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Evaluation of Practical Feed Formulations with Different Protein Levels for Juvenile Red Claw Crayfish (*Cherax quadricarinatus*)

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ABSTRACT

A 5-week feeding trial was conducted in aquaria with juvenile (0.022 g) red claw, *Cherax quadricarinatus*, to examine the effects of dietary protein levels on growth and survival. Four practical diets were formulated to contain 25, 35, 45, and 55% protein. Finished diets had analyzed protein percentages of 23, 33, 43, and 52%. A commercial shrimp diet (45% protein) was also fed for comparative purposes. After 5 weeks, final whole body weight and percentage weight gain of red claw fed diets containing 33, 43, and 52% protein were significantly ($P < 0.05$) higher than red claw fed the commercial shrimp diet. No significant differences ($P > 0.05$) were found in final whole body weight and percentage weight gain of red claw fed a diet containing 23% protein compared to red claw fed either the other test diets or the commercial shrimp diet. Percentage survival was not significantly different ($P > 0.05$) among treatments. These results indicate that these diets formulated for the red claw appear to be suitable and that a diet containing 33% may be adequate.

INTRODUCTION

Interest in the culture of the Australian red claw crayfish, *Cherax quadricarinatus*, has increased during the last several years. The red claw shares many of the attractive attributes of marron, *C. tenuimanus*, including a comparatively non-aggressive and non-burrowing behavior. A simplified life-cycle in which relatively advanced juvenile crayfish are released directly from the female, eliminates the requirement for expensive and sophisticated hatcheries for larval rearing (1). An advantage of red claw is a wider temperature tolerance (15–30°C) compared to marron (17–25°C) (2). Greater temperature tolerance may increase the potential of red claw as an aquaculture species in a larger geographical area in the United States, including Kentucky, than marron.

Masser and Rouse (3) stated that a two-phase culture system will be needed for red claw if cultured in the southeastern United States. An intensive, indoor phase would be used in the winter months to spawn adults and raise juveniles to a 0.5–2.0 g stocking size and would be followed by an outdoor phase to raise juveniles to market size (30–50 g).

Formulation of diets suitable for production of red claw in intensive culture requires determination of its nutritional requirements. Lack of such information may impede red claw aquaculture in the United States. Currently, expensive shrimp diets are fed to juvenile red claw (D. B. Rouse, pers. comm.). The purpose of the present study was to evaluate the growth and survival of juvenile red claw fed practical diets containing various percentages of protein.

MATERIALS AND METHODS

Experimental Diets

Four experimental diets were formulated to contain increasing percentages of protein (25, 35, 45, and 55%) (Table 1). Menhaden fish meal was added to each diet as a constant percentage of total protein (51%). A commercial shrimp diet (Zeigler Shrimp diet, Zeigler Bros., Gardners, Penn.) which is commonly

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TABLE 1. Ingredient composition of four experimental diets fed to red claw crayfish reared in aquaria.

Ingredient	Diet (% protein)			
	23%	33%	43%	52%
Menhaden fish meal	19.0	28.0	36.5	44.5
Soybean meal	3.0	15.0	28.0	30.5
Shrimp head meal	13.0	13.0	13.0	10.0
Ground corn	51.0	31.0	11.5	0.0
Soybean lecithin	0.5	0.5	0.5	0.5
Cod liver oil	4.0	4.0	3.0	2.0
Cholesterol (analytical)[1]	0.5	0.5	0.5	0.5
Dicalcium phosphate	3.0	2.0	1.0	1.0
Vitamin and mineral mix[2]	3.0	3.0	3.0	3.0
Casein	0.0	0.0	0.0	5.0
CMC[3]	3.0	3.0	3.0	3.0

[1] Cholesterol (analytical grade) was purchased from Sigma Chemical, St Louis, MO.

[2] Vitamin and mineral mix provided the following (in mg or IU/kg of diet): A, 5,280 IU; D_3, 2,640 IU; E, 66 IU; B_{12}, 0.11 mg; K, 13.2 mg; riboflavin, 16 mg; pantothenic acid, 42 mg; thiamine, 13.2 mg; niacin, 106 mg; B_6, 11.2 mg; folic acid, 2.6 mg; choline chloride, 619 mg; ascorbic acid, 935 mg; NaH_2PO_4, 10 g; $CaHPO_4$, 20 g; KCL, 10 g; Se, 0.4 mg; Zn, 207 mg; Fe, 72 mg; Mn, 216 mg; Cu, 9 mg; I, 4.5 mg; Co, 1.9 mg.

