FORMULATION OF FISH FEEDS WITH HIGH LEVEL OF PROTEIN-BOUND METHIONINE AND LYSINE FOR AFRICAN CATFISH (*Clarias gariepinus*) FINGERLINGS' GROWTH

by

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A thesis submitted in fulfillment of the requirements for the degree of Master of Science in Bioprocess Engineering

School of Bioprocess Engineering
UNIVERSITI MALAYSIA PERLIS

2017

GRADUATE SCHOOL

UNIVERSITI MALAYSIA PERLIS

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ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisor, Mr. Muhammad Faiq Bin Abdullah, my co-supervisors, Associate Prof. Dr. Zarina Zakaria, Madam Siti Jamilah Hanim Binti Mohd Yusof and Madam Rozaini Abdullah for their assistance, patience and continued support throughout my project. Their guidance has been essential to the progress of this project.

Special thanks to my beloved mother, Madam Fatimah Binti Salleh and my family members who always motivate, support and prayed for me to work harder until complement of my thesis. Not forget to my fellow friends who keeps accompany me during thick and thin, thank you so much for all kindness and supports.

Siti Nurhafa Imra Naqtahnain Binti Hamid, 2017

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LIST OF ABBREVIATIONS

AABA	α-aminobutyric acid
ADC	Apparent Digestibility Coefficient
ATP	Adenosine Triphosphate
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CCD	Central Composite Design
DOE	Design of Experiment
DOF	Department of Fisheries Malaysia
DOSM	Department of Statistics Malaysia
EAA	Essential Amino Acid
FAO	Food and Agriculture Organization of the United Nations
FBW	Final Body Weight
FCR	Feed Conversion Ratio
FDA	United States Food and Drug Administration
FID	Flame-ionisation detector
GC	Gas Chromatography
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IU	International Unit
kcal	kilo calorie
MARDI	Malaysian Agricultural Research and Development Institute
NaOH	Sodium hydroxide

Na_2CO_3	Sodium carbonate
NRC	National Research Council
PBM	Poultry by-products meal
RSM	Response Surface Methodology
SAM	S-adenosylmethionine
SGR	Specific Growth Rate
SR	Survival Rate
WG	Weight Gain

LIST OF SYMBOLS

^{o}C	Degree Celcius
mg / L	Milligram per liter
$\%$	Percentage
α	Alpha
ε	Epsilon
ppm	Parts per million
MT	Million tones
β	Beta
g	gram
mg / kg	Milligram per kilogram
μm	Micrometer
N	Normality
μL	Microliter
nm	Nanometer
$\%w/w$	Percentage weight per weight
M	Molarity
psi	Per square inch
R^2	Coefficient of determination

Formulasi Makanan Ikan Menggunakan Kandungan Protein-terikat Methionine dan Lysine yang Tinggi untuk Pertumbuhan Anak Ikan Keli Afrika (*Clarias gariepinus*).

