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Growth performance, nutrient utilisation and body composition of *Macrobrachium rosenbergii* **fed graded levels of phytic acid**

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Abstract

Information relating to the effects of phytic acid (PA), an anti-nutritional factor (ANF) commonly found in plant protein ingredients, on the growth performance, feed conversion ratio (FCR), nutrient utilisation and whole proximate composition in the Malaysian giant freshwater prawn, *Macrobrachium rosenbergii,* is lacking. Replicate groups of *M. rosenbergii* (mean initial carapace length of 6.03 ± 0.30 mm; mean initial weight of 0.29 ± 0.02 g; n = 20 per replicate group) were fed graded levels of PA for 140 days. The basal diet, to which different levels of PA were added to obtain 0.26 (control), 6.48, 11.28, 16.53, 21.45 and 26.16 g PA kg^{-1}, contained fishmeal, soy protein concentrate and wheat meal. Terminal sampling indicated that the growth performance, FCR and survival did not differ significantly between the groups receiving the different inclusions of PA within their diets. The apparent protein, lipid and energy utilisation responded negatively, decreasing significantly (p<0.05) with an increasing inclusion of PA, particularly within the groups of prawns fed diets with the highest inclusions of PA (*i.e.* 21.45–26.16 g PA kg^{-1}). The whole body composition of protein (p<0.04), lipid (p<0.01) and gross energy (p<0.05) also decreased significantly with an increasing supplementation of PA, while the ash content significantly increased (p<0.01), most notably in the groups of prawns receiving the highest levels of dietary PA (26.16 g PA kg^{-1}). In conclusion, inclusions of up to 11.28 g PA kg^{-1} appear safe but levels above this have negative impacts. The results show that PA had significant effects on the nutrient utilisation and body compositions (p<0.05) but not on growth performance and FCR (p>0.05) on the *M. rosenbergii* reared under the experimental conditions that were employed here.

Key words: Malaysian giant river prawn; anti-nutritional factors; aquafeed; crustacean diet; sodium phytate; sustainable feeds.

1. Introduction

Over the last four decades, attention has been devoted to the possibility of increasing the inclusion of plant protein sources into the artificial diets given to aquaculture species because of the unpredictable and limited supply of fish meal. A major proportion of current research focuses on freshwater prawn production, where the cost of feed is one of the major expenditures. The current study sets out to explore sustainability by focusing on the substitution of fishmeal with sustainable ingredients, *i.e*. plant proteins. Importantly, an increase in using plant protein ingredients as an alternative protein source does not appear to be a viable option for the future unless the feed formulation is addressed. One of the major problems limiting the use of plant protein is the presence of anti-nutritional factors (ANFs), such as phytic acid (*myo*-inositol hexakisphosphate, PA), commonly found in most plant protein ingredients such as soybean meal, wheat meal etc (Storebakken *et al.*, 2000; Francis *et al*., 2001). Phytic acid is a concern because of its ability to form complexes with proteins potentially rendering them unavailable with the consequential impacts on growth performances of the target species.

Civera & Guillaume (1989) reported poor feed efficiency and growth rate in *Penaeus vannamei* which was markedly depressed by phytic phosphorus and sodium phytate (SP) in this particular diet (*i.e.* 15 g kg^{-1}). Sodium phytate, however, had no detrimental effect on the growth rate or survival of *Penaeus* [s*yn. Marsupenaeus*] *japonicus* (see Civera & Guillaume, 1989) when incorporated into diets at levels of 20 g PA kg^{-1}. The review of Francis *et al*. (2001) on the effects of ANFs indicated that the growth and feed efficiency in commonly cultured fish species were negatively affected by the inclusion of phytate containing ingredients in the diets. Similar reductions in growth performance have also been seen in fish-based studies with both herbivorous and carnivorous species. For example, rainbow trout, *Oncorhynchus mykiss*, fed purified diets containing casein and gelatine with an inclusion of 5 g PA kg^{-1} for 150 d were found to have a reduced growth rate (Spinelli *et al*., 1983). Depressed growth was also reported for common carp (*Cyprinus carpio*), Chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*) when fed 15 g kg^{-1} sodium phytate for 105 d, 28.5 g PA kg^{-1} for 56 d, and 20.7 g kg^{-1} of SP for 80 d, respectively (Richardson *et al*., 1985; Hossain & Jauncey, 1993; Denstadli *et al.*, 2006).

To date, there has been no published work on the effects of PA on the freshwater prawn, *Macrobrachium rosenbergii*. The nutritional implications and interference of PA in the diets on growth and health of *M. rosenbergii*, therefore, are still not known. Hence, the aim of the present

study was to investigate the effects of graded levels of PA included in experimental diets presented to *M. rosenbergii* on their growth performances, feed conversion ratio (FCR), nutrient utilisation and whole body chemical composition.

2. Material and methods

2.1. Diets

The experimental diets were prepared within the feed processing room at the Institute of Aquaculture (IoA), University of Stirling (UoS), Scotland. Six diets, nominally control-PA0, PA5, PA10, PA15, PA20 and PA25, differed only in their PA and corn starch content, which was added to obtain nominal inclusion rates of 0, 5, 10, 15, 20 and 25 g PA kg^{-1} in the diet. Fishmeal, soy protein concentrates and wheat meal were selected as the main ingredients in the present study. The dry ingredients were ground to a powder using a hammer mill prior to mixing them in a A200 Hobart Ltd mixer and adding PA, fish oil and water. The mixture was steam-pelleted using a California Pellet Mill (Model CL2) with a 1.0 mm die. Pellets were dried for 24 h at 40°C, once cool, the diets were packed in labelled polythene bags and kept at 4°C. A chemo-attractant, an aqueous solution of 15% trimethylamine (TMA), was then sprayed on to a week's ration of each diet prior to feeding. The formulation and chemical analyses of the experimental diets are shown in Table 1.

