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Use of Alfalfa Hay, Compared to Feeding Practical Diets Containing Two Protein Levels, on Growth, Survival, Body Composition, and Processing Traits of Australian Red Claw Crayfish, *Cherax quadricarinatus*, Grown in Ponds

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Abstract

Juvenile red claw, *Cherax quadricarinatus*, were obtained from a commercial supplier and stocked into twelve 0.04-ha ponds with an average water depth of approximately 1.1 m at a rate of 1100 red claw per pond (27,170 red claw/ha). Mean stocking weight (±SD) was 6.25 ± 3.0 g. Three replicate ponds were randomly assigned to each of the four treatments. In Treatment 1 (TRT1), red claw were stocked into ponds to which dried alfalfa hay (forage) was added at a rate of 500 kg/ha/mo; Treatment 2 (TRT2) consisted of red claw being fed a prepared diet containing 13% protein; in Treatment 3 (TRT3), red claw were fed a diet containing 13% protein at which had dried alfalfa hay been added at a rate of 500 kg/ha/mo; and in Treatment 4 (TRT4; control), red claw were fed a complete diet containing 28% protein. Water quality measurements were made three times weekly for dissolved oxygen and temperature (AM, PM), pH, total ammonia nitrogen, nitrite, and alkalinity averaging 7.17 mg/L, 8.96 mg/L, 25.5 C, 27.3 C, 8.68, 0.39 mg/L, 0.012 mg/L, and 106.5 mg/L, respectively. After 113 d, the final mean weight of red claw fed TRT3 was significantly ($P < 0.05$) higher (68.10 g) compared to that of red claw fed hay only (49.40 g) but not different from red claw fed TRT2 (56.03 g) and TRT4 (62.10 g). Likewise, red claw fed TRT3 had significantly higher percent weight gain (990%) compared to that of red claw fed hay only (690%) but not different from TRT2 (796%) and TRT4 (893%). Feed conversion ratio of TRT2, TRT3, and TRT4 and percent survival among all treatments did not differ significantly ($P > 0.05$), averaging 2.85 and 66.8%, respectively. Total yield of red claw fed TRT3 and TRT4 (968 and 952 kg/ha) was significantly ($P < 0.05$) higher compared to that of red claw fed hay only (617 kg/ha) but did not differ from red claw fed TRT2 (882 kg/ha). Tail meat yield of male red claw in TRT3 was significantly ($P < 0.05$) higher (17.3 g) compared to that of male red claw in TRT1 (12.7 g) and TRT2 (14.9 g) but not significantly different ($P > 0.05$) from male red claw in TRT4 (16.9 g). Tail meat yield of female red claw in TRT3 and TRT4 was significantly ($P < 0.05$) higher (14.2 and 13.9 g, respectively) compared to that of female red claw in TRT1 (10.5 g) and TRT2 (10.4 g). Tail muscle proximate compositions showed no significant differences ($P > 0.05$) in protein (wet weight basis) between males and females among treatments (diet) which averaged 16.2 and

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14.6%; however, there were little to no significant differences in fat and ash between males and females among treatments. Results from this study indicate that pond-cultured red claw stocked at 27,170 red claw/ha can be fed a practical diet containing 13% protein, with or without forage (alfalfa hay), compared to red claw fed a complete diet containing 28% protein; however, if alfalfa hay is added to the pond at 500 kg/ha/mo as the sole source of added nutrients, growth is reduced. Therefore, the use of alfalfa hay, in combination with a low-protein pelleted diet may be a production method for pond-grown red claw that may reduce costs for producers and thereby increase profits.

Globally, interest in the culture of the Australian red claw crayfish, *Cherax quadricarinatus*, has increased during the past several years and is commercially cultured in several countries, including China, Mexico, and Australia. It has been shown that red claw possess many favorable culture traits essential for commercial culture (Rouse et al. 1991). Red claw can be fed prepared diets and grow rapidly (65–90 g) in a limited (117-d) growing season in temperate-climate ponds (Thompson et al. 2004a; Webster et al. 2004). Preliminary marketing studies conducted at our facility show that red claw are highly desired by consumers because of their excellent tail meat flavor, of their lobster-like appearance, they are larger than shrimp, and they have excellent storage quality (Tseng et al. 2002, 2003, 2004; Kong et al. 2006). Further, there is no larval phase as found for some other crustacean species (Jones 1995), and this negates the need for expensive and sophisticated hatcheries.

However, because red claw are a subtropical species, culture of red claw in temperate climate ponds is constrained by a growing season that lasts 5–7 mo in length (Medley 1991; Masser and Rouse 1993). Further, red claw juveniles are not readily available in the USA; therefore, producers must purchase these individuals from hatcheries in other countries (Australia or Mexico) and costs can range from \$0.40 to \$0.50 (US) per juvenile (with transport costs). Likewise, diet costs can represent up to 70% of the total operating expenses of an aquaculture enterprise. Thus, recent efforts to determine specific nutrient requirements and evaluate inexpensive practical diets have been devoted to reduce diet costs and possibly increase profits (Jacinto et al. 2003, 2005; Thompson et al. 2003a, 2003b, 2004a, 2005, 2006; Muzinic et al. 2004).

