

Evaluation of growout diets with varying protein and energy levels for red drum (*Sciaenops ocellatus*)

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Abstract

The augmentation of nutrient retention and subsequent reduction of effluent waste generated by aquaculture production facilities are important factors in lowering production costs while simultaneously minimizing pollution and environmental impact resulting from intensive growout culture. Consequently, the present study was designed to evaluate the influence of dietary protein and energy levels on growth and nutrient utilization of the red drum in the initial stages of growout culture. Four practical diets were formulated to contain 44, 40, 36, and 32% protein with practical energy:protein (E:P) ratios. Additionally, the E:P ratio of the 44% protein diet was increased and the E:P ratio of the 32% protein diet was reduced. Each diet was fed to juvenile red drum (mean initial weight 50 g) to apparent satiation in triplicate tanks containing 670 l of seawater at 35‰, 28.8 ± 1.4°C for 13 weeks. Significant differences ($P < 0.05$) in final weights, protein consumption, feed efficiency (FE), protein conversion efficiency (PCE) and intraperitoneal fat ratios (IPFR) were observed due to the dietary treatments. Weight gain and FE generally increased with protein and energy content of the diet. Increasing the lipid content of the 44% protein diet resulted in significantly higher FE values but also doubled the amount of fat deposited in the peritoneal cavity. Reduction of digestible energy from 335 to 279 kcal/100 g diet in the 32% protein diet did not significantly influence body composition or feed consumption. Based on regression analyses of feed consumption data, consumption rates appear to be primarily influenced by the weight of the fish and not the energy content of the diet. Consequently, reduced growth rates of fish offered the low protein feeds appear to be due to reduced protein intake and not imbalances in

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E:P ratios. These data indicate that red drum reared in seawater require at least 44% dietary protein (dry weight basis) for maximum growth and FE. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *Sciaenops ocellatus*; Protein; Growout

1. Introduction

As mariculture operations continue to expand there is increased pressure to reduce nutrient loading and minimize production costs associated with supplemental feed systems. Optimization of feed efficiency is one mechanism that can be utilized to help reach both of these goals. To facilitate reductions in nutrient loading within the culture system the concept of a nutrient-dense, low-pollution diets has been suggested as a means to minimize waste output while maximizing the mass of fish produced (Cho et al., 1994). Dietary nutrient density and the positive effects on growth rates, nutrient retention, and water quality have been evaluated with juvenile red drum, *Sciaenops ocellatus*, in closed systems (Jirsa et al., 1997). Results from these studies indicate that high protein and energy diets can have a positive effect on growth rates, nutrient retention and water quality.

Dietary protein constitutes one of the primary nutrient costs of the feed and is the initial source of nitrogen waste products entering a culture system. Consequently, optimization of dietary protein levels along with increasing nutrient retention by the fish could reduce nitrogen loading and positively influence production costs. Protein retention is influenced by a variety of factors including the digestibility of feed ingredients, protein content of the diet, amino acid balance and dietary energy to protein (E:P) ratios. The optimization of E:P ratios is widely accepted as a means to spare protein, thereby increasing growth at a reduced cost (Lee and Putnam, 1973; Machiels and Henken, 1985; Ellis and Reigh, 1991; Serrano et al., 1992; Shiau and Peng, 1993).

Currently, considerable information on the nutritional requirements of juvenile red drum exists; however, there is little data on the nutritional requirements of larger fish. Given that the investment in feeds and nutrient loading of the culture system is highest during the final stages of production, it is appropriate that feeds and feeding strategies should be optimized for growout. The present study was, therefore, conducted to evaluate the optimum protein and energy levels in a practical diet formulation for red drum in the initial stages of growout culture. Additionally, the paradigm that fish eat to satisfy an energy requirement was examined with respect to feed consumption.

2. Materials and methods

Six practical diets (Table 1) were formulated to contain varying levels of protein and energy. Four protein levels (44, 40, 36, and 32% protein) were formulated and energy levels were altered in both the high and low protein diets. The energy content of the

