

The Nutritive Value of Palm Kernel Meal and the Effect of Enzyme Supplementation in Practical Diets for Red Hybrid Tilapia (*Oreochromis* sp.)

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Abstract

A 9-week feeding trial was conducted to evaluate the nutritive value of palm kernel meal (PKM) as a dietary ingredient in pelleted feed for red hybrid tilapia. Three commercially available feed enzymes were also assessed in an attempt to enhance the nutritive value of PKM. Seven isonitrogenous (30% crude protein) and isoenergetic ($15.1 \text{ kJ} \cdot \text{g}^{-1}$) practical diets were formulated and fed to triplicate groups of 10 fish (mean initial weight $8.0 \pm 0.1\text{g}$). The control diet contained no PKM, and three other diets contained 10, 20 and 40% PKM, respectively, replacing soybean meal in the basal diet formulation. Three additional diets were formulated with 40% PKM and supplemented with increasing dosage of commercial feed enzymes known to improve growth performance in poultry fed plant proteins. Growth performance, feed conversion ratio and protein efficiency ratio of tilapia fed up to 20% PKM was not significantly different ($P>0.05$) compared to fish fed the control diet. The addition of PKM in the diets of red tilapia resulted in lower dry matter, protein, lipid and energy digestibility coefficients at all levels of dietary inclusion. Enzyme supplementation significantly improved dry matter and energy digestibility coefficients of the 40% PKM diet; however, no beneficial effects on the growth and feed utilization efficiency of red tilapia were observed. It was concluded that PKM is a potential partial substitute for soybean meal in tilapia diets.

Introduction

Palm kernel meal (PKM) is a by-product of palm kernel oil extraction from the nut of the palm tree, *Elaeis guineensis*. The global production of PKM is increasing due to the tremendous growth of the oil palm industry in many parts of Asia and Africa. About 2 million tons of PKM are produced in

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Malaysia, the current global leader in the oil palm industry (PORLA 2000). PKM has been successfully used as a feed ingredient for ruminants (Siew 1989; Hassan and Yeong 1999) and research have shown that it could also be incorporated at low levels (10 to 30%) in the diets of nonruminant livestock such as poultry (Yeong 1985; Panigrahi and Powell 1991) and pigs (Rhule 1996; Agunbiade et al. 1999). The detailed characteristics and nutrient content of PKM have been reviewed by Siew (1989).

The low cost and availability of PKM have recently generated much interest in its potential use in fish diets. Very little information is currently available on the use of PKM in fish diets (Omoregie and Ogbemudia 1993; Saad et al. 1997; Lim et al. 2001; Ng and Chen, in press). Research into alternative locally available feedstuffs such as PKM is crucial for the continued expansion of the aquaculture industry in Malaysia and in other developing countries. Soybean meal, a common ingredient used in commercial fish diets, is imported into many tropical countries, which cannot grow soybeans. Therefore, the first objective of the present study is to evaluate PKM as a possible substitute for soybean meal in an attempt to reduce the impact of rising costs to feed tilapia.

The low digestibility of feed ingredients of plant origin is commonly attributed to the high level of nonstarch polysaccharides (NSP) found in the cell wall materials. These NSP impair the digestibility and utilization of nutrients present in the plant feedstuff either by direct encapsulation of the nutrients or by increasing the viscosity of the intestinal content thereby reducing the rate of hydrolysis and absorption of nutrients in the diet (Choct and Annison 1992). The NSP composition of PKM had been reported by Dusterhoft and Voragen (1991) and consisted mainly of mannans (major NSP), cellulose and xylans.

Exogenous enzymes are routinely used in poultry and swine feeds to improve the nutritive value of feed ingredients of plant origin (Campbell and Bedford 1992; Cowan et al. 1996; Marquardt et al. 1996; Kitchen 1997). The current trend of increased use of plant proteins in fish diets had generated much interest in the use of enzymatic supplements in aqua feeds. To date, the few studies conducted on the addition of proteolytic, fibrolytic or carbohydrate-degrading enzymes to fish and shrimp diets had resulted in contradictory findings (Carter et al. 1992; Carter et al. 1994; Buchanan et al. 1997; Divakaran and Velasco 1999). More research needs to be carried out on the effectiveness of various exogenous enzymes in fish and shrimp diets before any recommendations can be made to the aqua feed industry. The use of enzyme supplements in PKM-based diets has not been investigated. Therefore, the second objective of the present study is to evaluate the efficacy of several commercial feed enzymes in improving the nutritive value of a PKM-based tilapia diet.

