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Effect of selected feed meals and starches on diet digestibility in the mud crab, *Scylla serrata*

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Running title: Digestibility of mud crab feed ingredients

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Abstract

The present study examined the capacity of the mud crab, *Scylla serrata* to digest experimental diets that contained different animal and plant-based feed meals or different levels or types of starch. The apparent dry matter digestibility (ADMD) coefficients for all feed meals tested in the first part of this study, except meat meal, were similar (78% to 88%). Crude protein digestibility (ACPD) coefficients for all feed meals were relatively high, with values ranging from 86% to 96%. Cotton seed meal, poultry meal, canola meal, fishmeal, soybean meal and lupin meal had similar gross energy digestibility (AGED) values ($p>0.05$) ranging from 84% to 89%. In the second part of this study, the impact of selected starches on the digestibility of fishmeal-based formulated diets was assessed. The apparent starch digestibility (ASD) of wheat starch decreased significantly as the inclusion level was increased from 15% to 60%, however, there was no significant effect on ACPD values. At a 30% inclusion level, the ASD of diets containing different starches decreased in the order corn > wheat > potato = rice. Moreover, ACPD values were significantly higher ($p<0.05$) in the diets containing corn or rice starch than in those containing wheat or potato starches.

1. Introduction

Mud crabs of the genus *Scylla* are a valuable source of nutrition and provide income for many coastal communities in the Asia-Pacific region. In recent years, however, many mud crab fisheries have experienced over harvesting which has threatened capacity to meet future demand from local and export markets. Aquaculture is now widely regarded as a key strategy for meeting increased demand for mud crab product and reducing pressure on wild stocks.

Typically, mud crabs kept in culture have been fed natural diets based on marine animals such as “trash fish”. The collection and use of such natural feeds has many disadvantages including depletion of local marine communities, fouling of culture production systems, variable feed availability and cost. It is now widely recognised that the future viability and expansion of mud crab aquaculture is dependant upon the development of low cost but nutritionally adequate formulated diets (Fielder 2004).

Formulated feeds designed for penaeid prawns have been used with some success for mud crab grow out (Mann and Paterson 2004). Many of these penaeid diets, however, are relatively expensive and there is little data available to indicate how effectively they meet the nutritional requirements of mud crabs. In particular, there is evidence that the nutritional requirements of mud crabs may not be as stringent as those reported for penaeid prawns and that the levels of some relatively expensive components in mud crab diets, such as protein, may be reduced. For example, many artificial prawn diets contain between 50% - 60% protein (Teshima and Kanazawa 1984) yet good growth rates for mud crabs in culture have been reported using substantially lower levels of dietary protein (Catacutan 2002; Tuan, Anderson, Luong-van, Shelley and Allan 2006). Another disadvantage of using artificial prawn diets for crab aquaculture is that most are based on marine animal meals, such as fishmeal. Typically, fishmeal is one of the most expensive ingredients incorporated into aquaculture diets (Hardy and Tacon 2002). It has been predicted that with future demand for fishmeal expected to increase as aquaculture production expands, only species of very high market value will be able to compete for this critical feed ingredient (Edwards, Tuan and Allan 2004). Reducing reliance on fishmeal is now recognised as a priority for reducing aquaculture feed cost.

Identification of feed ingredients with the potential to replace fishmeal in formulated aquaculture diets requires data on the nutritional requirements and digestive capacity of candidate species. Traditionally, mud crabs have been viewed as carnivores that show a preference for natural diets containing molluscs, crustaceans, and fish (Hill 1979). Interestingly, however, the presence of significant amounts of plant-based material in the mud crab digestive system has been reported (Hill 1976; Tacon and Akiyama 1997). Moreover, Prasad and Neelakantan (1988) demonstrated that detritus was the main food source for crabs less than 70mm carapace width. In recent years, evidence has emerged that the mud crab digestive system possesses a significant capacity to digest plant-based materials. Investigations into the digestive physiology of *S. serrata* have confirmed that this species possesses the necessary enzymes to digest many plant-based carbohydrates. For example, Pavasovic, Richardson, Anderson, Mann and Mather (2004) detected significant amylase, cellulase and xylanase activity in soluble extracts from the mid gut gland. Findings such as these provide a rationale to investigate the use of relatively cheap plant-based ingredients in mud crab diets. Supplying nutrients from plant-based sources also offers potential opportunities to reduce reliance on fishmeal and therefore lower diet costs.