Salame and Rouse (2000) stated that forage materials added to ponds accommodate changing feeding habits of crayfish. Addition of forage should stimulate zooplankton and macroinvertebrates that are utilized by young crayfish and then consumed by larger (>5 g) red claw as they grow. Addition of forage materials to the pond may allow for the use of less expensive supplemental diets, which may help reduce diet costs and increase producer's profits. While information about specific nutritional requirements and practical diet formulations for red claw is increasing (Jacinto et al. 2003, 2005; Thompson et al. 2003a, 2003b, 2004a, 2005; Hernandez et al. 2004; Muzinic et al. 2004), there is little information on feeding strategies for red claw when comparing forage-based and diet-based culture methods when red claw are grown in ponds.

The purpose of the present study was to evaluate various feeding strategies for pond-raised red claw and to examine a least-cost approach, because production cost is currently high in the USA. Growth, survival, body composition, and processing characteristics were evaluated on red claw when fed a dried forage (alfalfa hay), with or without a low (13%) -protein diet, compared to feeding red claw a prepared diet containing either a low (13%) or a high (28%) level of protein with no forage.

Materials and Methods

Description and Stocking of Ponds

Juvenile red claw were obtained from a commercial supplier (Central Queensland Crayfish, Biloela, Queensland, Australia). Red claw were placed into aerated water, and actively moving individuals were stocked into twelve 0.04-ha ponds with an average water depth of approximately 1.1 m on June 2, 2005, at a rate of 1100 red claw per pond (27,170 red claw/ha) or 3.0/m^2. Groups of 100 live red claw were hand counted and stocked into ponds at random until all ponds had received 1100 red claw. Mean stocking weight (±SD) was 6.25 ± 3.0 g. Three replicate ponds were randomly assigned to each of the four treatments. In Treatment 1 (TRT1), red claw were stocked into ponds to which dried alfalfa hay (forage) was added at a rate of 500 kg/ha/mo; Treatment 2 (TRT2) consisted of red claw being fed a prepared diet containing 13% protein; in Treatment 3 (TRT3), red claw were fed a diet containing 13% protein at which had dried alfalfa hay been added at a rate of 500 kg/ha/mo; and in Treatment 4 (TRT4), red claw were fed a complete diet containing 28% protein.

Experimental Diets and Feeding Rates

Experimental diets were formulated to contain 13 and 28% protein (as-fed basis) based upon the nutrient composition of ingredients supplied by the feed mill (Table 1). Dietary ingredients were processed into 1-cm sinking pellets by a commercial feed mill (Integral Fish-Foods, Grand Junction, CO, USA). Two feedings, each consisting of one-half of the total daily ration, were distributed over the entire surface area of each pond between 0800 and 0830 h and between 1530 and 1600 h for 113 d. All red claw (regardless of protein level) were fed the same amount of diet based upon a percentage of estimated body weight and a feeding schedule devised by C. D. Webster (unpublished data) with survival assumed to be 65%. Amount of diet fed was adjusted every 2 wk based upon an estimated body weight. Red claw were fed 10% of estimated body weight the first 2 wk, 6% the following 2 wk, and 4% for the duration of the study, with assumed harvest weight of 80 g based upon previous published growth of red claw (Rouse and Kahn 1998; Thompson et al. 2004a). Total diet fed for treatments-fed pellets (TRT2, TRT3, and TRT4) was 117.3 kg/ha/pond.

Dried diets were also evaluated for pellet stability in water. Ten grams of pellets of equal length were distributed uniformly on a 100-cm² brass screen (2-mm mesh size) having raised sides. Samples were lowered into static water (approximately 10 cm under the water surface) for 30 min and then dried in an oven (100 C) for 24 h. Diets were agitated slightly by hand every 10 min or three times in total. The residue left on the screen was recorded as dry solids not leached in water. The percentage of dry solids 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.

TABLE 1. *Ingredient and chemical composition (%) of two practical diets fed to Australian red claw crayfish.*

Ingredient	Diet (% crude protein) 1 (13%)	2 (28%)
Full-fat soybean meal	10.0	20.0
Solvent-extracted soybean meal	0.0	29.0
Wheat midds	10.0	36.4
Whole wheat	76.4	10.0
Menhaden fish oil	1.0	2.0
Vitamin mix[1]	1.0	1.0
Mineral mix[2]	0.5	0.5
Stay C (35% active)[3]	0.08	0.08
Choline chloride	0.20	0.20
Dicalcium phosphate	0.8	0.8
Ethoxyquin	0.03	0.03
Chemical analysis		
Moisture[4]	10.1	9.8
Crude protein[5]	16.7	32.2
Crude lipid[5]	5.6	9.8
Fiber[5]	3.0	5.1
Ash[5]	3.8	5.9
NFE[6]	70.9	47.0
Available energy[7]	1.7	1.7
Pellet water stability[8]	95 ± 0.1[a]	88 ± 0.7[b]

Proximate analysis values are means of two replicates per diet.

[1] Vitamin mix was the Abernathy vitamin premix number 2 and supplied the following (mg or IU/kg of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as alpha-tocopheryl acetate), 50 IU; folic acid, 16.5 mg; myo-inositol, 132 mg; K (as menadione sodium bisulfate complex), 9.2 mg; niacin, 221 mg; pantothenic acid, 106 mg; B_6, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU; and ethoxyquin, 99 mg.

[2] Mineral mix was Rangen trace mineral mix F1 for catfish with 0.3 mg selenium/kg diet added.

[3] Vitamin C (Roche's Stay C at 35% active).

[4] Wet weight basis.

