THE USE OF PROTEIN FROM PALM KERNEL MEAL IN DIETS OF SEABASS (*Lates calcarifer*)

MOHAMMED SUHAIMEE BIN ABD. MANAF

UNIVERSITI TEKNOLOGI MALAYSIA

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MOHAMMED SUHAIMEE BIN ABD. MANAF

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ABSTRACT

Malaysia has an abundant supply of palm kernel meal (PKM) as a plant protein source as it contains up to 20 % crude protein. It has the potential to at least partly reduce the dependency on the soybean meal presently used in fish diets. However, the nutritive value of PKM limits its incorporation in fish diets due to the presence of high fibre content. A preliminary study on the use of raw PKM indicated that it could be included up to 15% inclusion level in the diets of juvenile seabass without affecting fish growth performance and feed efficiency. A series of problem-solving experiments were conducted to optimise the utilization of PKM as a feed ingredient in the diets of seabass (*Lates calcarifer*). Initially a chemical extraction of protein involving acid and bases was conducted. Less than 3% (w/w protein) soluble protein from PKM was obtained after 30 min in either acid (pH 2.7) or alkaline (pH 12.5) conditions. However, this amount increases to about 50% (w/w) when left for a longer period of time (3 h) at 28 ^{o}C and in alkaline condition (pH 10 and above). Increasing the extraction temperatures (60 ^{o}C, 80 ^{o}C, 105 ^{o}C or 121 ^{o}C) did not substantially improve the amount of extracted protein. The low recovery by the chemical extraction method could probably be due to the protein being hindered by layers of cell wall. Thus, pre-treatment on the raw PKM by physical elements such as pressure and heat using the fibre explosion process were attempted. However, it also did not substantially improve protein recovery compared to untreated raw PKM ($P > 0.05$). Hence, another strategy to expose the binding or entrapment of protein by polysaccharides was to treat PKM with urea at 80:750 g kg^{-1} (urea:water). A series of feeding trial were then conducted to evaluate the effect of using raw PKM and chemically-treated PKM using urea (UTPKM) as feed ingredients in diets on growth performance of juvenile seabass *L. calcarifer*. Seabass juveniles fed 7.5% PKM and 15% PKM or 7.5% UTPKM, 15% UTPKM and 22.5% UTPKM diets showed similar growth performance ($P > 0.05$) with control diet. However, fish fed 22.5% PKM diet showed poor growth performance and feed efficiency ($P < 0.05$) than fish fed the control diet. On the other hand, there were significant differences ($P < 0.05$) in terms of protein efficiency ratio (PER) as fish fed the control diet giving the best PER compared to fish fed either 22.5% PKM, 15.0% UTPKM or 22.5% UTPKM. Net protein utilization (NPU) of fish fed 15.0% PKM and 22.5% PKM diets were substantially lower ($P < 0.05$) than fish fed the control diet. In general, fish growth performance and feed efficiency were shown to be better when fish fed diets with up to 22.5% inclusion of UTPKM than fish fed 22.5% PKM diets. It was concluded that raw PKM has the potential to partially replace soybean meal in the diets of juvenile seabass and that urea treatment on PKM may further improve the utilization of this by-product from the oil palm industry.