One of the first steps in estimating the potential of a new ingredient for use in formulated aquaculture diets is to test its digestibility in candidate species. Recent digestibility studies have confirmed that the mud crab has a significant capacity to utilise feed ingredients from a variety of terrestrial animal or plant-based sources. For example, Catacutan, Uusebio and Teshima (2003) showed that plant-based feed ingredients such as soybean meal, corn meal and copra meal were all highly digestible to mud crabs while Tuan *et al.* (2006) reported that the protein digestibility of blood meal and soybean meal was not significantly different to that of fishmeal.

In a recent study, Pavasovic *et al.* (2004) demonstrated that mud crabs have a relatively high capacity to digest purified carbohydrates derived from plants. Specifically, high digestibility coefficients were obtained for soluble cellulose derivatives and starch in formulated feeds. The inclusion of digestible carbohydrate has already been recommended for diets formulated for carnivorous fish and some crustacean species (Wilson 1994; Catacutan and Coloso 1997; Kaushik 2001). Nevertheless, the digestibility of plant based materials such as starch can vary with botanical origin and treatment. Furthermore, the inclusion of starch in aquafeeds can affect the digestibility of other dietary elements, such as protein, which are essential for growth (Cousin, Cuzon and Guillaume 1996: Sales and Britz 2002; Stone, Allan and Anderson 2003). As a consequence, digestibility coefficients for plant-based carbohydrates should be determined before they are included routinely in formulated diets for candidate species.

The objective of the current study was to evaluate the potential of a range of feed ingredients for use in diets formulated for *S. serrata*. In the first part of this study, digestibility coefficients for selected commercial animal feed meals were determined. Feed meals tested were derived from either terrestrial animal (meat or poultry) plant (soybean, lupin, canola or cotton seed) or single cell (yeast) sources. The aim of the second part of the study was to examine the digestibility of experimental diets containing selected plant-based starches. Specifically, digestibility coefficients were determined for fishmeal-based formulated diets containing different levels (15%, 30%, 45% or 60%) or types (wheat, potato, rice or corn) of purified starch.

2. Materials and Methods

2.1 Animals

Experimental animals were supplied by Bribie Island Aquaculture Research Centre Station (BIARC), Bribie Island, QLD Australia. For all experimental treatments, crabs were supplied with recirculated, aerated seawater that was gravity fed through an electrically heated overhead tank. Water temperature was maintained within a range (27.5 + 0.5^{o}C) suggested for optimal growth of mud crabs.

2.2 Study 1: Digestibility of Selected Feed Meals

A digestibility trial was conducted to evaluate the digestibility coefficients of Brewer's yeast (BY) (Swift and Co, Australia) and selected animal feed meals: South American fish meal (FM) (Ridley Aqua Feed, Australia), meat and bone meal (MBM) (Southern Meats, Australia), poultry meal (PM) (AJ Bush, Australia), lupin meal (LM) (MC Croker, Australia), soybean meal (SBM) and canola meal (CM) (Radford Park Aquafeed, Australia). The reference diet was used as a control which consisted of a commercial *P.monodon* diet (Turbo, Thailand). Experimental diets were formulated by combining test ingredients and the reference diet in a 30%:70% ratio, on a dry weight basis. A complete list of ingredients for all test diets is presented in Table 1. The proximate nutrient content of the reference diet and test ingredients shown in Table 2. Proximate composition of diets and faecal material were determined at the Animal Research Institute (Brisbane, Australia) according to AOAC protocols (1984).