ABSTRAK

Pengeluaran ikan keli yang tinggi akan mewujudkan permintaan terhadap makanan ikan. Bahan makanan tempatan yang banyak boleh memenuhi kehendak keperluan sumber protein dalam makanan ikan. Kajian ini melaporkan mengenai formulasi makanan ikan baharu menggunakan kandungan protein-terikat methionine dan lysine yang tinggi dan kesannya terhadap kadar pertumbuhan, kadar survival, penggunaan makanan ikan, dan penghadaman methionine dan lysine oleh anak ikan keli Afrika. Makanan ikan diformulasi dengan menggunakan bahan makanan ikan yang berpotensi: serbuk cacing tanah, ikan baja, sisa soya, daun *Leucaena leucocephala* dan dedak padi. Setiap bahan makanan ikan dinilai untuk menentukan anggaran komposisi termasuk profil asid amino, protein, lemak, karbohidrat, kelembapan dan abu. Makanan ikan diformulasi dengan menggunakan perisian Design Expert. Setiap formulasi makanan ikan dianalisis kandungan asid amino terhad (methionine dan lysine). Makanan ikan komersial bertindak sebagai diet kawalan. Makanan ikan dengan kombinasi 25.0 g serbuk cacing tanah, 20.0 g ikan baja, 25.0 g sisa soya, 10.0 g daun *Leucaena leucocephala* dan 14.0 g dedak padi, memberikan paras protein-terikat methionine dan lysine tertinggi (2.08 dan 8.11% asid amino dalam diet protein, masing-masing) dalam kalangan semua diet dan dinamakan sebagai LyMet A. Kajian memberi makanan ikan dijalankan selama 12 minggu untuk mengenal pasti penggunaan formulasi makanan ikan terpilih terhadap anak ikan keli Afrika. Anak-anak ikan keli, dengan purata berat awal 3.10±0.10 g diberi makan dua kali sehari pada kadar 5.00% daripada berat badan. LyMet A memberikan kenaikan berat (1.68×10^{3}±13.60%), kadar pertumbuhan spesifik (3.20±0.01% per hari) dan kebolehadaman methionine dan lysine (92.3±0.11% dan 98.4±0.01%, masing-masing) paling tinggi. Hasil ujikaji menunjukkan bahawa LyMet A mempunyai paras asid amino terhad (methionine dan lysine) yang mencukupi untuk memenuhi diet ikan keli tanpa penambahan asid amino sintetik.

Formulation of Fish Feeds with High Level of Protein-Bound Methionine and Lysine for African Catfish (*Clarias Gariepinus*) Fingerlings' Growth

ABSTRACT

The high production of catfish will create demands on the fish feeds. The availability of local feedstuff ingredients in abundant can fulfill the needs of protein source in fish feeds. This research reports the new formulation of fish feeds with high level of protein-bound methionine and lysine and the effects on the growth rate, survival rate, feed utilization and digestibility of methionine and lysine on African catfish (*Clarias gariepinus*) fingerlings. Fish feeds were formulated by using potential feedstuff ingredients; earthworm powder, fish meal, soybean wastes, *Leucaena leucocephala* leaves, and rice bran. Each of the feedstuff ingredients was evaluated for their proximate composition including amino acid profile, crude protein, crude lipid, carbohydrate, moisture and ash content. The fish feed was formulated by using Design Expert software. Each of the formulated fish feeds was analyzed for their limiting amino acids (methionine and lysine). The commercial fish feeds act as a control diet. Fish feed with combination of 25.0 g earthworm powder, 20.0 g fish meal, 25.0 g soybean wastes, 10.0 g *Leucaena leucocephala* leaves and 14.0 g of rice bran, give the highest protein-bound methionine and lysine level (2.06 and 8.11% of amino acids in dietary protein respectively) among all diets and named as LyMet A. Feeding trial was conducted for 12 weeks to determine the utilization of selected formulated fish feeds towards African catfish fingerlings. The fingerlings, with a mean initial weight of 3.10±0.10 g were fed twice a day at 5.00% of their body weight. The fingerlings fed with LyMet A showed the highest weight gain (1.68×10^{3}±13.6%), specific growth rate (3.20±0.01% per day) and methionine and lysine digestibility (92.3±0.11% and 98.4±0.01%, respectively). The results indicate that LyMet A has an adequate amount of limiting amino acids (methionine and lysine) to fulfill African catfish fingerling diets without supplementation of synthetic amino acids.

CHAPTER 1

INTRODUCTION

1.1 Background

Fishery sectors play important roles as major source of protein to the Malaysian population for decades. The fisheries can be divided into two major sectors namely marine capture fisheries and aquaculture (Merino et al., 2012). Aquaculture can be defined as the proliferation of fish seed or raising of fish through fish farming during whole or part of its life cycle (Department of Statistics Malaysia, 2013). Marine capture fisheries refer to the harvesting activities of naturally occurring living resources in marine ecosystems. However, marine capture fisheries production is inconsistent and unpromising due to overexploited and overfishing activities (Food and Agricultural Organization, 2012).

