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Effects on growth, survival, body composition, processing traits and water quality when feeding a diet without vitamin and mineral supplements to Australian red claw crayfish (*Cherax quadricarinatus*) grown in ponds

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Abstract

To be profitable, producers must reduce diet costs, which can be as high as 80% of the variable costs of an aquaculture expense. As vitamin and mineral premixes represent a significant cost, eliminating addition of these premixes could reduce diet costs if no adverse effects were observed for growth and production parameters. A 105-day feeding trial was conducted with juvenile Australian red claw crayfish (*Cherax quadricarinatus*) to evaluate the effects of growth, survival, body composition, processing traits and water quality when red claw were fed a supplemental diet containing 28% crude protein (CP) without vitamin and mineral premixes (and supplemented alfalfa hay) compared with red claw fed a diet (control diet) containing 42% CP, vitamin and mineral premixes, and with supplemented alfalfa hay, and compared with red claw only fed alfalfa hay when grown in ponds. Juvenile red claw (mean weight of 15.7 ± 1.0 g) were randomly stocked into nine 0.02-ha ponds at a rate of 640 per pond (3.2 per m^2), and each treatment was used in three ponds. There were two feedings per day, each consisting of one-half of the total daily ration. At harvest, individual weight, percentage weight gain, specific growth rate, survival and total yield of red claw fed a control diet was significantly higher ($P < 0.05$) (83.0 g, 398%, 1.53% day^{-1}, 65.1%,and 1708 kg ha^{-1} respectively) compared with red claw only fed alfalfa hay (44.9 g, 202%, 1.04% day^{-1}, 30.3% and 431 kg ha^{-1}, respectively), but not different ($P > 0.05$) from red claw fed the supplemental diet without vitamin and mineral premixes (76.2 g, 367%, 1.47% day^{-1}, 57.2% and 1378 kg ha^{-1}). There were no significant differences ($P > 0.05$) in feed conversion ratio (FCR) among treatments, which averaged 5.0 (based on prepared diet input). These results indicate that vitamin and mineral supplementation in a diet is not necessary when diet containing 28% CP and alfalfa hay are used in combination for pond grown red claw. These results may help reduce diet costs and possibly increase producers' profits which might allow for industry expansion.

Keywords: red claw, growth, diets, pond culture

Introduction

Australian red claw crayfish (*Cherax quadricarinatus*) is a freshwater crayfish species native to the tropical region of Queensland, Australia and southeastern Papua New Guinea. Aquaculture of red claw is increasing because this species has numerous attributes that make them an ideal choice for aquaculture. Red claw can tolerate a wide range of temperatures and fluctuating water quality environments which includes: low dissolved oxygen concentrations (as low as <1 ppm), hardness and alkalinity (20–300 ppm) and pH (6.5–9) (Masser & Rouse 1997). Red claw exhibit rapid growth rates achieving weights of 50–100 g in a three-to-4-month growing period (Metts, Thompson, Xiong, Kong, Webster & Brady 2007). Approximately 30% of the total body weight of red claw is edible tail meat (Thompson, Muzinic, Yancey, Webster, Rouse & Xiong 2004), while the red swamp crayfish (*Procambarus clarkii* has a tail meat yield of between 15–20% (Masser & Rouse 1997). Also, red claw have a simple life cycle, meaning that the adult morphology is achieved without passing through free-living larval phases (Jones 1995a). Other positive aspects include: a non-burrowing and non-aggressive behaviour, straightforward production technology and tolerance to high stocking densities if sufficient shelter is available (Jones 1995b; Masser & Rouse 1997).

Red claw are very flexible in their feeding habits consuming decaying plant and animal matter (referred to as detritus), as well as macrophytes, benthic invertebrates, algae, bacteria and fungi (Saoud, Graza de Yta & Ghanawi 2012). This may allow expensive prepared diets to be supplemented or replaced by natural foods or forages. Combining pellet-based feeding with forage has the potential to reduce diet costs. Application of forage may stimulate the natural food chain in the pond as well as serve as refuge for protection against predation (Salame & Rouse 2000; Metts *et al.* 2007). McClain, Neill and Gatlin (1992a) demonstrated that small red swamp crayfish grew better by feeding on a combination of formulated diets with forage compared with those fed only detrital forage. Salame and Rouse (2000) also found better survival rates and total yield of red claw in ponds receiving a combination of prepared diets and forage compared with those receiving prepared diets only. Metts *et al.* (2007) reported that the protein content of the prepared diet can be lowered if combined with supplemented alfalfa hay as similar growth was achieved in red claw fed a diet containing 13% protein compared with red claw fed a high protein (28%) diet without hay. While red claw seem to use prepared diets efficiently, additional research is necessary to understand the use of feed forage combinations to reduce production costs.

Another option in which costs can be reduced is to eliminate more expensive dietary components, such as vitamin and mineral premixes. Vitamins and minerals are essential micronutrients (Saoud *et al.* 2012); however, they can contribute up to 1.2–3.1% of the total diet cost (David Brock, Rangen, Buhl, ID, USA; personal communication). Currently, there is no data on vitamin and mineral requirements for red claw. Consequently, feed manufactures add vitamin and mineral pre-mix supplements based on requirements for other crustacean species. The exclusion of vitamin and mineral supplements on other crustacean species has been conducted where natural food organisms were available (Trino, Penaflorida & Bolivar 1992; Trino & Sarroza 1995; Trino, Millamena & Keenan 2001).

The objective of this pond-based study was to evaluate the growth performance of red claw fed a moderately low (28%) protein diet devoid of vitamin and mineral supplements when combined with alfalfa hay and compare this treatment with either a diet containing a high protein content (42%) with alfalfa hay, or red claw fed only alfalfa hay.

