($P>0.05$) data for different genders was combined and reported by diet. Means and standard errors were computed for ovule and semen data and qualitative comparisons were made.

Table 5. Analyzed total lipid (% of tissue), lipid classes (g/100 g lipid), and selected fatty acids (g/100g lipid) of plasma of *Piaractus brachypomus* (paco) broodstock fed diets differing in protein content.[a]

Lipid or Lipid Class	40% Protein	30% Protein
Total Lipid	2.72 ± 0.41	1.47 ± 0.33
Sterols	4.61 ± 0.68	5.33 ± 0.40
Sterol Esters	2.47 ± 0.35	3.26 ± 0.20
Triglycerides	55.59 ± 7.55	44.52 ± 3.74
Phospholipids	24.42 ± 5.91	37.53 ± 3.28
Free Fatty Acids	3.31 ± 0.85	2.28 ± 0.21
16:0	24.50 ± 0.94	21.63 ± 1.46
18:0	10.39 ± 0.61	9.53 ± 0.75
18:1	31.82 ± 3.06	37.56 ± 3.13
18:2n-6	9.02 ± 2.53	8.44 ± 1.86
18:3n-3	0.97 ± 0.31	0.91 ± 0.27
20:4n-6	0.72 ± 0.11	0.99 ± 0.19
20:5n-3	2.05 ± 0.53	1.71 ± 0.36
22:5n-3	0.61 ± 0.13	0.49 ± 0.17
22:6n-3	7.16 ± 1.16	9.43 ± 2.43
n-3/n-6 ratio	1.06 ± 0.14	1.16 ± 0.28

a. Values are means + SE of 4 individual fish per diet. There were no significant differences between treatments as determined by ANOVA (P>0.05).

Results

The calculated lipid and fatty acid composition of the diets is shown in Table 2. Diets contained more n-6 than n-3 fatty acids, as expected since palm oil contains 9–10% 18:2n-6, less than 0.5% 18:3n-3 and no n-3 or n-6 HUFAs. The ratio of n-3 to n-6 fatty acids was less than one for both diets. The amino acid composition is shown in Table 3. Diets met or exceeded the published (NRC, 1993) essential amino acid requirements of warmwater omnivorous fishes including channel catfish (*Ictalurus punctatus*), tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*). The analyzed total lipid and lipid class composition for plasma of gamitano fed diets differing in protein content is shown in Table 4. There were also no significant differences in total lipid, lipid classes or fatty acids due to diet. The analyzed total lipid and lipid class composition for plasma of paco fed diets differing in protein content is shown in Table 5. There were no significant differences in total lipid, lipid classes or fatty acids due to diet. The primary non-essential fatty acids in both species were 16:0, 18:0, and 18:1n-9. Linoleic acid (18:2n-6) and 22:6n-3 were the main n-6 and n-3 fatty acids, respectively, in the serum.

Mean total lipid was 0.4 ± 0.1 g 100 g^{-1} in sperm and 14.4 ± 1.0 g 100 g^{-1} in ovules. The predominant lipid classes in both ovules and sperm were phospholipids (PL) and sterols (ST). Sterol esters (SE) were much more prominent in semen than in ovules, and the reverse was true for ST. Triglycerides (TG) were present only in trace amounts in both ovules and semen. The main non-essential fatty acids in ovules and semen were 16:0, 18:0, and 18:1n-9. The ratio of n-3 to n-6 fatty acids was 1.2 ± 0.2 and 2.7 ± 0.5 for ovules and semen, respectively. Levels of 20:5n-3 were similar in ovules and sperm. Eicosapentaenoic acid (20:5n-3) and 20:4n-6 were present in approximately equal amounts in ovules. Arachidonic acid (20:4n-6) was 3–4 times higher in semen than in ovules, and 22:6n-3 was about three times higher in semen than in ovules. Docosahexaenoic acid (22:6n-3) was the predominant fatty acid in most semen samples, constituting as much as 39 g 100 g^{-1} of total lipids.

Discussion & Conclusions

Adult fish require more energy than juveniles for maintenance, and even more energy for production of gametes. The calculated E:P ratio (kcal of energy per gram of protein) of the 32% protein broodstock diet used previously at IIAP was about 8.7. This is lower than the range of values reported for good growth of characid species (10.7–13.9) (Castagnolli, 1991). Even though lipid was added to diets for this workplan (4% palm oil) in an effort to increase the total energy in both diets, the calculated E:P ratios of the diets are still low, depending on the data used to determine the estimates. The total lipid in each diet was around 8%, which is only 1% higher than the total lipid in the previous (32% protein) diet. Dietary lipid is a crucial nutrient for characids, as their eggs are lipid-rich as an adaptation to support metabolism of newly hatched larvae during prolonged periods without feeding (Araujo-Lima and Goulding, 1997). Both of the diets in this study had calculated E:P ratios and n-3 fatty acid contents below optimal levels reported for spawning success in other fishes (Izquierdo et al., 2001; Sargent et al., 2002).

