Comparison of Flaxseed and Cottonseed Meals on Water Quality, Zooplankton, and Red Drum Fingerling Production in Plastic-lined Ponds

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Abstract: A 35-d growth trial was conducted to evaluate the effect of fertilization with flaxseed meal versus cottonseed meal on fingerling red drum (*Sciaenops ocellatus*) production characteristics (individual weight, total length, and percent survival), water quality parameters, and zooplankton densities in plastic-lined ponds. Red drum fry were stocked into 16 randomly allocated 0.40-ha ponds, with eight ponds per fertilization treatment. Mean individual weight of red drum fingerlings was significantly larger in ponds fertilized with flaxseed meal than in ponds fertilized with cottonseed meal throughout the growth trial. Similarly, red drum fingerlings had significantly greater mean length in ponds fertilized with flaxseed meal during the first 25 days of culture; however, no difference in total length was observed at the termination of the growth trial. No differences were observed for water quality parameters between treatments except for pH, which was significantly higher in ponds fertilized with cottonseed meal. Rotifer populations were significantly greater in ponds fertilized with CSM than FSM; however, no differences in total copepods, total copepod nauplii, or total zooplankton densities were observed between pond treatments. Our results indicated the growth of red drum fingerlings was enhanced in ponds fertilized with flaxseed meal. Furthermore, at the rate used in this study (392 kg/ha), flaxseed meal did not negatively impact water quality or zooplankton populations in fingerling red drum production ponds. However, initial cost-benefit analysis suggests that utilizing flaxseed meal for red drum production is cost prohibitive compared to cottonseed meal. Further research is needed to determine specific impacts of the flaxseed meal application to marine ponds as well as the economic feasibility of adding flaxseed meal to production ponds before this procedure can be recommended for red drum fingerling production.

Key words: flaxseed, cottonseed, meal, red drum, fingerlings

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Use of various organic fertilizers to increase phytoplankton production and consequent zooplankton populations has been investigated thoroughly for aquaculture in freshwater systems (Gieger 1983, Kurten et al. 1995, Boyd 1997). However, very little information is available with respect to optimization of organic fertilizers for marine fish production.

Successful production of red drum (*Sciaenops ocellatus*) fingerlings depends on many factors; perhaps the most important of these is the establishment of an adequate forage base for the fish. Red drum, like other planktivorous fry, rely on zooplankton as the primary source of exogenous food once the yolk sac has been absorbed (Holt et al. 1987). As such, fertilization is an important management tool to ensure a proper forage base for production of red drum juveniles.

Fertilizers used in the aquaculture industry fall into two classes: inorganic and organic. Inorganic fertilizers, as sources of nitrogen (N) and phosphorous (P), enhance phytoplankton blooms that serve as the basis for zooplankton production. However, the proportion of N to P can influence the type of phytoplankton community produced. Previous research has indicated that fertilizers with a high N:P ratio produce a phytoplankton bloom conducive to fingerling production (Swingle and Smith 1939, Smith 1983, Boyd and Tucker 1998). Conversely, fertilizers with low N:P ratios result in undesirable production of nitrogen-fixing blue-green algae and filamentous algae (Darley 1982, Smith 1983).

Organic fertilizers, such as cottonseed meal, alfalfa meal, hay, and manure, are utilized to stimulate phytoplankton production, but they are also capable of supporting heterotrophic production of bacteria and protozoa (Boyd 1990, Barkoh 1996, Kurten et al. 1999). Typical recommendations suggest organic fertilizers with low carbon:nitrogen ratios because greater proportions of nitrogen in organic fertilizers affects microbial decay rate of organic matter in ponds, which ultimately dictates the utilization rate of nutrients in the pond by organisms in higher trophic levels (Boyd 1979). Although adequate pond fertilization can be achieved through either inorganic or organic sources, previous research has indicated that effective production of zooplankton communities is optimized when both classes are utilized (Anderson 1993).