Materials and methods

Description and stocking of ponds

Juvenile red claw were obtained from a domestic supplier (Alma Bryant High School Aquaculture Program, Irvington, AL, USA) and shipped without water in insulated containers with moist cool packs. Red claw (average individual weight ±SD of 15.7 ± 1.0 g) were placed into aerated pond water for acclimation. Animals which appeared healthy and active were hand-counted into each of nine 0.02-ha ponds at a rate of 640 red claw per pond (3.2 red claw m^{-1}). Ponds had an average water depth of 1.1 m and were located at the Aquaculture Research Center, Kentucky State University, Frankfort, KY, USA. Three replicate ponds were randomly assigned to each of the three treatments. In Treatment 1 (TRT1) red claw were fed a complete (control) diet containing 42% crude protein (CP) and dried alfalfa hay was added as potential forage at a rate of 500 kg ha^{-1} $month^{-1}$; in Treatment 2 (TRT2) red claw were fed a supplemental diet containing 28% CP without vitamin and mineral supplements, and dried alfalfa hay was added as potential forage at a rate of 500 kg ha^{-1} $month^{-1}$ and Treatment 3 (TRT3) received only dried alfalfa hay at a rate of 500 kg ha^{-1} $month^{-1}$.

Experimental diets and feeding rates

Two experimental diets were formulated (Table 1): the complete diet contained 42% CP with vitamin and mineral premixes added, and the supplemental diet contained 28% CP and no vitamin and mineral premixes. Dietary ingredients were processed into 4-mm sinking pellets by a commercial feed mill (Melick Aquafeed, Catawissa, PA, USA). Two-feedings, each consisting of one-half of the total daily ration, were distributed over the entire surface area of each pond between 08:00 and 08:30, and between 15:30 and 16:00 hours for 105 days. Amount of diet was adjusted weekly based upon estimated body weight. Red claw in all ponds were fed 10% of estimated body weight the first 2 weeks; 8% the following 2 weeks; 6% the following 2 weeks; 4% the following 2 weeks and 3% for the remainder of the study, with assumed growth rate of 4.2 g per week based upon previous published data (Metts *et al.* 2007).

Prepared diets were also examined for pellet stability in water. Ten grams of pellets of equal length were spread uniformly on a 100-cm^2 brass screen (2-mm mesh size) with raised sides, lowered into static water for 30 min, then dried in an oven (100°C) for 24 h (Metts *et al.* 2007). The dry solids as a percentage of original weight were used as a comparative measure of pellet stability in water.

Hay and application rates

All nine ponds had dried alfalfa hay (14% CP) added at a rate of 500 kg ha^{-1} $month^{-1}$ based on rate used in Metts *et al.* (2007). Hay additions were conducted every 2 weeks and evenly distributed over the pond bottom. Hay bales were placed into each of the nine ponds for approximately 2 weeks prior to distribution and/or were weighted with bricks to ensure that the hay would sink to the pond bottom and would be accessible for red claw before distribution.

Data analysis

Experimental diets were analysed for moisture, protein, lipid and ash (Table 1). Moisture was determined by placement of a 2-g sample into a convention oven (135°C) for 2 h (AOAC procedure 930.15); protein was determined by combustion (AOAC procedure 990.03); lipid was determined by the acid hydrolysis method (AOAC procedure 954.02); and ash was determined by placing a 2-g sample in a muffle furnace (600°C) for 2 h (AOAC procedure 942.05) (AOAC 1995). Amino acid compositions of diets (Table 2) were determined at Texas A&M University, College Station, TX, USA.

Table 1 Ingredient, chemical composition and pellet stability of two practical diets fed to Australian red claw crayfish *Cherax quadricarinatus*. Proximate analysis values are means of two replicates per diet. Each replicate was analysed in triplicate. Values in parentheses are percentage protein of ingredient

	Diet (% crude protein)	
Ingredient	1 (42%)	2 (28%)
Wheat midds	35.35	72.0
Soybean meal (50%)	35.0	6.0
Alfalfa meal (17%)	15.0	15.0
Poultry by-product	6.0	0.0
Wheat gluten	4.0	4.0
Soybean oil	3.0	3.0
Vitamin mix*	0.4	0.0
Mineral mix†	0.1	0.0
Stay C (35%)‡	0.05	0.0
Choline chloride	0.1	0.0
Dicalcium phosphate	1.0	0.0
Chemical analysis		
Moisture (%)§	6.7	9.8
Crude protein (%)¶	42.0	28.4
Crude lipid (%)¶	4.7	5.5
Ash (%)¶	6.4	5.3
Pellet stability**	67 ± 2.2^a	62 ± 2.0^a

*Vitamin mix contained: thiamin (B_1), 1.01%; riboflavin (B_2), 1.32%; pyridoxine (B_6), 0.9%; nicotinic acid, 8.82%; folic acid, 0.22%; cyanocobalamine (B_{12}), 0.001%; pantothenic acid, 3.53%; menadione (K), 0.2%; ascorbic acid (C), 33.1%; retinol palmitate (A), 4409 IU kg^{-1}; cholecalciferol (D_3), 2204600 IU kg^{-1}; α tocopherol (E), 66.2 IU kg^{-1}; ethoxyquin, 0.66%.
†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 $CoSO_4$); Ca as carrier.
‡Vitamin C.
§Wet weight basis.
¶Dry-matter basis.
**Pellet stability = percentage of dry solids retained after 30 min in static water; means within a row having the same superscripts are not significantly different ($P > 0.05$).