Diets used in the experiment were prepared by thoroughly mixing dry ingredients, followed by wet ingredients, until a crumbly dough consistency was achieved. Diet mixture was pressure pelleted using a meat grinder with a 3mm die. Pellets were steamed in a rice steamer in a microwave oven (Sanyo) for 10 min, prior to drying at 50°C in a drying oven, overnight. All experimental diets were stored at -20°C until required. All diets contained 0.5% Chromic oxide (Cr_2O_3) as an inert indicator to allow the calculation of digestibility coefficients for dry matter (ADMD), crude protein (ACPD) and energy (AGED) using formulae suggested by Jones and De Silva (1997). Pilot studies determined that there was no significant loss of detectable chromium (Cr) in feed pellets immersed in water at 26^{o}C for 1h (data not shown).

At the commencement of this study, crabs were selected that had an average body weight of 89g ± 18g. These crabs were then assigned randomly into ten groups and housed individually in plastic containers (19.5cm x 28cm x 22cm). Crabs were kept in these containers for one week to acclimate to the culture conditions prior to the start of the experiment and fed a commercial prawn diet (Turbo; for analysis see Table 2). Nine dietary treatments (n=12 crabs / treatment) were utilised in this study (Table 1). Crabs were fed experimental diets twice daily at a feeding rate of 5% body weight (BW) per day until approximately 1.5 to 2g of faecal material (dry weight) was collected. A daily record was kept of mortalities in each test group. To allow crabs sufficient time to acclimate to experimental diets, faecal collection did not commence until day 7 of the experiment. Faecal material at the bottom of the tank was collected by syphoning into a plastic sieve and gently rinsed for one minute in distilled water before removing individually using forceps. To collect sufficient material for analysis, faecal material from three crabs in each treatment was pooled (n=4 / treatment). All samples were lyophilized and stored at -20°C until required for analysis.

The indirect method of Furukawa and Tsukahara (1966) was used to calculate the digestibility coefficients of all diets tested. Briefly, 0.5g of feed or faecal material was added to 4.0mL of concentrated nitric acid (AnalaR grade, 16 M HNO_3) and incubated overnight at room temperature. Samples were then heated to 150^{o}C for an additional 60 min. After cooling, samples were mixed with 5.0mL of concentrated perchloric acid (AnalaR grade, 70% $HClO_4$) then heated to 220^{o}C for 30 min and 245^{o}C for a further 30 min. After cooling, the absorbance of each sample was read at 346.5nm. For calibration purposes, the above protocol was repeated using known quantities of Cr_2O_3..

Coefficients for ADMD were determined using the formula:

ADMD = 100 – 100 (%Cr_2O_3 in feed / % Cr_2O_3 in faeces).

Coefficients for crude protein (ACPD) or gross energy (AGED) were determined using the formula:

APD = 100 – 100 [(%Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% protein or Mj/kg energy in faeces/ % protein or Mj/kg energy in feed)].

Apparent digestibility coefficients (ADC) of the test ingredients were calculated using the following equations described by Bureau, Harris and Cho (1999).

$ADC_I = ADC_T + ((1 - s)\ D_R\ /\ s\ D_I)\ (ADC_T - ADC_R)$; where: ADC_I = apparent digestibility coefficient of test ingredient; ADC_T = apparent digestibility coefficient of test diet; ADC_R = apparent digestibility coefficient of the reference diet; D_R = % nutrient (or kJ/g gross energy) of the reference diet mash; D_I = % nutrient (or kJ/g gross energy) of the test ingredient; s = proportion of test ingredient in test diet mash (i.e. 0.3 in this study); $(1 – s)$ = proportion of reference diet mash in test diet mash (i.e. 0.7 in this study).