2.2. Prawns and experimental conditions

The experimental feed trial was conducted over a period of 140 days within the Prawn Unit, Tropical Aquarium at IoA, UoS. A total of 120 specimens of *M. rosenbergii* (mean initial weight of 0.29 ± 0.02 g; mean initial carapace length of 6.03 ± 0.30 mm) that had been reared from a single brood of fertilised eggs (taken from a population of *M. rosenbergii* originally from Malaysia but bred and reared in the Tropical Aquarium, Institute of Aquaculture, University of Stirling) were randomly distributed between tanks. Twenty-four experimental tanks each with a capacity of 26.4 L were used, with four tanks being assigned to each treatment (n = 6 treatments). Each prawn was placed in cylindrical mesh pot, 18 cm tall × 10.5 cm in diameter with a volume of 1.53 L, with five such pots per experimental tank.

The recirculating system was supplied with aerated water (*i.e.* dissolved oxygen consistently >5 mg L^{-1}) regulated at 28.7 ± 0.4°C and delivered at a fixed rate of 1 L min^{-1} such that a water depth of 17 cm was maintained in all experimental tanks. Water quality parameters (ammonia, nitrite and nitrate) were maintained within acceptable levels for the culture of *M. rosenbergii* (see Boyd & Zimmerman, 2000) using mechanical and biological filters. Total water hardness was 120–180 mg l^{-1} and pH was 7.2-7.6. Photoperiod within the experimental facility was set at 12 h light. Moulting events and mortalities were recorded on a daily basis. Exuviae were noted and left to allow the animal to naturally feed on throughout the experiment.

2.3. Sampling

Prior to the allocation of the prawns to the experimental tanks, 120 animals were selected at random and taken as an initial sample (*i.e.* T0) to determine the proximate composition. The prawns were euthanased by submerging them in iced water for 1 min; the samples were subsequently frozen (-20°C) and kept until analysis. All the experimental prawns were individually weighed and the carapace lengths were measured at the start of the trial and then every 20 days thereafter. Excess water was gently blotted with soft paper towel from each animal which was then weighed using a top pan balance (Combics 1, Sartorius) to the nearest 0.01 g. The carapace length was measured from the posterior margin of the eye orbit to the base of the carapace using a calibrated vernier calliper to the nearest 0.01 mm. The same procedure was used throughout the entire experimental period to reduce sampling error. Prawns were starved for 24 h prior to each weighing event. All experimental animals were individually hand fed to apparent satiation twice a day at 09:00 am and 16:00 pm. The daily intake of feed was recorded and uneaten food was collected by siphoning each pot after each feed and used to precisely calculate feed intake and their FCR. At the end of the experimental period, the prawns were euthanased by immersion in iced water and then frozen (-20°C) until analysed. Faecal matter was collected by siphoning twice per day and was pooled for each treatment. Faeces were centrifuged (Centaur 2 Sanyo) at 2,268 × g for 10 min to separate and discard the supernatant and then were frozen at -20°C and kept until the samples could be analysed.

2.4. Chemical analyses

The nutrient composition analyses, including moisture, crude protein, crude lipid, crude fibre and the ash contents of the experimental diets, faeces, whole body samples were conducted following AOAC procedures (1995). The dry matter of diets and the homogenates of prawn whole body were determined by drying at 105°C for 24 h. Crude protein was determined by the Kjeldahl method (Tecator Kjeltec^{TM} 2300, Foss, Warrington, UK (total nitrogen × 6.25)), while crude lipid was determined after acid hydrolysis followed by petroleum ether extraction according to the Soxhlet method (Tecator Soxtec 2050, Foss, Warrington, UK). Ash was determined gravimetrically after combustion at 600°C for 12 h. The measurement of crude fibre was determined using the FOSS fibercaps system. Gross energy was analysed using an adiabatic bomb calorimeter (Parr 6200, USA) and benzoic acid as a standard. For elemental analysis, the samples were digested in 5 ml of 69% nitric acid using a MarsXpress microwave (CEM Corporation, USA) for 30 min and then allowed to cool. Once cool, the samples were diluted to 10 ml with distilled water and then prepared for analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Thermo Scientific, USA). The phosphorus (P) content of each tissue, however, was determined using the protocol detailed by Allen (1989). The phytic acid analysis was determined by Megazyme, K-Phyt 05/07 (Megazyme Inc., USA), a method that involves acid extraction of inositol phosphates followed by treatment with a phytase that is specific for PA (IP6) and the lower *myo*-inositol phosphates (*i.e*. IP2, IP3, IP4, IP5). Subsequent treatment with alkaline phosphatase ensures the release of the final phosphate from *myo*-inositol (IP1) which is relatively resistant to the reaction of phytase. The total phosphate released was measured using a colorimetric method as described by Lowry & Lopez (1946).

2.5. Calculations

All experimental animals were weighed and measured at the beginning of the trial and then every 20 days thereafter until the end of the 140-day trial (see Figure 1). The data obtained were analysed for growth performance, feed intake, FCR, protein efficiency ratio (PER) and nutrient utilisation. Growth performance was calculated as weight gain (mean final body weight – mean initial body weight) and carapace length increment (mean final carapace length – mean initial carapace length).