Water quality management

Water temperature and dissolved oxygen (DO) were measured in all ponds twice daily (09:00 and

15:30 hours) using an YSI Model 58 oxygen meter (Yellow Spring Instruments, Yellow Spring, OH, USA). A 1/2-HP electric aerator (Air-O-Lator, Kansas City, MO, USA) was located in the centre of each pond and run continuously throughout the duration of the study. Total ammonia-nitrogen (TAN), nitrite-nitrogen and alkalinity were measured three times per week. Nitrite and TAN were measured using a DREL/2800 spectrophotometer (Hach Company, Loveland, CO, USA), while alkalinity was measured by titration using a digital titrator (Hach Company).

Harvest

Ponds were harvested between 26 September and 2 October 2012. Three days prior to harvest, the water level in each pond was lowered approximately 0.5 m at the drain end. On the day of harvest, water was completely drained and red claw were manually removed. Total weight and number of red claw from each pond were recorded. The sex of all individuals was determined and each gender was bulk weighed and counted.

Growth parameters and feed efficiency were calculated as follows:SGR(specific growth rate) $(\%\text{day}^{-1}) = [(\ln W_t - \ln W_i)/T] \times 100$, where W_t and W_i are the final and initial individual weight of the red claw, respectively, and T is the length of the culture period in days;

$$\text{Weight gain}(\%) = 100[(W_t - W_i)/W_i]$$

Feed conversion ratio (FCR) = total diet fed (kg)/ total wet weight gain (kg).

Ten males and 10 females from each pond (30 males and 30 females per treatment) were randomly sampled after weighing and chill-killed using an ice water bath, and the tail muscle meat was removed from each body and stored in ziplock bags and frozen (−15°C) until analysed. Weight of tail muscle, the percentage tail meat with shell, and the percentage tail meat without shell contributed to total individual body weight were calculated from both male and female red claw. Moisture, protein, lipid and ash of the tail muscle were analysed by a commercial analytical laboratory (Eurofins Scientific, Des Moines, IA, USA). Analysis procedures were as described for the diets except for moisture (AOAC procedure 950.46), and lipid was determined by ether extraction (AOAC procedure 960.39) (AOAC 1995). Amino acid composition of tail muscles were analysed at Texas A&M University, College Station, TX, USA.

Table 2 Amino acid composition of two practical diets fed to Australian red claw crayfish

	Diet	
Amino acid	1 (42%)	2 (28%)
Alanine	1.37 ± 0.03	0.92 ± 0.09
Arginine	1.27 ± 0.07	0.81 ± 0.13
Aspartic acid	1.70 ± 0.1	1.32 ± 0.17
Cystine	0.10 ± 0.0	0.21 ± 0.06
Glutamic acid	3.07 ± 0.23	2.49 ± 0.10
Glycine	1.60 ± 0.20	0.66 ± 0.06
Histidine	0.63 ± 0.18	0.39 ± 0.04
Isoleucine	1.17 ± 0.12	0.61 ± 0.05
Leucine	2.23 ± 0.12	1.48 ± 0.14
Lysine	1.33 ± 0.09	0.77 ± 0.09
Methionine	0.68 ± 0.25	0.28 ± 0.04
Phenylalanine	1.37 ± 0.07	0.76 ± 0.08
Proline	2.13 ± 0.30	1.10 ± 0.13
Serine	1.48 ± 0.16	0.80 ± 0.07
Taurine	0.03 ± 0.03	0.0 ± 0.0
Threonine	1.1 ± 0.13	0.64 ± 0.02
Tyrosine	0.7 ± 0.07	0.48 ± 0.05
Valine	1.5 ± 0.22	0.74 ± 0.07

Values are percentage (±SE) of the diet. Amino acid analysis values are means of three replicates per diet.

Statistical analysis

Response data was subjected to a mixed model analysis of variance (PROC MIXED) with contrasts using Tukey's studentized range (honestly significant difference) test. All statistical analyses were performed using the SAS software version 9.3 (SAS 2011). Data were log-transformed prior to analysis (Zar 1984). Significant level α was set at 0.05. One pond from TRT2 was excluded from all data analysis as there were few red claw in the pond at harvest.

Results

Water quality

All ponds were continuously aerated throughout the duration of the study, and dissolved oxygen (DO) concentration averaged 8.0 mg L^{-1} for the morning and 9.1 mg L^{-1} for the afternoon (Table 3). There were no significant differences ($P > 0.05$) in morning and afternoon water temperature, which

Table 3 Means (±SE) of water quality parameters of ponds stocked with Australian red claw crayfish and fed a diet containing 42% protein with vitamin-mineral premixes and alfalfa hay (Treatment 1), a diet containing 28% protein without vitamin-mineral premixes and alfalfa hay (Treatment 2), or alfalfa hay only (Treatment 3)

	Treatment		
Parameters	1 (42%)	2 (28%)	3 (Hay only)
Dissolved oxygen (mg L^{-1}; AM)	7.86 ± 0.06[b]	8.02 ± 0.05[ab]	8.20 ± 0.04[a]
Dissolved oxygen (mg L^{-1}; PM)	9.28 ± 0.09	9.09 ± 0.05	9.04 ± 0.04
Temperature (°C; AM)	23.1 ± 0.21	22.8 ± 0.18	23.0 ± 0.17
Temperature (°C; PM)	25.2 ± 0.23	24.7 ± 0.22	25.0 ± 0.18
TAN (mg L^{-1})	0.99 ± 0.03	0.95 ± 0.03	1.30 ± 0.04
Nitrite (mg L^{-1})	0.019 ± 0.0	0.004 ± 0.0	0.000 ± 0.0
Alkalinity (mg L^{-1})	122 ± 1.9	114 ± 1.0	108 ± 1.5

TAN, total ammonia nitrogen.
Means within a row having different superscripts are significantly different ($P < 0.05$). One replicate in TRT2 was excluded from the data analysis.

averaged 23.0°C and 25.0°C respectively (Table 3). Additionally, average morning and afternoon water temperature were analysed by month. Morning water temperatures for June, July, August and September were 23.1, 25.5, 22.9 and 20.0°C respectively. Afternoon water temperatures for June, July, August and September were 25.8, 27.3, 24.4 and 21.5°C respectively. There were no significant differences ($P > 0.05$) in total ammonia-nitrogen (TAN), nitrite-nitrogen and alkalinity among three treatments, which averaged 1.08 mg L^{-1}, 0.008 mg L^{-1} and 115 mg L^{-1}, respectively, and were within acceptable limits for red claw (Table 3).

