Potential of Cowpea (*Vigna unguiculata* L.) Meal as an Alternative Protein Source in Diets for Giant Freshwater Prawn (*Macrobrachium rosenbergii*, de Man 1879)

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Abstract

Growth trials were conducted to evaluate cowpea *Vigna unguiculata* (L.) meal as a potential protein source in diets for giant freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879), reared in tank and lake-based cages. Five isonitrogenous (approximately 37% crude protein) and isocaloric diets were formulated where fish meal (FM) protein was replaced with 0%, 15%, 30%, 45% and 60% cowpea meal protein (or CP0, CP15, CP30, CP45, and CP60, respectively). Results of an 8-week tank trial showed that the final body weight (FBW), percent weight gain, specific growth rate (SGR) and survival of prawns were not significantly influenced by dietary treatments ($P > 0.05$), although the highest values, except for survival, were observed with CP45. In a lake-based cage trial that lasted for 16 weeks, prawns fed CP30 and CP45 had significantly higher FBW (13.1 and 14.4 g, respectively) compared to other treatment groups ($P < 0.05$). SGR (4.52–5.00%/day), survival rates (53-77%), yield (98.5-116.5 g m-2) and feed conversion ratio (FCR; 2.0-2.7) were not affected by increasing levels of cowpea meal in the diets. Based on these results, cowpea meal can be considered as an alternative protein source in diets for *M. rosenbergii*.

Keywords: *Vigna unguiculata*, giant freshwater prawn, growth, Laguna de Bay

Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii* de Man) is an economically important species for aquaculture in Asian countries such as China, India, Thailand and Malaysia. It is a promising alternative to black tiger shrimp (*Penaeus monodon*) due to its high market value and relatively low susceptibility to diseases. Presently, in the Philippines, farming of *M. rosenbergii* in natural inland water bodies such as lakes and reservoirs could be a sustainable option for the growth of aquaculture in lake-shore fish farming communities (Cuvin-Aralar *et al.*, 2007), similar to other well-known species such as bighead carp *Aristichthys nobilis*, milkfish *Chanos chanos* and Nile tilapia *Oreochromis niloticus*. Farming of this species requires a nutritionally-balanced diet for optimal growth and survival. However, the rising cost of feed hinders profitability of production due to the use of expensive protein sources such as fishmeal (McCoy, 1990; Tacon and Metian, 2015). Feed constitutes 40-60% of the operational costs for *M. rosenbergii* culture (Mitra *et al.*, 2005). Therefore, assessment of locally available sources such as plant proteins for use in feed formulations needs to be tapped and explored.

Among the alternative protein sources for fishmeal, cowpea (*Vigna unguiculata* (L.)) meal has been used to replace fishmeal in crustacean diets because of its high nutritional value and digestibility (Eusebio, 1991; Eusebio and Coloso, 1998; Rivas-Vega *et al.*, 2006). An important legume crop in the Philippines and in other Southeast Asian countries, cowpea seeds are known for their crude protein content of 23–26%, high levels of essential amino acids such as lysine and tryptophan and digestible energy. Likewise, the successful use of *V. unguiculata* has been reported for tilapia feeding (Keembiyehetty and de Silva, 1993; Olvera-Novoa *et al.*, 1997). The present study evaluated the response of *M. rosenbergii* to diets containing cowpea meal (*Vigna unguiculata*).

Materials and Methods

Experimental Diets

The chemical composition of *Vigna unguiculata* is shown in Table 1. Five experimental diets were formulated by replacing 0%, 15%, 30%, 45% and 60% of the FM protein with cowpea meal (CP0, CP15, CP30, CP45 and CP60). All diets were formulated to be isonitrogenous (approximately 37% dietary protein) and isocaloric. The experimental diets were tested in both tank and lake-based feeding trials.

Feeding trials

Tank trial

Fifteen day-old postlarvae (0.029 ± 0.008 g mean weight) were stocked in 60-l polyethylene tanks at 15 prawns per tank and acclimatized for one week prior to actual feeding trial. Tanks were half-filled with freshwater which was maintained throughout the experiment. Tanks were provided with nets as substrates where PL

Table 1. Proximate composition (% dry matter) of cowpea meal *Vigna unguiculata.*

	Cowpea meal
Moisture	
Crude protein	23.03
Crude fat	0.28
Crude fiber	4.38
NFE*	68.65
Ash	3.66

*Nitrogen Free Extract

adhered after feeding. Experimental diets were fed at 20–30% of estimated biomass three times daily at 0800, 1300, and 1600 h for an eight-week period. Each diet treatment was replicated thrice. Water temperature, dissolved oxygen (DO) and pH ranged from 26.3–28.4°C, 5.62–8.88 mg L^{-1} and 8.6–9.4, respectively during the rearing period.