Key nutrients such as long-chain HUFAs and amino acids are implicated consistently in the spawning performance and production of quality offspring in many fish species (De Silva and Anderson, 1995). Amino acid data was not obtained for tissues in this study, but the essential amino acid content of both diets met or exceeded the dietary amino acid requirements of other warmwater omnivorous fishes such as channel catfish, tilapia and common carp (NRC, 1993). Animal protein may be important for reproduction in some species (Cumaranatunga and Thabrew, 1989) and both diets contained ≥ 20% fish meal.

The analyzed total lipid, lipid classes, and fatty acid profiles of the plasma did not reveal any differences due to diet in either paco or gamitano. Varibability in the data was fairly high, possibly because the total lipid and lipid classes in the blood are subject to rapid changes in relation to stress, activity and time of feeding (Henderson and Tocher, 1987). Gender was another source of variability, although the effects were not statistically significant at P<0.05. Levels of the n-3 and n-6 fatty acids were well above dietary levels in most cases. The n-3 to n-6 ratio was similar across species and diets (1.1–1.2), despite the fact that this ratio was 0.7 or lower in the diets. Tacon (1987) suggested that a ratio of 1.0 is optimal for fish performance.

Total lipid of the eggs (14%) was slightly above the range reported for freshwater fish (2.5–10.0% of wet weight)(Henderson and Tocher, 1987). Lipid-rich eggs frequently contain high levels of triglycerides (Weigand, 1996), but eggs in the present study contained almost no triglycerides and sterols were the predominant neutral lipid class. Similar to other freshwater fish the eggs were also rich in phospholipid, which is concentrated in the yolk. As the

Table 6. Analyzed total lipid (% of tissue), lipid classes (g/100 g lipid), and selected fatty acids (g/100g lipid) of ovules and semen of paco (*Piaractus brachypomus*) and/or gamitano (*Colossoma macropomum*) broodstock. There were not enough samples to determine diet or species effects so data was pooled by tissue type.[a]

Lipid or Lipid Class	Ovules [b]	Sperm [c]
Total Lipid	14.43 ± 1.04	0.44 ± 0.08
Sterols	24.63 ± 2.28	14.58 ± 2.67
Sterol Esters	2.49 ± 0.89	17.25 ± 6.48
Triglycerides	0.05 ± 0.04	0.01 ± 0.01
Phospholipids	38.99 ± 4.91	38.24 ± 3.57
Free Fatty Acids	13.17 ± 4.30	10.53 ± 3.49
16:0	31.97 ± 1.26	24.08 ± 1.44
18:0	10.20 ± 0.56	14.73 ± 2.28
18:1	29.52 ± 0.88	13.88 ± 0.89
18:2n-6	6.00 ± 0.44	1.63 ± 0.21
18:3n-3	ND [d]	ND [d]
20:4n-6	1.46 ± 0.05	7.32 ± 1.18
20:5n-3	1.60 ± 0.25	2.64 ± 0.40
22:5n-3	0.53 ± 0.11	1.55 ± 0.31
22:6n-3	8.40 ± 0.68	25.07 ± 3.57
n-3/n-6 ratio	1.19 ± 0.16	2.71 ± 0.50

a. Statistical analysis of the data was not possible. Data is presented for qualtitative comparison only.
b. Values are means + SE of 3 individual fish (1 paco, 1 gamitano, 1 unknown).
c. Values are means + SE of 11 individual fish (10 paco, 1 gamitano).
d. ND=Not detected.

phospholipids are catabolized during embryonic development and the yolk-sac stage, the essential fatty acids they released may be incorporated into the fish's body. Alternatively, the phospholipids may be catabolized for energy, depending on the fish species (Sargent et al., 2002). Major seasonal changes in cholesterol, phospholipids and free fatty acids of semen were observed in the catfish (*Clarias batrachus)* in relation to cycles in steroid hormone synthesis (Singh and Joy, 1999). Diet-induced fluctuations in lipid classes of semen are less well known. Diet did not affect the cholesterol or phospholipid contents of rainbow trout semen (Labbe et al., 1993). However, the species of phospholipids in semen can be affected since the fatty acid components of phospholipids are affected by diet (Bell et al., 1996).

