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Effects of complete and supplemental diets and organic pond fertilization on production of *Macrobrachium rosenbergii* and associated benthic macroinvertebrate populations

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Abstract

Growth and population characteristics of freshwater prawns, *Macrobrachium rosenbergii*, fed either a complete diet, a supplemental diet (with fish meal replaced and vitamin and mineral supplements deleted), or the supplemental diet with an adjunct organic pond fertilization regime were examined. Effects of these treatments on potential forage organisms (benthic macroinvertebrates) were also evaluated. Juvenile prawns averaging 0.46 ± 0.49 g were stocked into nine 0.02 ha earthen ponds at 39 520 ha^{-1}. Treatments were evaluated in triplicate ponds. There were significant differences ($P<0.05$) between the regression lines for sample weights over time (growth) in prawns fed the complete diet and supplemental diet without organic pond fertilization and between those fed the supplemental diet with and without fertilization. There was no significant difference ($P>0.05$) between regression lines for prawns fed the complete diet and those fed the supplemental diet with an organic pond fertilization regime. At harvest, yield, survival, individual weight, and feed conversion averaged 943 kg ha^{-1}, 76%, 31 g, and 3.9, respectively over the three treatments. Deletion of vitamin and mineral supplements and replacement of fish meal in prawn diets was associated with a significant reduction ($P<0.05$) in total benthic macroinvertebrate populations, possibly due to increased predatory pressure by prawns. In ponds in which prawns were fed the supplemental diet, organic fertilization resulted in a significant increase ($P<0.05$) in total benthic macroinvertebrates and a 15%

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increase in average prawn weight at harvest. Results indicate that prawns may increase predation when essential nutrients are deleted from prepared diets and that the supply of potential forage organisms may be increased by organic fertilization.

Keywords: *Macrobrachium rosenbergii*; Supplemental diets; Organic fertilization; Complete diet; Benthic macroinvertebrates

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1. Introduction

Feed costs constitute 40–60% of operational costs in production of the freshwater prawn *Macrobrachium rosenbergii* (D'Abramo and Sheen, 1991). Initial efforts to reduce feed costs for aquaculture species often include replacement of expensive animal protein with plant protein meals, which are generally less expensive (Piedad-Pascual et al., 1990; Tidwell et al., 1993), or elimination of excess vitamin and mineral premixes from the diet (Trino et al., 1992).

Castille and Lawrence (1989) investigated the effects of deleting certain dietary ingredients from nutritionally complete pelleted diets on growth of pond reared shrimp (*Penaeus vannamei*). Growth was reduced by deletion of mineral supplements from the diet but no effect was observed when dietary vitamin supplements were removed. Trino et al. (1992) reported that elimination of dietary vitamin supplements did not reduce growth in *P. monodon* and that the resulting decrease in feed costs increased potential profitability.

Corbin et al. (1983) suggested that the major portion of macronutrients (i.e. protein) for freshwater prawns needs to be supplied by prepared feeds, but that required levels of micronutrients (i.e. vitamins and minerals) could be derived from natural productivity, or stimulated productivity of natural foods, in the culture ponds. A better understanding of the role of natural productivity in prawn nutrition and development of methods for selective management of desirable components are needed (MacLean et al., 1989). This could result in decreased production costs based upon providing a combination of pelleted rations and pond organisms to satisfy nutrient requirements (Corbin et al., 1983). The present study was designed to evaluate production and population characteristics of pond raised prawns fed a complete diet, a supplemental diet (a similar formulation but with fish meal and vitamin and mineral supplements removed), or the supplemental diet with an adjunct organic pond fertilization regime to enhance secondary productivity within the ponds. The relationships of treatment to type and quantity of potential prawn forage organisms (benthic macroinvertebrates) were also examined.