Materials and Methods

Diet preparation

PKM was obtained from a local palm oil distillery (Palmco Oil Mill, Penang) and ground to fine powder with a Wiley mill before being used in the

experimental diets. Seven isonitrogenous (30% crude protein) and isoenergetic ($15.1 \text{ kJ}\cdot\text{g}^{-1}$) practical diets were formulated (Table 1). The control diet contained no PKM, and three other diets contained 10, 20 and 40% PKM, respectively, replacing soybean meal (as a source of dietary protein) and corn flour (as a source of dietary energy). Three additional diets were formulated with 40% PKM and supplemented with increasing dosage of commercial feed enzymes known to improve growth performance in poultry and swine fed vegetable proteins (Cowan et al. 1996; Kitchen 1997). Diet 5 was supplemented with an enzyme mix consisting of Allzyme VegproTM (Alltech Inc., KY), RonozymeTM VP (Hoffmann-La Roche Ltd., Basel) and pure mannanase (Alltech Inc., KY) at inclusion levels of 0.1%, 0.05% and 0.01%, respectively. This inclusion level (E1) was recommended by the respective companies for poultry and swine feed. Since these enzymes have not been tested in PKM-based fish feeds, we also formulated two other diets (Diet 6 and Diet 7) to contain five (E5) and ten times (E10) the recommended inclusion levels, respectively (Table 1). Allzyme Vegpro contains protease ($7700 \text{ HUT}\cdot\text{g}$) and cellulose ($75.1 \text{ CMC units}\cdot\text{g}$) whereas Ronozyme VP has endo-1, 3(4)- β -glucanase ($50 \text{ FBG}\cdot\text{g}$) and pectinase ($3.5 \text{ U}\cdot\text{g}$) activities.

All ingredients were thoroughly mixed in a Hobart mixer and chromic oxide was added as an inert marker for nutrient digestibility determination.

Table 1. Formulation of experimental diets ($\text{g}\cdot 100^{-1} \text{ g dry diet}$).

Ingredients	Diet no. / % Palm kernel meal inclusion						
	1 0	2 10	3 20	4 40	5 40+E1 ^a	6 40+E5 ^b	7 40+E10 ^c
Fish meal ^d	25.01	25.01	25.01	25.01	25.01	25.01	25.01
Soybean meal ^e	24.13	20.69	17.25	10.37	10.37	10.37	10.37
Palm kernel meal ^f	-	10.00	20.00	40.00	40.00	40.00	40.00
Corn flour	26.28	21.87	17.46	8.63	8.63	8.63	8.63
Cod liver oil	2.16	2.16	2.16	2.16	2.16	2.16	2.16
Palm kernel oil	4.72	4.01	3.30	1.89	1.89	1.89	1.89
Vitamin premix ^g	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix ^h	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl cellulose	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Allzyme Vegpro ^{TMi}	-	-	-	-	0.10	0.50	1.00
Ronozyme TM VP ^j	-	-	-	-	0.05	0.25	0.50
Mannanase ^k	-	-	-	-	0.01	0.05	0.10
Cellulose	9.70	8.26	6.82	3.94	3.78	3.14	2.34

^aE1 = 0.1% Allzyme Vegpro + 0.05% Ronozyme VP + 0.01% mannanase.

^bE5 = 0.5% Allzyme Vegpro + 0.25% Ronozyme VP + 0.05% mannanase.

^cE10 = 1.0% Allzyme Vegpro + 0.5% Ronozyme VP + 0.1% mannanase.

^dDanish fish meal contains ($\text{g}\cdot 100^{-1} \text{ g dry weight}$): 72.0 crude protein, 11.3 crude lipid, 13.9 ash.