Although there are many available sources for organic fertilizers, perhaps the most commonly used is cottonseed meal (CSM). Cottonseed meal has been used to fertilize ponds both for freshwater and marine finfish species (Colura 1990, Barkoh 1996). Although the use of CSM is commonplace in the freshwater aquaculture industry, it may not be as desirable in the mariculture industry. This is because organic fertilizers differ in their physical properties, decomposability, release of available nutrients, and nutritional value to zooplankton (Barkoh and Rabeni 1990). The nutritional value to zooplankton is an especially important aspect in red drum fingerling production because zooplankton is the primary food forage for red drum fry.

Alternative sources of organic fertilizers, such as rice bran and alfalfa meal, have been investigated to determine their suitability for phytoplankton, zooplankton, and fish production. Studies using these fertilizers have yielded conflicting results regarding fish production characteristics. Subsequently, there is no clear answer as to their suitability as a fertilizer. Another potential organic fertilizer for marine fish production is flaxseed meal (FSM). Previous research has shown that FSM contains significantly higher levels of omega-3 (n-3) fatty acids that could enrich the nutritional value of heterotrophic zooplankton in culture ponds (Lane and Kohler 2007). This may lead to a source of zooplankton enhanced with long-chained n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which would be beneficial to the growth and development of red drum fingerlings.

Because very little information is available concerning the effect of organic fertilizers on the production of red drum fingerlings, the objective of the present study was to compare the effects of FSM versus CSM on the dynamics (zooplankton and water quality parameters) and production characteristics of fingerling red drum ponds.

Materials and Methods

Experimental Design and Protocol

A 35-d performance trial was conducted during the 2006 and 2007 production seasons in the Texas Parks and Wildlife Sea Center Texas fish-hatchery facility located at Lake Jackson, Texas. Trials were conducted using typical pond culture conditions, utilizing 16, 0.40-ha plastic-lined ponds (six ponds during year 1; ten ponds during year 2). Ponds were selected for treatment using a completely randomized design, with eight replicates per treatment (CSM or FSM). Two ponds were filled at a time, one pond for each treatment, to maximize fill rates. Once ponds were filled (Day 1), the fertilization schedule utilized was adapted from Colura (1990) for fingerling red drum production as follows: an initial application of inorganic fertilizers (6-L of liquid ammonia nitrate and 2.5-L of phosphoric acid) the day after ponds were filled (Day 2). On Day 3, an initial application of either CSM or FMS (224 kg/ha) for each pond was added to the ponds. Thereafter, supplemental applications of inorganic fertilizers were added on Day 5 and 10 and supplemental applications of organic fertilizers (28 kg/ha) were added to the ponds on days 8, 13, 15, 22, 27, and 34. Total amount of organic fertilizer added to each pond was 392 kg/ha.

Zooplankton Monitoring and Pond Stocking

Prior to stocking the ponds with red drum larvae, zooplankton densities were measured daily between 0700 and 0800 using a Turtox plankton tow net with a 64-µm mesh netting (Wildlife Supply Company, Buffalo, New York). A 100-mL sample from each pond was collected and preserved with Lugol's solution. A 1-mL aliquot from each sample was placed onto a Sedgwick-Rafter counting cell slide (Pyser-SGI Unlimited, Edenbridge, Kent, United Kingdom) and rotifers, copepod nauplii, and copepods were identified and enumerated via microscope under 40X magnification. Once zooplankton densities in ponds had a minimum of 600 organisms/L, each pond was stocked with 310,506 ± 7,430 (± SE) 3-day old red drum fry. After experimental ponds were stocked, zooplankton tows were conducted three times a week between 0700 and 0800 CDT throughout the study.

Ichthyoplankton sampling was conducted in the ponds on the 10th day after stocking. During this sampling period, a total of 200 fish (four subsamples of 50 fish) from each pond were collected for size measurements (total length and weight). Thereafter, salmon starter was applied to each pond, at 3% estimated total body weight of fish per day. On the 16th day, a total of 20 fish were collected from each pond, weighed collectively, and their lengths were measured to determine growth rate. On the 25th and 35th day, 20 fish from each pond were sampled and individual fish weight and length were recorded. At the termination of the growth trial, ponds were drained, fish were harvested, and samples collected to determine percent survival.

