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Nutritive value of termite as fish meal supplement in the diet of freshwater prawns (*Macrobrachium rosenbergii* de Man) juveniles

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Abstract. A six-week feeding trial was conducted in a recirculating system in the laboratory to evaluate termite meal as a possible replacement for fish meal as a protein source in the diet of *M. rosenbergii* juveniles. Four experimental diets of varying percentages of Peruvian fishmeal/termite meal; 100/0%, 65/35%, 35%/65% 0/100% as Diets 1, 2, 3, 4, respectively, were used. The results showed a decreasing order in growth with increasing termite meal. Significantly the best specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) were exhibited by prawns fed diet 1 (100% Peruvian fishmeal) while diet 2 (65% FM/35% TM) resulted in second best values and were significantly different and the least values were recorded in diet 4 (0% FM/100% TM). Using the tail muscle of juvenile *M. rosenbergii* as reference protein, the EAAI of the termite was found to be 0.85% while that of the Peruvian fish meal to be 0.93. Termite (*Nasutitermes* sp.) meal was not adequate to be used solely as protein source for *M. rosenbergii* juveniles, however, it can still be considered for amino acid supplementation since its inclusion in the diet can support growth and better survival.
Key Words: termite meal, *Macrobrachium rosenbergii*, protein source, protein replacement.

Introduction. *Macrobrachium rosenbergii* is an omnivorous freshwater crustacean that consumes a wide variety of plants and animals, either living or decomposing, and also accepting balanced artificial diets. Favorable production technology has been available for over 50 years (Pérez-Fuentes et al 2013). Over the past 20 years farming techniques for *M. rosenbergii* have been studied and developed throughout the world (New 2000). The freshwater prawn larvae can be raised on a diet of only *Artemia nauplii* (Devresse et al 1990; Lavens et al 2000). It is the largest of the freshwater prawns and has been classified as a benthophagic omnivore based on analysis of foregut contents (Cohen & Ra'anan 1983; Weidenbach 1980).

Soybean meal (SBM) is probably the most promising and most studied alternative protein source to replace fish meal (FM). Use of SBM as a substitute for FM for many fish and marine crustaceans has been reported (Davis & Arnold 2000; Tacon & Akiyama 1997; Webster & Lim 2002). However, the presence of various anti-nutritional factors such as protease inhibitors, lectins, antigenic or estrogenic factors, oligosaccharides etc. Liener (1989) limits its use in crustacean diets. Also, most if not all of the soybean supply in the Philippines is imported and thus presents problems of fluctuating prices and unsustainable supply. Other potential replacements, whether partial or full, should also be evaluated. Termite could be one of alternative protein feedstuff that has yet to be evaluated in the diet of crustaceans.

Termites are social insects that swarm seasonally especially at the onset of rainy season or after heavy rainfall (Sogbesan & Ugwumba 2008b). The long winged reproductive termite is edible and highly sought after as a delicacy. During swarming, a lot of these termites are wasted and could be utilized for fish feed production since fish had been reported to consume them live when they fall into fish pond (Madu et al 2003). Termite is a regular feed ingredient in fish farms in Cambodia and Thailand (Sermwatanakul et al 2004) and in Nepal (SUPPORT 2009). The nutritive potential and utilization of this insect as crustacean feed ingredient have not been adequately documented. Thus, the objective of the present study was to evaluate the nutritive value of termite *Nasutitermes* spp. as a cheaper replacement for fish meal in the diet of the giant freshwater prawn *Macrobrachium rosenbergii* De Man.

Material and methods

Termites. *Nasutitermes* spp, were collected from a dilapidated structure at the Miagao Campus of the University of the Philippines Visayas. About 2 kg of the nest was taken at a time and washed twice in a basin half-filled with water. Those that floated were collected by scoop, blotted dry and kept frozen until use. The termites were a mix of soldiers, workers and a few queens, both mature and immature ones.