[5] Dry matter basis.

[6] NFE = nitrogen-free extract (by difference).

[7] Available energy was calculated as 4.0, 4.0, and 9.0 kcal/g of protein, carbohydrate, and lipid, respectively.

[8] Pellet water stability = percentage of dry solids retained after 30 min in static water; means within a row having different superscripts are significantly different ($P < 0.05$).

Hay and Application Rates

Six randomly selected ponds had dried alfalfa hay (14% protein) added at a rate of 500 kg/ha/mo. There were two hay additions per month, each consisting of one-half of the total monthly ration, and hay was distributed over the entire bottom surface area. Before distribution, hay bales were placed into each of the six ponds for approximately 2 wk to ensure that the hay would sink to the pond bottom and would be accessible for red claw.

Diet Analysis

Experimental diets were analyzed to determine moisture, protein, lipid, fiber, and ash. Moisture was determined by placement of a 2 g sample into a convection oven (135 C) for 2 h until constant weight (Association of Official Analytical Chemists [AOAC] procedure 930.15, 1995); protein was determined by combustion (AOAC procedure 990.03, 1995); lipid was determined by the acid hydrolysis method (AOAC procedure 954.02, 1995); fiber was determined by using the fritted-glass crucible method (AOAC procedure 962.09, 1995); and ash was determined by placing a 2 g sample in a muffle furnace (600 C) for 2 h (AOAC 942.05, 1995). The nitrogen-free extract (NFE) was determined by difference (NFE = 100 − [% protein + % lipid + % fiber + % ash]). Available energy was calculated from estimated physiological fuel values of 4.0, 4.0, and 9.0 kcal/g for protein, carbohydrate (NFE), and lipid, respectively (Garling and Wilson 1977; Webster et al. 1999). Proximate and amino acid composition of each of the two practical diets are presented in Tables 1 and 2 and were determined by a commercial analytical laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA).

Water Quality Management

Water temperature and dissolved oxygen (DO) were measured in all ponds twice daily (0900 and 1530 h) using an YSI Model 58 oxygen meter (YSI, Yellow Springs, OH, USA). A 1/2-HP electric aerator (Air-O-Lator, Kansas City, MO, USA) was positioned in the center of each pond and run continuously throughout the duration of the study. The pH was measured daily (1530 h) using an electric YSI Model 60 pH meter (YSI). All other water quality parameters were measured three times weekly (1300 h), which included total ammonia and nitrite using a DREL/2000 spectrophotometer (HACH, Loveland, CO, USA). Alkalinity was determined by titration using a digital titrator (HACH).

TABLE 2. *Amino acid composition of two practical diets fed to Australian red claw crayfish.*

Amino acid	Diet (% crude protein) 1 (13%)	2 (28%)
Alanine	0.68 (4.1)	1.3 (4.0)
Arginine	0.8 (4.8)	1.9 (6.0)
Aspartic acid	1.1 (6.3)	2.9 (9.0)
Cystine	0.3 (1.6)	0.4 (1.3)
Glutamic acid	3.0 (17.7)	5.5 (17.1)
Glycine	0.7 (4.2)	1.3 (3.9)
Histidine	0.5 (2.9)	0.9 (2.7)
Isoleucine	0.5 (2.9)	1.2 (3.6)
Leucine	1.0 (5.7)	2.0 (6.3)
Lysine	0.6 (3.6)	1.5 (4.8)
Methionine	0.2 (1.3)	0.4 (1.2)
Phenylalanine	0.6 (3.8)	1.3 (4.2)
Proline	0.9 (5.4)	1.4 (4.2)
Serine	0.7 (4.2)	1.5 (4.6)
Threonine	0.4 (2.3)	0.9 (2.7)
Tyrosine	0.4 (2.2)	0.8 (2.5)
Valine	0.7 (4.1)	1.3 (4.1)

Values in parentheses are expressed as amount of amino acid of the dietary protein (%). Amino acid analysis values are means of two replicates per diet.

Harvest

Ponds were harvested between September 15, 2005, and September 22, 2005. Three days prior to harvest, the water level in each pond was lowered to approximately 0.5 m at the drain end. On the day of the harvest, the standpipe in each pond was lowered so that all water was completely drained and red claw were then manually removed. Total weight and number of red claw (by sex) from each pond were recorded at harvest. Growth parameters and feed efficiency were calculated as follows: specific growth rate (SGR; % day) = $[(\ln W_t - \ln W_i)/T] \times 100$, where W_t and W_i are the final and initial individual weights of the red claw, respectively, and T is the length of the culture period in days; weight gain (%) = 100 $[(W_t - W_i)/W_i]$; feed conversion ratio (FCR) = total diet fed (kg)/total wet weight gain (kg).

After weighing, 20 males and 20 females from each pond (60 males and 60 females per treatment) were randomly sampled and chill killed using an ice water bath, and the tail muscle was removed from each body and stored in ziplock bags and frozen (−15 C) until analyzed. Weight of tail meat and the percentage it contributed to total body weight were calculated from both male and female red claw (Table 5). Moisture, protein, lipid, and ash of the tail muscle were analyzed, analysis procedures were as described for the diets except for moisture (AOAC procedure 950.46, 1995), and lipid was determined by ether extraction (AOAC procedure 960.39, 1995) (Table 6).

