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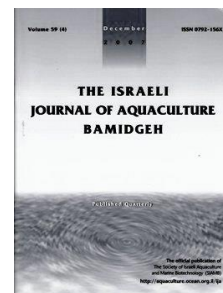
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Growth Performance and Apparent Digestibility Coefficients of Selected Feed Ingredients for Red Swamp Crayfish *Procambarus clarkia*.

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Key words: *Procambarus clarkii*, apparent digestibility coefficient, fish meal, plant protein sources

Abstract

In this study, the growth performance and apparent digestibility coefficients (ADCs) of dry matter (DM), crude protein (CP), crude lipid (EE), gross energy (GE), phosphorus (P), essential amino acids (EAA), and nonessential amino acids (NEAA) for fish meal (local) (FM), soybean meal (SBM), rapeseed meal (RM), cottonseed meal (CM), peanut meal (PM), and extruded soybean (ESB) were determined for red swamp crayfish *Procambarus clarkia*, (weight 5.43 ± 0.21 g), using a reference diet (RF) and test diets (70% RF diet plus 30% of tested ingredient) containing 0.1% Yttrium oxide as an inert marker. The results showed that there was no significant difference in SGR, FCR, and survival among all groups ($P > 0.05$). Apparent digestibility of DM, CP, EE, P, and GE of six feed ingredients ranged from 62.8 to 71.4%, 71.2 to 90.3%, 44.2 to 92.1%, 70.1 to 83.9% and 40.3 to 63.5 %, respectively. From the data for the six feed ingredients, SBM was found to contain the highest ADCs levels in DM (71.4%) and GE (83.9%). FM recorded the highest ADC in EE (92.1%), while PM and CM had the highest ADCs of CP (90.3%), and P (63.5%), respectively. The lowest apparent digestibility of DM, CP, and GE was found in RM, the lowest apparent digestibility of EE was found in ESB, and FM had the lowest ADC of amino acids. Based on the current findings, SBM, CM, and PM could be considered potential substitutes for FM in diets for red swamp crayfish.

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Introduction

Red swamp crayfish (*Procambarus clarkii*) is the most widespread species of crayfish originating from south-central United States and northeastern Mexico. It was introduced to Nanjing, China from Japan in 1929 (Li et al., 2007). The red swamp crayfish has become an important economic crustacean species in China, due to its fast growth, easy management, high yield, high market value, and consumer demand, especially in Jiangsu Province, with gross production value of more than 200 million US dollars in 2009 (Ding et al., 2012). In 2012, the total production of crayfish was approximately 554,821 tons (Anonymous, 2013). Due to increase in annual intensive aquaculture, cost of production has increased. Formulated diets for crayfish must be both nutritionally and economically efficient. Useful accurate and reliable information obtained from measuring the apparent digestibility coefficient (ADC) of nutrients in an ingredient should facilitate the optimization of the nutritional value and cost of formulated feeds (Terrazas-Fierro et al., 2010).

Apparent digestibility coefficient (ADC) determination of feedstuffs is an essential prerequisite not only for accurate feed formulation, but also for regulating and controlling aquaculture waste (Zhou et al., 2004; Liu et al., 2009). Protein is the major and most expensive nutrient in shrimp feeds and is also considered a growth limiting factor (Fernández-Gimenez et al., 2009). The most common source of protein in aquaculture is usually fish meal (FM), however since supply is unstable and price of FM has increased (Falaye et al., 2011; Sevgili et al., 2011), a search for alternative protein sources has become an international research priority, and is critical in China where high-quality FM is lacking.

In a previous study, we reported that 270 g/kg protein, and 40-70 g/kg of lipid with a digestible energy ratio of 17-21 g protein/MJ appeared optimal for rapid growth rate, muscle quality, and high yields (Xu et al., 2013). One study on ADCs for phosphorus and amino acids in red swamp crayfish diets has been carried out (Reigh et al., 1990), however research in this area is scarce. The purpose of this study was to determine ADCs for dry matter, crude protein, crude lipid, energy, phosphorus, and amino acids in local fish meal (FM), soybean meal (SBM), rapeseed meal (RM), cottonseed meal (CM), peanut meal (PM), and extruded soybean (ESB) for red swamp crayfish.