Specific growth rate (SGR) represents the average weight change per day between any two measurement time points and is based on the natural logarithm of weight and was calculated as [*ln* (final weight) – *ln* (initial weight)] / no. of days × 100. Hepatosomatic indices (HSI) were calculated using the formula of (weight of hepatopancreas / total weight of animal) × 100. Feed conversion ratio (FCR) was determined as feed intake (g) / weight gain (g), while protein efficiency ratio (PER) was calculated as weight gain (g) / crude protein intake. Nutrient utilisation was calculated using the following formula:

$$\text{Apparent net protein utilisation (ANPU) \%} = \frac{\text{(final prawn body protein – initial prawn body protein)}}{\text{crude protein intake}} \times 100$$

$$\text{Apparent net lipid utilisation (ANLU) \%} = \frac{\text{(final prawn body lipid – initial prawn body lipid)}}{\text{crude lipid intake}} \times 100$$

$$\text{Apparent net energy utilisation (ANEU) \%} = \frac{\text{(final prawn carcass energy – initial prawn carcass energy)}}{\text{gross energy intake}} \times 100$$

2.6. Statistical analyses

All data were subject to normality (*i.e.* Kolmogorov-Smirnov) and homogeneity of variance (*i.e.* Levene's) tests. The data sets of apparent nutrient utilisation and whole body composition, which were identified as non-homogeneous (Levene's test), were subjected to arcsine transformation before analysis (Zar, 1999). One-way ANOVA analyses were performed, followed by a Tukey's *post hoc* test, when appropriate, to rank significantly different ($p<0.05$) means (SPSS version 18.0, SPSS Inc, 2010).

2.7 Ethics statement

The project design and its objectives were appraised by an internal ethics review committee and strict codes of practice were exercised and monitored under the daily observation of a University appointed named animal care welfare officer assigned to the Prawn Unit.

3. Results

3.1. Diets

The main nutrients were consistent among the diets with approximately 908.0 g kg^{-1} dry matter, 427.8 g kg^{-1} crude protein, 70.9 g kg^{-1} crude lipid and 19.5 kJ g^{-1} gross energy (Table 1). The dietary PA content of the six experimental diets when analysed were close to the nominal target values and were determined to be 0.26, 6.48, 11.28, 16.53, 21.45 and 26.16 g PA kg^{-1}, respectively.

3.2. Growth performance

Data on the growth, feed intake, FCR, PER and survival are presented in Tables 2 and 3. The dietary inclusion of PA of up to 26.16 g kg^{-1} had no significant effects on the growth (*i.e.* in a comparison of the increase in carapace length, weight gain and SGR) of *M. rosenbergii* when compared to the controls. Differences in the weight of animals fed different inclusions of phytic acid (PA) at each time point were tested for using one-way ANOVAs (Table 2). Dietary treatments containing PA did not show any significant differences on the hepatosomatic index (HSI) (Table 2). Survival over the 140-day trial was high at 85.0–95.0% regardless of the level of dietary PA inclusion. The mortalities that did occur, however, were principally after weighing events. Overall no significant effects of dietary PA level on feed intake and FCR were identified. A one-way ANOVA comparing the different dietary treatments in each sample point showed no significant effects between dietary PA and PER.

3.3. Apparent nutrient utilisation

Increasing dietary PA inclusions significantly decreased the nutrient utilisation of protein ($p<0.05$), lipid ($p<0.05$) and energy ($p<0.05$; see Table 4). Prawns fed diets containing high levels of dietary PA at 21.45–26.16 g PA kg^{-1} demonstrate significant lower ANPU ($p<0.05$) and ANLU ($p<0.05$) values compared to prawns in the other groups and those in the controls. Similar trends were found for ANLU with a decrease from 4.25% to −27.27% with increasing inclusion levels of PA. Prawns fed the PA enriched experimental diets (16.53–26.16 g PA kg^{-1}) resulted in significantly lower ANLU values where negative values indicate lower levels of lipid that were

retained in the carcass. Likewise, an increasing inclusion of dietary PA decreased ANEU from 13.51 to −2.74 kJ kg^{-1} (p<0.05). A dietary inclusion rate of 26.16 g PA kg^{-1} resulted in the lowest, negative values of ANEU.

3.4. Whole body composition

There were significant effects on the protein, lipid, gross energy and ash content between the treatment groups of *M. rosenbergii* fed the different levels of dietary PA diets while the moisture content was not affected by the varied inclusion rates of PA (Table 5). The protein content of the prawn receiving the experimental diet containing 26.16 g PA kg^{-1} were significantly affected (p<0.05), with lower protein content than those prawns receiving the diet containing 6.48 g PA kg^{-1}. Likewise, the lipid content of the prawns receiving between 16.53 to 26.16 g PA kg^{-1} in their diets decreased significantly (p<0.01) and had lower levels of lipid than the prawns in the other test and control groups. Prawns fed diets containing high levels of dietary PA at 21.45–26.16 g PA kg^{-1} demonstrated significant lower (p<0.05) gross energy content in the final carcass. In contrast to these results, the ash content of the whole body was found to increase with an increasing inclusion of PA. Prawns fed the diet containing 16.53 to 26.16 g PA kg^{-1} had a significantly higher ash content (p<0.01) than those prawns fed the diets containing no inclusion of PA (control group) and between 6.48 to 11.28 g PA kg^{-1}.

