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*Full Length Research Paper*

# Nutritional evaluation of fermented palm kernel cake using red tilapia

**F. B. Iluyemi[1], M. M. Hanafi[2]*, O. Radziah[1] and M. S. Kamarudin[3]**

[1]Depatment of Land Management Faculty of Agriculture, Universiti Putra Malaysia, 43400-Serdang, Selangor, Malaysia.
[2]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400-Serdang, Selangor, Malaysia.
[3]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400-Serdang, Selangor, Malaysia.

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**The use of palm kernel cake (PKC) and other plant residues in fish feeding especially under extensive aquaculture have been in practice for a long time. On the other hand, the use of microbial-based feedstuff is increasing. In this study, the performance of red tilapia raised on *Trichoderma longibrachiatum* fermented PKC (TL-PKC) was evaluated. Seven isonitrogenous and isocaloric diets were formulated. Reference diet, D1 had no PKC, while the other diets, D2 to D5 contained 10, 20, 30 and 40% fermented PKC (TL-PKC), respectively. All diets contained 1% chromic oxide as an inert marker. Red tilapias of average weight of 2.5 g were fed on these six diets for a period of 8 weeks. The fish were fed at 4% of their body weight, twice a day. There was no difference in mortality rate of fish on the various diets used. However, weight gain decreased with an increase of TL-PKC in diets. There were significant ($P \leq 0.05$) differences in the apparent digestibility coefficient (ADC) of protein and dry matter between the reference diet and diet containing TL-PKC. The ADC of both protein and dry matter generally decrease when the percentage TL-PKC was increased in the test diets. There was also no significant difference in carcass protein content among fish on the various diets. However, there was a significant ($P \leq 0.05$) increase in the levels of phosphorus, calcium and copper in the carcass of fish raised on TL-PKC, but the level of lipids was significantly reduced. Decreasing dry matter and protein digestibility with corresponding weight reduction may have resulted from increased crude fibre content of diets with TL-PKC.**

**Key words:** Palm kernel cake, *Trichoderma longibrachiatum*, red tilapia, fermentation.

## INTRODUCTION

Tilapia constitutes the third largest group of farmed finfish after carps and salmonids (El-Sayed, 1999). They are widely cultured in the tropical and subtropical regions of the world. Generally categorized as herbivorous under an extensive system, tilapia species feed on plankton, vegetation and algae. This short-chained feeding habit is advantageous in the development of supplementary or complete feed for farmed tilapia. Incorporation of plant proteins for farmed tilapia has been a research focus for quite some time. Apart from legume seeds, oilseed and fruit by-products, such as palm kernel, groundnut, sunflower, rapeseeds and copra have been found to be potentially good sources of protein for farmed tilapia (Jackson et al., 1982; Davies et al., 1990; Omoregie and Ogbemudia, 1993; Saad et al., 1997). In the case of palm kernel cake (PKC), a number of authors have reported its inclusion in fish feeds (Jauncey and Ross 1982; Omoregie and Ogbemudia 1993; Saad et al., 1997).

On the other hand, the use of microbial-based ingredients in aquaculture feeds is becoming quite popular due to their high biomass production within a relatively short period. Avnimelech et al. (1989) found that the growth of fish fed with single cell protein produced using cheap carbon and nitrogen sources is identical to that of those fed a protein-rich diet. Similarly, Chamberlain and Hopkins (1994) reported that spraying a source of carbon,

*Corresponding author. E-mail: mmhanafi@agri.upm.edu.my. Tel: +603-89467842.

**Abbreviations: D1, D2, D3, D4, D5;** Diets 1, 2, 3, 4 and 5, **PKC;** palm kernel cake, **TL-PKC;** *Trichoderma longibrachiatum* fermented palm kernel cake.