[3] CMC = carboxymethylcellulose.

used in red claw production (D. B. Rouse, pers. comm.) was also used for comparison with the diets formulated in this study. Chemical composition of the diets is presented in Table 2.

In preparing diets, dry ingredients were first ground to a small particle size (approximately 250 μm) in a Wiley mill. Dry ingredients were then thoroughly mixed with water to obtain a 30% moisture level. Diets were passed through a mincer with die to form 0.4-mm diameter strands and dried (20°C) for 16 hr using a convection oven. After drying, diets were broken and sieved into 4-mm pellets. Cod liver oil was sprayed onto the dried pellets immediately prior to storage. All diets were kept frozen (−15°C) until fed.

Percentage protein of the diets was determined by the Kjeldahl method, percentage fat was determined by acid hydrolysis and percentage moisture was determined by drying a 10-g sample in a convection oven at 95°C until constant weight (4). Due to possible differences between the proximate compositions of the diet ingredients and published values (5), the finished diets were 23, 33, 43, and 52% protein. Actual protein values will be used throughout the rest of this paper. Diets were also analyzed for amino acid composition by a commercial analytical laboratory (Woodson-Tenent Labs, Dayton, Ohio) and are presented in Table 3.

Since neither digestible nor metabolizable energy values are available for red claw, dietary energy values were calculated (based on proximate analysis of diets) from physiological fuel values of 5.65 kcal/g of protein, 4.1 kcal/g of carbohydrate (NFE), and 9.5 kcal/g of lipid as stated by El-Sayed and Teshima (6).

Dried diets were also evalnated for pellet stability in water. Ten grams of pellets of equal length were distribnted uniformly on a 100-cm^2 brass screen (2-mm mesh size) having raised sides. Samples were lowered into static water (approximately 10 cm under the water surface) for 30 minutes and then dried in an oven (100°C) for 24 hr. The residue left on the screen was recorded as dry solids not leached in water. The percentage of dry solids

TABLE 2. Chemical composition (dry-matter basis) of four experimental diets and a commercial shrimp diet fed to red claw reared in aquaria. Percentage moisture, protein, fat, fiber, and ash values are means of two replicates.

	Diet (% protein)				
	23%	33%	43%	52%	Zeigler
% Moisture	10.4	10.7	7.7	7.6	8.8
% Protein	23.3	33.0	43.4	51.6	45.2
% Fat	10.6	10.7	11.1	10.8	11.1
% Fiber	4.5	5.0	5.4	4.6	3.0
% Ash	16.5	18.1	19.7	20.1	14.4
NFE[1]	45.2	33.2	20.4	13.0	26.3
Energy (kcal/100 g diet)[2]	417.7	424.3	431.3	447.4	465.0
P:E[3]	55.8	77.8	100.6	115.3	97.2
Pellet water stability[4]	79 ± 1[a]	78 ± 2[b]	53 ± 6[c]	75 ± 1[b]	90 ± 0[d]

[1] NFE = 100 − (% protein + % fat + % fiber + % ash).

[2] Energy values are based on physiological fuel values used by El Sayed and Teshima (1992).

[3] P:E = protein to energy ratio; calculated as mg of protein/kcal.

[4] Pellet water stability = percentage of dry solids retained after 30 minutes in static water.

on the screen after 30 min in water to total solids in the pellets was used as a comparative measure of pellet stability in water.

Experimental System and Animal Maintenance

The feeding trial was conducted in 20 37.5-liter acrylic aquaria (50 × 25 × 30.5 cm; L × W × H). Water was recirculated through biological and mechanical filters. The recirculating system was a 2,000-liter vertical screen filter system utilizing high-density polyester screens and polyethylene "bio-balls" to remove particulate materials and provide substrate for nitrifying bacteria (Red Ewald, Inc., Karnes City, Texas). Continuous aeration was provided by a blower and air-stones. All aquaria were cleaned by siphon once daily (1330) to remove uneaten diet. Each aquarium was supplied with water at a rate of 0.5 liter/min. Approximately 4% of the total water volume of the system was exchanged per day.