Table 1
Composition of experimental diets (g/100 g dry weight)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Menhaden fish meal ¹	30.00	30.00	27.27	24.55	21.82	21.82
Soybean meal ²	23.40	23.40	21.27	19.15	17.02	17.02
Soy protein isolate ³	10.00	10.00	9.09	8.18	7.27	7.27
Wheat starch ⁴	19.00	23.00	29.58	36.16	42.74	22.74
Wheat gluten ⁴	5.00	5.00	4.55	4.09	3.64	3.64
Menhaden fish oil ⁵	8.00	4.00	3.64	3.27	2.91	2.91
Soy lecithin ⁶	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral premix ⁷	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix ⁸	3.00	3.00	3.00	3.00	3.00	3.00
Stay C ⁹	0.10	0.10	0.10	0.10	0.10	0.10
KH ₂ PO ₄ ¹⁰	0.50	0.50	0.50	0.50	0.50	0.50
Alpha-cellulose	–	–	–	–	–	20.00
<i>Formulated to contain:</i>						
Protein	44.0	44.0	40.0	36.0	32.0	32.0
Lipid	13.2	9.2	8.4	7.6	6.9	6.9
Digestible energy (kcal/100 g diet) ¹¹	384	359	351	343	335	279
Digestible Energy:Protein ratio	8.7	8.2	8.8	9.5	10.4	8.7
<i>Measured to contain:</i>						
Protein	44.6	44.0	39.4	36.3	32.6	32.8
Percent dry matter	91.9	92.0	89.7	91.9	91.2	89.6

¹Special Select TM, Zapata Haynie, Hammond, LA, USA.

²Solvent extracted, Producers Co-operative Association, Bryan TX, USA.

³NURISH 3000, Protein Technologies International, St. Louis, MO, USA.

⁴United States Biochemical, Cleveland, OH, USA.

⁵Zapata Haynie, Reedville, VA, USA.

⁶Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, IN, USA.

⁷g/100 g premix: cobalt chloride 0.004; cupric sulfate pentahydrate 0.250; ferrous sulfate 4; magnesium sulfate heptahydrate 28.398; manganous sulfate monohydrate 0.650; potassium iodide 0.067; sodium selenite 0.010; zinc sulfate heptahydrate 13.193; filler 53.428.

⁸g/kg premix: thiamin HCl 0.5; riboflavin 3; pyroxidine HCl 1; D-calcium pantothenate 5; nicotinic acid 5; biotin 0.05; folic acid 0.18; vitamin B₁₂ 0.002; choline chloride 100; inositol 5; menadione 2; vitamin A acetate (20,000 IU/g) 5; vitamin D₃ (400,000 IU/g) 0.002; DL- α -tocopherol acetate (250 IU/g) 8; α -cellulose 856.266.

⁹Stay C, L-ascorbyl-2-polyphosphate, Hoffman-LaRoche, Nutley, NJ, USA.

¹⁰Spectrum Chemical, Gardendale, CA, USA.

¹¹Digestible energy values are based on values reported by Lovell (1989) and Gaylord and Gatlin (1996).

32% protein diet was reduced by replacing wheat starch with cellulose (Diet 6). In the 44% protein diet, 4 g menhaden fish oil was supplemented in place of wheat starch to increase the energy content of Diet 2 (Diet 1). Diets were prepared by mixing the dry ingredients and oil in a food mixer (Hobart, Troy, OH, USA) for 30 min. Hot water was then blended into the mash to attain a consistency appropriate for pelleting. Each feed was passed through a 3-mm die in a meat grinder, and pellets were dried to a moisture content less than 10%. Protein content was confirmed by micro-Kjeldahl analysis (Ma

and Zuazago, 1942). Diets were stored in a refrigerator and were crumbled and sieved to the desired size before use.

Red drum (*S. ocellatus*) eggs were obtained from broodstock maintained at the University of Texas Marine Science Institute, Fisheries and Mariculture Laboratory, and the larvae were reared under controlled culture conditions similar to those described by Holt et al. (1990). Prior to the initiation of the growth trials, juvenile red drum were hand-graded to a uniform size, and stocked in a semi-closed recirculation system consisting of 18 culture chambers (670 l), subsurface aeration, sand filter, biological filter and circulation pump. Fish were acclimated to experimental conditions for 14 days during which they were offered a maintenance diet containing 45% protein derived primarily from menhaden fish meal and 8.5% lipid. Twelve fish (mean weight 50.0 g/fish) were stocked into each tank and diets were randomly assigned to three replicate tanks per treatment. Ten fish from the remaining population were collected and frozen at -60°C for subsequent proximate analysis. Each diet was fed twice daily to the fish to apparent satiation over the 13-week feeding trial. Satiation in each tank was established to be the point during a 1-h feeding regime when fish ceased to actively ingest the sinking feed at or near the surface of the culture tank. Photoperiod was set for 12:12 h light:dark cycle. System maintenance, such as siphoning of settled solids and water exchanges, were conducted as needed. Fish were counted, dipped in dechlorinated fresh water, weighed, and the culture chambers scrubbed weekly through week three then bi-weekly thereafter. System temperature, dissolved oxygen level, and salinity were measured every other day. Ammonia–nitrogen, nitrite–nitrogen, and pH were measured twice weekly using photometric methods (Spotte, 1979) and a Fisher Accumet pH Meter Model 900 (Pittsburgh, PA). System temperature and dissolved oxygen levels were measured using a YSI Model 55 (Yellow Springs, OH) dissolved oxygen meter.