**Table 1.** Dietary nutrient levels (g/kg on as fed basis) for tilapia.

| Nutrients | Minimum requirement |
|---|---|
| Crude protein | 300.0 |
| Phenyalanine/tyrosine | 16.5 |
| Valine | 08.1 |
| Threonine | 6.9 |
| Isoleucine | 8.2 |
| Methionine/cystine | 14.5 |
| Histidine | 6.9 |
| Arginine | 9.8 |
| Leucine | 11.1 |
| Lysine | 12.3 |
| Crude fibre | < 100 |
| Phosphorus | 10.0 |
| Lipids | ≤ 100 |
| Digestible energy | 11 kJ/g |

such as wheat bran and cellulose on the surface of pond water with continuous aeration increases the bacterial biomass that is used as fish food. Recently, the mass production of microbes, such as the photosynthetic bacterium *Rhodopseudomonas palustris* and *Candida utilis* for protein sources in aquaculture feeds has been studied (Joong and Bum-Kyu, 2000; Bum-Kyu and Joong, 2001).

Previous laboratory studies on the fermentation of PKC with various fungal species showed that the process could produce a relatively high protein ingredient that could partially substitute fishmeal in a fish diet. This study was therefore conducted to evaluate the performance of fungus enriched PKC produced under solid-state fermentation with *Trichoderma longibrachiatum* in red tilapia diet.

## MATERIALS AND METHODS

### Preparation of experimental diets

All ingredients used for feed formulation were obtained from a local supplier. Diets were formulated to meet the amino acid requirements of the red tilapia, used as the experimental fish. The amino acid requirements were determined by carrying out the amino acid profile of the fillet of red tilapia fingerlings of an average weight 1.0 g according to the method of Stein and Moore (1963). Five isonitrogenous and isocaloric diets were formulated based on both the analysed amino acids and recommended levels of protein, lipids, fibre, phosphorus and energy for tilapia (Jauncey and Ross, 1982) (Table 1). Using a least cost feed formulation software, composition of the five diets were calculated and formulated using ingredients listed in Table 2. D1 which was used as the reference diet had no PKC. D2 to D5 contained 10, 20, 30 and 40% *T. longibrachiatum* fermented PKC (TL-PKC), respectively. A chromic oxide inert marker (1%) was included in all diets. Diet ingredients were thoroughly mixed and extruded using a meat mincer (ø = 2 mm). However, diets containing TL-PKC were extruded using a 5 mm mesh size because of their gummy consistencies. The feeds were extruded in a "spaghetti" form, oven dried at 100°C for 24 h and crumbled into small granules. Dust-like particles were sieved out and 2 mm-sieved granules were fed to the fish. The moisture, crude protein, ether extract, crude fibre, ash and mineral content analysis of samples were carried out according to the methods of Association of Official Analytical Chemists (AOAC, 1997).

### Feeding trial

An 8-week feeding trial was carried out at the Aquatic Resources Technology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia. The experimental fish, red tilapia (*Oreochromis* sp.), were hatchery produced at the unit. Two hundred and ten red tilapias (mean weight 2.50 g) were randomly distributed into twenty-one 24 l capacity experimental glass tanks of 40 cm in length, 25 cm width and 24 cm in height. Each tank had 10 fish and a constant volume of 10 l of water was maintained in each tank throughout the experimental period. Tanks were randomly assigned to different diets in triplicates. The fish were acclimatized to trial diets for 7 days at a daily rate of 3% of their body weight prior to the start of the experiment. At the start of the experiment, the fish were weighed individually and subsequently were bulk-weighed at every 14 days for growth measurement and daily ration adjustment. During the feeding trial, fish were fed at 4% body weight $day^{-1}$ at 10:00 and 16:00h. The afternoon feeding was preceded by the siphoning of fecal materials and left over feed. After the cleaning, 75% of the total water volume was replaced in each tank.

The entire water in each tank was completely changed once a week during the bulk weighing of fish. Ground water was used during the entire 8 weeks of the experiment. The water was stored in round plastic overhead tanks and aerated for at least 24 h before use. Each experimental tank was also aerated continuously via an air stone and the ammonia-N concentration was maintained below 0.14 mg/l throughout the experimental period. The pH remained between 6.8 and 7.6.