Chloride levels were maintained at approximately 1,000 mg/liter by addition of food-grade NaCl. Hardness and alkalinity levels were maintained at approximately 400 mg/liter by addition of sodium bicarbonate (baking soda) and analytical-grade calcium phosphate (dibasic; $CaHPO_4$). Black plastic covered the back and sides of all aquaria to minimize disturbances caused by personnel entering the laboratory.

Water temperature was measured daily using an electronic thermometer. Dissolved oxygen was measured twice weekly using a YSI Model 58 (YSI Industries, Yellow Springs, Ohio). Total ammonia, nitrite, total alkalinity, and chlorides were monitored twice weekly using a DR/2000 spectrophotometer (Hach Company, Loveland, Colorado). pH was monitored twice weekly using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio). Over the duration of the study these water quality parameters averaged (±SD): water temperature, 27.5 ± 1.1°C; dissolved oxygen, 6.7 ± 0.5 mg/liter; total ammonia, 0.25 ± 0.18 mg/liter; nitrite, 0.03 ± 0.02 mg/liter; total alkalinity, 419.5 ± 51.5 mg/liter; chlorides, 1,065 ± 100 mg/liter; pH, 8.62 ± 0.81.

Juvenile red claw, *Cherax quadricarinatus* (mean individual weight of 0.022 g), were obtained from the research hatchery at Auburn University, Auburn, Alabama and randomly stocked into 20 aquaria at a rate of 15 juveniles per aquarium, with 4 replications per treatment (diet). Individual weight of the juveniles was measured on an electronic scale (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland) prior to stocking. On day 2, stocking mortalities were replaced. No subsequent replacement of mortalities was performed. Red claw were counted every other week. It was decided prior to the start of the study that if mortality in any treatment reached 50%, the study would be terminated. Red claw were fed to excess twice daily (0800 and 1500) for 5 weeks. Diet was evenly distributed over the bottom of each aquarium to ensure availability to all individuals. Each aquarium had one nylon-mesh substrate structure and 10 tubes (2.5-cm sections of 2.0-cm diameter garden hose) for shelters. At the conclusion of the feeding trial, red claw were individually weighed (wet weight).

TABLE 3. Amino acid composition (% of diet) of diets containing different protein levels fed to juvenile red claw crayfish. Values are means of two replications. Tryptophan levels were not determined.

	Diet (% protein)				
Amino acid	23%	33%	43%	52%	Zeigler
Alanine	0.92	1.53	2.10	2.26	2.35
Arginine	1.14	1.69	2.47	2.90	2.17
Aspartic acid	1.61	2.53	4.15	4.96	3.81
Cystine	0.17	0.24	0.35	0.41	0.38
Glutamic acid	2.66	3.46	5.94	7.00	5.57
Glycine	1.28	1.81	2.37	2.77	2.17
Histidine	0.61	0.83	1.52	1.92	1.33
Isoleucine	0.78	1.22	1.46	1.86	1.52
Leucine	1.66	2.19	2.93	3.72	3.56
Lysine	1.20	1.82	2.48	3.15	2.33
Methionine	0.45	0.64	0.91	1.09	0.75
Proline	1.71	1.30	1.80	2.24	2.31
Phenylalanine	0.85	1.22	1.64	2.00	1.88
Serine	0.76	0.97	1.66	2.09	1.70
Threonine	0.69	0.91	1.54	1.95	1.48
Tyrosine	0.42	0.62	1.41	1.59	0.83
Valine	0.95	1.41	1.77	2.29	2.11

Statistical Analysis

Final individual weight (g), survival (%), specific growth rate (SGR), and weight gain (%) were calculated at the conclusion of the study. Specific growth rate was calculated as follows: SGR (%/day) = (ln W_t − ln W_i/T) × 100 where W_t is the weight of the juvenile at

TABLE 4. Final weight, survival, specific growth rate (SGR), and weight gain of juvenile red claw fed diets containing various percentages of protein and a commercial shrimp diet (Zeigler). Values are means ± SE of four replications. Means within a column having different superscripts were significantly different ($P < 0.05$).