Chemical analysis of the termites. About 25 g of frozen termites were freeze-dried and used for proximate analysis (AOAC 1984). Crude protein was analyzed using Kjeldahl method, moisture by HH53 Halogen moisture analyzer (Metler Co.). The meal was also subjected to amino acid analysis in a high-performance liquid chromatography (HPLC) with *o*-phthaldialdehyde (OPA) as hydrolyzing agent. Approximately 3 mg termite meal was hydrolyzed in 1 mL 6N HCl at 110 ^{o}C for 24 h (Penaflorida 1989). Pierce reacti-therm heating module and hydrolysis tubes (10 x 150 mm) were used for hydrolysis. The hydrolysate was made up to 5 mL with pH 2.2 sodium buffer and passed through a 0.45 mm filter. Samples of each hydrolysate were analyzed using stepwise elution of either lithium or sodium buffers in a Shimadzu HPLC-10A post-column derivatization with fluorometric detection using o-phthalaldehyde/N-acetylcystine reagent. EAAI value was calculated by Oser equation (1951) *cit.* Mitchell & Block (1946) with mathematical model:

$$\log \text{EAAI} = \frac{1}{10}\log\left(100\times\frac{A_1}{At_1}\right)+\log\left(100\times\frac{A_2}{At_2}\right)+\Lambda\ \log\left(100\times\frac{A_n}{At_n}\right)$$

Where A1: first, ...(1,2,3,4...n) essential amino acid rate from experiment sample
At1: first, ...(*1,2,3,4...n*) essential amino acid rate from standard (usually from whole chicken egg but in the present study, the *M. rosenbergii* tail muscle determined by Farmafarmaian & Lauterio (1980) was used as the standard.

Feed formulation and preparation. Frozen termites were thawed, oven-dried at 60^{o}C for 24 h, pulverized in a grinder (Cyclotec, Tecator Hoganes, Sweden) and sieved through a 350-µ mesh. All the other ingredients were also powdered and sieved. Table 1 shows the composition and proximate composition of the experimental diets. All the powdered ingredients were mixed thoroughly after which slurry of cooked bread flour was added, mixed in an electric mixer. The dough formed was flattened on trays to about 0.5 mm thick and were cut into squares (~4 cm^2), steamed for 5 min, air-dried for 20 min and oven-dried at 60^{o}C for 24 h. The dry feeds were ground into crumbles and sieved to about 0.5 mm diameter sizes and kept at 4^{o}C in a refrigerator until use.

Weaning postlarvae. The feeding trial was done at Philippine National Freshwater Fisheries Technology Research Center at Munoz, Nueva Ecija, Philippines. Four hundred postlarval *Macrobrachium* (15-day old postlarvae (PL-15), 0.01-0.03 g) were distributed randomly into 12 epoxy-painted wooden chamber (0.14 m^3 water volume) in which pieces of bamboo serve as refuge. They were fed the control diet (Diet 1) 4-5 times daily between 0700 to 2200 h. Cleaning and water change (50%) were done every morning before feeding. The postlarvae were weaned for 18 days to PL 33 with a size range of 0.02-0.13 g and total length range of 11-32 mm.

Experimental set up. The feeding trial was done in a recirculating system with an average water flow rate of 5 mL sec^{-1} under continuous aeration. Bamboo twigs and PVC pipes cut into 6 cm served as refuges in the various chambers. Eighteen PL 33 were stocked in each chamber filled with 20L of water. The experiment was conducted using a randomized complete block design (CRBD). The animals were grouped into similar average sizes due to variability and the group were assigned to each treatment randomly. There were 4 treatments in 4 blocks. All water quality parameters were relatively constant throughout the feeding trial period (Table 2).

Table 1

Composition and proximate composition of the experimental diets

Ingredient	*Diet 1 (g)*	*Diet 2 (g)*	*Diet 3 (g)*	*Diet 4 (g)*
Fishmeal (Peruvian)	48.09	31.26	16.83	0
Termite meal	0	21.60	40.12	61.73
Bread flour	8.00	8.00	8.00	8.00
Rice bran	12.00	12.00	12.00	12.00
Acetes meal	4.00	4.00	4.00	4.00
Cod liver oil	2.00	2.00	2.00	2.00
Soybean oil	2.00	2.00	2.00	2.00
Vitamin and mineral mix*[1]	4.00	4.00	4.00	4.00
Dextrin	14.86	10.09	6.00	1.22
Carageenan	5.00	5.00	5.00	5.00
Ethoxyquin	0.05	0.05	0.05	0.05
Total	100.00	100.00	100	100.00
Proximate composition	*%*	*%*	*%*	*%*
Crude protein	38.45	38.58	39.76	39.44
Crude fat	9.32	12.58	13.8	14.87
Crude fiber	1.54	3.87	6.48	8.06
Ash	16.60	12.85	10.14	9.50
NFE	34.09	32.14	29.82	28.13
Estimated gross energy (kcal kg^{-1})*[2]	3780	3979	4025	4041