### Carcass analysis

At the conclusion of the trial, whole fish were homogenized, freeze-dried and stored at -20°C prior to analysis. Fresh samples were analysed for moisture before freeze-drying. Freeze-dried samples were analysed for ash, lipids, crude protein, phosphorus, calcium and copper. All analyses were carried out in duplicates.

### Determination of ash

Porcelain crucibles were cleaned, dried and cooled in a desicator. The empty crucibles were weighed and about 2 g of samples were taken to determine the ash content. The crucibles, with samples were placed in a muffle furnace. The temperatures were increased gradually to 550°C and the samples were ignited for about 6 h. The crucibles were then transferred from the furnace to the desicator, allowed to cool and then weighed.

$$\text{Ash (\%)} = (\text{Weight of ash} / \text{Sample weight}) \times 100$$

### Crude protein

About 0.1 g of sample was weighed into Kjeldahl digestion tubes. One half of Kjeldahl catalyst tablets were added to each tube. About 5 ml of concentrated sulphuric acid was also added. The tubes were placed in the digestion block under a fume hood and the temperature of the digestion block was increased gradually to 370°C. Samples were allowed to heat until the solution became

**Table 2.** Composition of test diets (g/kg on as fed basis).

| Feed ingredients | D1 | D2 | D3 | D4 | D5 |
|---|---|---|---|---|---|
| TL- PKC | - | 10 | 20 | 30 | 40 |
| Soybean meal | 364.0 | 355.4 | 346.9 | 290.4 | 233.8 |
| Fishmeal | 132.0 | 91.1 | 50.1 | 50.0 | 50.0 |
| Rice bran | 378.7 | 339.5 | 300.2 | 267.2 | 234.3 |
| Fish/palm oil (50:50) | 94.2 | 82.8 | 71.5 | 61.3 | 51.1 |
| Mineral mix | 10 | 10 | 10 | 10 | 10 |
| Vitamin mix | 10 | 10 | 10 | 10 | 10 |
| Threonine | 0.6 | 0.7 | 0.8 | 0.7 | 0.5 |
| Histidine | 0.4 | 0.5 | 0.6 | 0.4 | 0.2 |
| Chromic oxide | 10 | 10 | 10 | 10 | 10 |
| **Proximate composition** | | | | | |
| Crude protein (%) | 33.34 | 31.26 | 32.19 | 31.36 | 31.17 |
| Crude fibre (%) | 6.54 | 7.43 | 8.58 | 8.78 | 9.52 |
| Ether extract (%) | 9.45 | 8.94 | 7.75 | 6.33 | 6.00 |
| Phosphorus (%) | 1.03 | 1.12 | 1.22 | 1.47 | 1.51 |
| Calcium (%) | 1.83 | 1.64 | 1.28 | 1.17 | 1.28 |
| Copper (mg/kg) | 92.14 | 96.23 | 105.93 | 127.05 | 148.74 |
| Ash (%) | 12.62 | 12.50 | 12.05 | 12.03 | 12.02 |
| Digestible energy (kJ/g) | 11.91 | 11.66 | 11.37 | 11.25 | 11.55 |

colourless indicating that digestion was completed. The digestion tubes were allowed to cool and the digests were quantitatively transferred to 100 ml volumetric flasks. The volumetric flasks were allowed to cool and then made up to volume with distilled water. The nitrogen (N) in the solution was determined by an auto-analyser. The auto-analyser was calibrated with standard samples of known nitrogen concentrations, while the nitrogen concentrations of the unknown samples were calculated by comparing their absorbance peaks with those of the standards. The crude protein product was calculated by the equation:

Crude protein (%) = N x (f)

Where, N = Total nitrogen; f = Constant (6.25).