Statistical Analysis

Data were calculated for final individual weight (g), percentage weight gain, SGR, FCR, percentage survival, total yield (kg/ha), proximate composition, and processing data. Pond average values were used as units of observation for statistical analysis. Data were analyzed by ANOVA, and Duncan's multiple-range test was used to compare treatment means using SAS software version 9.1 (SAS Institute 2003). All percentage and ratio data were transformed to arcsine values prior to statistical analysis (Zar 1984). All statistical computations were performed at the $P < 0.05$ probability level.

Results

Water Quality

All ponds were continuously aerated throughout the duration of the study, and DO concentration averaged 7.17 mg/L for the morning and 8.96 mg/L for the afternoon (Table 3). Although there were significant differences ($P < 0.05$) in pH and temperature, the minimum and maximum values were within acceptable limits for red claw (Masser and Rouse 1993). The average pH and morning/afternoon water temperatures were similar among the four dietary treatments, which averaged 8.68, 24.5 C (AM), and 27.3 C (PM), respectively (Table 3). Average morning and afternoon water temperature were also examined by month. Data indicate that the morning water temperatures for June, July, August, and September were 25.6, 26.2, 25.7, and 22.9 C, respectively. Afternoon water temperatures for June, July, August, and September were 27.7, 27.9, 27.2, and 25.0 C, respectively. There were significant differences ($P < 0.05$) in total ammonia nitrogen (TAN), nitrite, and alkalinity; however, the minimum and maximum values were also within acceptable limits for red claw (Masser and Rouse 1993). TAN was significantly ($P < 0.05$) higher in ponds in TRT4 (0.43 mg/L) compared to that of ponds in TRT1 (0.36 mg/L) and TRT2

TABLE 3. *Means (±SE) of water quality parameters of ponds stocked with Australian red claw crayfish and fed with alfalfa hay (Treatment 1), a diet containing 13% protein (Treatment 2), a diet containing 13% protein and alfalfa hay (Treatment 3), and a diet containing 28% protein (Treatment 4).*

	Treatment			
Parameters	1	2	3	4
DO (mg/L; AM)	7.22 ± 0.04[a]	7.12 ± 0.04[b]	7.27 ± 0.04[a]	7.05 ± 0.04[b]
DO (mg/L; PM)	9.05 ± 0.07[ab]	8.89 ± 0.09[bc]	8.69 ± 0.07[c]	9.19 ± 0.10[a]
pH	8.82 ± 0.09[a]	8.58 ± 0.2[b]	8.53 ± 0.06[b]	8.80 ± 0.1[a]
Temperature (C; AM)	25.4 ± 0.10[b]	25.7 ± 0.10[a]	25.2 ± 0.10[b]	25.7 ± 0.10[a]
Temperature (C; PM)	27.2 ± 0.12[ab]	27.4 ± 0.13[a]	27.0 ± 0.12[b]	27.4 ± 0.11[a]
TAN (mg/L)	0.36 ± 0.03[b]	0.37 ± 0.08[b]	0.39 ± 0.04[ab]	0.43 ± 0.04[a]
Nitrite (mg/L)	0.011 ± 0.0[ab]	0.010 ± 0.0[a]	0.015 ± 0.0[b]	0.012 ± 0.0[a]
Alkalinity (mg/L)	103 ± 4.7[b]	111 ± 2.3[a]	108 ± 2.2[b]	104 ± 4.2[a]

DO = dissolved oxygen; TAN = total ammonia nitrogen. Means within a row having different superscripts are significantly different ($P < 0.05$).

(0.37 mg/L) but not different ($P > 0.05$) from ponds in TRT3 (0.39 mg/L). Nitrite was significantly ($P < 0.05$) higher in ponds in TRT3 (0.015 mg/L) compared to that of ponds in TRT2 (0.010 mg/L) but did not differ ($P > 0.05$) from ponds in TRT1 (0.011 mg/L) and TRT4 (0.012 mg/L). Total alkalinity was significantly ($P < 0.05$) higher in ponds in TRT2 (111 mg/L) compared to that of ponds in TRT1 (103 mg/L) and TRT4 (104 mg/L) but did not differ ($P > 0.05$) from ponds in TRT3 (108 mg/L) (Table 3).

Growth and Production

After 113 d, the final mean weight of red claw fed a diet containing 13% protein and receiving alfalfa hay was significantly ($P < 0.05$) higher (68.10 g) compared to that of red claw which were in ponds receiving alfalfa hay only (49.40 g) but not different from red claw fed a diet containing 13% protein (56.03 g) and red claw fed a diet containing 28% protein (62.10 g) (Table 4). Likewise, red claw fed a diet containing 13% protein and receiving alfalfa hay had significantly higher percent weight gain (990%) compared to that of red claw in TRT1 (690%) but not different from red claw in TRT2 (796%) and TRT4 (893%). FCR and percent survival did not differ significantly ($P > 0.05$) among treatments, averaging 2.85 and 66.8%, respectively. Total yield of red claw in TRT3 and TRT4 (968 and 952 kg/ha) was significantly ($P < 0.05$) higher compared to that of red claw in TRT1 (617 kg/ha) but did not differ ($P > 0.05$) from red claw in TRT2 (882 kg/ha) (Table 4).