While average TAN levels were not different among treatments for the entire experimental period, TAN was significantly ($P < 0.05$) higher in ponds with red claw fed only hay in the month of August (Fig. 1). However, higher ($P < 0.05$) nitrite levels were recorded in September for ponds with red claw fed a diet containing 42% protein with levels peaking at 0.05 mg L^{-1} (Fig. 2). All other months had similar TAN and nitrite values among treatments.

Growth and production

After 105 days, final average weight, per cent weight gain and specific growth rate of red claw fed the complete diet was significantly higher ($P < 0.05$; 83.0 g, 398% and 1.53% day^{-1}, respectively) compared with red claw fed only alfalfa hay (44.9 g, 202% and 1.04% day^{-1}, respectively), but did not differ ($P > 0.05$) from red claw fed the supplemental diet (76.2 g, 367%, and 1.47% day^{-1} respectively; Table 4). There was no significant difference ($P > 0.05$) in FCR between red claw fed complete and supplemental diets, which averaged 5.01. Percentage survival did not differ significantly ($P > 0.05$) between red claw fed complete and supplemental diets (65.1% and

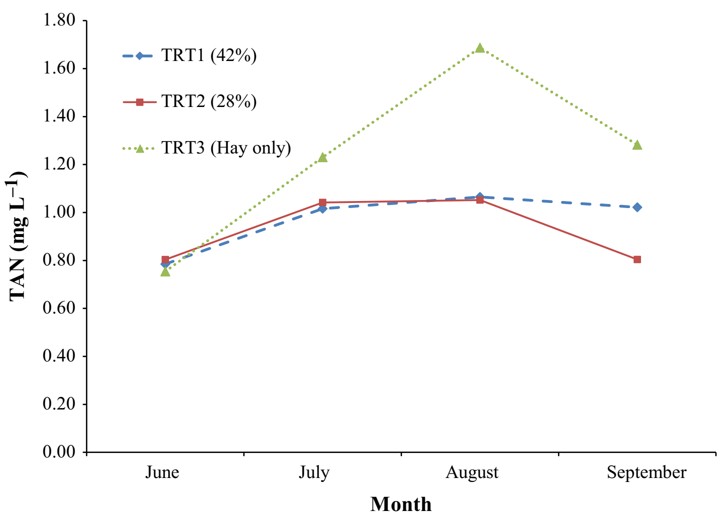

Figure 1 Changes in TAN (mg L^{-1}) in ponds (by month) over the course of the 105-day feeding trial.

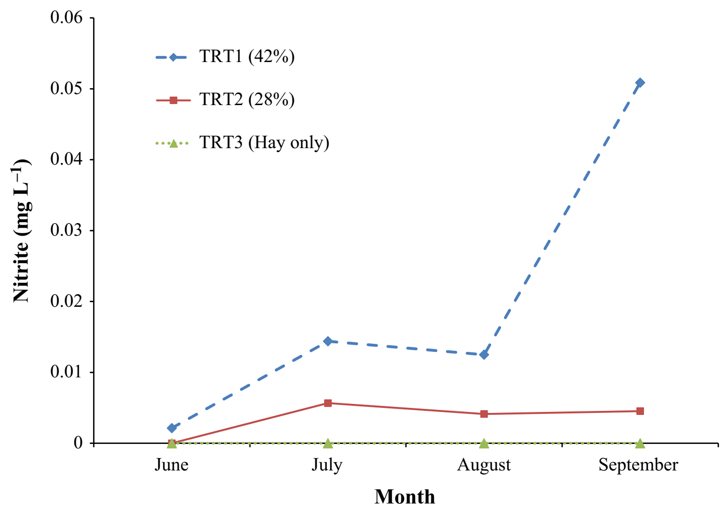

Figure 2 Changes in nitrite (mg L^{-1}) in ponds (by month) over the course of the 105-day feeding trial.

57.2%, respectively); however, red claw fed only alfalfa hay had significantly lower percentage survival ($P < 0.05$; 30.3%) compared with the other two treatments. Likewise, red claw fed complete and supplemental diets had significantly higher ($P < 0.05$) total yield (1708 and 1378 kg ha^{-1}, respectively) compared with red claw fed only alfalfa hay (431 kg ha^{-1}).