Materials and Methods

Diet preparation. Determination of nutrient and energy digestibility in feedstuffs was achieved with experimental diets composed of 70% reference diet (RF) and 30% of each of the test ingredients, on an as-fed basis, as described by Yuan et al., 2010, (Table 1).

Table 1. Ingredients composition (g per kg) of the reference and experimental diets

| Ingredients | Reference diet | Experimental diet | |
|--------------------------|----------------|-------------------|---|
| Fish meal | 100.00 | 70.00 | ^a Premix (mg or g kg ⁻¹ diet): CuSO ₄ ·5H ₂ O, 2 g; FeSO ₄ ·7H ₂ O, 25 g; ZnSO ₄ ·7H ₂ O, 22 g; MnSO ₄ ·4H ₂ O, 7 g; CoCl ₂ ·6H ₂ O, 0.1 g; Na ₂ SeO ₃ , 0.04 g; KI, 0.026 g. Vitamin E, 150 IU; Vitamin K, 50 mg; Vitamin B ₁ , 80 mg; Vitamin B ₂ , 50 mg; Vitamin B ₆ , 50 mg; Vitamin B ₁₂ , 0.02 mg; niacin acid, 150 mg; pantothenic acid, 150 mg; biotin, 1 mg; Folic acid, 10 mg; inositol, 300 mg; Vitamin C, 300 mg; Vitamin A, 10,000 IU; Vitamin D ₃ , 2,000 IU. |
| Soybean meal | 300.00 | 210.00 | |
| Rapeseed meal | 80.00 | 56.00 | |
| Cottonseed meal | 80.00 | 56.00 | |
| Flour | 310.00 | 217.00 | |
| Soybean oil | 20.00 | 14.00 | |
| Fish oil | 20.00 | 14.00 | |
| Attapulgate | 15.00 | 10.50 | |
| Salt | 3.00 | 2.10 | |
| Ecdysone premix | 1.50 | 1.05 | |
| Shrimp shell meal | 30.00 | 21.00 | |
| Calcium biphosphate | 24.50 | 17.15 | |
| Carboxyl-methy cellulose | 5.00 | 3.50 | |
| Premix ^a | 10.00 | 7.00 | |
| Y2O3 | 1.00 | 1.00 | |

The RF was formulated to contain 28% crude protein and 4.77% crude lipid. Yttrium oxide (Y2O3) was used as the inert marker at 0.1% concentration in the diet. Test ingredients for ADCs were FM, SBM, RM, CM, PM, and ESB which are common protein sources and often included in feeds for red swamp crayfish. All feed ingredients were obtained from commercial sources and subjected to chemical analysis (Table 2).

Table 2. Proximate and amino acid compositions of ingredients used to test diets (n = 3)