Percentages of Males and Females at Harvest and Final Weight

There were no significant ($P > 0.05$) differences in the percentages of males and females harvested from ponds among treatments. Overall, the percentage of males averaged 47.2% and the percentage of females averaged 51.4%. However, the percentage of intersex of red claw in TRT3 was significantly ($P < 0.05$) higher (2.0%) compared to that of red claw in TRT2 (1.2%) and TRT4 (1.2%) but not different ($P > 0.05$) from those red claw in TRT1 (1.3%).

Final individual mean weight of males and females in TRT3 was significantly ($P < 0.05$) higher (75.0 and 61.1 g, respectively) compared to that of red claw in TRT1 (53.4 and 45.3 g, respectively) but not different ($P > 0.05$) from red claw in TRT2 (63.6 and 48.6 g, respectively) and TRT4 (68.7 and 55.4 g, respectively). Final individual mean weight of intersex red claw was significantly ($P < 0.05$) higher in ponds fed TRT2 (98.8 g) compared to that of TRT1 (56.9 g) but not significantly different ($P > 0.05$) from TRT3 (85.7 g) and TRT4 (80.1 g). When comparing the final individual weights of males and females for each treatment, males in TRT4 were significantly ($P < 0.05$) higher (68.7 g) compared to females (55.4 g). However, there were no significant ($P > 0.05$) differences in the final weights between males

TABLE 4. *Means (±SE) of final weight, percent weight gain, specific growth rate (SGR), feed conversion ratio (FCR), percentage survival, and total yield of Australian red claw crayfish fed with alfalfa hay (Treatment 1), a diet containing 13% protein (Treatment 2), a diet containing 13% protein and alfalfa hay (Treatment 3), and a diet containing 28% protein (Treatment 4).*

	Treatment			
	1	2	3	4
Final weight (g)	49.4 ± 4.2^{b}	56.0 ± 4.3^{ab}	68.1 ± 6.8^{a}	62.1 ± 3.1^{ab}
Weight gain (%)	690 ± 68^{b}	796 ± 68^{ab}	990 ± 108^{a}	893 ± 50^{ab}
SGR (%/d)	1.83 ± 0.07^{b}	1.93 ± 0.07^{ab}	2.10 ± 0.09^{a}	2.03 ± 0.05^{ab}
FCR	N/A	2.99 ± 0.28^{a}	2.72 ± 0.34^{a}	2.83 ± 0.48^{a}
Survival (%)	58.3 ± 3.6^{a}	72.8 ± 1.0^{a}	65.8 ± 5.6^{a}	70.2 ± 6.9^{a}
Yield (kg/ha)	617 ± 15^{b}	882 ± 68^{ab}	968 ± 127^{a}	952 ± 132^{a}

N/A = not available.

Means within a row having different superscripts are significantly different ($P < 0.05$).

TABLE 5. *Mean (±SE) harvest percentage, final individual weight, tail meat weight, and percent of tail meat yield from the total body of male and female Australian red claw crayfish harvested from ponds and fed with alfalfa hay (Treatment 1), a diet containing 13% protein (Treatment 2), a diet containing 13% protein and alfalfa hay (Treatment 3), and a diet containing 28% protein (Treatment 4) for 113 d.*

	Treatment			
	1	2	3	4
Males				
Harvest (%)	48.5 ± 1.19^{a}	45.2 ± 1.31^{a}	46.9 ± 1.53^{a}	48.1 ± 0.91^{a}
Final weight (g)	$53.4 \pm 5.8^{b,x}$	$63.6 \pm 4.7^{ab,x}$	$75.0 \pm 8.0^{a,x}$	$68.7 \pm 3.2^{ab,x}$
Tail meat (g)	$12.7 \pm 0.6^{c,x}$	$14.9 \pm 0.8^{b,x}$	$17.3 \pm 1.0^{a,x}$	$16.9 \pm 0.6^{ab,x}$
Tail meat (%)	$23.5 \pm 0.5^{a,y}$	$23.2 \pm 0.5^{a,x}$	$23.8 \pm 0.5^{a,x}$	$23.4 \pm 0.5^{a,x}$
Females				
Harvest (%)	50.1 ± 0.95^{a}	53.7 ± 1.25^{a}	51.1 ± 1.54^{a}	50.7 ± 0.95^{a}
Final weight (g)	$45.3 \pm 3.0^{b,x}$	$48.6 \pm 3.9^{ab,x}$	$61.1 \pm 5.5^{a,x}$	$55.3 \pm 3.0^{ab,y}$
Tail meat (g)	$10.5 \pm 0.4^{b,y}$	$10.4 \pm 0.5^{b,y}$	$14.2 \pm 0.6^{a,y}$	$13.9 \pm 0.5^{a,y}$
Tail meat (%)	$24.9 \pm 0.4^{a,x}$	$23.6 \pm 0.6^{a,x}$	$23.8 \pm 0.5^{a,x}$	$23.6 \pm 0.5^{a,x}$

Values in a row with different superscripts (a, b) were significantly different ($P < 0.05$) among treatments. Means between males and females in the same column (treatment) for each respective variable with different superscripts (x, y) are significantly different ($P < 0.05$).

and females in the other three treatments (Table 5).

Tail Meat Yield, Percentage, and Composition

Tail meat yield and tail meat percentages of males and females are presented in Table 5. Tail meat yield of male red claw in TRT3 was significantly ($P < 0.05$) higher (17.3 g) compared to that of male red claw in TRT1 (12.7 g) and TRT2 (14.9 g) but not significantly different ($P > 0.05$) from male red claw in TRT4 (16.9 g). Tail meat yield of female red claw in TRT3 and TRT4 was significantly ($P > 0.05$) higher (14.2 and 13.9 g, respectively) compared to that of female red claw in TRT1 (10.5 g) and TRT2 (10.4 g). There were no differences in tail meat percentages among treatments between males and females, which averaged 23.5 and 24.0%, respectively.