^eSolvent-extracted soybean meal contains ($\text{g}\cdot 100^{-1} \text{ g dry weight}$): 49.7 crude protein, 1.2 crude lipid, 10.1 ash.

^fPalm kernel meal contains ($\text{g}\cdot 100^{-1} \text{ g dry weight}$): 17.1 crude protein, 7.5 crude lipid, 6.1 ash, 13.8 crude fiber.

^g, ^hComposition according to Ng et al., 2000.

ⁱ, ^j, ^kCommercial feed enzymes. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply endorsement or recommendation.

The experimental diets were prepared following the procedure described by Ng et al. (1998). All pelleted diets were stored in a freezer at -20°C until needed.

As an indicator of whether enzyme activity was retained after processing, the reducing sugar content of the various diets was measured by the Somogyi-Nelson method (Nelson 1944; Somogyi 1952). Samples of the various diets were obtained before and after pelleting, and after 2 months of storage. The diet samples were ground to powder and incubated with distilled water (1:2) overnight at room temperature. Samples (1 g each) of the various diets were then diluted in 10 mL distilled water, vortexed and 1 mL of the resultant supernatant subjected to the reducing sugar test. The reducing sugar concentration of the diets at various stages is shown in table 2.

Experimental procedure

Malaysian red hybrid tilapia (*Oreochromis* sp.) fingerlings were obtained from a local fish hatchery, and reared to experimental size in our Fish Nutrition Laboratory. The experimental fish were fed a commercial feed (34% crude protein, KT Feed, Thailand) during the 4-week acclimatization period. Prior to starting the feeding trial, all fish were acclimated to the control diet (Diet 1) for one week.

At the start of the experiment, 10 red tilapia fingerlings (mean weight of 8.0 ± 0.1 g) were stocked into each aquarium. The description and operation of the aquaria system were similar to that described in Ng et al. (2000). The seven dietary treatments were fed to randomly assigned triplicate groups of fish. Tilapia were fed close to apparent satiation at 4% of their body weight per day in two equal feedings and this feeding rate was subsequently reduced to 3.5% body weight per day from the fourth week onwards to eliminate feed wastage.

Fish were batch-weighed by aquarium once every week and the daily ration adjusted accordingly. The feeding trial was conducted for 9 weeks.

Table 2. Proximate composition (% dry weight) and reducing sugar concentration (mg·g⁻¹) of the experimental diets.

Component	Diet no. / % Palm kernel meal inclusion						
	1 0	2 10	3 20	4 40	5 40+E1 ^a	6 40+E5 ^b	7 40+E10 ^c
Moisture	13.8	13.5	13.2	12.2	14.9	12.5	12.6
Crude protein	29.9	30.5	31.0	29.2	30.1	30.6	30.6
Crude lipid	9.0	9.6	9.0	9.6	9.3	9.8	9.5
Ash	8.0	8.6	8.3	9.3	9.0	9.5	9.3
Crude fiber	9.0	8.0	7.9	8.9	9.0	8.4	8.6
NFE ^d	44.1	43.3	43.8	43.0	42.6	41.7	42.0
Gross energy ^e	15.8	15.8	15.9	15.7	15.6	15.6	15.7
Reducing sugar concentration (mg . g ⁻¹ diet)							
Before pelleting	nd ^f	nd	nd	0.93	1.42	2.00	2.75
After pelleting	nd	nd	nd	0.93	1.44	2.01	2.82
After 2 months	nd	nd	nd	0.93	1.56	2.20	2.86

a, b, ^cEnzyme supplementation. See footnotes a, b, c, Table 1.
^dNitrogen-free extract = 100 – (crude protein + crude lipid + crude fibre + ash).
^eGross energy (kJ/g) determined using a bomb calorimeter (C5000, IKA).
^fNot determined.

Sample collection and analysis

At the start of the feeding trial, 20 fish were sacrificed, weighed and kept frozen for subsequent initial whole-body composition analysis. At the end of the feeding trial, all fish were starved for 48 h, killed, and weighed. Liver from each fish were removed and weighed to determine hepatosomatic index. All fish samples were stored frozen at -20°C for subsequent whole-body composition analysis. Fish carcasses were blended, dried and ground into powder before proximate analysis.