Aquaculture has sustained global growth at present and is expected to increasingly fill the shortfall in captured fish products. Aquaculture activity is considered as the alternative for development and improvement of fisheries resources and revitalization of the ecosystems (Okechi, 2004). Aquaculture industry is also imperative for food security and the easiness of protein source supply. Apart from contribution on food security, aquaculture activity also helps to increase the income of the people in rural areas through aquaculture entrepreneurship and reduce the income gaps within the

country. Further grow in Malaysia aquaculture industry could eventually lead Malaysia to reach high-income nation by 2020 (Khalid, 2012).

Aquaculture sector can be divided into freshwater aquaculture and brackish water aquaculture. The major brackish water aquaculture species in Malaysia are shrimps, mollusks and crustaceans while tilapia, catfish and climbing perch are the major freshwater species farmed in Malaysia (Hashim, 2008). The average annual growth for aquaculture production of freshwater during 2008 to 2012 was 14.4 %, slightly lower than aquaculture production of brackish water which is 16.1 % (Department of Statistics Malaysia, 2013). Marine captured fish have contributed the largest amount for fish production as compared to aquaculture industry (Food and Agricultural Organization, 2010). However, the production of marine captured fish is inconsistent mainly due to weather and overfishing activities. This situation has driven fish farmers to produce more freshwater species to accommodate the inconsistent supply of marine captured fish.

In Malaysia, the freshwater fish industries are growing rapidly due to massive demands, technology advancement and government supports (Sara, Ismail, Kamarulzaman, & Mohamed, 2014). Aquaculture activities have been identified as one of the critical component since Seventh Malaysia Plan (RMK-7), which is imperative to ensure food security (Skonhoft, 2005). Freshwater aquaculture has contributed RM155.4 million or approximately 10.5% of Malaysia's gross output in 2011 and this amount is anticipated to increase in the foreseeable future (Department of Statistics Malaysia, 2011). Figure 1.1 portrays the annual production of freshwater fish in Malaysia until 2012. The production of freshwater fish is increasing from 2007 until 2010 with almost 55% increment. However, the production of freshwater fish has

dropped by 21% in 2011 due to rising in production costs, difficulties for land acquisition, threat of diseases and lack of skilled labor (Hashim, 2008). In 2012, the production of freshwater fish has risen again and its production has surpassed the annual production of the previous 5 years. The increase in production of freshwater fish annually creates demand in fish feeds to fulfill the requirements of fish diet.