| | <i>Ingredients¹</i> | | | | | |
|----------------------------------|--------------------------------|------------|-----------|-----------|-----------|------------|
| | <i>FM</i> | <i>SBM</i> | <i>RM</i> | <i>CM</i> | <i>PM</i> | <i>ESB</i> |
| Dry matter | 90.22 | 89.20 | 89.88 | 89.04 | 88.79 | 90.13 |
| Crude protein | 61.12 | 43.96 | 32.30 | 43.10 | 45.63 | 33.90 |
| Crude lipid | 11.76 | 1.05 | 1.14 | 1.30 | 0.93 | 21.33 |
| Ash | 15.54 | 6.81 | 10.02 | 8.29 | 6.02 | 5.46 |
| Gross energy (kJ/g) | 14.75 | 18.22 | 16.88 | 14.62 | 14.83 | 22.56 |
| Phosphorus | 3.14 | 0.65 | 0.90 | 1.36 | 0.96 | 0.76 |
| <i>Amino acids</i> | | | | | | |
| <i>Essential amino acids</i> | | | | | | |
| Thr | 2.373 | 1.719 | 1.105 | 1.572 | 1.179 | 1.437 |
| Val | 2.591 | 1.946 | 1.211 | 1.953 | 1.581 | 1.593 |
| Met | 1.589 | 0.409 | 0.276 | 0.537 | 0.292 | 0.415 |
| Ile | 2.207 | 1.869 | 0.942 | 1.412 | 1.32 | 1.498 |
| Leu | 3.929 | 3.179 | 1.72 | 2.696 | 2.572 | 2.704 |
| Phe | 2.212 | 2.09 | 0.985 | 2.51 | 1.955 | 1.7 |
| Lys | 4.139 | 2.698 | 1.231 | 2.066 | 1.447 | 2.148 |
| His | 1.499 | 1.102 | 0.676 | 1.326 | 0.912 | 0.919 |
| Arg | 3.005 | 3.379 | 1.449 | 5.714 | 4.454 | 2.535 |
| <i>Non-essential amino acids</i> | | | | | | |
| Asp | 6.139 | 5.979 | 2.355 | 5.402 | 5.554 | 4.671 |
| Ser | 2.104 | 2.153 | 1.084 | 2.047 | 1.949 | 1.748 |
| Glu | 7.547 | 8.226 | 4.596 | 10.188 | 8.269 | 6.657 |
| Pro | 2.37 | 2.105 | 1.512 | 1.783 | 1.789 | 1.861 |
| Gly | 3.706 | 1.803 | 1.301 | 1.934 | 2.271 | 1.508 |
| Ala | 3.536 | 1.816 | 1.128 | 1.801 | 1.562 | 1.565 |
| Cys | 0.405 | 0.443 | 0.272 | 0.588 | 0.432 | 0.375 |
| Tyr | 1.779 | 1.622 | 0.982 | 1.502 | 1.587 | 1.448 |

Mean (n = 3 samples), ¹ Fish meal (native) (FM), Tong Wei Co., Ltd., Wuxi branch, Jiangsu Province, China; soybean meal (SBM), Tong Wei Co., Ltd., Wuxi branch, Jiangsu Province, China; rapeseed meal (RM), Tong Wei Co., Ltd., Wuxi branch, Jiangsu Province, China; cottonseed meal (CM), Tong Wei Co., Ltd., Wuxi branch, Jiangsu Province, China; peanut meal (PM), Tong Wei Co., Ltd., Wuxi branch, Jiangsu Province, China; extruded soybean (ESB), Nanjing Jin Xiang specific feed Company, Nanjing, China.

Various feedstuffs were ground separately, sieved through 60 µm mesh, and mixed for 15 min using a food mixer. Y2O3 was dissolved in 20 ml distilled water and the mixture was sprayed with an atomizer during mixing. Fish oil and soybean oil were then mixed into the mixture, and water added to form dough containing 15% to 20% water. The dough was then ground into 2 mm diameter feed pellets using a small feed machine, and dried in a ventilated oven at 40°C for 12 h. All diets were stored at 4°C until use.

Experimental animals and culture conditions. Experimental crayfish were obtained from Lukou fish farm of Freshwater Fisheries Research Institute of Jiangsu province, China. Prior to the experiment, red swamp crayfish (*P. clarkia*) were acclimated to the experimental conditions for 2 weeks in a 25,000-l PVC tank. During acclimation, the crayfish were fed the reference diet (RF) three times daily to satiation. Thereafter, 315 uniform-sized healthy crayfish (initial mean weight, 5.43 ± 0.21 g) were randomly distributed into 21 plastic tanks (1.0 m × 0.4 m × 0.4 m). Each tank was stocked with 15 crayfish. In order to reduce aggression between crayfish, 4 PVC tubes (15 cm length) were placed in each tank. Tanks were cleaned daily at 0700 h and 1500 h before feeding. Triplicate groups of fish were fed the reference and experimental diets by hand to visual satiety twice per day at 0800 h and 1600 h. The amount of feed supplied daily was recorded, and uneaten feed was removed from the tanks 1 h after feeding in order to avoid contamination of uneaten feed with feces. After a 7-day feeding period, feces were collected at 0900 and 1900 using a Pasteur pipette and then siphoned into a bucket and then transferred with a pipette into a glass dish. The collected fecal matter was rinsed gently in distilled water for a few seconds to remove other materials (e.g. dirt particles), then oven-dried (50°C) and stored in a sealed bag at -20°C for analysis. Successive fecal samples collected from the same tank were collected and stored in bulk in a valve bag until a sample of at least 5 g dry weight of feces had been collected. This was sufficient for the intended chemical analyses. Crayfish production was evaluated after 35 d growth trial, during which specific growth ratio, feed efficiency ratio, and survival rate were measured.