*[1] Composition (mg 100 g^{-1}): Vitamin A (beta carotene 5000 IU; Vit. D, 4000 IU; Vit. E, 30 IU; Vit. K, 25 µg; Vit. B1, 1.5 mg; Vit. B2, 1.7 mg; Niacinamide 20 mg; Vit. B6, 2 mg; Vit. B12, 6 µg; Vit. C, 60 mg; Folic acid, 400 µg; Biotin, 30 µg; Pantothenic acid, 10 mg; Calcium, 162 mg; Phosphorus, 125 mg; Iodine 150 µg; Iron, 18 mg; Magnesium, 100 mg; Copper, 2 mg; Zinc, 15 mg; Manganese, 2.5 mg; Potassium, 40 mg; Chloride, 36 mg; Chromium, 25 µg; Molybdenum, 25 µg; Selenium 25 µg; Nickel, 5 µg; Tin, 10 µg, Vanadium, 10 µg.

*[2]Energy was estimated using the ff. physiological fuel values: 4 kcal g^{-1} crude protein, 9 kcal g^{-1} crude fat and 4 kcal g^{-1} carbohydrate.

Sampling. Sampling and cleaning of the chambers were done every 14 days; the shrimp were bulk-weighed and the length of selected samples was measured. Weight gain was expressed as mean ± standard error of the mean (SEM). Food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), condition factor (K) were calculated on a per fish basis as follows:

$$\text{FCR} = \frac{feed\ consumed\ (g)}{wet\ weight\ gain\ (g)}$$

$$\text{SGR (\% day)} = \frac{(\ln final\ weight\ - \ln initial\ weight) \times 100}{feeding\ period}$$

$$\text{PER} = \frac{\textit{wet weight gain by prawn}}{\textit{protein}\ \text{intake}}$$

$$\text{K} = \frac{\textit{weight}\,(g)}{\text{L}^3\ (\text{cm}^3)} \times 1000$$

Statistical methods. All data were analyzed using analysis of variance (ANOVA) in a randomized complete block design (RCBD) and if significance was detected, Duncan's Multiple Range Test (DMRT) was used to compare treatment means ($P<0.05$). Standard error of the mean (SEM) was calculated for all mean values.

Results and Discussion. Water temperature range measured was 28-30 ^oC, pH 8.0-8.5, dissolved oxygen 5.0-6.2 mg L^{-1}, nitrate below 0.1 mg L^{-1}, phosphate 0.5 mg L^{-1} and all the rest such as copper nitrate, and iron were all below detection. Thus, water quality parameters were within the optimum range for *M. rosenbergii* postlarvae.

The crude protein content of the termite meal in the present study was high (55.4 %) but was lower than in the Peruvian fish meal (Table 2). The level in the present study was higher than that from the same genus of termite (*Nasutitermes* spp.) (44.12 %, Oyarzun et al 1996; 43.65 %, Oyarzun et al 1996) and from other species of termites: 46.3 % in *Macrotermes subhyalinus* (Sogbesan & Ugwumba 2008b), 37 % and 48.80 % for *Macrotermes* spp. (Aduku 1993; Fadiyimu et al 2003), 41.80% for *Macrotermes falciger alates* (Phelps et al 1975), 20.4% and 22.1% in *Macrotermes bellicosus* and *M. notalensis*, respectively (Banjo et al 2006). In contrast to the crude protein content, crude fat was higher in the termite meal than in fish meal in the present study. The lipid content analysis of this animal confirms the work of Sogbesan & Ugwumba (2008b), Oyarzun et al (1996) and Aduku (1993) that termites contain very high amounts of lipid. As indicated by the very low ash content, termite meal was poor in mineral composition. This result was in accordance with the report of Barker et al (1998) that insects are low in major mineral compositions most especially in calcium and phosphorus. Ash content of the termite meal was more than three times lower than that of fish meal but the nitrogen-free extract was more than twice higher in the termite meal.

Table 2

Proximate analysis of termite *(Nasutitermes spp.)*

Proximate composition (%, dry matter basis)	*Dried termite meal*	*Fish meal (Peruvian)*
Crude protein	55.4	71.1
Crude fat	14.8	9.8
Crude fiber	15.5	0.9
Ash	4.3	13.9
Nitrogen-free extract	10.0	4.3
Total	100.0	100.0