Table 4 Means (±SE) of final individual weight, per cent weight gain, weight gain per week, specific growth rate (SGR), feed conversion ratio (FCR), percentage survival and total yield of Australian red claw crayfish fed a diet containing 42% protein with vitamin-mineral premixes and alfalfa hay (Treatment 1), a diet containing 28% protein without vitamin-mineral premixes and alfalfa hay (Treatment 2), or alfalfa hay only (Treatment 3)

	Treatment		
	1 (42%)	2 (28%)	3 (Hay only)
Final weight (g)	83.0 ± 0.1[a]	76.2 ± 0.7[a]	44.9 ± 4.2[b]
Weight gain (%)	398 ± 2.0[a]	367 ± 12[a]	202 ± 27[b]
Weight gain (g $week^{-1}$)	4.42 ± 0.03[a]	3.99 ± 0.06[a]	2.00 ± 0.27[b]
SGR (% day^{-1})	1.53 ± 0[a]	1.47 ± 0.03[a]	1.04 ± 0.09[b]
FCR	3.99 ± 0.03[a]	6.18 ± 1.65[a]	N/A
Survival (%)	65.1 ± 0.4[a]	57.2 ± 7.4[a]	30.3 ± 1.0[b]
Yield (kg ha^{-1})	1708 ± 11[a]	1378 ± 179[a]	431 ± 49[b]

N/A, not available.

Means within a row having different superscripts are significantly different ($P < 0.05$). One replicate in TRT2 was excluded from the data analysis.

Percentages of males and females at harvest and final weight

There were no significant differences ($P > 0.05$) in the percentages of males and females harvested from ponds among treatments (Table 5). The percentage of males averaged 50.7%, while the percentage of females averaged 49.3%.

Final individual mean weights of males and females fed the complete diet were significantly higher ($P < 0.05$) (89.5 and 79.5 g, respectively) compared with that of red claw fed only alfalfa hay (48.1 and 42.1 g, respectively), but not different ($P > 0.05$) from red claw fed the supplemental diet (82.8 and 69.7 g respectively). When comparing the final individual mean weight for each treatment, males in the supplemental treatment were significantly larger ($P < 0.05$; 82.8 g) compared with females (69.7 g). However, there were no significant differences ($P > 0.05$) in the final mean weights between males and females in the other two treatments (Table 5).

Tail meat yield, percentage and composition

Weight of tail muscle meat and percentage of tail meat, with and without shell, of males and females are presented in (Table 5). Tail muscle meat of male red claw fed the complete diet was significantly higher ($P < 0.05$; 19.2 g) compared with that of male red claw fed only alfalfa hay (12.4 g), but not significantly different ($P > 0.05$) from male red claw fed the supplemental diet (18.9 g). Also, tail muscle meat of female red claw fed the

Table 5 Means (±SE) harvest percentage, final individual weight, tail meat weight, percentage of tail meat with shell and per cent of tail meat without shell from the total body of male and female Australian red claw crayfish harvested from ponds and fed a diet containing 42% protein with vitamin-mineral premixes and alfalfa hay (Treatment 1), a diet containing 28% protein without vitamin-mineral premixes and alfalfa hay (Treatment 2), or alfalfa hay only (Treatment 3) for 105 days

	Treatment		
	1 (42%)	2 (28%)	3 (Hay only)
Males			
Harvest (%)	51.8 ± 4.26^{a}	52.8 ± 6.20^{a}	47.4 ± 1.52^{a}
Final weight (g)	$89.5 \pm 3.2^{a,x}$	$82.8 \pm 2.8^{a,x}$	$48.1 \pm 5.3^{b,x}$
Tail meat (g)	$19.2 \pm 1.6^{a,x}$	$18.9 \pm 0.9^{a,x}$	$12.4 \pm 0.7^{b,x}$
Tail meat with shell (%)	$33.9 \pm 0.6^{ab,x}$	$32.3 \pm 0.6^{b,y}$	$34.9 \pm 0.5^{a,y}$
Tail meat without shell (%)	$25.7 \pm 0.6^{a,x}$	$24.2 \pm 0.6^{a,y}$	$25.8 \pm 0.5^{a,x}$
Females			
Harvest (%)	48.2 ± 4.26^{a}	47.2 ± 6.20^{a}	52.6 ± 1.52^{a}
Final weight (g)	$76.5 \pm 2.4^{a,x}$	$69.7 \pm 0.8^{a,y}$	$42.1 \pm 3.4^{b,x}$
Tail meat (g)	$18.5 \pm 1.0^{a,x}$	$16.8 \pm 0.6^{a,x}$	$11.0 \pm 0.6^{b,x}$
Tail meat with shell (%)	$35.1 \pm 0.5^{b,x}$	$35.4 \pm 0.5^{ab,x}$	$36.6 \pm 0.4^{a,x}$
Tail meat without shell (%)	$26.2 \pm 0.6^{a,x}$	$26.0 \pm 0.6^{a,x}$	$26.7 \pm 0.4^{a,x}$

Means in a row with different superscripts (a, b) are 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 ($P < 0.05$). One replicate in TRT2 was excluded from the data analysis.

complete diet was significantly higher ($P < 0.05$; 18.5 g) compared with that of female red claw fed only alfalfa hay (11.0 g), but not different from female red claw fed the supplemental diet (16.8 g). There were no significant differences ($P > 0.05$) in the tail muscle meat between males and females for either complete or supplemental dietary treatments.

When comparing the percentage tail meat without shell among treatments between genders, there was no significant difference ($P > 0.05$), which averaged 25.2% and 26.3% respectively. The percentage tail meat without shell of females in the supplemental treatment was significantly higher ($P < 0.05$; 26.0%) compared with males (24.2%), while there were no significant differences ($P > 0.05$) between males and females in the other two treatments (Table 5).