At the conclusion of the growth trial, three fish were randomly selected from each tank for proximate analyses. Three fish from each tank were individually weighed, scaled and dissected to determine hepatosomatic index ($\text{HSI} = \text{liver weight} \times 100/\text{fish weight}$) and intraperitoneal fat ratio ($\text{IPFR} = \text{intraperitoneal fat weight} \times 100/\text{fish weight}$). The fish and dissected material were then homogenized as a group and frozen for subsequent whole body analyses. Samples from the start of the experiment were analyzed with samples obtained at the conclusion of the growth trial. Dry matter was determined by drying to a constant weight at 90°C . Protein content was determined by the micro-Kjeldahl method. All analyses were conducted with duplicate sub-samples from each tank. Feed efficiency (FE) was calculated as $(\text{wet weight gain} \times 100/\text{dry weight feed offered})$. Protein conversion efficiency (PCE) was calculated as $(\text{dry protein gain} \times 100/\text{dry protein offered})$. Feed consumption was calculated as the average feed consumed per day divided by the average body weight of the fish per treatment as measured during the preceding routine weighing $(\text{dry feed consumed} \times 100/\text{wet weight of fish})$.

All data were subjected to one-way analysis of variance to determine significant ($P < 0.05$) differences among treatment means, and Student–Neuman Keuls' multiple-range test (Steel and Torrie, 1980) was used to distinguish significant differences among treatment means. To evaluate the influence of dietary protein and digestible energy (DE) content of the various diets on final weight and feed consumption, stepwise regression

analyses ($P < 0.10$) was conducted. Statistical analyses were conducted using the SAS System for Windows (v6.1, SAS Institute, Cary, NC).

3. Results

The red drum utilized in this study readily adapted to the reported culture conditions and experimental protocol. However, it should be noted that the fish were initially weighed on a weekly basis. Depressed consumption rates were observed one to two days post handling and this observation was presumed to result from the stress induced by handling. Hence, routine weighings were reduced to a bi-weekly schedule. Water quality parameters (mean \pm standard deviation) were as follows: temperature, $28.8 \pm 1.4^\circ\text{C}$; salinity, $35 \pm 1.4\text{‰}$; dissolved oxygen, 6.1 ± 0.33 mg/l; total ammonia–nitrogen, $0.10 \pm .04$ mg/l; nitrite–nitrogen, 0.04 ± 0.03 mg/l; pH, 7.7 ± 0.13 . In general, the fish performed very well in the research system and responded suitably to manipulations in dietary protein and energy content (Table 2). At the conclusion of the 13-week growth trial, significant differences in final weight, protein intake, FE, PCE and IPFR were observed. No statistical differences were observed in the percent survival which ranged from 91.7 (Diet 3) to 100% (Diets 1 and 6) or whole body proximate composition in terms of dry weight or protein content.

Diets 2–5 were formulated to contain decreasing levels of protein (44, 40, 36, and 32% protein, respectively) and increasing DE:P ratios. In general, final weights of the fish decreased with decreasing protein content of the diets. The final weights of fish offered the 44% protein diet were significantly higher than fish offered the 32% protein

Table 2
Response of red drum (mean initial weight 50.0 g) offered the test diets over a 13-week growth trial¹

Diet	Final weight (g)	Total feed consumed ² (g)	Protein consumed ² (g)	FE ³ (%)	PCE ⁴ (%)	IPFR ⁵
1 (44H) ⁶	388.9 ^a	344 ^a	151.4 ^a	98.7 ^a	38.8 ^{ab}	1.91 ^a
2 (44)	357 ^{ab}	338.6 ^a	149 ^a	90.3 ^b	36.1 ^{ab}	0.70 ^b
3 (40)	305.8 ^{bc}	301 ^a	120.4 ^b	84.7 ^c	41.4 ^a	0.76 ^b
4 (36)	300.4 ^{bc}	328.6 ^a	118.3 ^b	76.2 ^d	35.7 ^b	1.17 ^b
5 (32)	271.7 ^c	311.2 ^a	99.6 ^{bc}	71 ^d	39.3 ^{ab}	0.92 ^b
6 (32L)	252.5 ^c	287.6 ^a	92 ^c	70.7 ^d	38.0 ^{ab}	0.97 ^b
PSE ⁷	16.72	15.89	6.31	1.76	1.2	0.2

¹ Means of three replicates. Numbers in the same column with different superscript are significantly different ($P < 0.05$).