During the experimental period, aeration was also provided to maintain enough dissolved oxygen (DO). During the rearing period, water temperature ranged from 25°C-2°C, the water quality was as follows: pH 6.6-7.3, DO > 7 mg/l, NH₃ < 0.05 mg/l, H₂S < 0.01 mg/l. Growth of crayfish was measured every 2 weeks and feeding rate was adjusted accordingly. Exuviation and dead crayfish were checked daily.

Analytical methods and calculation formula. Specific growth ratio, feed conversion ratio, and survival were calculated as follows:

Specific growth ratio (SGR, %) = $100 \times (\ln W_t - \ln W_0) / t$;

Feed conversion ratio (FCR) = $F / (W_t - W_0)$;

Survival (%) = $100 \times N_t / N_0$.

where W_t = final mean weight; W_0 = initial mean weight; t = test days; F = feed consumption; N_t = final amount of fish; N_0 = initial amount of fish.

Feed ingredients, experimental diets, and fecal samples (from each tank) were analyzed in triplicate by standard methods of AOAC (1995). Samples were dried to a constant weight at 105 °C for the determination of dry matter; ash was determined by incineration in a muffle furnace at 550°C for 24 h; crude protein (% nitrogen×6.25) by the Kjeldahl system method using a BUCHI nitrogen determinator (model K-360, BUCHI Corporation, Flawil, Sweden.); crude lipid by ether extraction using Soxhlet method; gross energy by a full-automatic calorimeter (model TYHW-V, Hebi Tianyu instrument and Meter Manufacturing Co., Ltd., Hebi, China.). Yttrium oxide and phosphorus were analyzed using an inductively coupled plasma-atomic emission spectrophotometer (model Prodigy, Leeman Labs Inc, New Hampshire, USA). Amino acid concentrations were analyzed using an automatic analyzer (model S-433D, SYKAM GmbH, Munich, Germany.), after acid hydrolysis using 6 N HCl for 22 h. Tryptophan could not be measured because of its degradation during acid hydrolysis.

The ADCs for the nutrients and energy of the test and reference diets were calculated using the following formula:

ADC of nutrient (%) = $100 \times [1 - (F/D \times DY/FY)]$,

Where F = % nutrient or gross energy in feces; D = % nutrient or gross energy in diet; DY = % Y₂O₃ in diet; and FY = % Y₂O₃ in feces.

The ADCs for ingredients were calculated using the following formulae:

$D_i = (DT - rDR) / (1 - r)$;

$r = (0.3 \times WT) / (WT \times 0.3 + WR \times 0.7)$.

where D_i = apparent digestibility of nutrient or gross energy in the test ingredient; DT = the ADCs of nutrient or gross energy concentration in test diet; DR = the ADCs of nutrient or gross energy concentration in the reference diet; WT = the nutrient or gross energy concentration in the test diet; WR = the nutrient or gross energy concentration in the reference diet. In all cases the average value of each specific reference diet was used to calculate ingredient ADCs.

Statistical analysis. All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of $P < 0.05$, and the results were presented as means \pm S.D. All statistical analysis was carried out using the SPSS 16.0 for windows (SPSS, Michigan Avenue, Chicago, IL, USA).

Results

Results of the growth study showed that crayfish fed with SBM and PM diets had higher specific growth rate (SGR) and lower feed conversion ratio (FCR) (Table 3), but there was no significant difference in SGR, FCR, and survival among all groups ($P > 0.05$).