Table 6 Means (±SE) percentage moisture, protein, fat and ash (wet weight basis) of tail meat of male and female Australian red claw crayfish fed a diet containing 42% protein with vitamin-mineral premixes and alfalfa hay (Treatment 1), a diet containing 28% protein without vitamin-mineral premixes and alfalfa hay (Treatment 2), or alfalfa hay only (Treatment 3)

	Treatment		
	1 (42%)	2 (28%)	3 (Hay only)
Males			
Moisture (%)	80.4 ± 0.5	79.0 ± 0.6	79.4 ± 0.7
Protein (%)	18.1 ± 0.3	18.9 ± 0.4	18.8 ± 0.6
Fat (%)	0.15 ± 0.04	0.14 ± 0.03	0.10 ± 0.0
Ash (%)	1.20 ± 0.04	1.24 ± 0.08	1.32 ± 0.06
Females			
Moisture (%)	80.6 ± 0.7	78.7 ± 0.8	80.3 ± 0.8
Protein (%)	17.9 ± 0.6	19.0 ± 0.5	18.4 ± 0.7
Fat (%)	0.12 ± 0.02	0.14 ± 0.02	0.11 ± 0.01
Ash (%)	1.26 ± 0.05	1.29 ± 0.03	1.28 ± 0.04

Means in each row are not significantly different ($P > 0.05$). Means between males and females in the same column (treatment) for each respective variable are not significantly different ($P > 0.05$). One replicate in TRT2 was excluded from the data analysis.

Proximate compositions of male and female red claw tail muscle are shown in (Table 6). There were no significant differences ($P > 0.05$) in percentage moisture, protein, lipid and ash (wet-weight basis) in the tail muscle of males among treatments, averaging 79.6%, 18.8%, 0.13% and 1.25% respectively. Likewise, there were no significant differences ($P > 0.05$) in percentage moisture, protein, lipid and ash (wet-weight basis) in the tail muscle of females among treatments, which averaged 79.9%, 18.4%, 0.12% and 1.28% respectively. There were no significant differences ($P > 0.05$) in proximate compositions of tail muscle between male and female when examined within the same treatment.

Discussion

Vitamin and mineral premixes cost approximately \$US15–35 tonne^{-1} in the United States, although the exact cost varies with geographic location and availability (David Brock; personal communication). This is the first study evaluating the production of red claw grown in ponds with the elimination of vitamin and mineral supplements and fed a moderately low CP diet. Results demonstrated that red claw grown in ponds at 3.2 per m^2 can be fed a diet containing 28% crude protein (CP), without vitamin and mineral premixes, with no adverse effects on growth, survival, tail muscle yield and body composition. These data differ from Lochmann, McClain and Gatlin (1992) who reported that dietary vitamin supplementation is needed for optimal growth in the red swamp crayfish. However, in that study, crayfish were grown in an indoor tank flow-through system devoid of supplemental food items. In addition, Catacutan and de la Cruz (1989) found that *Penaeus monodon* juvenile fed diets without vitamin supplementation in tanks had significantly lower weight gain compared with shrimp fed a complete diet.

It has been reported that penaeids raised in ponds may be able to meet their mineral requirements entirely from the intake of natural food organisms (Trino *et al.* 1992; Trino & Sarroza 1995). Davis and Gatlin (1996) suggested that a complete mineral premix may not be needed for some culture organisms under certain culture conditions because practical diets normally contain a considerable amount of endogenous minerals. Kanazawa, Teshima and Sasaki (1984) also stated that shrimp can absorb some minerals, such as calcium, magnesium, manganese and iron, directly from the rearing water. Tidwell, Webster, Sedlacek, Weston, Kinght, Hill, D'Abramo, Daniels, Fuller and Labrenty Montanez (1995) reported that prawn (*Macrobrachium rosenbergii*) grown in ponds fed a supplemental diet, without FM and vitamin and mineral supplements, combined with organic pond fertilizer, had higher yield and individual weight than those fed only a supplemental diet. However, yield and individual weight were not different from those fed a complete diet. Chen (1993) suggested that more carnivorous species of crustaceans may have higher dietary vitamin requirements than omnivorous species. Thus, red claw may have lower vitamin and mineral requirements, since they are considered omnivores/detritivores and dietary sources may be sufficient.

While the dietary treatments of the present study may not seem balanced since different protein levels were used, it has been shown that a diet containing 28% protein provides for the same growth in red claw grown in ponds as diets containing higher percentages of protein (Thompson, Muzinic, Yancey *et al.* 2004; Metts *et al.* 2007) so both diets met the nutrient requirements of red claw. Average final weights of red claw fed the supplemental diet in the present study (76.2 g) were similar to, or higher than, weights reported previously for red claw grown in ponds (Rouse & Kahn 1998; Thompson, Muzinic, Engler, Morton & Webster 2004; Webster, Thompson, Muzinic, Yancey, Dasgupta, Xiong, Rouse & Manomaitis 2004; Thompson, Muzinic, Engler & Webster 2006; Metts *et al.* 2007). However, percentage weight gain of red claw in the present study were lower than previous reports (Thompson, Muzinic, Yancey *et al.* 2004; Thompson, Muzinic, Engler *et al.* 2004, 2006; Webster *et al.* 2004; Metts *et al.* 2007). This is due to the much larger (15.7 g) size at stocking compared with the other studies which resulted in lower percentage weight gains.

In the present study, ponds had no observable plant material and any addition of nutrients had to be from either the alfalfa hay or prepared diets fed to the red claw. As red claw are considered omnivores/detritivores, the addition of forage (detritus) may have served as a source of vitamins and minerals. As defined, detritus is decomposing plant material and its associated microbial biomass. As decomposition progresses, nutrient composition of the detritus decreases while the percentage of ligno-cellulose increases. McClain, Neill and Gatlin (1992b) reported that protein and lipid percentages of forage/detritus are lower than found in prepared diets, especially after increased decomposition time. It has been hypothesized that microbes and larger organisms living on the detrital material served as supplemental sources of nutrients, not the plant residue (Huner & Barr 1984; D'Abramo & Robinson 1989). This has been questioned because as detritus decomposes, protein (amino acids) is modified to form non-amino acid organic nitrogen which form indigestible products with lignin and humin and would provide too little protein for microbial growth (Bowen 1987). However, while not providing macronutrients (such as amino acids) to red claw, detritus may be able to provide micronutrients (such as vitamins and minerals) in sufficient quantities that allow for suitable growth. Further, red claw may be able to obtain at least some intact protein from alfalfa hay, which is 17% protein.