The collection of fish feces was carried out during the last three weeks of the feeding trial. The collection, handling and preparation of fish feces for chemical analysis were previously described by Lim et al. (2001). The moisture, crude protein, lipid, ash, and crude fiber contents of feed ingredients, experimental diets, fish carcasses and feces samples were determined following standard AOAC methods (AOAC 1997). Gross energy content of diets and feces were determined using a C5000 bomb calorimeter (IKA, Malaysia). For nutrient digestibility measurements, the chromic oxide content of feces and experimental diets were determined by using the wet-digestion technique of Furukawa and Tsukahara (1966). Dry matter, protein, lipid and energy digestibility of the diets were calculated following the formulas given in Cho et al. (1982).

Statistical methods

Data collected from the feeding trial were all subjected to one-way analysis of variance (ANOVA) (SAS Institute, Cary, NC) to determine if significant differences occurred among dietary treatments. Data expressed as percentages were arc sine-transformed before analysis. Differences between means were assessed using Duncan's multiple range test (Duncan 1955). All differences were regarded as significant at $P < 0.05$.

Results

The analyzed proximate composition of the experimental diets is presented in table 2 and the values were as expected from the ingredient formulations used. The concentration of soluble reducing sugars of aqueous extracts of diets, as an indicator of enzyme activity, increased with increasing dietary enzyme inclusion level of E1 to E10 (Table 2). Based on the reducing sugar concentration of diets before and after pelleting, and after 2 months of storage, no loss in enzyme activity in the diets was observed throughout the feeding trial.

All experimental diets were highly palatable and were immediately consumed by the red tilapia fingerlings throughout the duration of the experiment. All fish were active and appeared healthy at the end of the experiment, and there was no fish mortality.

Red tilapia fed diets with up to 20% inclusion of PKM did not show any significant difference in growth performance ($P > 0.05$) compared to fish fed the control diet without any PKM (Table 3). However, at 40% PKM, growth

performance was significantly poorer ($P < 0.05$) and this reduced growth was not alleviated with the supplementation of feed enzymes at all levels of inclusion. Similar trends to those observed for the growth responses were also observed for the effect of dietary PKM inclusion on feed and protein utilization efficiencies (Table 3). With the exception of net protein utilization (NPU), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish fed up to 20% PKM in their diets showed no significant differences compared to fish fed the control diet. Feed and protein utilization efficiencies were significantly poorer when tilapias were fed 40% PKM diets. The addition of feed enzymes to the 40% PKM diet did not improve feed utilization efficiency of tilapia.

The addition of PKM in the diets of red tilapia resulted in significantly lower dry matter, protein, lipid and energy digestibility even at the lowest level of inclusion tested (Table 4). A gradual decline in dry matter, protein and energy digestibility was observed when fish were fed diets with increasing levels

Table 3. Growth performance and feed utilization efficiency of red hybrid tilapia fed palm kernel meal-based diets for 9 weeks¹.

Parameter	Diet no. / % Palm kernel meal inclusion							
	1 0	2 10	3 20	4 40	5 40+E1 ²	6 40+E5	7 40+E10	s.e.m ³
Final weight (g)	32.1 ^a	31.8 ^{ab}	30.5 ^{abc}	27.9 ^{bcd}	24.8 ^d	25.8 ^d	27.5 ^{cd}	1.1
Weight gain ⁴ (%)	300.3 ^a	300.2 ^a	280.4 ^{ab}	247.1 ^c	207.3 ^c	220.3 ^c	243.7 ^{bc}	14.1
SGR ⁵ (%·day ⁻¹)	2.16 ^a	2.16 ^a	2.09 ^{ab}	1.94 ^{bc}	1.75 ^c	1.82 ^c	1.93 ^{bc}	0.06
FCR ⁶	1.63 ^c	1.67 ^c	1.73 ^{bc}	1.81 ^{abc}	1.97 ^a	1.92 ^{ab}	1.80 ^{abc}	0.05
PER ⁷	2.06 ^a	1.90 ^{ab}	1.89 ^{ab}	1.81 ^b	1.55 ^c	1.70 ^{bc}	1.77 ^b	0.06
NPU ⁸ (%)	31.5 ^a	28.3 ^{ab}	27.3 ^{bc}	26.0 ^{cd}	22.7 ^d	23.5 ^{cd}	26.7 ^{bcd}	1.1