Proximate compositions of male and female tail muscle are presented in Table 6. There were no significant differences ($P > 0.05$) in protein (wet weight basis) in the tail muscle of males among treatments (diet) which averaged 16.2%.

TABLE 6. *Means (±SE) moisture, protein, fat, and ash (wet weight basis) of tail muscle of male and female Australian red claw crayfish fed with alfalfa hay (Treatment 1), a diet containing 13% protein (Treatment 2), a diet containing 13% protein and alfalfa hay (Treatment 3), and a diet containing 28% protein (Treatment 4) for 113 d.*

	Treatment			
	1	2	3	4
Males				
Moisture (%)	81.2 ± 0.5^{a}	79.0 ± 0.7^{b}	79.7 ± 0.5^{ab}	78.9 ± 0.6^{b}
Protein (%)	16.0 ± 1.0^{a}	16.6 ± 0.6^{a}	16.2 ± 0.4^{a}	16.0 ± 0.5^{a}
Fat (%)	0.10 ± 0.0^{b}	0.11 ± 0.0^{b}	0.13 ± 0.0^{a}	0.10 ± 0.0^{b}
Ash (%)	1.30 ± 0.03^{b}	$1.33 \pm 0.01^{b,y}$	1.36 ± 0.06^{ab}	1.47 ± 0.05^{a}
Females				
Moisture (%)	82.0 ± 0.6^{a}	80.7 ± 0.3^{ab}	80.1 ± 0.2^{ab}	79.2 ± 1.1^{b}
Protein (%)	13.7 ± 1.0^{a}	14.1 ± 1.1^{a}	14.8 ± 0.7^{a}	15.6 ± 1.0^{a}
Fat (%)	0.10 ± 0.0^{a}	0.12 ± 0.01^{a}	0.12 ± 0.02^{a}	0.10 ± 0.0^{a}
Ash (%)	1.38 ± 0.04^{ab}	$1.45 \pm 0.01^{a,x}$	1.31 ± 0.04^{b}	1.43 ± 0.04^{a}

Means within a row having different superscripts (a, b) were significantly different ($P < 0.05$) among treatments. Means between males and females in the same column (treatment) having different superscripts (x, y) for each respective variable were significantly ($P < 0.05$) different.

However, moisture, fat, and ash were significantly different among treatments. Percentage moisture of male red claw tail meat from TRT1 was significantly ($P > 0.05$) higher (81.2%) compared to that of tail meat of red claw in TRT2 (79.0%) and TRT4 (78.9%) but not different from tail meat from male red claw in TRT3 (79.7%). Fat was significantly ($P > 0.05$) higher in tail meat from male red claw in TRT3 (0.13%) compared to males in TRT1 (0.10%), TRT2 (0.11%), and TRT4 (0.10%). Ash was significantly ($P > 0.05$) higher in tail meat of male red claw from TRT4 (1.47%) compared to males in TRT1 (1.30%) and TRT2 (1.33%) but not different from tail meat of male red claw from TRT3 (1.36%).

There were no significant differences in protein and fat (wet weight basis) in tail muscle of females among treatments, which averaged 14.5 and 0.10%, respectively. However, there were significant differences in the moisture and ash among the treatments. Percentage moisture in the tail meat of female red claw from TRT1 was significantly ($P > 0.05$) higher (82.0%) compared to females from TRT4 (79.2%) but not significantly different from females from TRT2 (80.7%) and TRT3 (80.1%). Percentage ash in the tail meat of female red claw from TRT2 and TRT4 was significantly ($P > 0.05$) higher (1.45 and 1.43%, respectively) compared to tail meat of females from TRT3 (1.31%) but not significantly different from tail meat of females from TRT1 (1.38%) (Table 6).

Discussion

Results from the present study indicate that red claw grown in ponds, at the size and stocking densities used, can be fed a diet containing 13% protein, with or without alfalfa hay (forage) added to the pond (TRT2 and TRT3), when compared to red claw fed a complete diet containing 28% protein (TRT4); however, when red claw were fed only forage at 500 kg/ha/mo (TRT1), growth was reduced. An unfed treatment of red claw was not used in the present study because previous reports clearly demonstrated that when crustaceans are not fed, a dramatic reduction in individual final weight is observed as compared to fed individuals (McClain et al. 1992a; Tidwell et al. 1997; Jones et al. 2002). As the objective of the present study was to determine different feeding strategies in the pond production of red claw, it was felt that not feeding red claw would not have been a particularly useful method of production. It appears that the addition of forage (detritus) alone does not support growth and survival in red claw as well as when they are fed a prepared diet.

Detritus can be defined as decomposing plant material with an associated microbial biomass. As detrital plant tissues decompose, the nutrient composition changes. Percentages of protein, lipids, and carbohydrates decrease, while levels of lignocellulose increase. Crude protein and lipid percentage of forage/detritus are lower than those found in prepared diets, especially after increased decomposition times (McClain et al. 1992b). Thus, it has been postulated that the microbes and larger organisms living on the decomposing detrital material provide supplemental sources of essential amino acids, lipids, vitamins, and minerals, not the plant residue (Fenchel 1972; Tenore et al. 1982; Huner and Barr 1984; D'Abramo and Robinson 1989).