Data from the present study indicates that red claw fed complete and supplemental diets had significantly higher mean final weight, weight gain percentage, total yield and tail muscle yield than red claw fed alfalfa hay only. These results are in agreement with Metts *et al.* (2007) who reported that red claw fed only alfalfa hay had reduced growth performance compared with red claw fed alfalfa hay plus prepared diets. Jones, Chavez and Mitchell (2002) also reported similar results as yabby (*Cherax destructor*) grew better with a prepared diet than those fed forage only. Salame and Rouse (2000) showed an increase in total yield of red claw grown in ponds when red claw were fed both a prepared diet and forage compared with crayfish fed diets only, although Metts *et al.* (2007) reported no benefit when red claw were fed a complete diet and alfalfa hay compared with red claw fed only a complete diet.

While decomposing hay may offer little in terms of nutrients, intact hay could be consumed directly and partially provide essential macronutrients. Metts *et al.* (2007) reported that red claw fed only hay had 80% of the final individual weight of red claw fed a diet containing 28% protein without hay added to the pond. In the present study, red claw fed only hay had 54% the final individual weight of red claw fed a diet containing 42% protein but with hay added. The addition of alfalfa hay in the present study could explain the difference observed between the two studies as the addition of hay could allow for red claw to obtain nutrients throughout the day. As final weights of red claw fed only hay were greater than 50% of crayfish fed prepared diets, some nutritional benefit may be obtained from addition of alfalfa hay. Unfed freshwater prawn and red claw had dramatically reduced final individual weights compared with fed organisms (Tidwell, Webster, Sedlacek, Weston, Knight, Hill, D'Abramo, Daniels & Fuller 1997; Jones *et al.* 2002). Although no data were collected in the present study on this, it is thought that zooplankton do not make a nutritional contribution for larger (>20 mm) red claw in a pond. Loya-Jevallana, Fielder and Thorne (1993) stated that these larger red claw had very little preference for zooplankton and concluded that it would comprise very little of their diet. As red claw used in the present study were much larger than 20 mm, it is doubtful that adult red claw could consume zooplankton and small bottom-dwelling invertebrates to sustain the growth observed.

While it has been argued that supplemental feeding on zooplankton provides for an important source of nutrients for yabby (Duffy, Godwin, Nolan & Purvis 2011), examination of the data reveals otherwise. Those authors reported growth of yabbies in tanks filled with potable water with zooplankton added had only 32–45% of the final weight of crayfish fed prepared diets. Furthermore, organisms were in tanks where chances of encountering live prey items may be dramatically increased compared with a pond environment. It appears that when only natural foods are present, crustaceans may only be able to derive one-third of the growth observed when fed prepared diets. This was stated for yabby (Duffy *et al.* 2011) and for *Macrobrachium rosenbergii* (Tidwell *et al.* 1997). In the present study, there were no unfed treatments, but addition of only alfalfa hay resulted in red claw growth of >50% compared with crayfish fed prepared diets and alfalfa hay.

Feed conversion ratio of red claw fed the supplemental diet (6.18) was lower, or similar to, previous reports (Brummett & Alon 1994; Rouse & Kahn 1998; Thompson, Muzinic, Engler *et al.* 2004; Webster *et al.* 2004), but higher than FCR values reported by (Jones & Ruscoe 2000; Thompson, Muzinic, Yancey *et al.* 2004, 2006; Metts *et al.* 2007). Metts *et al.* (2007) reported FCR values ranging between 2.8 and 3.0. The higher FCR values in the present study are probably due to several factors. The actual survival at harvest was lower than the estimated survival used to determine feeding rates prior to stocking as a 70% survival rate was assumed. Secondly, water stability of the diets (62–67%) was lower than other published data (88–95%; Metts *et al.* 2007). As red claw are bottom feeders and typically slow eaters compared with finfish, water stability of a diet is very important (Webster, Goodgame-Tiu, Tidwell & Rouse 1994). However, while the percentage of diet fed should be reduced from the levels used in the present study, the *a priori* calculation of growth rate (4.2 g week^{-1}) appears to be accurate.

Survival rate of red claw in the present study (51%) was lower, or similar to, previously reported values (Brummett & Alon 1994; Rouse & Kahn 1998; Salame & Rouse 2000; Thompson, Muzinic, Yancey *et al.* 2004; Thompson, Muzinic, Engler *et al.* 2004, 2006; Metts *et al.* 2007). It has been previously reported that stocking density does not affect growth and survival in red claw stocked in ponds (Webster *et al.* 2004), thus the lower survival percentage may have been partially due to shipping stress prior to stocking, with undetected delayed mortality. Also, there was an electric power failure during the week 7 and aerators were not working for approximately 24 h. Dissolved oxygen (DO) concentration reached as low as 1.20 mg L^{-1}. This may have contributed to lower survival in the present study compared with previous reports at our research facility. The recommended range of total ammonia nitrogen for hatchery production is 0.5 mg L^{-1} or less for red claw (Masser & Rouse 1997). Levels of TAN in the present study were higher than a previous study (Metts *et al.* 2007), but similar to Thompson, Muzinic, Yancey *et al.* (2004) except for the treatment receiving only hay, which was higher than previous reports. We cannot explain why TAN in ponds where red claw were fed only alfalfa hay had the highest average levels (1.30 mg L^{-1}; Fig. 1) as all ponds had hay added at identical rates.