**Ether extract**

The ether extract (lipid content) was determined by means of Soxtec System (Soxtec System HT 1043 Tecator). About 2 g ($W_1$) of samples were weighed into the extraction thimbles. The extraction cups were previously cleaned, dried, cooled in the desicator and weighed ($W_2$). About 50 ml of petroleum ether was poured into each extraction cup. The extraction cups containing the thimbles were then placed in the extraction unit. The samples were allowed to extract for the first 30 min in the “boiling position” followed by another 30 min in the “rinsing position”. The petroleum ether was recovered by evaporation and the cups were taken out and dried in the oven at 150°C for 2 h. After being cooled in the desicator, the cups containing the extracts were reweighed ($W_3$). The percentage of the ether extract was then calculated.

$$\text{Ether extract (\%)} = [(W_3 - W_2)/W_1] \times 100$$

Where $W_2$ = Weight of empty dry cup; $W_3$ = weight of dry cup + ether extract; $W_1$ = Sample weight.

**Determination of minerals**

For the determination of the inorganic constituents, it was necessary to remove the organic matter. The organic matter can be eliminated by either wet or dry ashing. In dry ashing, samples were ignited in a furnace to burn off the organic components, forming ash. For the determination of minerals, samples from ash determination were used. Crucibles were placed on a hot plate and 2 ml of concentrated hydrochloric acid was added to each crucible. Samples were heated to evaporate the acid. About 10 ml of 20% nitric acid were added and boiled for about 30 min, or until ⅔ of the acid had evaporated. The crucibles were then cooled and their contents transferred to 100 ml volumetric flasks and made up to volume. The samples were filtered and analysed for calcium and copper by atomic absorption spectrophotometer, while phosphorus was determined by the auto-analyser.

**Protein digestibility studies**

Feces and uneaten food were collected one hour after each feeding and oven dried at 60°C for a digestibility study. Analysis of uneaten feed and feces were carried out for crude protein using Kjeldahl nitrogen procedure and moisture content by the infra-red moisture balance procedure. Chromic oxide was determined for uneaten feed and feces according to the method of Kimura and Miller (1957). Dry matter and apparent protein digestibility were calculated based on Cho et al. (1982).

**Statistical analysis**

All data were analysed by ANOVA (SAS version 6.12, 1996) and mean differences were compared by the Least Significant Difference (LSD) test at 5% level of confidence. All percentage values were arc sin transformed prior analysis.

**Table 3.** Mean weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and survival rate.

| Diet | BW (g) | | BW gain | | SGR | FCR | PER | Survival (%) |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | (g) | (%) | | | | |
| D1 | 2.96 | 6.36 | 3.40 | 114.38$^{b}$ | 1.36 | 2.86$^{cd}$ | 1.03$^{bc}$ | 95.00$^{a}$ |
| D2 | 2.82 | 5.80 | 2.98 | 105.63$^{b}$ | 1.28 | 3.03$^{bcd}$ | 1.07$^{b}$ | 93.33$^{a}$ |
| D3 | 3.02 | 5.34 | 2.32 | 77.14$^{c}$ | 1.02 | 3.76$^{b}$ | 0.82$^{d}$ | 93.33$^{a}$ |
| D4 | 3.03 | 5.34 | 2.31 | 76.14$^{c}$ | 1.01 | 3.76$^{b}$ | 0.85$^{cd}$ | 100$^{a}$ |
| D5 | 3.18 | 4.88 | 1.70 | 53.54$^{d}$ | 0.76 | 5.34$^{a}$ | 0.60$^{e}$ | 100$^{a}$ |

Means within columns with common superscript are not significantly different (P ≤ 0.05); SGR (specific growth rate) = (ln final weight - ln of initial weight)/56 days *100; FCR (feed conversion ratio) = dry feed fed/body weight gain; PER (protein efficiency ratio) = body weight gain/crude protein fed; BW = Body weight.