2.3 Study 2: Impact of starch on diet digestibility

As described previously, other workers have recommended the inclusion of plant-based carbohydrates in formulated diets to supply energy for aquatic animal species. A second digestibility trial was therefore conducted in this study to assess the impact of starch included in formulated mud crab diets based on fishmeal. Adult sibling crabs (164.3 ± 27.7g) of the population used for study 1 were selected from a grow out recirculating cellular system at Bribie Island Aquaculture Research Centre for this trial. Each individual crab was weighed at the start and end of each experiment. Crabs were assigned randomly into nine groups with twelve crabs in each group and held in individual containers (19.5cm x 28cm x 22cm) which were covered by plastic net lids. Crabs were allowed to acclimate to experimental conditions and diets as described previously (2.2).

To ensure dietary protein levels in all test diets were set above those reported to promote good growth rates in culture (Catacutan 2002), a reference diet based on high quality South American fishmeal was formulated (RF Diet; Table 3). Eight other diets were also formulated where fishmeal in the reference diet was replaced with different amounts (15%, 30%, 45% or 60%) or types (wheat, corn, rice and potato) of purified starch (Sigma product). Diets used in the experiment were prepared as described previously (2.2), with the exception of the WSU45 Diet (45% wheat starch) which was not steam cooked (uncooked) prior to drying. All diets contained 0.5% Cr_2O_3 as an inert indicator to allow calculation of apparent nutrient digestibility coefficients.

Faecal material was collected as described previously (2.2) after which proximate composition of diets and faecal material was determined at the Animal Research Institute (Brisbane, Australia) according to AOAC protocols (1984). Proximate composition of diets is shown in Table 4. A total starch assay (amyloglucosidase/ α-amylase method, AOAC method 996.11) was also performed on all diets and faecal material collected. Briefly, 100mg of sample was wetted with 0.2mL of aqueous ethanol (80%v/v) to aid dispersion, stirred on a vortex mixer and then immediately 3mL of thermostable α-amylase (300Units) in MOPS buffer (50mM, pH 7.0) was added; vigorously stirred on a vortex mixer. The tube was then incubated in a boiling water bath for 6 minutes, placed in a bath at $50^{o}C$, then sodium acetate buffer (4mL, 200mM, pH 4.5) was added. This was followed by addition of amaloglucosidase (0.1mL, 20U), stirred on a vortex mixer and incubated at $50^{o}C$ for 30 min. The test tube was mixed thoroughly and the volume adjusted to 10mL with distilled water, then samples centrifuged at 3000g for 10 minutes. Duplicate aliquots (0.1mL) of this solution were transferred to the bottom of glass test tubes and 3.0mL of glucose oxidase-peroxidase (GOPOD) reagent was added to each tube (including the glucose controls and reagent blanks). The tube was then incubated at $50^{o}C$ for 20 min. A glucose control consisted of 0.1mL of glucose standard solution (1gL-1) and 3.0mL of GOPOD reagent. A reagent blank solution consisted of 0.1mL of distilled water and 3.0mL of GOPOD reagent. Absorbance of the supernatant solution and the glucose control was read against the reagent blank at 510nm using a Novospec spectrophotometer (LKB). Values of starch contained in diets and faecal were calculated according to the following formula:

Calculation of starch $= \Delta E \times F \times 1000 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 90$

Starch % (Dry weight basis) = Starch % (as is) x 100/ [100 – moisture content (%)]

Where:

ΔE	= Absorbance (reaction) read against the reagent blank
F	= 100 (μg of glucose)/ absorbance of 100μg of glucose
1000	= Volume correction (0.1mL taken from 100mL)
1/1000	= Conversion from micrograms to milligrams
100/W	= Factor to express “starch” as a percentage of sample weight
W	= Weight in milligrams (“as is” basis) of the sample
162/180	= Adjustment from free glucose to anhydro glucose (as occurs in starch)

2.4 Statistical analyses

The significance of data were determined by one- way ANOVA (SPSS version 13.0) and *post hoc* comparison by Tukey’s HSD. For all analysis the significance level of $p<0.05$ was used as standard.