ABSTRAK

Malaysia mempunyai bekalan *palm kernel meal* (PKM) yang banyak sebagai sumber protein, yang mempunyai potensi untuk mengganti sekurang-kurangnya sebahagian tepung soya yang biasanya digunakan dalam makanan ikan. Walaupun begitu, kandungan serat kasar yang tinggi menghadkan penggunaannya dalam makanan ikan. Kajian awal dalam penggunaan PKM menunjukkan sehingga 15% tahap pencampuran dalam diet ikan siakap (*Lates calcarifer*) boleh dilakukan tanpa mengganggu kadar tumbesaran dan keberkesanan makanan ikan. Beberapa siri kajian telah dijalankan untuk mengoptimumkan penggunaan PKM sebagai ramuan dalam makanan ikan siakap. Kurang dari 3% (w/w protein) protein larut didapati daripada PKM setelah 30 min pencampuran samada dalam larutan berasid (pH 2.7) atau beralkali (pH 12.5). Walau bagaimanapun, jumlah protein larut didapati meningkat sekitar 50% (w/w) apabila PKM dicampurkan dalam larutan beralkali (pada pH 10 dan ke atas) dalam masa 180 min (3 jam) pada suhu bilik (28^oC). Peningkatan suhu pengekstrakan kepada 60^oC, 80^oC, 105^oC atau 121^oC tidak dapat meningkatkan kandungan protein larut. Ini adalah kerana, kemungkinan protein PKM dihalangi oleh lapisan-lapisan dinding sel. Oleh itu, kaedah perawatan awal melalui elemen fizikal seperti tekanan tinggi dan pemanasan menggunakan proses letupan serat-amonia diuji. Hasilnya, penggunaan kaedah perawatan tersebut tidak dapat meningkatkan jumlah pengekstrakan protein berbanding dengan protein larut yang didapati sebelumnya (P>0.05). Maka, kaedah lain adalah untuk mendedahkan protein PKM melalui kaedah perawatan dengan urea pada kadar 80:750 g kg^{-1} (urea:air). Satu siri percubaan makanan telah dijalankan untuk menilai kesan penggunaan PKM dan PKM selepas rawatan urea (UTPKM) sebagai ramuan terhadap pencapaian tumbesaran dan keberkesanan makanan ikan siakap. Ikan siakap yang diberi diet-diet 7.5% PKM dan 15% PKM atau diet-diet 7.5% UTPKM, 15% UTPKM dan 22.5% UTPKM menunjukkan pencapaian tumbesaran yang sama di antara satu dengan yang lain (P>0.05) berbanding diet kawalan tanpa PKM. Walaupun begitu, kadar tumbesaran dan keberkesanan makanan ikan yang diberi diet 22.5% PKM merosot (P<0.05) berbanding ikan yang diberi diet kawalan. Dalam lain hal, terdapat perbezaan ketara (P<0.05) dari segi nisbah keberkesanan protein (PER) di mana ikan yang diberi diet kawalan menunjukkan nilai PER yang terbaik (P<0.05) berbanding ikan yang diberi diet 22.5% PKM, 15.0% UTPKM atau 22.5% UTPKM. Penggunaan tetap protein (NPU) ikan yang diberi diet 15.0% PKM dan 22.5% PKM adalah rendah (P<0.05) berbanding ikan yang diberi diet kawalan. Pada amnya, pencapaian tumbesaran dan keberkesanan makanan siakap adalah lebih baik apabila diberi diet yang mengandungi sehingga 22.5% UTPKM berbanding diet yang mengandungi 22.5% PKM. Kesimpulannya, PKM berpotensi untuk menggantikan sebahagian dari tepung soya dalam diet ikan siakap dan perawatan dengan urea ke atas PKM berkemungkinan dapat meningkatkan lagi penggunaan bahan sampingan dari industri minyak sawit ini.

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LIST OF SYMBOLS / ABBREVIATIONS

ADF	-	Acid detergent fibre
ADL	-	Acid detergent lignin
AFEX	-	Ammonia fibre explosion
AFEXTPKM	-	Ammonia fibre explosion treated palm kernel meal
cm	-	centimetre
CP	-	Crude protein
DFM	-	Danish fish meal
DL	-	Dietary lipid
DM	-	Dry matter
DO	-	Dissolved oxygen
DP	-	Dietary protein
EAA	-	Essential amino acids
FCR	-	Feed conversion ratio
FO	-	Fish oil
h	-	hour
ht	-	height
pI	-	Isoelectric point
ME	-	Metabolizable energy
min	-	minute
um	-	micron
mm	-	millimetre
NDF	-	Neutral detergent fibre

NFE	-	Nitrogen-free extract
NPU	-	Net protein utilization
NPN	-	Non protein nitrogen
NSP	-	Non-starch polysaccharide
PER	-	Protein efficiency ratio
PKM	-	Palm kernel meal
PKMPE		Palm kernel meal protein extract
ppm	-	Parts per million.
ppt	-	Parts per thousand.
SBM	-	Soybean meal
SGR	-	Specific growth rate
^{o}C	-	Degrees Celsius
TCA	-	Trichloroacetic acid
UTPKM	-	Urea treated palm kernel meal