² Expressed on a per fish basis.

³ Feed efficiency (FE) = wet weight gain \times 100/dry weight of feed offered.

⁴ Protein conversion efficiency (PCE) = dry protein gain \times 100/dry protein offered.

⁵ Intraperitoneal fat ratio (IPFR) = visceral fat wet weight \times 100/whole body wet weight.

⁶ Protein level is shown in parentheses. Energy level is indicated where applicable (H = high energy; L = low energy).

⁷ Pooled standard error.

diet; however, there were no significant differences in final weights between fish offered the 44% protein diet vs. fish offered diets containing 40 or 36% protein. Although there were no statistical differences in feed consumption, varying protein levels of the diets resulted in significant differences in protein consumption. The amount of protein consumed by the fish decreased significantly as the protein content of the diet decreased from 44 to 40% but did not significantly change between treatments containing dietary protein level of 40–32%. Feed efficiency decreased significantly as dietary protein content decreased from 44 to 36% protein. Reductions in dietary protein from 36 to 32% did not result in significant decreases in FE. There was no observable trend, or significant differences in PCE values for fish offered Diets 2–5 containing 44% to 32% dietary protein. Similarly, there were no significant differences in protein efficiency ratio (grams wet weight gain/grams protein fed; data not shown) which ranged from 2.24 to 2.05.

Fish offered the high-protein, high-energy diet (Diet 1) performed the best and had the highest final weight, feed intake, protein intake, FE and IPFR values when compared to all diets tested. With the exception of FE and IPFR values, which were significantly higher in the high-lipid diet, there were no significant differences observed between the two diets containing 44% dietary protein with either 13.2 or 9.2% lipid (384 and 359 kcal DE/100 g diet, respectively).

Conversely, red drum offered 32% protein with reduced energy (Diet 6) performed the poorest with respect to growth and had the lowest final weight, feed intake, protein intake and FE compared to all other diets tested. However, there were no significant differences in the aforementioned parameters, when comparing the response of fish offered the 32% protein diets containing 335 kcal DE/100 g diet (Diet 5) or 279 kcal DE/100 g diet (Diet 6). Reduction of digestible energy in the diet did not significantly affect the total feed or protein intake.

There were no significant differences in percent dry matter (27.7–30.1%), percent protein (57.7–63.8%), or HSI (1.57–1.83) from final whole body analyses conducted upon termination of the growth trial. The IPFR values for fish maintained on the 44% protein diet containing 13.2% lipid and an estimated DE content of 384 kcal/100 g diet (Diet 1) were significantly greater than those observed for fish offered the other diets. IPFR values did not significantly change as a result of reduction in DE from 335 to 279 kcal/100 g diet in the 32% protein diet or in diets containing variable dietary protein levels ranging 32 to 44% protein with similar DE levels (335–359 kcal/100 g).

To evaluate the influence of dietary protein and DE content of the diets on final weights of the fish, the growth data was analyzed by stepwise regression analyses. Analyses indicated a significant influence of the protein content of the diet but not DE values. The model, mean final weight = $8.82 \times (\text{g protein}/100 \text{ g diet}) - 22.54$, had an adjusted $R^2 = 0.6935$.

Consumption rates for juvenile red drum offered the various diets to apparent satiation were recorded over the 13-week feeding trial. Consumption rates ranged from 4.9%/day/fish (Diet 5) for a 51 g fish in the beginning of the experiment to 1.4%/day/fish (Diet 3) for a 269 g fish at the end of the experiment. To evaluate the influence of fish weight, dietary protein content, and dietary DE values on feed intake, regression analyses were conducted using the weekly and bi-weekly averages for feed