3. Results

3.1 Digestibility of Selected Feed Meals

Table 5 displays the apparent digestibility coefficients for dry matter, crude protein and gross energy obtained using selected animal feed meals. Overall, the ADMD coefficients for most feed meals tested were not significantly different from the value obtained for fishmeal, ranging from 79% to 88%. The ADMD value of meat meal, however, was significantly lower ($p<0.05$) than those obtained for all other test ingredients. ACPD coefficients for all feed meals tested were relatively high, with values ranging from 86% to 97%. Interestingly, the ACPD value for yeast was significantly higher ($p<0.05$) than those obtained for all other test ingredients. Cotton seed meal, poultry meal, canola meal, fishmeal, soybean meal and lupin meal had AGED values that were not significantly different ($p>0.05$) from one another, ranging from 84% to 89%. The AGED coefficient for meat meal, however, was significantly less ($p<0.05$) than values obtained for all other ingredients except cotton seed meal and poultry meal. By contrast, the highest value for AGED was obtained for yeast which was significantly higher ($p<0.05$) than those obtained for meat meal, cotton seed meal and poultry meal. Survival rates for all treatments were high, ranging from 92% to 100% (data not shown).

3.2 Impact of starch on diet digestibility

Apparent digestibility coefficients for dry matter, crude protein, gross energy and starch for diets tested in this study are presented in Table 6. ADMD values ranged from 72% to 85.3%. Inclusion of wheat, potato, rice and corn starch in cooked diets had no significant impact on dry matter digestibility when compared with the reference diet

(RF). By contrast, the inclusion of 45% wheat starch in the uncooked diet (WSU45) significantly increased dry matter digestibility. Apparent digestibility of crude protein was relatively high for all treatments, ranging from 86 % to 92%. Interestingly, ACPD values for diets containing rice starch (RS30), corn starch (CS30) or uncooked wheat starch (WSU45) were significantly higher ($p<0.05$) than the values obtained for all other experimental diets containing between 15% and 60% starch. AGED values for test diets ranged from 73% to 92%. The highest value obtained was for the uncooked diet containing wheat starch (WSU45) which was significantly higher ($p<0.05$) than for all other experimental diets, excepting diets RF and WS30. By contrast, the AGED value obtained for the diet containing potato starch (PS30) was significantly lower ($p<0.05$) than the values obtained for all other test diets. A consistent and significant ($p<0.05$) decline in starch digestibility in cooked diets was recorded as the level of wheat starch included was progressively raised to a maximum of 60% (WS60). The type of starch incorporated into test diets also appeared to significantly impact on ASD values. Specifically, at a 30% inclusion level in cooked diets the apparent digestibility of starch was in the following order (from most to least digestible) corn > wheat > rice = potato. Interestingly, for all parameters tested, significantly higher ($p<0.05$) ASD values were obtained for the diet containing 45% wheat starch which was not cooked (WSU45) than was obtained using the same amount of wheat starch in the cooked diet (WS45).

4. Discussion

Many plants and animal-based feed ingredients have been reported to have potential as replacement components for fishmeal in formulated aquafeeds (Tacon 1994). In the present study, we observed relatively high digestibility coefficients for a broad range of animal, plant and single sell- based ingredients, reflecting the ability of mud crabs to utilize a wide range of nutrient sources. These findings are consistent with other studies reporting high digestibility coefficients for a wide variety of animal and plant-based ingredients in mud crab diets (Catacutan *et al*. 2003, Tuan *et al*. 2006). In the current study, the ADMD coefficients for most high protein feed meals tested were similar, with the exception of meat meal. Similar results using some terrestrial animal-based meals in diets for mud crab have been reported by Catacutan *et al.* (2003) and Tuan *et al.* (2006). Reduced digestibility coefficients have also been associated with use of meat meal in *C. destructor* (Jones and De Silva 1997) and *P. setiferus* (Brunson, Romaire and Reigh 1997).