Table 3. Growth performance and survival of the reference and the test diets for red swamp crayfish

| Diets | Final mean weight (g) | Initial mean weight (g) | SGR (%) | FCR | Survival (%) |
|----------------|-----------------------|-------------------------|-----------------|-----------------|------------------|
| Reference diet | 12.01 \pm 0.84 | 5.52 \pm 0.21 | 2.22 \pm 0.09 | 1.98 \pm 0.11 | 88.89 \pm 2.78 |
| FM | 12.62 \pm 0.93 | 5.59 \pm 0.14 | 2.33 \pm 0.10 | 1.84 \pm 0.09 | 88.89 \pm 5.56 |
| SBM | 13.17 \pm 1.07 | 5.50 \pm 0.18 | 2.49 \pm 0.03 | 1.71 \pm 0.05 | 91.67 \pm 4.81 |
| RM | 11.47 \pm 1.11 | 5.52 \pm 0.20 | 2.08 \pm 0.14 | 2.11 \pm 0.15 | 88.89 \pm 2.78 |
| CM | 12.49 \pm 0.84 | 5.54 \pm 0.17 | 2.30 \pm 0.24 | 1.91 \pm 0.05 | 91.67 \pm 4.81 |
| PM | 12.99 \pm 0.87 | 5.53 \pm 0.17 | 2.44 \pm 0.12 | 1.76 \pm 0.13 | 89.68 \pm 5.20 |
| ESB | 11.77 \pm 1.02 | 5.51 \pm 0.16 | 2.16 \pm 0.13 | 2.02 \pm 0.14 | 89.68 \pm 5.20 |

Values as mean \pm S.D. of three replicate tanks and values within the same row with different letters are significantly different (Duncan's multiple-range test, $P < 0.05$).

Apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, energy, and phosphorus in the test ingredients were significantly ($P<0.05$) affected by the composition of the test ingredients (Table 4).

Table 4. ADCs (%) for dry matter, crude protein, crude lipid, energy and phosphorus of the test ingredients for red swamp crayfish

| Test ingredients | Dry matter | Crude protein | Crude lipid | Gross energy | Phosphorus |
|------------------|-------------------------|------------------------|------------------------|-------------------------|------------------------|
| FM | 66.8±0.90 ^b | 78.6±2.10 ^b | 92.1±5.00 ^d | 75.2±2.24 ^b | 40.3±1.60 ^a |
| SBM | 71.4±1.88 ^c | 82.8±1.63 ^b | 79.3±2.40 ^c | 83.9±1.51 ^c | 54.9±1.50 ^b |
| RM | 62.8±2.51 ^a | 71.2±0.07 ^a | 83.7±3.88 ^c | 70.1±2.70 ^a | 58.6±0.82 ^c |
| CM | 65.9±0.51 ^{bc} | 86.9±1.49 ^c | 73.6±3.13 ^b | 75.1±2.87 ^b | 63.5±2.25 ^d |
| PM | 69.4±1.38 ^{bc} | 90.3±2.29 ^c | 80.5±1.23 ^c | 79.3±3.67 ^{bc} | 62.4±2.30 ^d |
| ESB | 68.0±1.21 ^b | 77.3±0.83 ^b | 44.2±1.88 ^a | 83.8±0.60 ^c | 54.2±1.61 ^b |

Values as mean± S.D. of three replicate tanks and values within the same row with different letters are significantly different (Duncan's multiple-range test, $P<0.05$).

ADCs of DM were observed to be the highest in SBM (71.4%) and the lowest in RM (62.8%) ($P<0.05$). In PM, the ADCs of crude protein exceeded 90%. The protein digestibility coefficient of RM was the lowest among the treatments ($P<0.05$). The highest crude lipid digestibility was observed in FM ($P<0.05$). Differences in energy digestibility followed differences in dry matter digestibility. The highest energy digestibility coefficient was observed in SBM (83.9%), followed by ESB (83.8%), and the lowest in RM ($P<0.05$). The apparent phosphorus digestibility coefficients ranged from 40.3-63.5 ($P<0.05$). The highest phosphorus digestibility was obtained in both CM and PM, but was similar to RM. The lowest phosphorus digestibility was observed in FM ($P<0.05$).