High PA levels (*i.e.* 21.45–26.16 g PA kg^{-1}) were found to have negative effects (p<0.05) on the whole body P concentration (Table 6). Linear regression analyses were conducted to examine the relationship between calcium (Ca) concentration in the whole body and increasing levels of dietary PA and an inverse trend was seen (p<0.005) with increasing PA inclusion. The results of the linear regression model indicated Ca content was evidently elevated and linked to the increasing PA inclusion (r^2= 0.61, F (1, 9) = 14.1, p<0.005). There were no significant differences between the groups for the zinc (Zn) (0.13%–0.19%), magnesium (Mg) (3.15%–5.35%), potassium (K) (16.77%–19.95%) and sodium (Na) (5.00%–6.56%) content of the whole body that varied little. The copper (Cu) content varied from 0.06 to 0.10% and the iron (Fe) content from 0.03 to 0.08% with the highest observed levels for both trace elements being seen in the *M. rosenbergii* group receiving the 11.28 g PA kg^{-1} diet. There were no significant differences between the groups for either of these elements when tested for using a one-way ANOVA.

For the trace element manganese (Mn), there were no differences between experimental groups and levels did not appear to be influenced by the level of PA within the diet. Graded inclusion of PA resulted in a significant increase ($p<0.05$) in the ash concentration in the whole body of *M. rosenbergii*, from 2.18% to 5.95%.

4. Discussion

Although the present study indicates that juvenile *M. rosenbergii* can tolerate up to 26.16 g PA kg^{-1} in their diet without adverse effects on growth, survival or on SGR and HSI, levels exceeding 16.53 PA kg^{-1} appear to exert a series of negative impacts, with levels >20 g PA kg^{-1} resulting in a significant decrease in nutrient utilisation. Civera & Guillaume (1989) working with juvenile *P. vannamei* found poor growth when 15 g kg^{-1} sodium phytate was added to their diets for 28 d, whereas the inclusion of up to 20 g kg^{-1} had no apparent detrimental effect on the growth performances or survival of juvenile *P. japonicus*. For the current trial, the growth of the freshwater prawns was assessed every 20 days, to explore the performance of each group of prawns throughout the 140-day trial. The regular collection of data (*i.e.* every 20 days) ensured data for analysis, as opposed to only taking start and end weights which may have missed some subtleties in growth between groups, but has an increased probability of complete data loss if animals are lost. With regular growth assessments, however, there is a concern that handling stress, despite the level of care exercised, may impact on growth. No significant differences in growth were seen here but the sampling methodology cannot be ruled out as possible contributory factor towards this. The findings from the current study suggest that *M. rosenbergii* are able to tolerate higher levels of PA than are suggested for *P. vannamei* (Civera & Guillaume, 1989) and for a variety of fish species (Spinelli *et al*., 1983; Richardson *et al.*, 1985; Hossain & Jauncey, 1993; Denstadli *et al*., 2006). This is probably because *M. rosenbergii* are omnivorous and can adjust to reductions in the nutritional value of prepared diets, *i.e*. in their protein source, vitamin and mineral content, by increasing their consumption of available vegetation (Ling, 1969; Weidenbach, 1982). Successful replacement of fishmeal with plant protein is, however, feasible in semi-intensive pond culture systems where there is a contribution from natural productivity as has been observed by Tidwell *et al.* (1995) and by Tacon & Akiyama (1997). A better understanding of the role of natural productivity to satisfy nutrient deficiencies particularly, when using high plant protein diets containing high levels of anti-nutrients, to decrease production cost is needed.

In the present study, feed intake did not indicate any apparent adaptation to PA during the experimental period. The feed intake pattern was approximately maintained for all groups. Civera & Guillaume (1989) reported that the feed consumption of *P. japonicus* and *P. vannamei* seemed to diminish markedly and demonstrated poor feed utilisation when fed diets containing sodium phytate (15–20 g kg^{-1}) for over 28 days. In the current study, diets were formulated to meet the nutritional requirements of *M. rosenbergii* and TMA was used as a feed attractant. The protein sources that were used were fishmeal and plant protein (*i.e.* soy protein concentrate and wheat meal) used in a ratio of 1:1.5. The high concentration levels of protein and energy in the diets possibly allowed for sufficient digestion and for metabolic purposes without causing significant deleterious effects. If a lower level of protein and energy in the diet had been used, then PA may have complexed with high quality dietary protein thereby reducing its availability. Whether the lack of an effect of PA on feed intake is attributable to maintained palatability of the formulated diet or physiological mechanisms remains to be elucidated.

By comparison, fish based studies, for example, that were conducted by Richardson *et al.* (1985) reported that an inclusion of 25.8 g PA kg^{-1} significantly depressed PER in Chinook salmon. In a marked contrast to this latter study, research conducted by Lin (2005) reported that an inclusion of up to 20 g PA kg^{-1} did not significantly affect PER in juvenile cobia, *Rachycentron canadum*. Despite the high inclusion of PA of up to 26.16 g PA kg^{-1}, no significant differences were observed for PER in *M. rosenbergii* (Table 3) among the treatment groups due to the provision of sufficient protein in the diets. It is also essential to note that the source of protein and therefore the variable inclusion of PA in various diets, as highlighted by Teskeredźić *et al*. (1995), may play a role in the diversified results that are seen. In the present study, fishmeal was used as the protein source as was also used in the fish-based studies conducted by Riche & Garling (2004), Denstadli *et al*. (2006) and by Lin (2005). In the fish-based studies of Spinelli *et al.* (1983), Richardson *et al*. (1985), Hossain & Jauncey (1993) and Sajjadi & Carter (2004), and in the crustacean-based studies by Civera & Guillaume (1989) and Davis *et al.* (1993), however, casein was the principal protein ingredient that was used. Intact proteins and amino acids differ in their

capacity for binding to PA as reviewed by Dendougui & Schwedt (2004). Casein forms "casein micelles" and these structures are linked together with phosphate groups, demonstrating that they may serve as a preferable substrate for binding to PA (IP6) (Horne, 1998). The extent to which protein availability and digestion is inhibited by PA–protein interactions, therefore, will vary between proteins due to differences in the total number of cationic groups available to participate in binding with phytate (Adeola & Sands, 2003).