Protein digestibility for all feed meals was relatively high, with values over 85%. This finding is in general agreement with the findings of Catacutan *et al.* (2003) who observed high ACPD in adult mud crab fed a range of animal and plant-based ingredients. The high capacity of mud crabs to digest protein is not surprising considering the high level of protease in the digestive system of this species (Pavasovic *et al*. 2004). Based on the findings of Pavasovic *et al.* (2004) and the current study we argue that the mud crab has a high capacity to digest protein in a wide range of single cell, terrestrial animal and plant-based ingredients. Further studies which attempt to exploit the potential of these feed ingredients to replace fishmeal as a source of dietary protein may help reduce feed costs for this species.

In the second part of this study the digestibility of different types and amount of starches in formulated mud crab diets was tested. Overall, digestibility coefficients for starch and the associated diets were high. This finding is in close agreement with many other investigations which have reported that cereals containing high levels of starch are readily digested by crustaceans (Shi-Yen and Chu-Yang 1992; Cousin *et al*. 1996). The high digestibility of starch demonstrated in this study is not surprising considering the detection of significant carbohydrase activity in the mud crab digestive system (Pavasovic *et al*. 2004). Specifically, amylase, cellulase and xylanase activity has been detected in extracts prepared from the mud crab midgut gland suggesting a significant capacity to digest plant-based nutrients. Furthermore, plant-based material and detritus has been demonstrated in the digestive system of mud crab juveniles sampled from the wild (Hill 1976; Prasad and Neelakantan 1988).

Another important finding of the current study was that diet digestibility was affected significantly by the origin of starch. These results are in close agreement with other study that have shown the digestibility of starch varies with botanical origin (Cousin *et al*. 1996; Sales and Britz 2002; Stone *et al*. 2003). The cooking process also had a significant impact on the digestibility of mud crab diets in the current study. Specifically, the wheat starch diet prepared without steam cooking (WSU45) demonstrated higher digestibility coefficients than the equivalent diet that was steam cooked (WS45). Such differences are not unexpected since there is abundant evidence that cooking processes can have a dramatic impact on the performance of artificial diets for vertebrates (cf. Singh et al, 2007). We suggest that the steam cooking process used in the current study may increase the degree of starch gelatinisation in mud crab diets. Elsewhere it has been shown that changes in the gelatinisation of carbohydrate-rich ingredients in artificial diets can significantly alter diet performance parameters such as apparent total tract digestibility (Vicente et al, 2008) intestinal viscosity, feed intake and body weight gain (Garcia et al, 2008). Based on such findings, we recommend that further studies be conducted to assess how different diet processing methods effect the gelatinisation of starch in formulated mud crab diets and if the degree of starch gelatinisation impacts on growth performance.

In the current study it was shown that the digestibility of corn starch was generally higher than that for wheat starch (at 30% inclusion level). This contradicts the findings of Davis and Arnold (1993) who reported that in other crustacean species wheat was more efficiently digested than corn. The reason for this apparent discrepancy is unclear but it may reflect species specific differences in the capacity to digest carbohydrates from different sources or differences in the purity or preparation of carbohydrates incorporated into crustacean feeds.

The findings of the second part of this study suggest that starch should be considered as a potential feed ingredient in formulated mud crab diets. Overall, most diets containing starch were readily digested. In particular, there were no negative impacts on the digestibility of major nutrients (e.g. protein) observed following the inclusion of wheat, rice or corn starch in formulated feeds. These results argue that further studies are warranted to investigate the potential of starch to supply energy in mud crab diets and reduce the requirements for more expensive feed ingredients such as fishmeal.

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Table 1: Composition (% dry matter of the diet) of the formulated diets for the digestibility trial using commercial feed ingredients.