Lake-based cage trial

Postlarvae (PL20) were stocked in hapa net cages (L × W × H: 2 × 2 × 1.5 m) in Laguna de Bay with 15 shrimps m^{-2} (0.04 ± 0.01 g body weight) and three replicate cages per treatment. Each cage was provided with two used A-nets (mesh size: 2 mm^2; dimension: 0.5 × 2.0 m) as shelters and suspended horizontally inside each cage. The prawns were fed experimental diets once daily (0900h) at 10, 8, 6 and 4% of estimated biomass for the 1st, 2nd, 3rd and 4th month of culture (Millamena and Triño, 1997). Total length, individual weight, weight gain and survival were monitored monthly. Production parameters such as final weight, percent weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival were used to evaluate the acceptability of cowpea meal in diets for *M. rosenbergii*.

Water quality was monitored inside the cages between 0800 and 0900 hours for the duration of the experiment. DO levels ranged from 3.47–6.95 mg L^{-1} and temperature was noted between 25.8–28.4°C. pH readings varied from 7.5–8.4 during the trial period.

Data analysis

The results for growth, feed conversion ratio (FCR) and survival were analyzed using one-way ANOVA followed by Tukey's post hoc test when significant differences were detected. Survival data were arcsine transformed prior to statistical analysis. All statistical tests were performed using the Number Cruncher Statistical System (NCSS 07.1.4 version) 2007 Software (Hintze, 2007).

Results

Tank trial

The results for survival and growth after an eight-week tank trial are shown in Table 2. The experimental diet CP45 gave the highest mean weight, percent weight gain and SGR, but there were no differences among treatments ($P > 0.05$). However, a gradual decrease in growth performance was observed at CP60. Survival rates ranged from 83 (CP45) to 93% (CP15) and no significant difference was detected among treatments.

Lake-based Trial

FBW ranged from 10.1 to 14.4 g with significantly higher FBW at CP30 and CP45 compared to other treatments ($P < 0.05$). SGR (4.52–5.00%/day) and survival rates (53.4–77.2%) did not differ significantly among treatments. The experimental diet CP60 gave the best survival rates but the poorest FBW and SGR among the experimental diets. Production ranged from 98.5 g m^{-2} (CP0) to 116.2 g m^{-2} (CP60) and feed conversion ratio (FCR) between 2.00 (CP30) and 2.72 (CP60). No significant differences were found among treatment means for yield and FCR (Table 3).

Table 2. Growth and survival parameters monitored in *Macrobrachium rosenbergii* postlarvae fed diets with varying levels of cowpea *Vigna unguiculata* meal for 8 weeks during the tank trial.

Diets	Parameters			
	FBW (g)	Weight gain (%)	SGR (% d^{-1})	Survival (%)
CP0	0.230 ± 0.045[a]	693 ± 155[a]	3.43 ± 0.35[a]	88.3 ± 7.6[a]
CP15	0.249 ± 0.038[a]	759 ± 129[a]	3.57 ± 0.24[a]	93.3 ± 5.8[a]
CP30	0.271 ± 0.064[a]	833 ± 221[a]	3.69 ± 0.40[a]	91.7 ± 7.6[a]
CP45	0.288 ± 0.037[a]	892 ± 128[a]	3.81 ± 0.21[a]	83.3 ± 11.5[a]
CP60	0.208 ± 0.029[a]	617 ± 99[a]	3.27 ± 0.24[a]	90.0 ± 5.0[a]

FBW = final body weight, SGR = specific growth rate
Initial prawn weight, 0.029 ± 0.008 g; [1]SGR = (ln wt_{final} – ln $wt_{initial}$)/days of culture × 100
Survival = actual count at harvest/initial stock × 100
Column means followed by different letter superscripts are significantly different at P < 0.05

Table 3. Production parameters for *Macrobrachium rosenbergii* fed diets with varying levels of cowpea *Vigna uinguiculata* meal for 16 weeks during the lake-based cage trial.

Diets	Parameters				
	FBW (g)	SGR (% d^{-1})	FCR	Survival (%)	Yield (g m^{-2})
CP0	10.6 ± 1.03[a]	4.55 ± 0.13[a]	2.36 ± 0.12[a]	62.2 ± 8.39[a]	98.5 ± 4.04[a]
CP15	11.1 ± 1.18[a]	5.00 ± 0.57[a]	2.13 ± 0.26[a]	62.8 ± 0.96[a]	104.0 ± 9.56[a]
CP30	13.1 ± 0.42[b]	4.97 ± 0.24[a]	2.00 ± 0.20[a]	55.0 ± 7.64[a]	108.4 ± 15.4[a]
CP45	14.4 ± 2.92[b]	4.97 ± 0.31[a]	2.18 ± 0.20[a]	53.4 ± 4.24[a]	109.3 ± 32.5[a]
CP60	10.1 ± 0.76[a]	4.52 ± 0.22[a]	2.72 ± 0.04[a]	77.2 ± 8.22[a]	116.2 ± 9.03[a]

Column means followed by different letter superscripts are significantly different at P < 0.05

Discussion

The present study was conducted to evaluate the potential use of *Vigna unguiculata* as an alternative protein source in diets for *M. rosenbergii*. Based on chemical composition, cowpea meal has a high nutritional value (23% crude protein). Likewise, the nitrogen free-extract (NFE) or the carbohydrate content of *V. unguiculata* showed that it can be an excellent source of energy in crustacean diets.