Results from the present study found that the inclusions of dietary PA statistically affected protein (ANPU), lipid (ANLU) and energy (ANEU) utilisations (Table 4). The inclusion of 26.16 g PA kg^{-1} evidently decreased protein utilisation by as much as three-fold when compared to the levels determined for the *M. rosenbergii* within the control group. This agrees with the early study of Hossain & Jauncey (1993) who determined that an inclusion of 10 g PA kg^{-1} in the diet presented to common carp, *Cyprinus carpio,* depressed protein utilisation. The results from Table 4 indicate that inclusion of PA in all the experimental diets did not affect growth performance, however, PA included in diets at a rate of 21.45 - 26.16 g PA kg^{-1} were seen to have an impact at the metabolic level. The chelation of PA with protein here does not make available sufficient dietary protein for utilisation, as seen by the decreasing trend in ANPU with an increasing inclusion of PA in the diet. The decrease in ANEU helps explain the observed drop in ANPU. Lipid is an alternative energy source when protein is not available, in the present study, however, it appears that the prawn are not able to utilise lipid as efficiently as protein and this is seen by the marked decrease in ANLU in the prawn fed diets containing >16.53 PA kg^{-1}.

Earlier studies conducted with Atlantic salmon by Denstadli *et al.* (2006) likewise found that the digestibility of lipid in Atlantic salmon fed the highest PA level, *i.e.* 20.7 g PA kg^{-1}, was significantly reduced although the differences that were reported were minor when compared the other treatment group and to the control. These latter authors suggested that the significant decrease in lipid digestibility exhibited by Atlantic salmon might have been due to the reduced bile acid concentration. There is, however, no evidence for the production of bile acids by crustaceans (Boonyaratpalin, 1996). This suggests that the metabolic processes of emulsification, digestion and transportation of lipids in crustaceans differ from fish species. Lipid transport in shrimp, however, is accomplished primarily through high density lipoproteins (Boonyaratpalin, 1996). The ternary binding between lipase–mineral–PA which could reduce the capacity of lipase to liberate fatty acids and thereby facilitate the digestion of lipids as indicated by Knuckles (1988), is therefore the most probable explanation.

The observed diminishment in the final gross composition of the whole carcass agrees with findings of Sajjadi & Carter (2004) who reported that PA had no significant effect on the dry weight of the final carcass of Atlantic salmon but was found to lower the lipid content and to significantly increase the ash content. Hossain & Jauncey (1993) also reported that the inclusion of 10 g PA kg^{-1} led to lower content levels of protein and lipid in the carcasses of common carp. Studies carried out by Richardson *et al*. (1985) also found that Chinook salmon fed 25.8 g PA kg^{-1} had consistently lower percentages of lipid and higher percentages of ash in their bodies. Complexing of PA with protein either through PA–protein or PA–protein–cation types of interaction may alter the protein structure as they closely pack around the negatively charged PA. This may in turn lead to decreased solubility, digestibility and functionality of the proteins (Cheryan, 1980; Cosgrove, 1980; Reddy *et al.*, 1982). The reduced lipid content could be correlated with the high levels of PA and with decreasing protein and energy levels which consequentially led to the mobilisation of body lipid reserves to meet protein and energy requirements for vital body functions. A number of earlier studies indicate that whole body element concentrations are homeostatically controlled (Satoh *et al*., 1987) and therefore less than normal whole body levels of a particular element is indicative of a sub-clinical element deficiency (Storebakken *et al*., 1998). An accompanying effect on whole body mineral composition and deposition due to PA interference with dietary mineral digestibility was expected. These effects were, however, less severe than anticipated. In the present study, the inclusion of PA in the diets presented to *M. rosenbergii* did not appear to cause detrimental effects to their other whole body mineral composition other than P. No severe pathology or prominent signs of disturbances in the mineral homeostasis of the experimental animals were observed, but there were several indications of a sub-optimal mineral availability in the prawns.

Phosphorus is expected to be a challenge in diets containing high levels of PA. Previous studies performed on *P. vannamei* reported that the presence of 15 g kg^{-1} PA in their diets resulted in a depressed P bioavailability (Davis *et al*., 1993). At the end of the present experimental period, the concentration of the important mineral P in the whole body analysis of the carcass showed a declining trend with an increasing inclusion of dietary PA. This was most probably due to PA binding strongly with P making it unavailable. Considering that P is an essential macro minerals for the hardening of the exoskeleton, a decrease in the amount of P as consequence of dietary PA appears to influence moult frequency and to some extent the somatic tissue growth of *M. rosenbergii.* As the P concentration declined in response to an increasing PA content in the

diet, there was an increase in the Ca content of the whole body of *M. rosenbergii*, suggesting a strong close correlation between the two minerals and dietary PA. The results of the present study are clearly in agreement with earlier studies conducted on shrimp species, *i.e. M. japonicus* and *P. vannamei*. Civera & Guillaume (1989) reported similar findings where the ash (P) content of the exoskeleton of *M. japonicus,* and for *P. vannamei* although but less clearly, was significantly reduced while there was a simultaneous elevation in the Ca content.The mechanisms involved are complex; it may involve calcification, absorption, and acid-base balance. In addition, *M. rosenbergii* can uptake minerals from its aquatic surroundings; an imbalance of these elements, therefore, could be compensated for immediately by an uptake by active transport via the gills, skin or mouth (NRC, 1993; Davis & Kurmaly, 1993; Guillaume *et al*., 2001).