	Diet								
Ingredient	1	2	3	4	5	6	7	8	9
Basal Diet (Turbo)	62.4	62.4	62.4	62.4	62.4	62.4	62.4	62.4	92.4
Fishmeal	30								
Meat meal		30							
Poultry meal			30						
Soybean meal				30					
Canola meal					30				
Lupin meal						30			
Cotton seed meal							30		
Yeast								30	
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Common ingredients[a]	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6

[a] Common ingredients (g/100g): mineral and vitamin premix (2%), (kg^{-1} of total diet - 4.68 g K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000 IU vitamin retinol; 500 mg thiamine; 1750 mg riboflavin; 1125 mg pyridoxine hydrochloride; 3750 mg cyanocobalamin; 25000 mg ascorbic acid; 500 000 mg colecalciferol; 20 000 IU d-alpha-tocopheryl acid succinate; 50 mg biotin); astaxanthin (0.1%); chromic oxide (Cr_2O_3) (0.5%).

Table 2: Proximate nutrient composition (%) in dry matter of basal diet and test ingredients used in the digestibility trial.

Ingredient	Dry Matter	Crude Protein (N x 6.25)	Crude Fat	Ash	Energy (Mj/kg)
Basal Diet; Turbo *P.monodon* feed	90.4	49.7	6.7	15.3	19.1
Fishmeal	91.7	75.5	8.7	17.2	15.9
Meat meal	97.7	59.6	13.4	20.4	16.9
Poultry meal	96.7	69.2	13.1	14.1	20.9
Soybean meal	88.3	53.2	1.9	7.2	20.6
Canola meal	90	44.1	3.8	7.4	21.5
Lupin meal	86.1	30.8	9.4	3.6	15.9
Cotton seed meal	89.2	48.4	2.4	7.3	19.3
Yeast	95.1	48.6	0.4	9.6	18.3

Table 3: Composition (% dry matter of the diet) of the formulated diets for the digestibility trial using different starches.

Ingredient (%)	Diet								
	RF[a]	WS15	WS30	WS45	WSU45	WS60	PS30	RS30	CS30
Fish meal	87.4	72.4	57.4	42.4	42.4	27.4	57.4	57.4	57.4
Wheat starch (WS)	0	15	30	45	45[b]	60			
Potato starch (PS)							30		
Rice starch (RS)								30	
Corn starch (CS)									30
Binder (Wheat gluten)	5	5	5	5	5	5	5	5	5
Common ingredients [c]	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6

[a] Reference diet

[b] Prepared without steam cooking (Uncooked)

[c] Common ingredients (g/100g): mineral and vitamin premix (2%), (kg^{-1} of total diet - 4.68 g K_2HPO_4; 7.12 g $MgSO_4.7H_2O$; 1.84 g $NaH_2PO_4.2H_2O$; vitamin premix (kg^{-1}) - 100000 IU vitamin retinol; 500 mg thiamine; 1750 mg riboflavin; 1125 mg pyridoxine hydrochloride; 3750 mg cyanocobalamin; 25000 mg ascorbic acid; 500 000 mg colecalciferol; 20 000 IU d-alpha-tocopheryl acid succinate; 50 mg biotin); dried squid (5%); astaxanthin (0.1%); chromic oxide (Cr_2O_3) (0.5%).

Table 4: Proximate nutrient composition (%) in dry matter of experimental diet used in the starch digestibility trial.