At the end of the six-week feeding, survival was low and exhibited no significant difference among dietary treatments (Table 3). Initial body weight of the larvae was statistically similar in all the dietary experiment. Final ABW, weight gain and SGR were significantly highest in prawns fed the control diet (100% FM, 0% TM) followed by those fed Diet 2 (65% FM and 35% TM) and those fed Diets 3 and 4 (35 % FM, 65% TM and 100% TM, 0% FM, respectively) showed the lowest values and were statistically similar. Prawns fed the diet containing 100% fish meal exhibited significantly the highest FCR followed by those fed Diet 2, while those fed Diets 3 and 4 exhibited significantly the lowest FCR. PER was higher in prawns fed Diet 1 and all diets containing the termite meal exhibited lower values and were statistically similar with each other. Condition factor was higher in all prawns fed diets containing the termite meal while those fed containing

100% fish meal showed significantly the lowest condition factor. This meant that prawns fed diets containing the termite meal developed a more ‘cubic’ body than did those fed the control diet.

The least inclusion of the termite meal at 35% resulted in a lower growth performance in the prawn than did the Peruvian fish meal diet and poorer results were exhibited when the inclusion was increased. One very important factor among several that should be considered was the termite meal bioavailability since a high crude fiber value of 15.5 % of the termite meal suggested high chitin (complex carbohydrate) content, due to the termite exoskeleton, which could be poorly digested by prawns. Oyarzun et al (1996) have made the observation that the crude protein values are based on determination of total nitrogen (N x 6.25) and include the N present as a component of the insect’s exoskeletons (chitin) which may not be available to the animals. Thus, the crude protein values may not represent a true digestible protein value. Phelps et al (1975) have reported that the digestibility of termite protein (*M. falciger alates*) was poor (<50%) compared to that of casein when fed to white rats. However, we can only speculate that the availability of these amino acids after digestion in the digestive tract of the freshwater prawn is probably not much higher.

Table 3

Growth performance and conversion efficiencies of *M. rosenbergii* juveniles fed varying percent replacement of fish meal (FM) with termite meal (TM).

Parameter	*Diet 1 (100% FM, 0% TM)*	*Diet 2 (65% FM, 35% TM)*	*Diet 3 (35% FM, 65% TM*	*Diet 4 (0% FM, 100% TM)*
n	18	18	18	18
Survival (%)	37.01 ± 3.8	36.0 ± 8.1	41.5 ± 7.3	48.5 ± 9.1
Initial ABW	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
Final ABW	0.32 ± 0.10^{a}	0.16 ± 0.04^{b}	0.12 ± 0.02^{c}	0.11 ± 0.02^{c}
Weight gain	0.25 ± 0.08^{a}	0.10 ± 0.02^{b}	0.07 ± 0.02^{c}	0.06 ± 0.00^{c}
FCR	1.26 ± 0.28^{c}	2.23 ± 0.30^{b}	3.13 ± 0.18^{a}	3.69 ± 0.54^{a}
SGR (% d^{-1})	3.44 ± 0.34^{a}	2.40 ± 0.26^{b}	2.10 ± 0.10^{b}	1.77 ± 0.24^{b}
PER	1.93 ± 0.52^{a}	1.22 ± 0.16^{b}	0.80 ± 0.04^{b}	0.73 ± 0.08^{b}
K	7.03 ± 0.24^{b}	7.43 ± 0.22^{ab}	7.75 ± 0.22^{ab}	7.95 ± 0.24^{a}

Means within rows with different superscripts are significantly different at $P<0.05$.

Table 4 presents the result of the essential amino acid (EAA) profile of the termite and Peruvian fish meal in the present study, together with that of the tail muscle of *M. rosenbergii* from Farmafarmaian & Lauterio (1980) and also the EAA of the termite *Macrotermes subhyalinus* by Sogbesan & Ugwumba (2008b) recalculated to the common unit of g 100 g^{-1} protein. The termite meal in the present study had essential and non-essential amino acid content. The highest essential amino acid of earthworm was dominated by lysine (2.04 % of protein). Lysine plays a role in growth, as well as the production of carnitine, which aids in the metabolism of fats and also assists in calcium absorption. The non-essential amino acid of the termite meal was dominated by glutamate (3.46 % of protein). Glutamate is involved in protein synthesis and as source of energy for cells lining the intestine and it facilitates immune function and improves anti-inflammatory effects.