5. Conclusion

The findings from the present study, which aimed to investigate the effects of increasing PA levels up to 26.16 g PA kg^{-1}, in diets fed to *M. rosenbergii*, resulted in significant decreases in the protein, lipid and energy utilisations particularly in those animals receiving the highest inclusion of PA. Inclusions of up to 11.28 g PA kg^{-1} appear safe to include, but levels exceeding this, *i.e.* >16.53 PA kg^{-1} appear to exert a series of negative impacts. Significant changes in the protein, lipid, gross energy and ash compositions of the carcass were also observed, again most notably in those animals fed the diet containing the highest level of PA. These changes may have a serious impact on meeting the nutritional requirements of *M. rosenbergii*, particularly when fishmeal is substituted with protein derived from plant sources. Care, therefore, must be taken to ensure that the levels of dietary PA in feeds for *M. rosenbergii* are sufficient to meet these nutritional demands and are appropriate for optimal production. As an extension to this work, trials exploring the addition of microbial phytase and its activity specifically in hydrolysing PA when added as a supplement to practical diets containing high levels of plant protein that are fed to *M. rosenbergii* were carried out and will be reported on in a future manuscript.

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Table 1. Feed formulations and analysed chemical composition of the experimental diets presented to *Macrobrachium rosenbergii*.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Formulations (g kg^{-1})						
Fishmeal[1]	320.0	320.0	320.0	320.0	320.0	320.0
Soy protein conc.[2]	285.0	285.0	285.0	285.0	285.0	285.0
Wheat meal	210.0	210.0	210.0	210.0	210.0	210.0
Corn starch	88.0	80.2	72.4	64.6	56.8	50.0
Fish oil[3]	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin premix[4]	15.0	15.0	15.0	15.0	15.0	15.0
Mineral premix[5]	15.0	15.0	15.0	15.0	15.0	15.0
Yttrium oxide[6]	2.0	2.0	2.0	2.0	2.0	2.0
Carboxymethyl-cellulose[7]	25.0	25.0	25.0	25.0	25.0	25.0
Phytic acid[8]	0.0	6.9	13.8	20.7	27.6	34.5
Chemical composition (g kg^{-1})						
Dry matter	908.5	908.3	908.1	908.4	907.5	907.4
Crude protein	429.4	426.7	425.9	428.8	427.3	428.5
Crude lipid	69.8	70.0	70.5	71.9	72.0	72.2
Crude fibre	20.6	20.4	19.5	20.1	18.8	19.0
Ash	68.5	69.2	69.7	70.2	70.7	71.1
NFE[9]	320.6	320.7	319.7	312.5	311.5	307.3
Gross energy[10]	19.7	19.6	19.6	19.6	19.5	19.0
Phytic acid	0.26	6.48	11.28	16.53	21.45	26.16

[1]Ewos Ltd; [2]soy protein concentrate, BioMar UK Ltd; [3]herring oil; [4]per kg of diet: 1000 IU retinol palmitate; 4 mg cholecalciferol; 7,000 mg tocopherol acetate; 1,500 mg vitamin K; 37,500 mg ascorbic acid; 4,250 mg vitamin B1; 3,000 mg vitamin B2; 1,250 mg vitamin B6; 1.25 mg vitamin B12; 5,250 mg calcium pantothenate; 12,500 mg niacin; 90 mg biotin; 1,000 mg folic acid; 74,050 mg choline chloride; 25,000 mg inositol; 200 mg ethoxyquin; [5]per kg of diet: 472.77 g $CaCO_3$; 0.3 g $CaIO_3.6H_2O$; 0.13 g $CrCl_3.6H_2O$; 0.48 g $CoSO_4.7H_2O$; 0.75 g $CuSO_4.5H_2O$; 25.0 g $FeSO_4.7H_2O$; 127.5 g $MgSO_4.7H_2O$; 2.54 g $MnSO4.4H_2O$; 50.0 g KCl; 60.0 g NaCl; 5.5 g $ZnSO_4.4H_2O$; [6]Sigma Aldrich (205168); [7]Sigma Aldrich (C5013); [8]phytic acid sodium salt hydrate from rice (Sigma Aldrich P8810); [9]Nitrogen free extract; 10 (kJ g^{-1}).

Table 2. The average weight (mean ± standard deviation) of the *Macrobrachium rosenbergii* in each dietary group at each weighing point throughout the 140-day trial. The number of animals surviving at each time point are shown in parentheses. Differences in the weight of animals fed different inclusions of phytic acid (PA) at each time point were tested for using one-way ANOVAs and differences were regarded as significant when $p<0.05$.