Diet	Dry matter	Protein (Nx6.25)	Crude fat	Ash	Starch	Gross Energy ($MJkg^{-1}$)	P/E ratio
RF	93.7	70.5	8.7	15.4	1.3	20.6	3.42
WS15	93.3	63.6	7.5	13.0	14.9	20.6	3.09
WS30	92.8	53.2	6.3	10.6	31.2	19.9	2.67
WS45	90.8	41.8	5.1	8.2	48.5	19.2	2.18
WSU45	92.3	41.9	5.1	8.2	47.6	19.3	2.17
WS60	87.6	33.9	3.9	5.8	60	19.0	1.78
PS30	89.0	49.1	6.3	10.6	33.2	19.7	2.49
RS30	93.4	53.3	6.3	10.6	28.6	19.9	2.68
CS30	93.8	53.6	6.3	10.6	29.8	19.9	2.69

Table 5: The apparent digestibility coefficients (%) of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) for yeast and selected animal feed meals.

Ingredient	ADMD	ACPD	AGED
Basal Diet; Turbo prawn feed	$83.2 + 0.5^{b}$	$90.4 + 0.5^{ab}$	$89.3 + 0.9^{cd}$
Fishmeal	85.4 ± 1.7^{b}	88.3 ± 0.7^{ab}	87.8 ± 1.3^{cd}
Meat meal	67.0 ± 1.3^{a}	86.3 ± 0.9^{ab}	78.2 ± 0.8^{ab}
Poultry meal	78.9 ± 3.0^{b}	88.2 ± 2.1^{ab}	85.2 ± 1.9^{bc}
Soybean meal	80.4 ± 1.2^{b}	91.7 ± 0.5^{bc}	89.1 ± 0.9^{cd}
Canola meal	83.5 ± 4.7^{b}	87.6 ± 2.7^{ab}	87.5 ± 2.9^{cd}
Lupin meal	88.1 ± 1.6^{b}	89.1 ± 0.9^{ab}	89.9 ± 1.4^{cd}
Cotton seed meal	80.5 ± 0.8^{b}	86.8 ± 0.6^{ab}	83.9 ± 0.4^{abc}
Yeast	85.7 ± 3.2^{b}	96.8 ± 1.6^{c}	93.5 ± 1.7^{d}

Values are means ± standard error (n = 4 replicates per treatment). Means in the same column with the same superscript are not significantly different ($p>0.05$) from one another

Table 6: Impact of starch on apparent digestibility coefficients (%) for dry matter (ADMD), crude protein (ACPD), gross energy (AGED) and starch (ASD) in fishmeal-based formulated mud crab diets

Diet	ADMD	ACPD	AGED	ASD
RF	75.4 ± 1.9^{ab}	89.2 ± 0.8^{abcd}	86.0 ± 1.1^{def}	97.0 ± 0.2^{h}
WS15	72.0 ± 2.8^{ab}	$86.5. \pm 1.1^{ab}$	83.7 ± 1.4^{de}	92.8 ± 0.1^{f}
WS30	75.2 ± 1.2^{ab}	88.0 ± 0.8^{abc}	87.7 ± 0.7^{ef}	90.0 ± 0.1^{de}
WS45	77.6 ± 0.3^{bc}	86.7 ± 0.1^{ab}	81.4 ± 0.7^{bcd}	87.2 ± 0.2^{b}
WSU45	85.3 ± 0.4^{d}	91.9 ± 0.5^{d}	91.8 ± 2.0^{f}	91.1 ± 0.1^{e}
WS60	80.7 ± 0.6^{bcd}	86.9 ± 0.4^{ab}	82.7 ± 0.9^{cde}	84.4 ± 0.4^{a}
PS30	76.4 ± 1.2^{abc}	88.0 ± 0.9^{abc}	73.1 ± 1.6^{a}	88.5 ± 0.1^{c}
RS30	83.0 ± 2.5^{bcd}	91.6 ± 1.3^{d}	84.8 ± 1.5^{de}	88.5 ± 0.1^{c}
CS30	83.4 ± 0.9^{bcd}	91.6 ± 0.6^{d}	85.2 ± 1.1^{de}	92.5 ± 0.1^{f}

Values are means ± standard error (n = 4 replicates per treatment). Means in the same column with the same superscript are not significantly different ($p>0.05$) from one another