The apparent essential amino acid (EAA) and non-essential amino acid (NEAA) availability coefficients of the test ingredients consumed by red swamp crayfish are presented in Table 5.

Table 5. Apparent availability coefficients (%) of amino acids in test ingredients for red swamp crayfish

| Ingredients | | | | | | |
|---------------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|------------------------|
| | FM | SBM | RM | CM | PM | ESB |
| Essential amino acids | | | | | | |
| Thr | 81.8±2.9 ^{bc} | 86.1±2.6 ^d | 70.8±1.5 ^a | 86.8±1.6 ^d | 81.9±0.9 ^{bc} | 80.3±1.9 ^b |
| Val | 78.4±2.1 ^b | 86.7±1.6 ^c | 69.3±1.4 ^a | 91.0±1.5 ^d | 87.0±1.4 ^c | 80.0±2.1 ^b |
| Met | 73.4±2.8 ^c | 67.6±1.4 ^b | 57.9±2.5 ^a | 77.1±1.5 ^d | 73.2±1.5 ^c | 71.1±3.0 ^{bc} |
| Ile | 76.3±2.2 ^a | 91.5±1.2 ^c | 73.7±2.1 ^a | 89.2±1.7 ^c | 88.1±2.0 ^c | 84.4±2.2 ^b |
| Leu | 79.0±2.0 ^b | 89.5±1.4 ^c | 73.6±2.8 ^a | 86.2±0.6 ^c | 87.4±1.6 ^c | 82.2±2.5 ^b |
| Phe | 79.6±2.0 ^a | 92.4±0.9 ^c | 76.7±2.5 ^a | 92.9±1.9 ^c | 94.5±1.3 ^c | 85.4±2.1 ^b |
| Lys | 90.3±2.5 ^d | 92.8±1.0 ^d | 76.5±0.8 ^a | 80.4±1.2 ^b | 83.8±1.3 ^c | 91.1±1.2 ^d |
| His | 89.4±1.7 ^{bc} | 92.0±3.1 ^{cd} | 78.6±0.7 ^a | 92.5±2.5 ^d | 88.3±1.9 ^b | 88.7±2.7 ^{bc} |
| Arg | 87.7±1.1 ^c | 92.8±2.4 ^b | 83.9±2.6 ^a | 96.0±2.5 ^c | 99.7±2.4 ^d | 85.9±1.8 ^b |
| Non-essential amino acids | | | | | | |
| Asp | 91.4±2.6 ^b | 98.1±1.4 ^c | 73.9±2.7 ^a | 99.4±1.4 ^c | 98.5±0.7 ^{bc} | 92.1±2.1 ^b |
| Ser | 78.5±2.6 ^b | 89.4±1.5 ^d | 74.7±2.1 ^a | 90.2±0.9 ^d | 88.9±0.6 ^d | 83.3±2.4 ^c |
| Glu | 82.1±1.5 ^a | 90.4±0.6 ^b | 83.4±3.2 ^a | 89.6±0.6 ^b | 91.0±0.4 ^b | 85.2±2.4 ^a |
| Pro | 81.5±1.5 ^b | 88.9±2.4 ^c | 76.3±1.1 ^a | 88.8±0.4 ^c | 90.2±0.8 ^c | 89.3±2.7 ^c |
| Gly | 82.1±2.0 ^c | 79.3±1.9 ^b | 74.8±1.9 ^a | 85.0±0.7 ^d | 78.4±0.4 ^b | 75.3±2.0 ^a |
| Ala | 86.3±2.8 ^{cd} | 83.1±2.3 ^c | 69.2±1.6 ^a | 89.5±1.1 ^d | 85.2±1.6 ^c | 78.0±2.4 ^b |
| Cys | 66.4±3.0 ^a | 77.1±1.4 ^c | 70.1±1.0 ^b | 96.8±0.4 ^f | 86.1±2.5 ^e | 82.4±2.6 ^d |
| Tyr | 66.2±0.4 ^{bc} | 71.8±1.6 ^d | 51.4±1.4 ^a | 69.0±3.3 ^{de} | 67.3±3.1 ^{bc} | 64.7±1.2 ^b |

Values as mean± S.D. of three replicate tanks and values within the same row with different letters are significantly different (Duncan's multiple-range test, $P<0.05$).