In terms of biological performance, results of the tank study indicated that the growth performance of *M. rosenbergii* PL fed the control diet was inferior to prawn fed cowpea meal-based diets. SGR, in particular, was comparable to or even higher than the findings of Du and Niu (2003) who achieved an SGR of 2.5% day^{-1} when soybean meal was used to replace FM in diets for the same species. Growth performance improved with increasing levels of cowpea meal protein, but the best results were obtained at 30 to 45% substitution. However, the inclusion above 45% resulted in diminished performance in terms of mean FBW, suggesting that mixing of cowpea meal with low levels of FM protein may have contributed to the slower growth of *M. rosenbergii* PL. Cowpea meal contains several inherent anti-nutritional factors such as trypsin inhibitor which may interfere with feed utilization. This however, may not be the case as the trypsin inhibitor activity (TIA) in the cowpea meal ranged from 23.7–31.6 TIU/mg protein as reported by Ologhobo and Fetuga (1984) and Rivas-Vega *et al.,* (2006) and even lower than those reported for soybean meal (106 TIU/mg of sample) (Kakade *et al.,* 1974), suggesting minimal impacts of any trypsin inhibition. On the other hand, while analysis of essential amino acids (EAAs) of experimental diets have not been determined, high inclusion level of cowpea meal at 60% is likely to be limiting in EAAs such as methionine and has resulted in poor growth . Survival rates (83–93%) of *M. rosenbergii* PL in the present tank study were comparable to or slightly lower than those reported by Roy *et al.,* (2009).

Similar trends in growth were also observed in the lake-based feeding trial with cowpea meal-based diets performing better than the control diet (CP0), which has FM and shrimp meal (*Acetes* sp.) as protein sources. *M. rosenbergii* are omnivore species which can efficiently digest both plants and animal protein sources (Ashmore *et al.,* 1985). This explains the best growth performance at CP45 experimental diet in this study. However, the mean weights of the prawns fed the best performing diet (CP45; 14.4 g) after 120 days are considerably lower than those obtained by Cuvin-Aralar *et al.,* (2007) for similar stocking density (15 prawns m^{-2}; the mean weight after 150 days is 26.3 g), but comparable with the mean sizes at higher stocking density (90 prawns m^{-2}; the mean weight at harvest is 14.3 g). Differences in the final size or weight at harvest were attributed to the initial size of PLs used at the start of the experiment (0.04 g, this study vs. 0.40 g in Cuvin-Aralar *et al.,* (2007). Nevertheless, SGRs obtained in the present study (4.52–5.00% d^{-1}) were considerably higher than those obtained by Cuvin-Aralar *et al.,* (2007) (2.68–3.02% d^{-1}) and Ghosh *et al.,* (2010) (3.55–3.75% d^{-1}).

The better survival of prawns in cages at CP60 (77.2%) followed by CP15 (62.8%) and CP0 (62.2%) has resulted to smaller size of prawns at harvest (10.1, 10.65 and 11.1 g for CP60, CP0 and CP15, respectively). Conversely, the relatively lower survival at CP30 (55.0%) and CP45 (53.4%) produced larger prawns (13.1–14.4 g), which were comparable to or even higher than those obtained by Cuvin-Aralar *et al.,* (2007), who reported survival rates ranging from 36.9–55.3%. Lower survival rates achieved in these diets maybe attributed to heterogeneous individual growth (HIG), possible entry of predators and competitors inside the experimental cages, and cannibalism (Ranjeet and Kurup, 2002; FAO, 2002; Cuvin-Aralar *et al.,* 2007). Nonetheless, FCRs obtained in the present study were comparable to those reported in other studies (FAO, 2002; Cuvin-Aralar *et al.,* 2007). Shrimp production varied from 98.5 to 116.2 g m^{-2}, which is generally higher than those reported in pond culture in Asian countries such as India (12–45 g m^{-2}) (Ghosh *et al.,* 2010).

In summary, the present study shows the potential of cowpea (*Vigna unguiculata*) meal as an alternative protein source in diets for *M. rosenbergii*. Cowpea meal can replace FM at 30–45% inclusion level with no adverse effects on growth and production of this species reared under laboratory and lake-based conditions.

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