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CHAPTER ONE
INTRODUCTION

CHAPTER 1

INTRODUCTION

1.1 The Importance of Aquaculture as a Source of Protein for Human Consumption

Aquaculture is the fastest expanding food producing sector in the world. According to Food and Agriculture Organization of the United Nation, Rome (FAO) statistics, aquaculture's contribution to global supplies of fish, crustaceans and molluscs continues to grow, increasing from 3.9 percent of total production by weight in 1970 to 27.3 percent in 2000. Worldwide, the sector has increased at an average compounded rate of 9.2 percent per year since 1970, compared with only 1.4 percent for capture fisheries and 2.8 percent for terrestrial farmed meat production systems [1]. In 2000, reported total aquaculture production (including aquatic plants) was 45.7 million tonnes by weight and USD56.5 billion by value. More than half of the total world aquaculture production in 2000 was finfish, and the growth of the major species groups continues to be rapid with no apparent slowdown in production to date. As production from wild fishery has been static at around 100 million tonnes for the last decade and unlikely to increase further, aquaculture production must at least double by the year 2030 if current per capita seafood consumption of between 19 and 21 kg is to be met [1 - 4]. Figure 1.1 shows the world fish production and food use consumption from 1976 and projected to 2030. Although much of this aquaculture expansion will have to come from inland water culture of herbivorous fish such as carp and tilapia, there will also be greater demand for high-value fish species that are grown on artificially-provided food. Global aquaculture feed requirements are estimated currently at between 3 and 4 million tonnes per annum and are predicted to double by the year 2010 with Asia alone expecting to consume at least 2.6 million tonnes [5, 6].

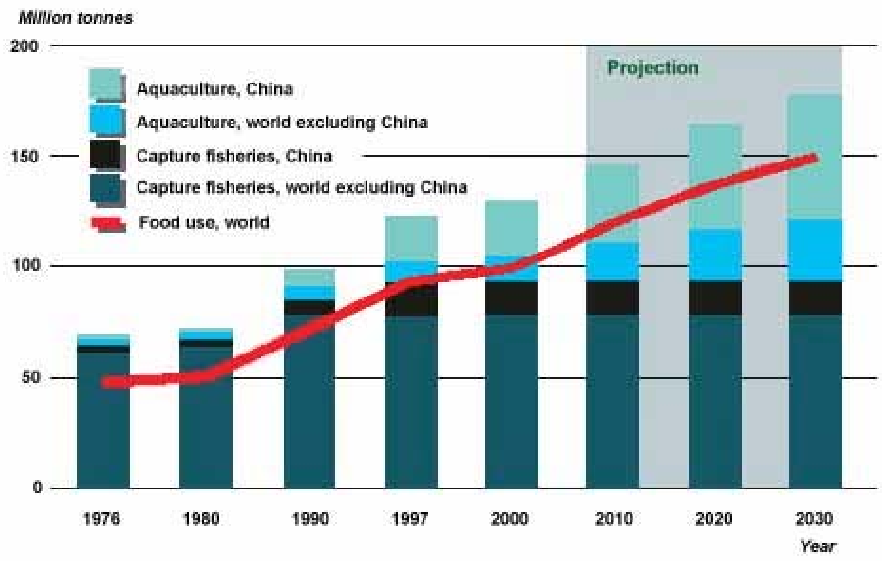

Figure 1.1: World fish production and food use consumption 1976 – 2030 (FAO, 2002)

1.2 The Need for Alternative Protein Sources in Aquaculture Feeds

One of the major factors limiting the expansion of aquaculture is the development of nutritionally adequate, cost-effective diets. Feeds and feeding can contribute up to 70% of the total operating costs for fish and shrimp farms [7]. The most expensive component of pelleted feed is protein, of which 25-55% is required, depending upon whether the species is herbivorous, omnivorous or carnivorous [8, 9]. The major protein source for most aquaculture diets is fishmeal [9, 10] and formulated diets can contain up to 60% fishmeal [7, 11]. Almost 30% of fishmeal available globally for export is being consumed by the aquaculture sector [5, 12, 13]. However, there are some major problems with fishmeal. Fishmeal and fish oil production is declining [14] and the aquaculture feed industry currently uses more than 3 million tonnes of the global fisheries catch [15] excluding 'trash fish' which is fed directly to aquaculture species. As aquaculture production increases, demand for fishmeal will also increase, inevitably forcing prices to rise. As higher quality fishmeal is generally required for aquaculture feeds, species of fish currently used for human consumption will increasingly be targeted by manufacturers for fishmeal production. In Malaysia, much of the cheap fish used to produce salted fish for human consumption is now instead used for aquaculture [12]. It is evident from the statistics that continued expansion of aquaculture would be curtailed unless suitable alternatives to fishmeal are found.