**Table 4.** Whole body protein, lipid, ash, phosphorus, calcium, copper and moisture content (% dry matter unless stated otherwise) of red tilapia at the end of 8-week feeding trials.

| Diet | Protein (%) | Lipids (%) | Ash (%) | P (%) | Ca (%) | Moisture (% wet) | Cu (µg/g) |
|---|---|---|---|---|---|---|---|
| D1 | 58.81$^{c}$ | 27.50$^{a}$ | 11.19$^{d}$ | 1.91$^{b}$ | 2.07$^{c}$ | 70.13$^{de}$ | 21.39$^{c}$ |
| D2 | 59.66$^{bc}$ | 25.00$^{ab}$ | 12.35$^{c}$ | 1.91$^{b}$ | 2.41$^{bc}$ | 70.12$^{e}$ | 27.95$^{b}$ |
| D3 | 58.38$^{c}$ | 22.50$^{ab}$ | 14.09$^{b}$ | 1.97$^{ab}$ | 3.49$^{a}$ | 73.13$^{c}$ | 30.86$^{b}$ |
| D4 | 60.90$^{b}$ | 20.00$^{ab}$ | 15.79$^{a}$ | 1.94$^{ab}$ | 3.52$^{a}$ | 77.28$^{a}$ | 36.71$^{a}$ |
| D5 | 58.42$^{c}$ | 20.00$^{ab}$ | 16.49$^{a}$ | 2.00$^{ab}$ | 3.44$^{a}$ | 75.57$^{b}$ | 37.00$^{a}$ |

Means within columns with common superscript are not significantly different (P≤ 0.05).

## RESULTS

### Feeding trial

All diets were well accepted as fish actively fed on them at each feeding time. The test diets did not cause significant or high mortality to red tilapia as survival for all experimental diets was above 90% (Table 3). Diets containing TL-PKC were harder than the reference diet. The pellets also remained floating for a longer period of time, as their rate of water absorption appeared to be slower. Weight gain of tilapia fed D2 with 10% TL-PKC was 105.63% and did not differ significantly (P ≤ 0.05) from that of the reference D1 (114.38%). However, weight gain generally decreased as dietary TL-PKC increased, but there was no significant difference in weight gain between fish fed on D3 (77.14%) and D4 (76.14%). Specific growth rate (SGR) of fish showed the same trend. Protein efficiency ratio (PER) of D2 (1.07) was however slightly better than that of the reference D1 (1.03). Feed conversion ratio (FCR) also increased with an increase in the level of TL-PKC incorporation (Table 3). The average value of FCR obtained from fish on D2 was 3.03, which was quite close to that of D1 (2.86) but differed significantly from other diets containing higher levels of TL-PKC. The FCR values of D3 and D4 were similar (3.76).

### Carcass analysis

There were little differences in body protein among fish on various dietary treatments. However, the highest value (60.90%) was obtained from fish fed on D4 (with 30% TL-PKC) and this was significantly higher than the value from the reference diet D1 (58.81%). Other diets gave protein values that were not significantly different from either D1 or D4 (Table 4). Body lipids decreased as TL-PKC level in diets increased while carcass moisture increased. The D4 and D5 both had the lowest lipid content of 20.0%, which was much lower than D1 with 27.50%. The inclusion of TL-PKC in diets also signify-cantly increased the ash content. Apart from fish that were fed on D2, there were no significant differences in the calcium and phosphorus contents among carcass from TL-PKC based diets. The copper content increased with an increase in the dietary TL-PKC level with the carcass of fish reared on D5 giving the highest value of 37 µg/g.

### Apparent protein and dry matter digestibility

Apparent dietary protein and dry matter (DM) digestibility decreased with the inclusion of TL-PKC (Table 5). Protein digestibility coefficient value for the reference diet (D1)

**Table 5.** Apparent digestibility coefficients of protein and dry matter of test diets.

| Diet | Apparent digestibility coefficients of | |
|---|---|---|
| | Crude protein | Dry matter |
| D1 | 0.81[a] | 0.54 [a] |
| D2 | 0.75[b] | 0.50 [b] |
| D3 | 0.59[d] | 0.33 [d] |
| D4 | 0.58[d] | 0.32 [d] |
| D5 | 0.55[e] | 0.36 [c] |

Means within column with common superscript are not significantly different ($P \leq 0.05$).

was 0.81. Among diets with TL-PKC, D5 with 40% TL-PKC gave the lowest protein digestibility coefficient (0.55), while the lowest value for DM digestibility coefficient (0.32) was given by D4 with 30% TL-PKC.