2. Materials and methods

2.1. Description, preparation, and stocking of ponds

Two weeks prior to the anticipated stocking date, ponds located at the Aquaculture Research Center, Kentucky State University, Frankfort, KY were drained, dried, and raked to remove organic debris (dried leaves and algae). Less than one week prior to stocking ponds were filled with water from a reservoir filled by runoff from the surrounding watershed. Water was filtered through a 385 μm mesh sock to prevent introduction of macroinvertebrates from the reservoir. The water surface area of all experimental ponds was 0.02 ha and average water depth was approximately 1.1 m. A 7.6 cm air-lift pump was located in the deepest area of each pond to prevent thermal stratification. Two applications of liquid fertilizer (NPK, 10:34:0) were added one week apart at a rate of 9.0 kg phosphorous ha^{-1} to each pond to achieve an algal bloom. Water for replacing evaporative losses and flushing of ponds was obtained from the reservoir.

Size-graded juvenile prawns were shipped by air from a commercial hatchery (Aquaculture of Texas, Weatherford, TX) on 4 June 1993. Prawns were held overnight in three 3000 l tanks containing plastic netting to provide substrate. On the stocking date (5 June 1993), the mean stocking weight was determined from a sample of 50 prawns that were blotted free of surface water and individually weighed ($\bar{x} \pm$ s.d. = 0.46 ± 0.49 g). Three replicate ponds were randomly assigned to each of the three experimental treatments consisting of different feeding strategies: a complete diet, a supplemental diet, or the supplemental diet with pond fertilization. Prawns were hand-counted and stocked in each pond at a density of 39,520 ha^{-1}.

2.2. *Samples*

A 3.2 mm square mesh seine was used to collect a sample of prawns from each pond every three weeks during the 109 day grow-out period. Prawns in the sample were counted, group-weighed (drained weight) to the nearest 0.1 g, and returned to the pond. At the sampling prior to harvest, prawns in the sample were also individually weighed and classified into either one of three female morphotypes: berried (egg carrying; BE), open (previously egg carrying; OP), and virgin (VG), or one of three male morphotypes, blue-claw (BC), orange-claw (OC), and small (< 20 g; SM) as described by Cohen et al. (1981).

2.3. *Feed and feeding rates*

Experimental diets were formulated to contain 32% protein. Ingredient composition of the complete diet (Table 1) was similar to that of the diet utilized by D'Abramo et al. (1989) and Tidwell et al. (1993), and contained 15% fish meal. In the formulation of the supplemental diet, fish meal was eliminated, distillers dried grains with solubles (DDGS) was added at 40% of the formulation, and soybean meal was increased slightly to maintain the crude protein level. The DDGS used in the study was a homogeneous composite from seven distilleries as provided by the Distillers Feed Research Council, Fort Wright, KY. Dietary ingredients were processed into 5 mm sinking pellets by a commercial feed mill (Farmers Feed Mill, Lexington, KY). The dietary protein level was determined using macro-Kjeldahl, dietary fat by acid hydrolysis, and moisture by drying to constant weight in a convection oven at 95 °C (AOAC, 1990). Ingredient composition, proximate analysis, and relative ingredient cost of each diet are provided in Table 1.

One-half of the daily ration of diet was distributed over the entire surface of each pond twice daily between 09.00 and 10.00 h and between 15.00 and 16.00 h. Prawns were fed at a set rate of 25 kg ha^{-1} day^{-1} until an average individual weight of 5 g was achieved. At

Table 1
Ingredient composition (%) and proximate analysis of experimental diets with and without fish meal and vitamin and mineral supplements (complete and supplemental, respectively) and fed to pond cultured freshwater prawns

Ingredient	Percent Composition	
	Complete diet	Supplemental diet
Menhaden fish meal (67%)	15.00	0.00
Soybean meal (44%)	25.00	26.25
DDGS	0.00	40.00
Wheat flour	13.00	13.00
Meat and bone meal (54%)	8.00	8.00
Ground corn meal	28.75	2.65
Mineral mix[a]	0.10	0.00
Vitamin mix[b]	0.10	0.00
Choline chloride	0.05	0.00
Cod liver oil	0.00	1.50
CMC[c]	5.00	5.00
Lignosulfonate binder	5.00	5.00
Analyzed composition (%)[d]		
Protein	33.70 ± 0.00	32.04 ± 0.00
Lipid	5.83 ± 0.39	8.15 ± 0.08
Moisture	9.98 ± 0.25	9.47 ± 0.06
Relative ingredient cost[e]	1.247	1.000