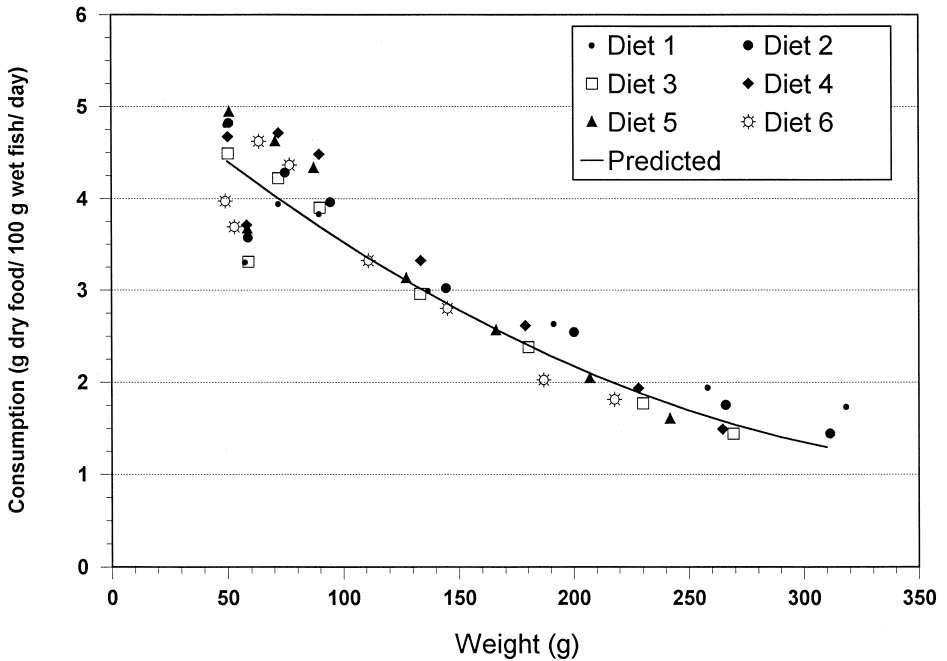


Fig. 1. Relationship of daily consumption (DC) rates for juvenile red drum of various sizes offered the test diets to apparent satiation. The predictive equation $DC = 0.00002648 Wt^2 - 0.021 Wt + 5.415$ had an adjusted R^2 of 0.7751.

intake. Stepwise regression analyses indicated that only fish weight ($P = 0.0001$, partial $R^2 = 0.7598$) significantly added to the model. The addition of DE to the model was not significant ($P = 0.1130$) and only resulted in a minimal increase in the R^2 value (partial $R^2 = 0.0043$). To further optimize this model, a polynomial model was applied which produced a slight improvement in the R^2 (0.7598 vs. 0.7751) and a better fit to the data. This predicted equation is presented in Fig. 1.

4. Discussion

In practical diet formulations for fish, protein constitutes one of the major costs in terms of nutrients and ingredients. To minimize feed costs, it is important to optimize both dietary protein level and utilization by the fish. Under the reported conditions, significant reductions in weight gain and FE values were observed as protein content was reduced from 44 to 32 g/100 g diet and E:P ratio was increased from 8.2 to 10.4 kcal/g protein (Table 2). These results are consistent with observations by Daniels and Robinson (1986) who reported increased feed utilization (total wet weight gain/dry weight feed fed) with increasing dietary protein from 34 to 44% of dry diet and maximum growth of juvenile red drum in brackish water at 26–33°C with 44% dietary

protein. Dietary protein level, however, did not affect whole fish protein content. This observation agrees with studies conducted on channel catfish (Page and Andrews, 1973) and red drum (Serrano et al., 1992). Results from the present study which were conducted in full strength seawater at 35‰, indicate that maximum growth and FE was achieved at a dietary protein level of at least 44 g protein/100 g diet.

Optimization of E:P ratios of feed formulations is generally intended to spare protein, thereby increasing the efficiency of protein retention (Lee and Putnam, 1973; Page and Andrews, 1973; Serrano et al., 1992). Protein sparing results when dietary lipid or carbohydrate replaces protein which could otherwise have been catabolized as an energy source for standard metabolism or lipid synthesis (Watanabe, 1982). In practical, high-protein feeds, lipids are often utilized as a dense source of energy to help increase the E:P ratio. In the present experiment, Diets 1 and 2 were formulated to contain 44% protein with either 13.2 or 9.2% lipid producing E:P ratios of 8.7 and 8.2 kcal DE/g protein. Comparison of the response of red drum to these diets indicated that there were no significant differences in terms of final weight, feed or protein intake, and PCE. However, increasing the lipid content of Diet 2 resulted in an increase in the FE from 90.3 to 98.7%. This result agrees with findings reported by Williams and Robinson (1988) who reported decreasing FCR (dry weight feed/wet weight gain) from 2.9 to 1.6 as dietary lipid content increased from 1.7 to 11.2%. Serrano et al. (1992) also reported an increase in feed efficiency (g weight gain/g dry diet fed) from 0.85 to 0.98 with increasing lipid levels from 3–10% with an optimal protein level at 40% dietary protein.