In general, amino acid (AA) availability reflected crude protein digestibility; however, there were major differences in the availability of different EAAs and NEAAs in some ingredients. The ADCs of EAAs and NEAAs were higher for SBM and PM, than ESB. The availability of EAAs and NEAAs in RM was the lowest among the treatments except for Cys ($P<0.05$). The ADCs of several amino acids, such as Arg, Asp, and Glu were generally high in all ingredients tested; their availability in PM was 99.7%, 98.51%, and 91.0%, respectively.

Discussion

In the present study, there was no significant difference in SGR, FCR, and survival rate among all groups ($P>0.05$), which indicated that the plant protein ingredients in this study replaced up to 30% of the FM without any adverse effect on crayfish performance. Several researchers also reported that there were no adverse effects when FM was replaced by plant products in crustaceans. The differences in SGR, FCR, and survival were not statistically significant for crab fed the diet with FM replaced by 30% of SBM and RM (Luo et al, 2012). The combination of SBM and CM produced good performance of juvenile kuruma shrimp, in which dietary FM was reduced by 30% without compromising growth, feed utilization and survival (Bulbul et al. 2013). This might be due to the blend of plant protein products which is sometimes beneficial to balance the nutrient profile, such as amino acids, allowing comparatively higher FM replacement in crustacean diets (Luo et al., 2011).

The ADCs of feed ingredients provide insight into nutrient utilization and evaluation of their suitability for a specific species. Stripping and dissection are common methods of collecting fecal material from fish for determining digestibility; however, these are not feasible methods in crustaceans (Smith and Tabrett, 2004). Nutrient loss from feces in the period between defecation and collection can cause an overestimation of digestibility. However, according to our observations and unpublished data, the values for the reference and experimental diets after extrusion remained stable up to 3 h of water exposure. In our study, uneaten feed was siphoned out 1 h after feeding. This short immersion time may result in leaching of nutrients from diets but it should not be significant. Cr₂O₃ and Y₂O₃ are common inert markers when determining digestibility. Various inert markers (including Cr₂O₃, Y₂O₃, and Ho₂O₃) on apparent digestibility in salmonids were compared, and it has been suggested that Y₂O₃ can be substituted for Cr₂O₃ in digestibility studies and can be used at lower concentrations without affecting accuracy (Austreng et al. 2000). In the present study, we found that siphoning, and using Y₂O₃ as the inert marker for measuring nutrient digestibility, to be reliable for red swamp crayfish.

DM digestibility coefficients provided a better estimate of the quantity of indigestible material present in feed than digestibility coefficients for individual nutrients (Brunson et al., 1997). In the present experiment, DM digestibility was highest in SBM (71.4%) and PM (69.4%), followed by ESB (68.0%); differences in energy digestibility followed differences in dry matter digestibility. In this study, ADCs of dry matter, and energy in FM, were significantly lower than those of SBM, PM and ESB (which had lower fiber and ash content). This trend may be due to high content of ash (10.02%) in FM, agreeing with results reported by Ming et al. (2012), Köprücü and Özdemir (2005). The low dry matter and energy digestibility of other plant-protein ingredients tested, such as RM and CM, appear to be related to their high fiber content, which is not utilized by crayfish (NRC, 1993). Several other studies also reported low dry matter and energy digestibility in plant proteins with high carbohydrate and fiber contents (Lee, 2002; Luo et al., 2008). The availability of dry matter and energy for ESB was high. This may be attributed to the anti-nutritional factors that were partially destroyed through fermentation (Yuan et al., 2010).