Water Quality

Dissolved oxygen (DO), water temperature, and pH were recorded three times daily (0400, 1400, and 2200 h) at a depth of about 110 cm near the pond kettle in each pond. Dissolved oxygen and temperature were measured with a YSI Model 550 meter (Yellow Springs Instrument Company, Ohio). The pH was measured with a pH pen, and nitrite (NO_2–N), nitrate (NO_3–N), and total ammonia levels were measured with a Hach DR 2800 spectrophotometer using colorimetric assay kits (Hach Company, Loveland, Colorado). Nutrients were measured three times weekly.

Statistical Analyses

A one-way analysis of variance (ANOVA) (SAS version 8.2; SAS Institute, Cary, North Carolina) was used to analyze production characteristics (individual weight, individual length, total yield, and percent survival) and pond characteristics (zooplankton population densities and water quality parameters). Percentage data were arcsine-square-root transformed prior to analysis. When significant differences were detected by ANOVA, Tukey's Studentized range test was used to compare treatment means. A significance level of $P < 0.05$ was used.

Results

The mean weight of red drum fingerlings was significantly greater in ponds fertilized with FSM than in ponds fertilized with CSM throughout the duration of the study (Table 1). Fingerlings reared in ponds fertilized with FSM grew 67% faster than fish cultured in ponds fertilized with CSM after 10 days post-stocked ($P < 0.001$). The total length was significantly greater for fish reared in ponds fertilized with FSM for the first 25 days; however, at the end of the study, no difference in total length was observed between treatments ($P = 0.7574$). Specifically, after the first 10 days post-stocking, total length of fish reared in the FSM treated ponds were 24% larger than their counterparts cultured in ponds fertilized with CSM. No significant difference in percent survival of fingerlings was observed between treatments ($P = 0.6987$)

No significant difference in zooplankton density (mean ± SE: 1,169.0 ± 346.6 CSM; 1573.2 ± 288.7 FSM; $P = 0.2839$) or zooplankton composition (percent copepod nauplii, copepods, rotifers) was observed to exist between ponds treated with FSM and those that received CSM on the day that ponds were stocked. However, total rotifer population in ponds was significantly greater in ponds fertilized with CSM than FSM for the duration of the growth trial ($P < 0.001$). In contrast, no significant differences were evident for total copepod nauplii ($P = 0.6795$), copepod populations ($P = 0.0682$), or total zooplankton density ($P = 0.1298$) (Figure 1) for the remainder of the growth trial.

No significant differences were observed in temperature, dissolved oxygen, total ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen between treatments (Table 2). However, overall mean pH values were significantly higher in ponds fertilized with CSM than in ponds fertilized with FSM ($P < 0.001$) (Figure 2).

Table 1. Production characteristics (mean ± SE) for red drum fingerlings reared in ponds fertilized with cottonseed meal (CSM) or flaxseed meal (FSM) for a period of 35 d. Values within each row with different letters are significantly different ($P < 0.05$).

		Fertilization regimen	
		CSM	FSM
Total length (mm)			
DPS[a]	10	9.4 ± 0.1b	11.7 ± 0.0a
	16	17.2 ± 0.2b	18.5 ± 0.1a
	25	23.8 ± 0.6b	25.8 ± 0.5a
	35	35.1 ± 0.3b	35.3 ± 0.4b
Weight (mg)			
DPS	10	12.3 ± 0.3b	20.5 ± 0.4a
	16	72.1 ± 2.4b	88.4 ± 2.1a
	25	138.6 ± 7.9b	161.6 ± 6.9a
	35	426.4 ± 9.8b	539.1 ± 24.8a
Survival (%)		51.0 ± 10.4	44.6 ± 12.2
Feed fed (kg)		68.6 ± 6.6	65.7 ± 10.3

a. Days post-stocked.