[a]Mineral mix contained: Mn, 10.0% (as $MnSO_4$); Zn, 10.0% (as $ZnSO_4$); Fe, 7.0% (as $FeSO_4$); Cu, 0.7% (as $CuSO_4$); I, 0.24% (as $CaIO_3$); Co, 0.10% (as $CoSO_4$). [b]Vitamin mix contained: thiamine (B_1), 1.01%; riboflavin (B_2), 1.32%; pyridoxine (B_6), 0.9%; nicotinic acid, 8.8%; folic acid, 0.22%; cyanocobalamine (B_{12}) 0.001%; pantothenic acid, 3.53%; menadione (K), 0.2%; ascorbic acid (C), 22.1%; retinolpalmitate (A), 4409 IU/kg; cholecalciferol (D_3), 2,204,600 IU/kg; α-tocopherol (E), 66.2 IU/kg; ethoxyquin, 0.66%. [c]CMC = carboxymethylcellulose, used in combination with lignosulfonate to further improve pellet stability. [d]Dry weight basis, mean ± s.d. of replicate analyses. [e]Ingredient costs based on prices quoted in Feedstuffs, 1993, 65(47).

sizes greater than 5 g, prawns were fed a percentage of body weight based on a feeding schedule reported by D'Abramo et al. (1989). Feeding rates were adjusted weekly based on an assumed 3.0 feed conversion (D'Abramo et al., 1989). Every three weeks biomass estimates for each pond were adjusted according to sample weights. Survival was assumed to be 100%. In ponds receiving organic fertilization, distillers dried grains with solubles (DDGS) was added at a rate of 75 kg/ha/week, split into three applications (every other day) based on rates and schedules adapted from Mims et al. (1991). Composition of the DDGS used as organic fertilizer is given in Table 2.

2.4. Macroinvertebrate sampling

Benthic samples were taken at two week intervals for two initial samples and every third week thereafter. Individual samples were taken from the deep and shallow end of each pond using a 0.09 m^2 Ekman dredge (Lind, 1979). Samples were rinsed and sieved (No. 30 US Series) according to procedures reported by Lind (1979). This mesh retains all specimens

Table 2
Percent composition (dry weight) of distillers dried grains with solubles used as an organic fertilizer in the study

Component	% of total
Protein[a]	25.83 ± 0.18
Lipid[a]	14.42 ± 0.08
Moisture[a]	16.51 ± 0.33
Fiber[a]	7.05 ± 0.07
Phosphorous[b]	0.71
Potassium[b]	0.44
Magnesium[b]	0.18
Calcium[b]	0.15

[a]Replicate analyses. [b]NRC (1983).

and particles > 583 μm. Macroinvertebrate were preserved in 70% ethanol until identification and enumeration.

2.5. *Water quality management*

Dissolved oxygen (DO) and temperature of all ponds were monitored twice daily (09.00 h and 15.30 h) using a YSI Model 57 oxygen meter (Yellow Springs Instruments, Yellow Springs, OH). When the DO level of any pond was predicted (graphically) to decline below 4.0 mg l^{-1}, overnight aeration was provided to that pond using an electric vertical pump aerator. Levels of total ammonia–nitrogen (TAN) and nitrite–nitrogen were determined weekly in water samples collected from each pond at approximately 13.00 h according to outlined procedures for a Hach DR/2000 spectrophotometer (Hach Col, Loveland, CO). The pH of each pond was determined daily at 13.00 h using an electronic pH meter (Hanna Instruments, Ltd., Mauritius). If afternoon pH was measured to be ≥ 9.5, the pond was slowly flushed overnight.

2.6. *Harvest*

One day prior to harvest, 20 September 1993, water levels in ponds were lowered to approximately 0.9 m at the drain end. On the following day, each pond was seined three times using a 1.3 cm square mesh seine and then completely drained. Remaining prawns were manually harvested from the pond bottom and purged in clean water. Total bulk weight and number of prawns from each pond were recorded. All prawns in each pond were then individually weighed and classified into one of the six previously described sexual morphotypes.