Total yield of red claw fed the supplemental diet (1378 kg ha^{-1}) was higher than other reports (Pinto & Rouse 1996; Salame & Rouse 2000; Thompson, Muzinic, Yancey *et al.* 2004; Thompson, Muzinic, Engler *et al.* 2004, 2006; Webster *et al.* 2004; Metts *et al.* 2007). This was due to the slightly higher final individual weight and survival percentages in the present study, but not density as stocking density of red claw in the present study (3.2 per m^2) was similar to other reports (Pinto & Rouse 1996; Salame & Rouse 2000; Webster *et al.* (2004); Metts *et al.* 2007).

At harvest, the percentage of male and female red claw from all treatments was 50.7% male and 49.3% female. As previous research (Webster *et al.* 2004; Thompson *et al.* 2006; Metts *et al.* 2007) has shown that there is only a small percentage of intersex red claw (animals that exhibit both male and female secondary sexual characteristics), no attempt was made in this study to identify them and no further examination of the organism was made once an individual was sexed and counted. In the present study, the percentage of male and female is consistent with other red claw pond studies (Pinto & Rouse 1996; Rouse & Kahn 1998; Thompson, Muzinic, Yancey *et al.* 2004; Thompson, Muzinic, Engler *et al.* 2004, 2006; Webster *et al.* 2004; Metts *et al.* 2007). Likewise, proximate composition of both male and female tail muscle in the present study is similar to other published reports (Thompson, Muzinic, Yancey *et al.* 2004, 2006; Metts *et al.* 2007).

Data from the present study indicates that final individual weight of males are higher than females and this is in agreement with other reports (Thompson, Muzinic, Engler *et al.* 2004, 2006; Metts *et al.* 2007); however, females fed the supplemental diet had a higher percentage of their body weight comprised of tail muscle (meat) with shell and tail muscle without shell compared with males. This is not consistent with previous red claw studies conducted in ponds (Thompson, Muzinic, Engler *et al.* 2004, 2006; Metts *et al.* 2007) and cannot be explained.

There were no significant differences ($P > 0.05$; Table 7) in amino acid composition of the tail muscle among treatments. Mente, Coutteau, Houlihan, Davidson and Sorgeloos (2002) also showed comparable results in the tail muscle and whole-animal tissues of *Litopenaeus vannamei*. They found that the total levels of amino acids were stable while there were some variation in the free pool concentration of individual amino acids. Cowey and Luquet (1983) suggested that the essential amino acid composition of fish muscle and the recommended dietary requirement

Table 7 Amino acid composition (wet-weight basis) in the tail meat from Australian red claw crayfish fed a diet containing 42% protein with vitamin-mineral premixes and alfalfa hay (Treatment 1), a diet containing 28% protein without vitamin-mineral premixes and alfalfa hay (Treatment 2), or alfalfa hay only (Treatment 3)

	Treatment		
Amino acid	1 (42%)	2 (28%)	3 (Hay only)
Alanine	1.07 ± 0.11	1.13 ± 0.15	1.23 ± 0.11
Arginine	1.98 ± 0.21	1.98 ± 0.27	2.32 ± 0.20
Aspartic acid	1.38 ± 0.14	1.38 ± 0.18	1.57 ± 0.14
Cystine	0.03 ± 0.02	0.03 ± 0.02	0.08 ± 0.02
Glutamic acid	2.12 ± 0.23	2.13 ± 0.26	2.43 ± 0.22
Glycine	1.00 ± 0.09	1.02 ± 0.11	1.32 ± 0.15
Histidine	0.43 ± 0.05	0.45 ± 0.07	0.50 ± 0.04
Isoleucine	0.88 ± 0.09	0.88 ± 0.10	1.02 ± 0.09
Leucine	1.57 ± 0.15	1.60 ± 0.20	1.80 ± 0.15
Lysine	1.75 ± 0.16	1.73 ± 0.24	1.93 ± 0.22
Methionine	0.47 ± 0.05	0.50 ± 0.06	0.60 ± 0.04
Phenylalanine	0.80 ± 0.08	0.87 ± 0.10	0.95 ± 0.06
Proline	0.70 ± 0.07	0.68 ± 0.08	0.73 ± 0.06
Serine	0.72 ± 0.07	0.75 ± 0.09	0.83 ± 0.08
Taurine	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.02
Threonine	0.08 ± 0.09	0.78 ± 0.10	0.90 ± 0.07
Tyrosine	0.52 ± 0.06	0.57 ± 0.07	0.60 ± 0.06
Valine	0.92 ± 0.09	0.93 ± 0.12	1.02 ± 0.09

Values are percentage of the diet. Values are means (±SE) of three replications containing two fish per replication. Means in each row are not significantly different ($P > 0.05$). One replicate in TRT2 was excluded from the data analysis.

were similar. However, amino acid concentration in tissues do not necessarily reflect dietary need, but can be used as an indicator of the amounts of each amino acid relative to each other. It appears that while red claw fed only hay were not as large as red claw fed prepared diets, they consumed sufficient nutrients for growth and protein deposition.

In conclusion, the results of the present study indicate that red claw perform as well with a supplemental diet (28% CP with no vitamin/mineral premixes) as with a complete diet (42% CP with added vitamin/mineral premixes) in ponds receiving forage (hay) input and both are superior to hay only. The use of diets without vitamin and mineral supplements and a moderately low crude protein level, plus alfalfa hay, may help reduce diet costs and thereby increase producers profits. Further research should be conducted using lower protein diets without vitamin and mineral premixes to determine minimum protein level for pond-grown red claw.

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