The termite meal in the present study contained only about 27% (11.4 g 100 g^{-1} protein) of the EAA of the Peruvian fish meal (42.3 g 100 g^{-1} protein). However the values were higher than the amino acid content in sexual and worker termites (6.77% and 4.66%, respectively) of *Macrotermes bellicosus* and *M. notalensis* (Banjo et al 2006). The low total EAA content was also observed by Sogbesan & Ugwumba (2008b) in the termite *Macrotermes subhyalinus.* According to them, the lowest total essential amino acids and highest crude fiber from termite meal could have resulted from the fact that high proportions of fiber has been associated with the reduction in total essential amino acid. This is so since crude fiber was a non-protein nutrient and has nothing to do with sparing of protein like lipids and nitrogen-free extract. Another reason for low total EAA was that the natural food of termites are poor in nitrogenous compounds with the carbon to nitrogen ratio being unfavorable for growth, the ratio higher in food than in the animal tissue (Higashi et al 1992). This situation is compensated by termites by ingesting bacteria and other micro-organisms as a source of fixed nitrogen.

The protein quality of a feed is related to the amino acid pattern and the availability of the amino acid present in the protein to the digestive process of the animal. The balance in the EAA of the termite meal in the present study as provided by the EAAI value was considered of medium quality at 0.85 compared to the 0.92 of the fish meal. The index in the present study was exactly the value estimated by Paoletti et al (2003) in the Venezuelan termite *Syntermes aculeosus* which they consider comparable with that of conventional meats.

Table 4

Essential amino acid composition (g 100 g^{-1} protein) of the termite meal and Peruvian fish meal in the present study and those of the tail muscle of *M. rosenbergii* and another termite (*Macrotermes subhyalinus*) in other studies

Essential amino acid	*Nasutitermes* spp.	Peruvian Fish Meal[*1]	*M. rosenbergii* tail muscle[*2]	*Macrotermes subhyalinus*[*3]
Indispensable				
Valine	1.51	5.20	2.63	1.79
Threonine	1.46	3.90	2.55	1.72
Methionine	0.69	2.80	2.26	0.96
Isoleucine	0.71	4.10	2.52	1.07
Leucine	1.46	7.10	5.15	1.51
Phenylalanine	1.19	3.80	2.58	1.84
Histidine	0.71	1.80	1.58	1.23
Tryptophan	nm[*4]	0.70	0.36[*3]	0.36
Lysine	2.04	7.40	6.04	3.23
Arginine	1.14	5.50	7.21	1.68
Dispensable				
Aspartate	2.65	8.70	7.41	
Tyrosine	2.00	3.20	2.35	
Serine	1.45	3.60	2.90	
Glutamate	3.46	13.20	10.0	
Proline	1.59	4.20	2.77	
Glycine	1.57	5.40	4.08	
Alanine	2.47	6.10	3.84	
Cysteine	nd	0.90	nd	
Total EAA (100 g^{-1}prot.)	11.4	42.3	32.88	15.39
EAAI	0.85	0.92		

*[1] From FDS (1994); [*2]Farmafarmaian and Lauterio, (1980); *[3] From Sogbesan & Ugwumba 2008);*[4]Assuminga Trp content of 0.36 g 100 g^{-1}protein from Sogbesan & Ugwumba (2008); TM = termite meal, PFM = Peruvian fish meal, nm= not measured; nd = not detected

The index might have also reflected the effectiveness of preparation of the meal (Istiqomah et al 2009). The amino acid balance (EAAI) could be increased by other physical and mechanical treatment such as fermentation and also pelleting (Mitchell &

Block 1946). Increase in the EAAI value could mean an increase in the biological value of feed material, absorption and also supporting the animal growth which has been shown in poultry (Rama Rao et al 1964; Raghunath & Narasinga Rao 1984).

Although the amount was lower, the balance of EAA was not detrimental since positive growths were still recorded in the present study. In fact, the estimated EAAI fell within what is considered a protein of medium quality (Lazo & Davis 2000). The only drawback could be that the limiting amino acids in the termite meal were the sulphur amino acids methionine and cysteine which is also the finding by Paoletti et al (2003). Tryptophan was not measured in the present study but Paoletti et al (2003) have documented an adequate amount in the termite *Syntermes aculeosus*; this amino acid is generally limiting in the food insects (DeFoliart 1989).

Conclusions. From the present results, although the crude protein level of termite was comparably high, its nutritional value was inferior to that of Peruvian fish meal. An inclusion of perhaps less than 35% could be used to replace fish meal with little or no decrease in growth performance or conversion efficiency. With reference to the amino acid profile of the termite meal, it could be used as a protein supplement for the culture of freshwater prawn since its amino acid balance indicated a protein source of medium quality and it had high lysine content, an amino acid found limiting in most plant protein sources. It could be a protein source that could complement other protein sources for maximum growth and health of the freshwater prawn *Macrobrachium rosenbergii*.

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