Diet	D1	D2	D3	D4	D5	D6	*p*
PA g kg^{-1}	0 g kg^{-1}	5 g kg^{-1}	10 g kg^{-1}	15 g kg^{-1}	20 g kg^{-1}	25 g kg^{-1}	
Initial	0.30 ± 0.08 (20)	0.30 ± 0.07 (20)	0.28 ± 0.07 (20)	0.31 ± 0.06 (20)	0.30 ± 0.07 (20)	0.28 ± 0.06 (20)	0.718
T_{20}	0.64 ± 0.25 (20)	0.59 ± 0.24 (20)	0.61 ± 0.23 (20)	0.66 ± 0.28 (20)	0.63 ± 0.24 (20)	0.64 ± 0.27 (20)	0.963
T_{40}	1.32 ± 0.31 (20)	1.39 ± 0.28 (20)	1.34 ± 0.37 (20)	1.51 ± 0.38 (20)	1.45 ± 0.34 (20)	1.24 ± 0.36 (19)	0.182
T_{60}	2.34 ± 0.46 (20)	2.28 ± 0.35 (19)	2.33 ± 0.69 (20)	2.49 ± 0.50 (20)	2.26 ± 0.69 (20)	2.14 ± 0.50 (19)	0.506
T_{80}	3.53 ± 0.60 (20)	3.42 ± 0.65 (19)	3.33 ± 1.05 (20)	3.58 ± 0.82 (20)	3.57 ± 0.75 (20)	3.07 ± 0.63 (19)	0.296
T_{100}	4.67 ± 0.74 (19)	4.53 ± 1.04 (19)	4.40 ± 1.28 (20)	4.95 ± 0.88 (20)	4.69 ± 1.06 (20)	4.18 ± 0.83 (18)	0.239
T_{120}	5.68 ± 0.97 (19)	5.36 ± 1.11 (19)	5.21 ± 1.50 (19)	5.82 ± 1.05 (20)	5.74 ± 1.28 (20)	4.94 ± 1.07 (18)	0.159
T_{140}	6.84 ± 1.11 (17)	6.74 ± 1.53 (19)	6.40 ± 1.77 (18)	7.20 ± 1.19 (19)	7.13 ± 1.58 (19)	6.36 ± 1.48 (18)	0.378

Table 3. Growth performance and feed efficiency of the experimental *Macrobrachium rosenbergii* fed graded levels of phytic acid in their diets for 140 days.

	Dietary phytic acid (g kg^{-1})					
	0.26 (Control)	6.48	11.28	16.53	21.45	26.16
Initial carapace length (mm)	6.17 ± 0.32	6.20 ± 0.13	5.70 ± 0.40	6.04 ± 0.11	6.12 ± 0.30	5.95 ± 0.27
Final carapace length (mm)	18.17 ± 2.56	20.07 ± 2.12	18.71 ± 2.40	20.76 ± 2.33	20.38 ± 1.74	18.73 ± 3.00
Carapace length increase (mm)	12.00 ± 2.65	13.87 ± 2.09	13.01 ± 2.59	14.72 ± 2.32	14.26 ± 1.51	12.79 ± 3.20
Initial weight (g)	0.30 ± 0.02	0.30 ± 0.03	0.28 ± 0.03	0.31 ± 0.01	0.30 ± 0.02	0.28 ± 0.02
Final weight (g)	5.81 ± 1.07	6.40 ± 1.01	5.76 ± 1.49	6.84 ± 0.84	6.77 ± 1.11	5.72 ± 1.24
Weight gain (g)	5.52 ± 1.07	6.10 ± 1.03	5.48 ± 1.48	6.53 ± 0.84	6.48 ± 1.12	5.44 ± 1.24
Av. daily weight gain	0.039 ± 0.01	0.044 ± 0.01	0.039 ± 0.01	0.047 ± 0.01	0.046 ± 0.01	0.039 ± 0.01
Specific growth rate (% day^{-1})	2.12 ± 0.16	2.18 ± 0.17	2.14 ± 0.20	2.21 ± 0.09	2.23 ± 0.15	2.14 ± 0.16
Feed intake (g prawn^{-1} day^{-1})	0.060 ± 0.00	0.061 ± 0.01	0.057 ± 0.01	0.068 ± 0.01	0.066 ± 0.01	0.054 ± 0.01
Feed conversion ratio (FCR)	1.57 ± 0.29	1.40 ± 0.05	1.48 ± 0.16	1.48 ± 0.18	1.42 ± 0.15	1.39 ± 0.28
Protein efficiency ratio	1.29 ± 0.25	1.42 ± 0.24	1.27 ± 0.34	1.52 ± 0.20	1.50 ± 0.26	1.25 ± 0.29
Hepatosomatic index (HSI) (%)	4.16 ± 0.37	3.35 ± 0.18	3.96 ± 0.17	4.34 ± 0.71	4.18 ± 0.42	3.30 ± 0.20
Survival (%)	85.0 ± 0.50	95.0 ± 0.50	90.0 ± 0.60	95.0 ± 0.50	95.0 ± 0.50	90.0 ± 0.60

Average daily weight gain (g prawn^{-1}).
Values are the means ± SD of four replicates.
Statistical analysis found no significant differences between the different diets for any of the measured parameters.

Table 4. Apparent nutrient utilisations (%) in the *Macrobrachium rosenbergii* fed the experimental diets for a period of 140 days.