2.7. *Statistical analyses*

Sample and harvest weights were regressed against time (days post-stocking) and tested for significance (Dowdy and Wearden, 1983). The general linear test was used to test the equality of regression lines for the three treatments (Neter and Wasserman, 1974). If

Table 3
Overall means (± s.e.) of daily morning and afternoon water temperatures and dissolved oxygen concentrations and weekly water quality determinations in ponds in which prawns were fed a complete diet, a supplemental diet, or a supplemental diet with organic pond fertilization. Treatments were replicated in triplicate ponds. Differences in treatments were not statistically different ($P > 0.05$) for any variable

Variable	Treatment		
	Complete	Supplememtal diet	Supplemental and fertilization
AM Temp (°C)	26.1 ± 2.1	26.3 ± 1.8	25.7 ± 1.9
PM Temp (°C)	28.1 ± 2.7	28.0 ± 2.1	27.6 ± 2.1
AM D.O. (mg l^{-1})	7.3 ± 0.7	8.0 ± 1.9	6.4 ± 0.5
PM D.O. (mg l^{-1})	10.9 ± 2.0	11.5 ± 1.8	10.6 ± 1.1
TAN (mg l^{-1})	0.92 ± 0.13	0.54 ± 0.10	0.76 ± 0.27
pH	8.8 ± 0.6	8.9 ± 0.6	8.8 ± 0.6
Nitrite (mg l^{-1})	0.047 ± 0.016	0.024 ± 0.013	0.052 ± 0.010
Chlorophyll-a (mg l^{-1})	21.0 ± 16.2	22.1 ± 20.0	28.6 ± 12.1
Phosphorous (mg l^{-1})	0.24 ± 0.00	0.35 ± 0.18	0.45 ± 0.09

differences in regression lines were detected, their slopes were compared using Student's t-test (Snedecor and Cochran, 1980). Harvest data were analyzed using the SAS ANOVA procedure (SAS, 1987). Duncan's multiple range test was used to compare treatment means if differences were indicated by ANOVA. Percentage and ratio data were converted to arcsin values prior to analysis (Zar, 1984).

3. Results

Overall mean values of water quality variables are presented in Table 3. There were no significant differences ($P > 0.05$) in overall means for morning and afternoon temperature, morning and afternoon dissolved oxygen, chlorophyll-a, total ammonia–nitrogen, nitrite–nitrogen, or phosphorous concentrations among the three treatments.

Regression lines for prawn weights over the 109 day culture period are presented in Fig. 1. The regression of sample weights on culture days was statistically significant ($P < 0.05$) in prawns fed the complete diet ($P < 0.01$, $r^2 = 0.95$), the supplemental feed ($P < 0.01$, $r^2 = 0.93$), and the supplemental feed with organic pond fertilization ($P < 0.01$, $r^2 = 0.95$). The regression line for prawns fed the complete diet was significantly different ($P < 0.05$) from the regression line for prawns fed the supplemental diet without pond fertilization. For prawns fed the supplemental diet, the regression line for those raised in organically fertilized ponds was significantly different ($P < 0.05$) from those raised in unfertilized ponds. There was no significant difference ($P > 0.05$) in the regression lines for prawns fed the complete diet or those fed the supplemental diet with organic fertilization. At final harvest mean wet weight of prawns fed the complete diet averaged 32.6 g. Elimination of vitamin and mineral supplements was associated with a 14% decrease in prawn weights to 28.4 g. Organic fertilization produced a 15% increase in average weight of prawns fed the supplemental diet, to 32.9 g, essentially identical to prawns fed the complete feed. However, these differences were not statistically significant ($P > 0.05$).