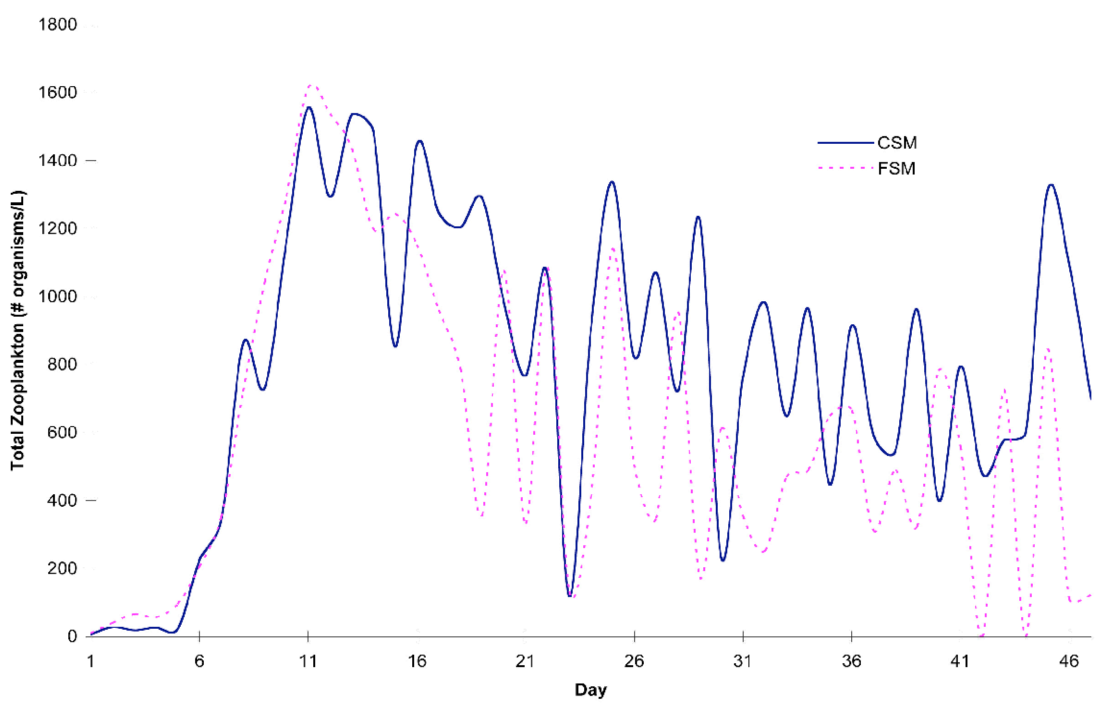

Figure 1. Mean daily total zooplankton population values (rotifers, copepod nauplii, and copepods) during a 35-day red drum fingerling growth trial utilizing CSM or FSM as organic fertilizers to enhance pond productivity.

Table 2. Water quality parameters (mean ± SE) of ponds fertilized with cottonseed meal (CSM) or flaxseed meal (FSM) during a 35-day production trial with red drum fingerlings. Values within each row with different letters are significantly different ($P < 0.05$).

Water parameters	Fertilization regimen	
	CSM	FSM
Temperature (C)	28.3 ± 0.5	27.5 ± 0.2
Dissolved oxygen (mg/L)	7.4 ± 0.1	7.3 ± 0.1
pH	8.92 ± 0.02a	8.75 ± 0.02b
Salinity (g/L)	23.3 ± 0.3	24.8 ± 0.4
TA-N (mg/L)	0.02 ± 0.0	0.02 ± 0.0
NO_2–N (mg/L)	0.01 ± 0.0	0.02 ± 0.0
NO_3–N (mg/L)	0.2 ± 0.1	0.2 ± 0.1