Although lipids are a good source of energy for fish, they are also easily stored in fat deposits. In the present experiment significantly higher IPFR values (1.91%) were measured in fish offered the 44% protein diet with 13.2% lipid (Diet 1) as compared to all other diets tested. A similar increase in IPFR values was measured by Serrano et al. (1992) in juvenile red drum offered diets containing 10% lipid vs. fish offered 3% lipid. Overall these observations are consistent with those of Watanabe (1982) who reported increasing whole body lipid in several species of teleosts as dietary lipid increased. Increasing dietary lipid content from 6.8 to 13.2% had no significant effect on HSI. This observation differs from the results of Daniels and Robinson (1986) and Serrano et al. (1992) who reported increasing HSI with increasing dietary lipid content. This difference may be due to the size of the fish employed in the studies.

Due to the cost of dietary protein, producers are often interested in minimizing dietary protein levels as a mechanism to reduce feed costs. However, the utilization of low protein diets has several disadvantages. These diets contain high levels of carbohydrates, which are poorly utilized by red drum (Ellis and Reigh, 1991) and hence add to the quantity of undigested material entering the culture system. Low protein feeds also generally have high E:P ratios which may reduce protein intake, resulting in reductions in growth. Assuming that red drum consume food to satisfy an energy requirement as has been demonstrated with rainbow trout (Kaushik and Luquet, 1983), then reducing the energy content of a low protein practical diets could minimize adverse effects on growth rates by increasing feed consumption. Consequently, Diets 5 and 6 were designed to evaluate the partial replacement of the carbohydrate source with indigestible fiber resulting in a reduction of the E:P ratio from 10.4 to 8.7 kcal/g protein.

Under the reported conditions, the reduction of the energy content of the 32% protein diet did not significantly influence fish performance with respect to growth or FE. Moreover, the reduction of dietary energy did not increase the amount of feed consumed, which was offered to a point of apparent satiation. A similar response has been reported for the Atlantic croaker, *Micropogonias undulatus*, offered practical diets with 15 or 30% alpha-cellulose replacing wheat starch (Davis and Arnold, 1997). These results indicate that the carbohydrate component of low protein diets are not being utilized and hence contribute to increased organic loading of the culture system.

Under the reported conditions and dietary manipulations, the red drum's response in terms of weight gain appears primarily driven by dietary protein level. Regression analyses of the final weight data against protein and energy content of the diets indicated that protein content of the feed was significantly correlated to the final weights of the fish. This response may indicate, that in a reasonably well balanced diet, protein intake is more limiting than energy. This would be expected to occur if the fish are not adjusting feed intake to meet a protein or energy requirement. Based on our observations in several satiation feeding trials, feed intake appears to vary with palatability of the feed and the size of the fish but not the nutrient content of the diet.

To evaluate the influence of fish weight, dietary protein and dietary DE on consumption, the collected feed intake data (graphically presented in Fig. 1) was evaluated using regression analyses. Pearson correlation coefficients indicated that percent consumption was significantly correlated with weight and poorly correlated to dietary protein and DE values. Stepwise regression analyses also indicated that only fish weight significantly added to the model. These results confirm our general observations that under standardized conditions, red drum will consume a given quantity of feed based on size of the fish and that the energy content of the diet has a relatively small impact on consumption rates.

5. Conclusion

Under the reported conditions, maximized growth and feed efficiency of juvenile red drum (mean initial weight 50 g) was observed in fish fed a diet containing 44% protein and 384 kcal/100 g DE. Although this diet produced the best growth, reducing the lipid content from 13.2 to 9.2 g/100 g diet resulted in a significant reduction in fat deposition. Since the use of high-lipid diets for red drum may compromise market quality by increasing the IPFR, reducing dress-out percentages and increasing the likelihood of oxidative degradation of the fish during storage, the diet containing 44% protein and 9.2% lipid is recommended.

Lowering the protein content of production diets often reduces feed costs. However, this will also result in reduction in weight gain and FE values. Albeit, reduced protein diets may support adequate growth rates for some commercial operation. Reductions in FE will result in increased feed costs per unit of fish produced and increase the nutrient loading of the culture system, possibly resulting in an increase in overall production costs. Based on the results of this research as well as investments in feed costs during growout, further research designed to evaluate nutrient requirements and practical feed formulations is warranted.

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