Diet (% protein)	Final wt (g)	Survival (%)	SGR	Wt. gain (%)
1 (23%)	0.516 ± 0.011^{ab}	58.4 ± 9.2^{a}	9.28 ± 0.06^{a}	2,301.7 ± 41.80^{ab}
2 (33%)	0.597 ± 0.074^{a}	58.3 ± 5.0^{a}	9.63 ± 0.32^{a}	2,611.8 ± 337.68^{a}
3 (43%)	0.563 ± 0.056^{a}	50.0 ± 8.1^{a}	9.49 ± 0.29^{a}	2,459.2 ± 252.84^{a}
4 (52%)	0.567 ± 0.029^{a}	61.8 ± 5.7^{a}	9.54 ± 0.15^{a}	2,475.6 ± 132.9^{a}
Ziegler	0.409 ± 0.017^{b}	71.7 ± 5.0^{a}	8.61 ± 0.11^{b}	1,760.6 ± 78.5^{b}

time t, W_t is the weight of the juvenile at time 0, and T is the culture period in days.

Data were analyzed by analysis of variance using the SAS ANOVA procedure (7). Duncan's multiple range test was used to compare differences among individual means. All percentage data were transformed to arc sin values prior to analysis (8).

RESULTS AND DISCUSSION

This is one of the first studies to evaluate prepared diets for juvenile red claw. Red claw juveniles fed experimental diets containing 33, 43, and 52% protein had significantly higher ($P < 0.05$) final individual weights (g) and specific growth rates (SGR) than those fed a commercial shrimp diet containing 45% protein (Table 4). Final body weight and SGR of red claw fed a diet containing 23% protein were not significantly different ($P > 0.05$) than those of red claw fed the other three experimental diets or the commercial shrimp diet. Growth of red claw fed the commercial shrimp diet in the present study was similar to growth rates observed in other studies (D. B. Rouse, pers. comm).

Red claw juveniles fed a commercial shrimp diet had a numerically higher percentage survival (71%) than red claw fed the experimental diets (58, 58, 50, and 61% for red claw fed diets containing 23, 33, 43, and 52% protein, respectively) (Table 4). However, differences in percentage survival were not statistically significant ($P > 0.05$), possibly due to variation within each treatment. Mortalities were associated with several factors including: several (15–20) red claw were found in the biofilter, probably after being removed from the aquarium through the standpipe. Some incidents of cannibalism were observed, but these occurred after week 3 of the study. However, survival values were comparable or greater than values reported in other studies with Australian crayfish (1, 9, 10, 11). Reduced water stability of experimental diets may have been a factor in the reduced survival percentages. Although survival was not significantly different ($P > 0.05$) among treatments, survival was lowest (50%) for red claw fed diet 3 (with poor water stability) and was highest (72%) among red claw fed the commercial shrimp diet (with good water stability). Water stability of diet pellets is an important factor when feeding crustaceans. Greater pellet stability minimizes leaching of water-soluble nutrients which may adversely affect crustacean growth. Fair and Fortner (12) compared growth of freshwater prawn, *Macrobrachium rosenbergii*, fed a water-stable pelleted diet to prawn fed pulverized pellets and reported that prawn fed water-stable pellets had twice the growth rate.

Data indicate that a diet containing 33–52% protein appears suitable for use in rearing juvenile red claw for the first 5 weeks after release from the female. This is in agreement with dietary protein levels reported for other crustaceans. Villarreal (11) reported no differences in growth of marron, *Cherax tenuimanus*, fed for 8 weeks with diets containing between 17–48% protein. Hubbard et al. (13) reported that a diet containing 30% protein was adequate for optimal growth of red swamp crayfish, *Procambarus clarkii*. Freshwater prawn, *Macrobrachium rosenbergii*, have a protein requirement between 32–40% (14, 15, 16). D'Abramo et al. (17) reported that growth of juvenile lobster, *Homarus americanus*, fed a diet containing 30% protein was not different from juveniles fed diets containing higher percentages of protein.

These data indicate that a formulated diet,

with a protein level of 33%, appear to be adequate for juvenile red claw. Feeding for longer duration, use of individual rearing containers, and use of a greater quantity of (or a different binder) may be useful in future nutritional studies. Diets used in this study appear to be a starting point for further studies to determine nutritional requirements of red claw.

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