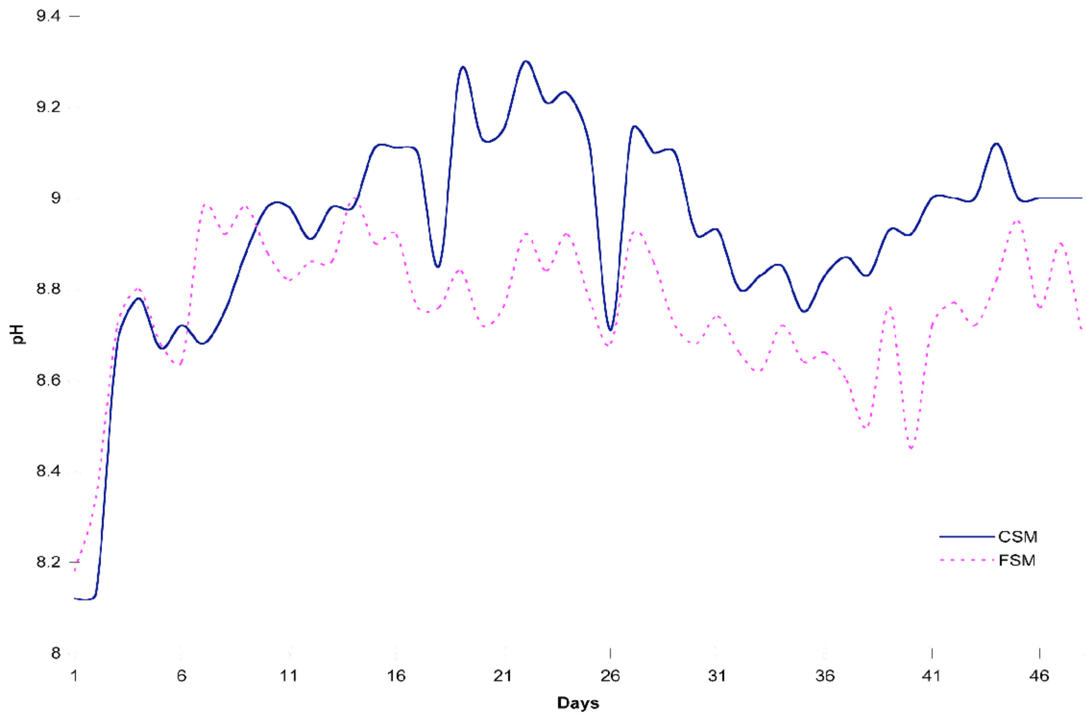

Figure 2. Mean daily pH values during a 35-day red drum fingerling growth trial utilizing CSM or FSM as organic fertilizers to enhance pond productivity.

Discussion

The use of fertilizers in culture ponds have been shown to enhance fish growth (Gieger et al. 1985). Further research has been conducted to ascertain optimal organic fertilizer sources that are conducive to fingerling fish production. For example, striped bass fingerlings (*Morone saxatilis*) grew larger in ponds fertilized with rice bran versus ponds fertilized with CSM (Ludwig and Tackett 1991). In contrast, sunshine bass (female *M. chrysops* X male *M. saxatilis*) attained greater growth in ponds fertilized with CSM than in ponds fertilized with ground rice bran or FSM (Lane and Kohler 2007). In the present study, red drum fingerlings had greater weight gain in ponds fertilized with FSM than in ponds fertilized with CSM, which contrasts with the findings of Lane and Kohler (2007).

Although not established in the present study, it is reasonable that the nutrients within FSM were imparted to the zooplankton population. Flaxseed meal, as well as flaxseed oil, has been shown to contain significant amounts of alpha-linolenic acid (ALA, 18:3n-3), which is the precursor for long-chain highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) (Arts et al. 2001). These fatty acids are beneficial to red drum fingerlings since marine fish require omega-3 HUFAs from exogenous sources for optimal growth (Craig 1994, Hardy 2001). Previous studies have shown that zooplankton have the ability to uptake excess ALA from their environment (Lane and Kohler 2007). Furthermore, research has determined that although rotifers are a poor source of n-3 HUFAs, copepod nauplii are an excellent source of these essential fatty acids (Cutts 2002). Although it is unclear if zooplankton, specifically rotifers, were able to modify ALA into longer chained HUFAs, it does appear that they are able to incorporate exogenous sources of ALA into their tissues (Lane and Kohler 2007). Therefore, zooplankton within ponds fertilized with FSM may have been able to incorporate the available ALA within their tissues and impart valuable n-3 HUFAs to the red drum fingerlings. This increase in n-3 HUFAs may have enhanced the growth of red drum fingerlings observed in the study.