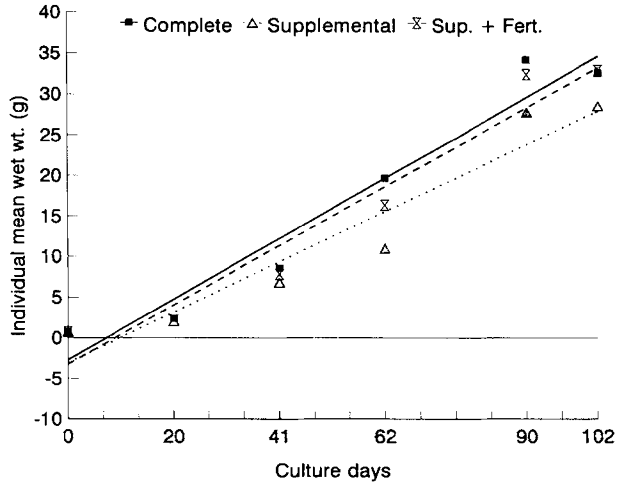

Fig. 1. Relationship between mean sample weight and culture period (time post stocking). Regression equations developed for the three treatments are: Complete feed, ———, $Y = -3.1373 + 0.3793\ X$; supplemental feed, · · ·, $Y = -3.6094 + 0.3205\ X$; supplemental feed with organic pond fertilization, – – –, $Y = -4.1561 + 0.3869\ X$. The final data points are based on individual weights of all prawns in the three treatments at harvest.

Production per unit area of prawns fed the complete feed averaged 990 kg ha^{-1}. Deletion of fish meal and vitamin and mineral supplements decreased production 14% (868 kg ha^{-1}). Organic fertilization of ponds in which prawns were fed the supplemental diet increased production 12%, to 970 kg ha^{-1}. These differences in production between treatments, however, were not statistically significant ($P > 0.05$). There was no significant difference ($P > 0.05$) in survival and feed conversion ratios (FCR) which averaged 75.9% and 3.9, overall. Nutritional regime did not significantly influence ($P > 0.05$) the proportion of different morphotypes in the total population by number or weight. Combined data for the three treatments are presented in (Fig. 2).

Overall mean numbers within different taxa of macroinvertebrates per sample (0.09 m^2) are presented in Fig. 3. Deletion of fish meal and vitamin and mineral premixes from the diet was associated with a 67% decrease in total number of invertebrates sampled (Fig. 3). This difference was statistically significant ($P < 0.05$). Organic fertilization was associated with a significant increase ($P < 0.05$) in the total number of macroinvertebrates in ponds where prawns were fed the supplemental diet, though counts were significantly lower ($P < 0.05$) than those in which prawns were fed the complete feed. The prevalent taxon in all ponds was Oligochaeta (Fig. 4), which numerically accounted for approximately 58% of the macroinvertebrates in ponds with prawns fed the complete feed. Deletion of dietary ingredients was associated with a significant ($P < 0.05$) decrease in oligochaete counts. Organic fertilization did not cause a significant ($P > 0.05$) increase in oligochaete numbers in ponds containing prawns fed the supplemental diet. Total insect numbers (Fig. 4) followed the same pattern as described for total macroinvertebrates relative to ingredient deletion and organic fertilization. The number of individuals representing other insect taxa (Belostomatidae, Ephemeroptera and Psychomyiidae) was significantly lower ($P < 0.05$) with ingredient deletion and was significantly higher ($P < 0.05$) with organic fertilization,

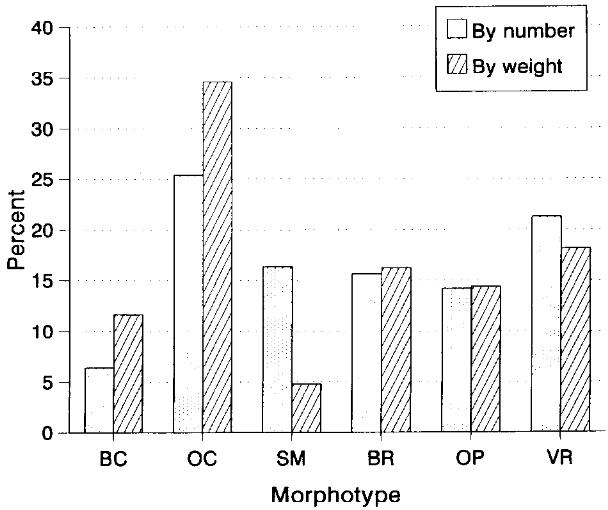

Fig. 2. Proportions of male and female morphotypes as percentage of total number and total biomass. BC, blue claw males; OC, orange claw males; SM, small males; BE, berried females; OP, non-berried females with open brood chambers; VG, non-breeding females with closed brood chambers.