However, Bowen (1987) reported that microorganisms compose only 1–5% of the organic matter in detritus. Because the organic nitrogen in living plants comprises amino acids, once the plant dies and decomposition begins, proteins are modified or lost. The modified proteins form non-amino acid organic nitrogen and form indigestible products with lignin, lignin-like substances, and humin, thus contributing little to organismal growth (Bowen 1987). When a prepared diet was fed to small (<0.5 g) red swamp crayfish, dramatically higher weight gains and survival percentages were reported compared to crayfish fed a variety of rice forages (McClain et al. 1992b). While plant material may have furnished some nutrients, McClain et al. (1992b) stated that their use as a mainstay food item for small crayfish was limited and that the nitrogen : carbon ratio is not a reliable indicator of the nutritive value of detritus. In the present study, ponds had no observable plant material, and any addition of nutrients had to be in the form of either the alfalfa hay or prepared diets fed to the red claw.

Very small ($<$0.6 g) red claw can consume zooplankton (Jones 1995), while small (5 g) crayfish have been classified as detritivores and/or herbivores but also appear to be opportunistic carnivores in that animal organisms associated with soil and peron on detritus can contribute nutrients to their growth (Huner and Barr 1984; Sanguanruang 1988; McClain et al. 1992b). However, as adults, crayfish feed predominately on vegetation and detritus (Goddard 1988). Loya-Javellana et al. (1993) reported that larger red claw crayfish (20–75 mm in total length) had a preference for decayed plant material, as opposed to zooplankton, and concluded that animal matter are of minor importance for red claw at this size. In the present study, red claw were much larger than used in the study by Loya-Javellana et al. (1993); thus, it appears possible that animal food items might make even less of a contribution to their overall nutrition.

It has been suggested that crustaceans do not consume prepared diets directly and that the diet serves as a fertilizer for organisms that will be consumed by the crustaceans (Tidwell et al. 1997). However, this assumption may be erroneous. It can be argued that red claw, as with other crayfish, successfully utilize a pelleted diet and do so efficiently. Tidwell et al. (1997) stated that the increases observed in macroinvertebrate populations in ponds stocked with prawn, *Macrobrachium rosenbergii*, and fed a prepared diet had to be because of the macroinvertebrates consuming uneaten diet, even if prawn were consuming the macroinvertebrates. However, no gut analysis data were offered to support this assumption. Gut analysis of prawn in which distillers-dried grains with solubles (DDGS) were added to the pond found that 47% of prawn had DDGS in the gut. This percentage is likely too low, as DDGS, once digested, would become unrecognizable. Therefore, if prawn consumed the diet and DDGS directly, fecal production by prawn could serve as a source of nutrients for macroinvertebrates and thereby lead to an increase in their numbers, as reported in Tidwell et al (1997).

It does not seem plausible that a large ($>$60 g) red claw could consume small macroinvertebrates to sustain rapid growth and size observed in the present study. Tidwell et al. (1997) reported that prawn stocked into ponds and not fed grew to 13 g after 117 d, while prawn fed a prepared diet grew to 36 g. While these smaller prawns may have consumed macroinvertebrates, it appears that consumption of natural food organisms in the pond only supported growth to 13 g. Red claw in the present study were much larger than prawn reported in Tidwell et al. (1997). McClain et al. (1992a) did not have any evidence that red swamp crayfish, *Procambarus clarkii*, utilize detrital microflora and microfauna effectively for growth. In contrast, parameter estimates generated by regression analysis indicated that between 65 and 72% of nutrition of small ($<$5 g) crayfish was because of consumption of the pelleted diet, whereas the percentages of nutrition provided by the soil substrate (4%), soil benthos (6%), and detrital forage (18–25%) were much lower.

Jones et al. (2002) stated that the increase in growth of yabby, *Cherax destructor*, with a prepared diet was a consequence of direct consumption, rather than from a fertilizer effect, and indicated that the best feed indexes were obtained when a prepared diet is the only source of nutrients. While ponds used in the present study did have soil, allowing for the potential that red claw could derive some nutrients from consumption of macroinvertebrates, there was no detrital material or nonliving sources of nutrients in the ponds other than what was supplied by the addition of alfalfa hay and/or prepared diet. The size of harvested red claw is much larger than organisms from previous reports (McClain et al. 1992a, 1992b; Tidwell et al. 1997; Jones et al. 2002) and casts doubt that the red claw in the present study were utilizing live food organisms as the basis for the majority of their growth and must be directly consuming the prepared diets fed to them.

Results of the present study are similar to those reported in Salame and Rouse (2000) who demonstrated that total yield of red claw was significantly higher when red claw were fed a prepared diet and provided with forage (889 kg/ha) than in ponds receiving diets only (594 kg/ha). In that study, red claw were stocked at a slightly higher stocking rate than the present study (4 red claw/m²), forage was applied at a lesser rate of 100 kg/ha/mo, and red claw were fed a higher protein level (35%) the first 2 mo of the study. These data are similar to those reported in red swamp crayfish (McClain 1995). McClain et al. (1992a) also reported that small red swamp crayfish, *P. clarkii*, fed a pelleted diet with forage were significantly larger than crayfish fed only detrital forages. Also shown in another study, yabby fed solely a pelleted diet were reported to be significantly larger than when the crayfish only had access to forage in tanks and ponds (Jones et al. 2002).