¹Values are the mean of triplicate groups of 10 fish. Mean values in the same row with different superscripts are significantly different ($P<0.05$). Average initial body weight of individual fish was 8.0 ± 0.1 g.
²Enzyme supplementation. See footnotes a, b, c, Table 1.
³Pooled standard error of means.
⁴Expressed as the percent of initial body weight at the end of 9 weeks.
⁵Specific growth rate (%/day) = $100 \times (\ln \text{ final} - \ln \text{ initial fish weight})/\text{days}$.
⁶Feed conversion ratio = total dry feed fed (g)/total wet weight gain (g).
⁷Protein efficiency ratio = wet weight gain (g)/total protein intake (g).
⁸Net protein utilization (%) = $100 \times [(\text{final} - \text{initial fish body protein})/\text{total protein intake}]$.

Table 4. Nutrient digestibility (%) of palm kernel meal-based diets fed to red hybrid tilapia for 9 weeks¹.

Nutrient	Diet no. / % Palm kernel meal inclusion							
	1 0	2 10	3 20	4 40	5 40+E1 ²	6 40+E5	7 40+E10	s.e.m ³
Dry matter	49.7 ^a	36.8 ^b	30.3 ^{cd}	25.5 ^d	30.7 ^{cd}	31.0 ^{cd}	32.1 ^{bc}	2.9
Protein	82.8 ^a	81.1 ^b	80.1 ^b	79.6 ^b	81.0 ^b	80.7 ^b	80.0 ^b	0.4
Lipid	89.8 ^a	87.3 ^b	87.1 ^b	88.0 ^{ab}	87.4 ^b	86.5 ^b	87.0 ^b	0.4
Energy	54.7 ^a	44.2 ^b	36.6 ^c	31.8 ^e	33.6 ^d	36.6 ^c	36.0 ^c	2.7

¹Values are the mean of triplicate groups of 10 fish. Mean values in the same row with different superscripts are significantly different ($P<0.05$).
²Enzyme supplementation. See footnotes a, b, c, Table 1.
³Pooled standard error of means.

of PKM. However, apparent lipid digestibility did not further decline when PKM was increased from 10 to 40% in the tilapia diet. Enzyme supplementation significantly improved dry matter and energy digestibility of the 40% PKM diet compared to fish fed a similar diet without supplementary enzymes (Table 4). Enzyme supplementation did not have any effect on protein and lipid digestibility of the diets.

Whole-body lipid of fish fed 40% PKM diets was significantly lower compared to the whole-body lipid of fish fed the control diet (Table 5). In general, the dietary inclusion of PKM did not affect whole-body moisture, protein and ash composition. Enzyme supplementation in the diets did not have any effect on the whole-body composition of tilapia. The hepatosomatic indexes of fish fed the various experimental diets were not significantly different (Table 5).

Discussion

The present study demonstrated that PKM is a potential partial substitute for soybean meal and could be included up to 20% (dry weight basis) in the diets of red tilapia without adversely affecting growth performance and feed utilization efficiency. Higher levels of PKM incorporation (30%) had been reported to be possible for tilapia raised in outdoor earthen ponds (Saad et al. 1997) and for tilapia fed diets with higher dietary protein levels (Lim et al. 2001). However, not more than 15% PKM should be included when PKM was used to replace fishmeal in tilapia diets (Omoregie and Ogbemudia 1993). The use of PKM, which can be obtained locally in large quantities as a by-product of the palm oil industry, will help reduce the cost of tilapia feeds. The decision on the amount of PKM to substitute for soybean meal will depend on economic grounds; whether any reduction in weight gain is compensated for by the use of a less-expensive ingredient.

Table 5. Whole body (% wet weight basis) and hepatosomatic index of red hybrid tilapia fed palm kernel meal-based diets for 9 weeks¹.