although in these groups counts in fertilized ponds were not significantly different ($P > 0.05$) from ponds in which prawns were fed the complete feed (data not shown). The number of gastropods, pelycypods, and nematodes was significantly lower ($P < 0.05$) in ponds in which prawns were fed the supplemental diet than in ponds in which were fed the complete diet (Fig. 3). For these taxa, however, organic fertilization was not associated with significantly greater ($P > 0.05$) numbers.

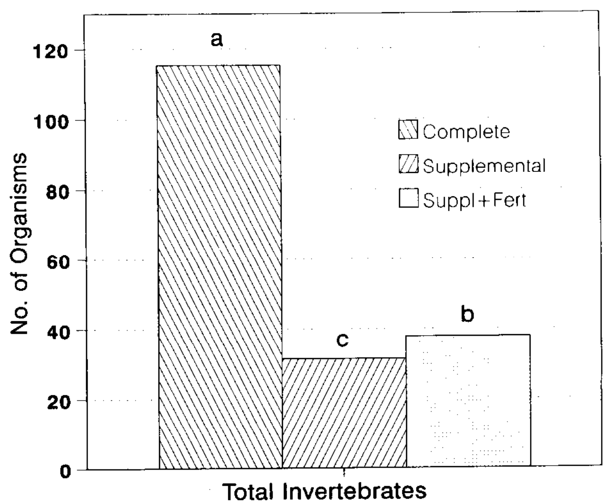

Fig. 3. Overall mean number of total macroinvertebrates per sample (0.09 m^2). Treatments were replicated in three ponds. Each pond was sampled six times (two biweekly then four triweekly samples) over the growing period so that each bar represents 18 samples over a 109 day period.

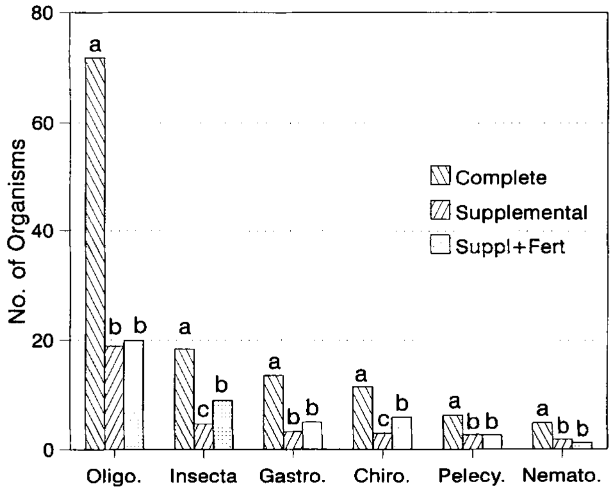

Fig. 4. Overall mean number of macroinvertebrates within different taxa per sample (0.09 m^2). Treatments were replicated in three ponds and each pond was sampled six times (two biweekly then four triweekly samples) over the growing period so that each bar represents 18 samples over a 109 day period.

4. Discussion

Replacement of fish meal and elimination of vitamin and mineral supplements in prawn diets resulted in a significant reduction of benthic macroinvertebrate populations, possibly due to increased predation by prawns. Past studies have demonstrated that natural productivity can be a significant food source in prawn production (Lilyestrom et al., 1987). Analyses of stomach contents led Weidenbach (1980) to conclude that prawns are able to adjust to the absence of feed pellets by increasing consumption of available vegetation. Data from the present study would suggest that prawns may be able to adjust to reductions in the nutritional value of prepared diets (i.e., protein source and vitamin and mineral content) by increasing predation on natural fauna (i.e., macroinvertebrates) in the pond.