## DISCUSSION

Feed utilization and growth parameters observed in this study showed that *T. longibrachiatum* fermented PKC (TL-PKC) resulted in a significant decrease in SGR and PER for tilapia (Table 3). Several authors had published the various merits and demerits of supplementing fish feeds with PKC. For example, Jauncey and Ross (1982) reported that palm kernel meal alone is not a good protein source in fish diets due to the high fibre content and its unpalatability. Omoregie and Ogbemudia (1993) showed that a good growth performance could be obtained with 15% level of PKC in fish feed. However, Saad et al. (1997) noted that the inclusion of up to 30% PKC in the diet of red tilapia did not cause any significant reduction in growth. In the present study, reduced weight gain and poor feed conversion ratio observed in fish raised on TL-PKC diets could be attributed mainly to decreased digestibility of TL-PKC, rather than the release of toxins since fish survival was not significantly affected and *T. longibrachiatum* has not been reported to secrete toxins. Several authors have reported a reduction in digestibility of fungal fermented products when fed to monogastric animals. Lim et al. (2001) reported that incorporation of *Aspergillus flavus* fermented PKC into tilapia diets caused growth depression as a result of decreased dietary digestibility and possibly because of the presence of aflatoxin $B_1$. Similarly, Mathot et al. (1992) reported a decrease in nitrogen digestibility, PER and weight gain in rats fed *Aspergillus niger* fermented barley. They attributed the poor performance of fermented barley to the presence of high proportion of non-protein nitrogen.

The poor digestibility of TL-PKC observed in this study may be primarily due to the presence of non-starch polysaccharides (NSP), which were reported to be the product of fermentation as well as part of fungal cell wall material. Eriksson et al. (1990) reported that during fermentation, galactomannans are broken down to soluble manno-oligosaccharides with a degree of polymerization ≥ 2. However, mixed D-mannose-oligosaccharides containing D-galactose and galactomannan is the major component of NSP in PKC (Stephan, 1983; Dusterhoft et al., 1991). Apart from galactomannan, filamentous fungi contain (1 - 3) β-glucan, a type of NSP that is a component of their cell wall (Kuhn et al., 1990). The presence of these soluble NSPs may have lead to the observed 'gummy' consistency of diets containing TL-PKC. Soluble NSP have been found to exhibit anti-nutritive properties when used in diets for monogastric animals (Storebakken, 1985; Bedford and Classen, 1992; Almirall et al., 1995; Choct et al., 1996).

In terms of carcass analysis, fish fed diets containing fermented PKC had higher whole body moisture, higher ash and lower lipids and dry matter contents. There was no significant difference in whole body protein among treatments. The lowering of fish body dry matter and lipid could also possibly be due to the presence of NSPs. Hossain et al. (2001) reported a reduction of growth and feed utilization in common carp fed diets containing *Sesbania* endosperm at 7.2% or higher. They suggested that *Sesbania* NSP increased the viscosity of the intestinal content and thereby affected nutrient absorption and utilization. Amongst Atlantic salmon and chicken, Refstie et al. (1999) noted that diets containing soybean meal (from flaked soybean husks) gave lower digestibility than those that contained soybean meal with reduced oligosaccharides. They concluded that the effect of the former soybean product could possibly be associated with anti-nutritional effects of the NSP fraction, which caused high viscosity in the gut and increased water content in the gut of salmon.

Overall, this experiment showed that the use of *T. longibrachiatum* fermented PKC (TL-PKC) produced the desired floating diets and eliminated the use of binders due to its 'gummy' consistency. However, its inclusion reduced dietary protein digestibility and fish growth. This indigestibility may be overcome by pre-treating the PKC before fungal fermentation. This pretreatment will remove lignin and possibly make the mannan less crystalline and so more accessible to fungal degradation. Additionally, carcass of fish on diets with fermented PKC had higher phosphorus and calcium content and reduced lipid content compared to unfermented PKC, which may be an advantage.

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