Results in the present study indicate that red claw stocked semi-intensively (3.0 red claw/m²) combined with forage at a rate of 500 kg/ha/mo may be able to utilize a lower protein diet (13%). The amount of forage added was higher than previously stated by Salame and Rouse (2000) for red claw so as to ensure that hay would not be limiting if used as a source of nutrients and/or as substrate. The amount of alfalfa hay added per month did not cause any detrimental effects on water quality.

The lack of differences in growth of red claw fed a diet containing 13 and 28% protein is in contrast to the previous reports that have been published on dietary protein needs for pond-grown red claw. Thompson et al. (2004a) reported that red claw fed a diet containing 22% protein had similar growth compared to red claw fed diets containing 32 and 42% protein, while Thompson et al. (2006) reported that red claw fed a diet containing 28% protein had higher final individual weight compared to red claw fed a diet containing 18% protein. However, the range of average final weights in the present study (49–68 g) was similar to those stated in other reports (Rouse and Kahn 1998; Thompson et al 2004a, 2006; Webster et al. 2004). All water quality parameters were within acceptable limits for red claw (Muzinic et al. 2004; Webster et al. 2004; Thompson et. al. 2004a, 2005, 2006).

Although it cannot be explained why growth of red claw fed a diet containing 13% protein (TRT2) was not different from that of red claw fed a diet containing 28% protein (TRT4), there are several factors that may have influenced the results that conflict with the previous red claw pond feeding studies (Webster et al. 1994; Muzinic et al. 2004; Thompson et al. 2004a, 2005, 2006) such as the use of different genetic stock and water temperature. Because of warmer water temperatures in the present study, as compared to the previous pond studies (Thompson et al. 2004a, 2006), red claw may have started breeding early after stocking, thus stunting the growth of females as they became berried. It was observed at harvest that a large number of small (<2 g) individuals were present upon harvest. These individuals were smaller than the individuals stocked and had to be a result of spawning activities within the pond.

While water stability of a prepared diet is important when feeding crustaceans (Webster et al. 1994), a pellet stability test on the diets showed that the 28% protein diet had a lower ($P < 0.05$) pellet water stability (88%) compared to the 13% protein diet (95%). However, although the 28% protein diet had a lower pellet water stability, it was similar to or higher than Webster et al. (1994) and Tidwell et al. (1997), and growth of red claw fed the 28% protein diet appeared to be similar to other published data (Webster et al. 2004; Thompson et al. 2004a, 2006).

Benefits on the use of forage in the culture of crustaceans have included the following: addition of forage helped stimulate the natural food chain in the pond and thus accommodated changing feeding habits of red claw as they matured (Salame and Rouse 2000) and forage provided beneficial cover (shelter) during crayfish molts (ecdysis) and may have provided a refuge for general protection against predation, thus influencing survival and production. Survival values (average of 66.8%) of red claw in the present study are similar to the previous red claw pond studies (Webster et al. 2004; Thompson et al 2004a, 2004b), so it appears that the addition of hay offers little benefit as substrate to enhance survival of the stocking density and culture conditions in the present study.

FCR values in present study (2.99–2.83) were lower, or similar to, previously reported values for pond-cultured red claw (Rouse and Kahn 1998; Jones and Ruscoe 2000; Webster et al. 2004; Thompson et al. 2006). Total yield average

(854.8 kg/ha) of red claw from the four treatments in the present study was similar to or higher than other reports (Salame and Rouse 2000; Webster et al. 2004; Thompson et al. 2004a, 2004b, 2006).

At harvest, the average percentage of males and females was 47.2% for males, 51.4% for females, and 1.4% for intersex animals for all treatments. Intersex red claw are those animals that exhibit both male and female secondary sexual characteristics (Medley and Rouse 1993). The low (<1.5%) percentage of intersex red claw in the present study has been reported in several published pond studies (Webster et al. 2004; Thompson et al. 2006). Harvest percentages of males and females in the present study were not significantly different among treatments and are consistent with earlier studies with red claw (Karplus et al. 1995; Pinto and Rouse 1996; Rouse and Kahn 1998; Webster et al. 2004; Thompson et al. 2006).

Data from the present study indicate that red claw from TRT1 and TRT2 had significantly lower tail meat weight and tail meat percentage compared to red claw in TRT3 and TRT4. When compared between sexes, males have higher tail meat yields than females; however, tail meat percentage did not differ between the sexes. These results are in agreement with other studies (Gu et al. 1994; Curtis and Jones 1995; Thompson et al. 2004a, 2004b, 2006).

In conclusion, the results of the present study indicate that juvenile red claw stocked at 27,170 red claw/ha can be fed a diet containing 13% protein diet, with or without forage (alfalfa hay), as growth and survival were similar to red claw fed a 28% protein diet, and it seems plausible that red claw directly utilize prepared diets for growth. Results may help reduce diet costs, increase profitability for producers, and allow for industry expansion. However, it appears that growth in red claw fed only hay is reduced, although this feeding practice may be acceptable for some red claw producers wishing for a low-input culture method. The use of lower protein diets in combination with added forage in ponds may help reduce operating costs, and thereby increase producer's profits. Future studies should continue to evaluate forage–pellet-based feeding and determine a least-cost feeding strategy to increase net returns.

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