The fatty acid profile of wild fish eggs can be used to indicate the appropriate essential fatty acid composition of the diet for broodstock (Tocher and Sargent, 1984). A deficiency or imbalance of the n-3 and n-6 HUFAs in broodstock diets has been identified or implicated in impaired spawning and/or reduced gamete and larval quality in rainbow trout (*Oncorhyncus mykiss*) (Fremont et al., 1984), fathead minnows (Pimephales promelas) (Cole and Smith, 1987), goldfish (*Carassius auratus*) (Wade et al., 1994), milkfish (*Chanos chanos*)(Ako et al., 1994), nile tilapia (Santiago and Reyes 1993), walleye (*Stizostedion vitreum*)(Czesny and Dabrowski, 1998), Atlantic salmon (*Salmo salar*)(Pickova et al., 1999), and many marine fishes (Izquierdo et al., 2001). Much less information is available on the effect of broodstock diets on the quality of semen. Fatty acid profiles of semen are affected by diet in rainbow trout (Labbe et al., 1993; Gasco et al., 1999) and sea bass (*Dicentrarchus labrax* L.)(Bell et al., 1996). Wild characid eggs and milt semen were not available during this study but the calculated fatty acid profiles of the diets indicated a possible deficiency in some of the essential fatty acids. There are well-established roles of n-3 fatty acids such as 20:5n-3 (EPA) and 22:6n-3 (DHA) in reproduction in fish. EPA and arachidonic acid (20:4n-6) are precursors of prostaglandins, which regulate ovulation in females (Goetz, 1983) and synchronization of reproductive behavior in males and females (Kobayashi et al., 1986a,b.). Docosahexaenoic acid (DHA) plays prominent roles in the development of the brain and other parts of the nervous system, especially the eye (Mourente and Tocher, 1998).

The lipid content of the broodstock diet influences the fatty acid profile of fish eggs and sperm (Watanabe et al., 1978, 1984; Labbe et al., 1993; Gasco et al., 1999). However, freshwater fish have varying capacities to elongate and desaturate 18-carbon n-3 and n-6 fatty acids to their respective HUFAs (Sargent et al., 2002). Therefore, identification of the fatty acids that are dietarily versus metabolically essential is more complicated. Diets in the present study appeared to contain very little 18:3n-3 (0.14–0.16%) and no more than 0.6% total n-3 HUFAs. The diets contained 1–1.4% 18:2n-6 and almost no 20:4n-6. The levels of 18:3n-3 in the ovules and semen were similar to those in the diet (essentially undetected). The level of 18:2n-6 was similar in the diets and in the semen. However, 18:2n-6 was about six times higher in ovules than in the diet. Levels of the HUFAs most important for reproduction (20:5n-3, 20:4n-6, and 22:6n-3) were several to many times higher in both ovules and semen than in the diets. There are several possible explanations for this trend: 1) The actual fatty acid composition of the diets was different than the calculated values; 2) The fish were getting nutrients from sources other than the experimental diets (i.e., natural foods in the ponds); and 3) The fish were metabolically converting the n-3 and n-6 18-carbon fatty acids to their respective HUFAs. These three explanations are not mutually exclusive. Analyzed fatty acid composition of the diets and of wild characid ovules and semen would be helpful to interpret the data further. The extent to which characids can metabolically convert the n-3 and n-6 18-carbon fatty acids to their HUFAs is not fully known. The silver dollar pirhana (*Mylassoma aureum*) is an herbivore that can convert 18-carbon n-3 and n-6 fatty acids to HUFAs and the carnivorous red pirhana (*Serassalmus natteri*) has similar capabilities (Henderson et al., 1996). Pacu and gamitano in this study showed evidence of the same ability to bioconvert fatty acids. However, if the preformed HUFAs are provided in the diet then the fish will not have to expend energy to synthesize them. Marine fish oils are excellent sources of these HUFAs but their use may be limited by environmental concerns, availability, or stability in tropical Amazonian regions. Therefore, additional research is needed to determine the best combination of oils/fatty acids to include in broodstock diets for characids.

ANTICIPATED BENEFITS

Improving the nutritional status of characid broodstock should increase spawning success and possibly, the quality

of resulting fry. These changes would enhance the economic viability of commercial characid farming in Peru. The impact indicator for this study is the number of broodstock diets for *Colossoma* and/or *Piaractus* resulting in biochemical composition of gametes and/or plasma consistent with reproductive success in fish. There were no differences in total lipid, lipid classes, or fatty acids of the plasma due to diet. However, fish spawning was limited in 2002 for unknown reasons, leading to small sample sizes of ovules and semen for analysis. Therefore, it is premature to state that either of the diets in this study were adequate to optimize reproduction. The primary impact of the present study was to delineate areas of broodstock nutrition for additional study.

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