To maximize optimal forage for larval red drum, it is important to ensure that the size of the zooplankton corresponds with the mouth gape of the larval red drum. Zooplankton succession has been shown to be dependent on several environmental factors. For instance, lower salinity levels have been shown to provide optimal zooplankton forage for larval euryhaline finfish when compared to zooplankton densities cultured in ponds with salinities ranging from 15 to 20 g/L (Colura et al. 1987). In the present study, salinity levels were slightly higher than the ranges required for optimal zooplankton forage; however, adequate zooplankton densities in both treatments did occur within a week after ponds were filled. Although not significantly different, ponds fertilized with FSM attained a greater total zooplankton density faster than ponds fertilized with CSM.

Even though there were no differences in zooplankton densities, it is interesting to note that ponds fertilized with FSM established a population of copepod nauplii in less time than in ponds fertilized with CSM; however, the FSM induced population quickly dropped off and never recovered to original density. This succession is important since copepod nauplii are the first prey of choice for many marine fish, including larval red drum (Schipp et al. 1999), and it is likely that the decrease in copepod nauplii populations within these ponds is indicative of larval fish actively seeking these zooplankters. In contrast, the adult copepod populations were maximized quickly in ponds fertilized with CSM compared to their counterparts in FSM treated ponds; in which copepod populations reached maximum levels several days after the CSM treated ponds. In both treatments, the copepod population never returned to original levels observed after the initial fertilization treatments. It is possible that the decrease in copepod density is a result of increased predation by the growing fingerling population, as suggested by Bass and Avault (1975) and Colura et al. (1976). These authors have reported that copepods are considered a substantial component of fingerling red drum diets and are actively sought by fingerling red drum throughout the culture period.

Mean pH level was significantly higher in ponds fertilized with CSM than in ponds treated with FSM. The pH levels in ponds fertilized with CSM reached the highest level three weeks post-flooding. In contrast, the mean highest level observed in ponds fertilized with FSM did not occur until six weeks post-flooding. High pH is best avoided in ponds since it has been reported to be detrimental to zooplankton production by reducing the growth rate and increasing the mortality rate of copepods and other crustacean zooplankters (O'Brien and Noyelles 1972, Ludwig et al. 2007). Indeed, in the present study, the elevated levels of pH appeared to reduce the population of copepods within CSM ponds after the initial fertilization rates, thereby resulting in the establishment of rotifers as the main source of food for red drum fingerlings. In contrast, the lower levels of pH within FSM ponds induced a greater proportion of copepods within the zooplankton population, which provided the fingerlings with their preferred food type. However, as previously mentioned, the pH levels within both treatments were slightly elevated which may explain the decrease in copepod population for the remainder of the growth trial. In addition to restricting preferred natural food forage, elevated levels of pH within ponds have been shown to increase mortality of larval finfish (Weiner et al. 1986, Anderson 1993, Barkoh 1996, Ludwig 2002). In addition, DO levels in the present study were significantly higher in ponds fertilized with CSM, which would correlate with increased pH levels associated with photosynthesis.

The results from the present study suggest that the growth of red drum fingerlings was enhanced when FSM was used as an organic fertilizer. Furthermore, these results indicate that FSM did not negatively affect the water quality parameters nor negatively affect the zooplankton population in production ponds. Although a beneficial effect was observed for fish growth, preliminary cost-benefit analysis suggests that utilizing FSM for red drum production is cost-prohibitive when compared to using CSM due to the fact that current market prices for CSM is U.S. $0.38/kg and FSM is $2.27/kg. Furthermore, utilizing a 35-day grow-out period does not produce a noticeable improvement in fingerling red drum production when FSM is used as an organic fertilizer. As such, further research is needed to determine the specific impacts of the application of FSM to marine ponds as well as the economic feasibility of adding FSM to production ponds before this procedure can be recommended for the production of red drum fingerlings.

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