Component	Diet no. / % Palm kernel meal inclusion							s.e.m ³
	1 0	2 10	3 20	4 40	5 40+E1 ²	6 40+E5	7 40+E10	
Whole body								
Moisture	73.7	73.7	73.6	74.5	74.1	74.9	74.1	0.2
Crude protein	15.8	15.4	15.4	15.1	15.4	14.9	15.5	0.1
Crude lipid	5.6 ^{ab}	6.1 ^a	5.4 ^{bc}	4.9 ^{cd}	4.9 ^{cd}	4.8 ^{cd}	4.7 ^d	0.2
Ash	4.4 ^c	4.4 ^c	4.8 ^{ab}	4.6 ^{abc}	4.8 ^{ab}	4.6 ^{abc}	4.9 ^a	0.1
Liver								
HSI ⁴	1.29	1.32	1.34	1.14	1.09	1.17	1.26	0.04

¹Values are the mean of triplicate groups of 10 fish. Mean values in the same row with different superscripts are significantly different ($P < 0.05$). Initial whole body composition was 71.4% moisture, 17.1% protein, 6.1% lipid and 5.0% ash.

²Enzyme supplementation. See footnotes a, b, c, Table 1.

³Pooled standard error of means.

⁴Hepatosomatic index.

The observed growth depression of tilapia when fed high levels of dietary PKM in the present study is consistent with other studies on the use of various oilseed meals in fish diets (Jackson et al. 1982; Shiau et al. 1987; Hasan et al. 1997). Other than anti-nutritional factors that may be present in some of these oilseeds, the marked decrease in diet digestibility, as the level of oilseed meal increased in the diet is a major contributing factor to the poorer growth performance of fish. In an attempt to increase the digestibility of PKM, various commercial feed enzymes were added into the 40% PKM diet in the hope that this will allow increased inclusion of this by-product in tilapia diet formulations. However, despite a significant improvement in dry matter and energy digestibilities of the 40% PKM diet, enzyme supplementation did not translate into improved growth and feed utilization efficiency for the red tilapia.

Despite using an enzyme dosage of up to ten times the recommended inclusion rate for poultry and swine feeds, the increase in energy digestibility of the 40% PKM diet was only about 4% and that of dry matter digestibility, about 7%. No improvement in diet protein and lipid digestibilities was observed. Even though enzyme activity was not destroyed after feed processing, the lack of enzyme effect may be due to the less than optimal conditions present in the digestive system of tilapia. The stomach pH of tilapia had been reported to be between 2.0 and 3.0 (Fish 1960) and even though the culture water temperature was around 28°C, tilapia are poikilotherms and this might have a negative effect on enzyme activity.

Cell wall materials constitute about 73% of PKM and NSP accounted for 75% of these water-insoluble cell wall materials (Dusterhoft and Voragen 1991). Dusterhoft and Voragen (1991) estimated that mannans (mannose units joined by β -1,4 linkages) formed about 76% of the total polysaccharides in the palm kernel followed by cellulose (11%) and xylans (4%). Therefore, it was logical to assume that incorporating a mannanase preparation in diets containing PKM given the high mannan content of PKM might enhance the nutritive value of PKM. However, it should be pointed out that the efficacy of mannose as an energy source for tilapia has not been evaluated and its absorption and utilization by tilapia is currently not known. Even if tilapia could effectively utilize the energy from mannose, which was previously unavailable in mannan, dietary available energy might not be the major limiting factor to fish growth in the present study. Other factors such as the amino acid composition of PKM (which is lacking in methionine and lysine) might have played an important role (Siew 1989).

Research is currently being carried out in our laboratory to optimize the inclusion of PKM in fish diets. Preliminary results from our lab (unpublished data) seems to indicate that tilapia fed diets incorporated with PKM pretreated with exogenous enzymes tended to show better growth performance compared to fish fed a similar dietary level of untreated PKM. Feeding trials carried out in Thailand also showed that tilapia fed diets with 21% PKM pretreated with 0.1% Ronozyme VP showed significantly better growth performance and net protein utilization compared to fish fed raw PKM (Boonyaratpalin et al. 2000). Pre-incubation of PKM with exogenous enzymes may be necessary for eliciting the maximum potential effect of these enzymes in PKM-based aquaculture feeds.

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