	Dietary phytic acid (g kg^{-1})					
	0.26 (Control)	6.48	11.28	16.53	21.45	26.16
Protein utilisation (ANPU)	13.53 ± 1.22^{a}	13.00 ± 1.43^{a}	11.77 ± 0.77^{a}	11.47 ± 0.92^{a}	6.98 ± 3.22^{b}	4.64 ± 1.99^{b}
Lipid utilisation (ANLU)	4.25 ± 0.93^{a}	3.53 ± 1.57^{a}	2.43 ± 0.90^{a}	−14.77 ± 4.61^{b}	−21.25 ± 1.26^{bc}	−27.27 ± 3.60^{c}
Energy utilisation (ANEU)	10.30 ± 0.72^{a}	13.51 ± 0.04^{b}	11.35 ± 0.66^{ab}	10.45 ± 0.72^{ab}	4.05 ± 0.07^{c}	−2.74 ± 0.96^{d}

Negative values indicate lower values of retained nutrient in the carcass when compared to the initial sample of *M. rosenbergii*.
Values are the means ± SD of three replicates.
Values within the same row with different letters are significantly different (p<0.05).

Table 5. Whole body proximate composition (% wet weight) of the *Macrobrachium rosenbergii* fed graded levels of phytic acid in the experimental diets presented to them over a period of 140 days.

	Dietary phytic acid (g kg^{-1})					
	0.26 (Control)	6.48	11.28	16.53	21.45	26.16
Moisture content	74.05 ± 2.26	73.55 ± 1.88	74.05 ± 1.31	75.79 ± 2.18	74.40 ± 0.45	76.05 ± 1.59
Crude protein	16.98 ± 1.82^{a}	17.35 ± 1.32^{a}	16.42 ± 0.63^{ab}	15.30 ± 0.99^{ab}	15.61 ± 0.10^{ab}	14.21 ± 0.81^{b}
Crude lipid	0.66 ± 0.04^{a}	0.61 ± 0.01^{a}	0.63 ± 0.08^{a}	0.31 ± 0.07^{b}	0.17 ± 0.03^{bc}	0.07 ± 0.07^{c}
Ash	2.21 ± 0.48^{a}	2.37 ± 0.22^{a}	3.92 ± 0.69^{b}	5.01 ± 0.37^{c}	5.42 ± 0.12^{c}	6.09 ± 0.35^{d}
Gross energy (kJ g^{-1})	4.67 ± 0.43^{a}	4.40 ± 0.24^{b}	4.54 ± 0.45^{ab}	4.61 ± 0.30^{a}	3.97 ± 0.11^{c}	3.95 ± 0.03^{c}

Values are the means ± SD of three replicates.

Values within the same row with different letters are significantly different (p<0.05). For parameters where there were no significant differences, no superscript values are provided.

Table 6. Whole body proximate mineral compositions (g kg^{-1} dry weight) and ash content (% wet weight) of the *Macrobrachium rosenbergii* fed experimental diets with increasing amounts of phytic acid (0.26 to 26.16 g PA kg^{-1}) for a period of 140 days.

		Dietary phytic acid, g PA kg^{-1}					
	Initial[1]	0.26	6.48	11.28	16.53	21.45	26.16
Phosphorus (P)	12.05 ± 0.53	12.82 ±0.76^{a}	12.56± 0.26^{a}	12.18± 0.49^{a}	12.06± 0.70^{a}	11.68± 1.24^{b}	11.51± 0.48^{b}
Calcium (Ca)*	14.37 ± 0.23	19.47 ± 1.74	19.93± 0.25	20.51± 1.07	21.08± 0.17	21.28± 1.49	23.95± 0.50
Zinc (Zn)	0.14 ± 0.01	0.13 ± 0.02	0.19± 0.01	0.16± 0.01	0.18± 0.02	0.19± 0.02	0.19± 0.01
Magnesium (Mg)	2.99 ± 0.06	3.87 ± 0.17	3.38± 0.08	4.11± 0.41	5.35± 0.40	3.15± 0.50	3.65± 0.69
Copper (Cu	0.11 ± 0.01	0.06 ± 0.03	0.08± 0.01	0.07± 0.10	0.10± 0.00	0.08± 0.00	0.09± 0.00
Iron (Fe)	0.03 ± 0.01	0.04 ± 0.00	0.03± 0.02	0.04± 0.04	0.04± 0.01	0.05± 0.04	0.04± 0.01
Manganese (Mn)	0.02 ± 0.00	0.02 ± 0.00	0.02± 0.00	0.02± 0.00	0.02± 0.00	0.02± 0.00	0.03± 0.03
Potassium (K)	15.69 ± 0.39	19.15 ± 0.08	19.03± 1.24	18.22± 0.22	19.95± 0.15	17.14± 0.91	16.77± 2.19
Sodium (Na)	7.20 ± 0.79	5.00 ± 1.38	5.44± 0.95	6.32± 0.60	6.43± 0.35	5.69± 0.89	6.56± 0.73
Ash (%, wet weight)	2.10 ± 0.15	2.18 ± 0.47^{a}	2.32± 0.16^{a}	4.02± 0.41^{b}	5.01± 0.23^{c}	5.36± 0.18^{c}	5.95± 0.15^{c}

Values presented represent the mean ± SD of three replicates.

Values within the same row with different letters are significantly different (p<0.05). For parameters where there were no significant differences, no superscript values are provided.

[1]Values not included in the one-way Anova.

*Significant differences using linear regression analyses.

Figure 1. Weight gained by *Macrobrachium rosenbergii* fed the experimental diets containing graded inclusion levels of phytic acid (nominally 0–25 g PA kg^{-1}) over 140 days.