Protein quality of dietary ingredients is the leading factor affecting fish performance, and the ADC of protein is the first measure of its availability in fish (Lee, 2002; Köprücü and Özdemir, 2005). In this study, the protein digestibility of most feedstuffs was high and ranged from 71.2-90.3%, suggesting the protein of FM, SBM, RM, CM, PM, and ESB was highly digestible in red swamp crayfish. Good feed with high protein content can promote utilization of dietary protein for shrimp (Halver and Hardy, 2002). However, protein digestibility was not correlated with protein content (Lee 2002). In our study, protein digestibility of PM and SBM was higher than that of FM which had the highest protein content. According to our observations and unpublished data, growth performance showed a similar trend. Different experimental conditions such as species, the frequency of feeding, processing treatments, feces collection, and digestible protein compounds of ingredients all contribute to variability in estimates of protein digestibility.

Protein quality of dietary protein sources depends on amino acid composition and availability (Lee, 2002). Although individual amino acid availability within a feed ingredient are variable, the data presented in this study suggest that the availability coefficients of EAAs and NEAAs tended to reflect the apparent protein digestibility coefficients of feed ingredients; this data is similar to data from other reports (Yuan et al., 2010; Zhou and Yue, 2012; Li et al., 2013). In this study, protein digestibility coefficients of SBM, CM, and PM showed that the highest values of availability for most amino acids, indicating some AAs of the test ingredients examined were efficiently utilized. High apparent digestibility of AAs has been reported for many aquatic animals (Terrazas-Fierro et al., 2010; Oujifard et al., 2012). On the other hand, ADCs of most amino acids were lowest in RM, in agreement with results reported by Luo et al. (2008). Among the ingredients tested, availability of several amino acids was also low, i.e., ADCs of Lys were 76.5, 80.4 and 83.8% in RM, CM, and PM, respectively demonstrating the importance of AA supplementation in soybean-based diets. Moreover, Met availability in SBM and His in PM were significantly lower than protein digestibility values for respective test proteins. There were large variations in availability of individual AAs within a protein source and among protein sources (Luo et al. (2008); Yuan et al. (2010), although there was no significant difference in protein digestibility between the protein sources. The above results suggest that ADCs of amino acid are more useful than protein digestibility values for comparison of protein quality.

Aquatic animals can utilize lipids efficiently (Zhou et al., 2004). In the present study, red swamp crayfish digested lipids in all feed ingredients tested, although the ADCs of lipids in ESB were significantly lower than others. This may be due to its high content of unsaturated fatty acids. The apparent phosphorus digestibility coefficients of FM (40.3%), SBM (54.9%), RM (58.6%), CM (63.5%), PM (62.4) and ESB (54.2%) for red swamp crayfish in the current study are higher than in previous studies with Nile tilapia (Köprücü and Özdemir, 2005), Chinese sucker (Yuan et al., 2010) and Senegalese sole (Dias et al., 2010), but lower than juvenile cobia (Zhou et al., 2004). Part of the variability in ADCs of phosphorus may be explained by differences in origin, processing of these various feed ingredients, and methods of feces collection. Moreover, in this study, the red swamp crayfish fed on FM protein sources had a lower ADC of phosphorus (40.3%) than crayfish. However, the apparent phosphorus digestibility coefficients of fish meal were significantly higher than those of plant products in juvenile white shrimp (Yang et al. 2009).

Although this study showed that red swamp crayfish can digest SBM very well, observed intestinal inflammation could have been caused by excessive feeding with SBM (unpublished data). This should be considered in diet formulation of red swamp crayfish.

In summary, red swamp crayfish appear to have the capacity to effectively digest a wide variety of ingredients of very different nutritional qualities. Moreover, great variability in the ADCs of dry matter, crude protein, crude lipid, phosphorus, energy, and amino acids of six feedstuffs was found. These variables could aid in preparing nutritionally balanced and cost-effective diets for intensive culture of red swamp crayfish *P. clarkia*.

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