The development of cost-effective diets, with reduced contents of fish- and other aquatic- meals is an urgent priority for most fish and crustacean aquaculture industries. The problem is particularly important in Malaysia, which has very poor supplies of aquatic meals. Malaysia is particularly vulnerable to any world shortage of fishmeal because of our reliance on imported fishmeal and other non-edible marine product. In Malaysia, under the Third National Agriculture Plan, aquaculture industry is going to be a major contributor of fish and shrimp to fulfil the protein demand of the nation. Aquaculture production of brackishwater fish species in year 2010 has been targeted at 110,000 metric tonnes from only around 8,000 metric tonnes in year 2001 [16]. Among the high-valued marine species are Asian seabass (*Lates calcarifer*), mangrove snapper (*Lutjanus argentimaculatus*) and grouper (*Epinephelus sp.*). At present, the aquaculture industry in Malaysia is still dependent on either trash fish or costly imported diets. The use of cost-effective aquaculture diets with reduced fishmeal usage will significantly enhance the aquaculture industry in Malaysia.

Future aquaculture expansion will be primarily dependent on intensification and feeding with nutritionally complete diets. With increasing costs and the uncertainty in the future supply of fishmeal, it is thus desirable for nutritionists and feed manufacturers to opt for less expensive, readily available plant protein as a substitute for fishmeal. Oilseed proteins such as soybean meal has been used with varying degrees of success as a replacement for fishmeal in the diets for many cultured fish species. Freshwater fish such as channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) [17, 18] as well as marine fish such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) [19], Australian snapper (*Pagrus auratus*) [20], cobia (*Rachycentron canadum*) [21] and Asian seabass (*L. calcarifer*) [22 - 24] were reported to be able to accept certain levels of soybean meal in their diets without affecting growth performance. However, the rising costs of conventional feed ingredients such as fishmeal and soybean meal, which are usually imported into tropical countries have stimulated much research into the use of alternative, locally available, plant protein sources [25].

Malaysia has an abundant supply of plant protein feed, which has the potential to at least partially reduce soybean meal presently used in compounded aquaculture diets. Palm kernel meal (PKM) is an oilseed protein source available in large quantities. The global production of PKM is increasing due to the remarkable growth of the oil palm industry in many parts of Asia and Africa. About 2 million tonnes of PKM are produced in Malaysia annually, the current global leader in the oil palm industry [26].

PKM has been successfully used as a diet ingredient for ruminants [27, 28] and can be incorporated at low levels (10-30%) in the diets of non-ruminant livestock such as poultry [29,30] and swine [31 - 34]. Generally, studies on the use of PKM by fish are very few and PKM is therefore seldom used in fish diets. In addition, the nutritive value of PKM limits its incorporation in fish diets due to the presence of high fibre content. It is worth to note that since PKM is very cheap, its incorporation in fish diets could considerably reduce the cost of feed. Thus, series of problem-solving experiments were conducted to determine the potential and improve the nutritional quality of PKM so that its use as a feed ingredient in the diets of seabass (*L. calcarifer*) could be optimised. This project provides the first attempt on isolating protein from PKM as the use of chemicals in protein isolation process from oilseeds such as soybean, cereal such as wheat and leaves such as alfalfa have successfully been practiced for decades. At present, the use of PKM in the diet of carnivorous marine fish species has not been reported.

1.3 Research Objectives

The main objective of this research is to evaluate the potential of PKM as a protein source in practical diets of seabass *Lates calcarifer* (Siakap) by enhancing its nutritional quality. This includes a series of experiments to determine:

1.3.1 The possibility of extracting protein from raw PKM in either acidic or alkaline condition, 1.3.2 The effect of pre-treating the cell wall of PKM using ammonia-fibre explosion method prior to protein extraction,

1.3.3 The effect of urea treatment on PKM.

1.3.4 The effect of using raw and treated PKM as feed ingredient in diets to partially replace soybean meal on growth performance and feed efficiency of seabass.

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