Alternatively, the benthic macroinvertebrate fauna may have benefited directly from the animal source protein and vitamin and mineral supplements in the complete diet. However, considering the very similar and consistent relationship among treatments over a wide variety of invertebrate taxa with vastly different food habits (i.e. carnivores vs. detritivores), such an occurrence appears less likely. It is also possible that a combination of these factors (reduced predatory pressure by prawns and direct benefit of high quality nutrients) account for the higher numbers of benthic macroinvertebrates in complete feed ponds.

The significant increase in the quantity of total benthic macroinvertebrates in ponds receiving an adjunct organic fertilization regime, relative to ponds in which prawns were fed the same diet without fertilization, indicates that organic fertilization increases the availability of some groups of benthic macroinvertebrates as forage for the prawns.

Relationships among the three treatments are consistent with these proposed treatment effects of nutritional quality of diets, utilization of forage organisms, and organic pond fertilization. Increased growth of prawns in ponds receiving organic fertilization with DDGS is likely due to increased availability of some forage organisms, but may also be partly due

Table 4
Estimated costs and net returns for a 4-water-hectare freshwater prawn farm using three alternative nutritional regimes. Based on a whole prawn price of \$12.10 kg^{-1}

Treatment	Estimated annual cost				
	Gross revenue[a] (\$)	Ownership cost[b] (\$)	Operating cost[b] (\$)	Total cost (\$)	Net revenue (\$)
Complete diet	44 322	14 813	19 740	34 553	9 679
Suppl. diet	38 748	14 813	18 186	32 999	5 749
Suppl. + Fert.	43 317	14 813	20 416	35 229	8 000

[a]Calculated by multiplying whole freshwater prawn yields by \$12.10 kg^{-1}. [b]For complete description see Montañez et al. (1992).

to direct feeding on the DDGS. Kohler (1987) observed prawns actively consuming unpelleted distillers solubles in laboratory studies and stated that direct consumption, in addition to enhancement of pond fertility, made the by-product more attractive for aquaculture. Ultimately the value of a feed, whether it represents an indirect or direct source of nutrients, is based upon the magnitude of the prawn standing crop it supports and its effect upon the biology of the pond (D'Abramo and Sheen, 1991). Production data also support presumed treatment effects on benthic macroinvertebrate populations and increased levels of prawn predation.

The major drawback to one annual crop of prawns in temperate climates is not overall production levels, but rather the large proportion of small individuals harvested which are difficult to market on a competitive basis (Smith et al., 1978). Harvestable weight for prawns has been defined as ≥ 20 g by MacLean et al. (1989) and ≥ 30 g by D'Abramo et al. (1989). For prawns fed the complete diet, supplemental diet, or supplemental diet with fertilization, the percentage of individuals with a weight > 20 g was similar (76, 76, and 79%, respectively). However, if marketable weight is defined as ≥ 30 g, a more profound treatment effect is evident. At harvest, 55% of the prawns fed the complete diet had reached marketable weight. The marketable proportion decreased to 44% when fish meal was replaced and vitamin and mineral supplements were eliminated from the diet. The combined use of supplemental diet with organic fertilization yielded an increase in the marketable proportion to 59%.

Input-output coefficients were applied to a synthesized model prawn farm of 4 ha developed by Montañez et al. (1992). A pond-bank sale price of \$12.10 kg^{-1} for whole prawns was assumed in the analysis which is consistent with small scale "niche marketing" situations in the region. Given the above assumptions and model, the farm strategy employing the supplemental diet realized the lowest net return (Table 4). Addition of an organic fertilization regime increased net return by 39% when the supplemental diet was used. The model farm employing the complete feed had the highest net return, approximately 21% greater than the farm using the supplemental diet with fertilization and 68% greater than the farm using supplemental diet without pond fertilization.

Under the conditions in this study, the complete feed treatment would still appear to be the most profitable. However, results also suggest that prawns are able to increase the level of predation on natural pond biota, to supplement their nutritional intake when expensive nutrients are deleted from the diet. Increasing our understanding of the role of natural productivity and its enhancement and the potential dual role of DDGS as fertilizer and feed may lead to management strategies that will further decrease production costs and increase profitability.

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