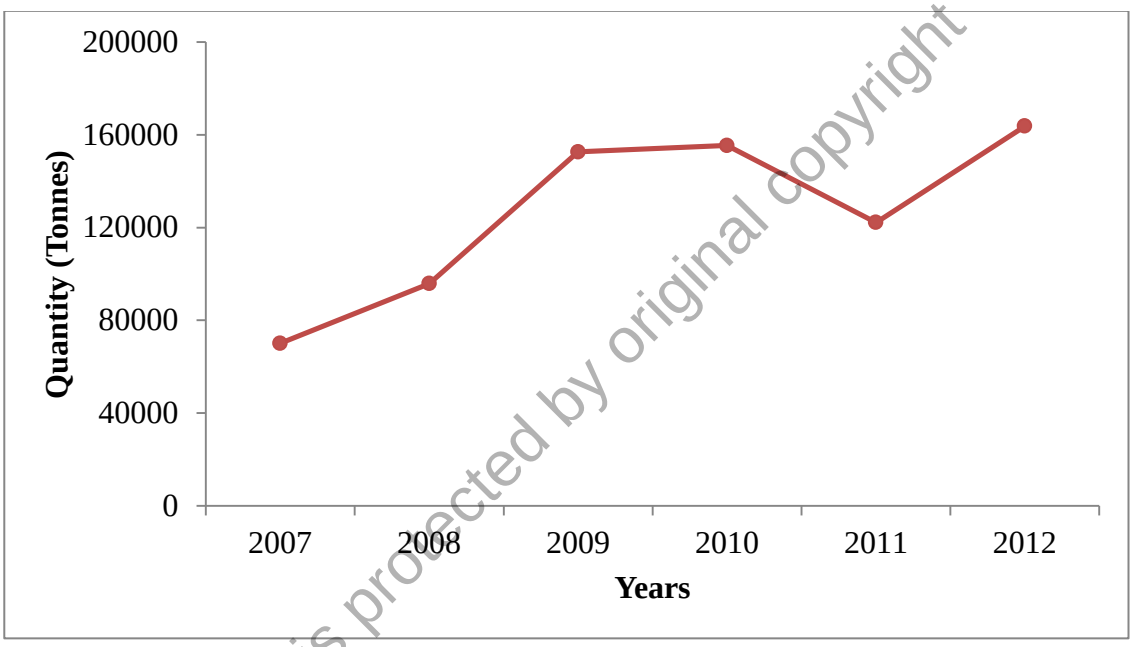

Figure 1.1**:** Annual production of freshwater fish in Malaysia (Department of Statistics Malaysia, 2013)

There are a few freshwater species that are widely farmed in Malaysia. Table 1.1 shows the most consuming fish in Malaysia according to their annual production in 2012. Freshwater catfish is the main freshwater species farmed in Malaysia with the total production nearly 47,000 tonnes. The delicious taste of catfish particularly African catfish and high market demands from food sector making it as a profitable commercial aquaculture species in Malaysia and Southeast Asia Region (Davies & Gouveia, 2008). Subsequently, the high production of African catfish is creating demands for the fish

feeds. Large productions of feeds are needed to fulfill the diets of the African catfish. A well-balanced and high quality of fish feeds will benefit both fish farmers and the consumers by producing a quality fish within short rearing times.

Table 1.1: Production of selected freshwater fish species in Malaysia

Species	Total production (thousand tonnes)
Freshwater catfish	46.5
Red tilapia	38.8
Patin	18.4
Black tilapia	12.7
Freshwater seabass	36.6

Source: (Department of Statistics Malaysia, 2013)

The nutritional content needed by African catfish is different for each stage of their development. There are three main stages of African catfish development namely fry, fingerlings, and grow-out. After few weeks of fry stage, catfish that reach 2.50 cm to 5.00 cm are known as fingerlings. High-protein fingerling feeds are needed during fingerlings stages to supply adequate protein and amino acids for fish growth and maintenances (Robinson, 1998).

Protein ingredients are the main concern during formulation of fish feeds as they represent the largest proportion of feed cost (Perez-Jimenez, Peres, & Oliva-Teles, 2014). The factor that contributes to the increase in fish feed cost is the increases of fish feeds ingredients cost particularly fish meal as the main protein sources. The price of fish meal has increased from 744.23 US$/metric tonnes in 2005 to 1921.47 US$/metric tonnes in 2014, an increment of almost 60.0% (Quandl, 2015). Nonetheless, the inconsistent supply of fish meal has affected the production of fish feed and subsequently, resulting in the decrease of production of commercial freshwater fish.

The inconsistent supply of fish meal has motivated researchers to explore the alternative ingredients for fish feed. The potential protein sources which are abundantly available, cost effective and have high composition of amino acids especially the limiting amino acids methionine and lysine are highly desired. The alternative and cost effective feedstuff ingredients could help to reduce dependency on fish meal as sole fish feed ingredients. Apart from that, the potential of several protein ingredients can be harnessed such as earthworm powder, soybean waste, *Leucaena leucocephala* leaves and rice bran.

Amino acids play important roles to enhance efficiency of protein utilization in fish. From the previous study, earthworm was proven to be a good source of amino acids particularly lysine and methionine which have been identified as two most important amino acids that influence the growth of African catfish (Li, Mai, Trushenski, & Wu, 2009; Sogbesan & Madu, 2008; Zakaria, Mohd Salleh, Mohamed, Anas, & Idris, 2012). Methionine and lysine are the limiting amino acid among existed amino acid thus, making them essential in fish feeds (Nunes, Sa, Browdy, & Vazquez-Anon, 2014).

1.2 Problem Statement

Protein sources are the major concern in fish feeds because it contributes the largest part in fish feeds. There are numerous studies on the nutritional content of agricultural wastes to be incorporated in fish feeds. This is due to their potential to be used as a protein source in animal feeds and also abundantly available. However, there are limited studies on the nutritional content of local agricultural wastes which are abundantly available and have potential to be incorporated in fish feeds.

On the other hand, most of the studies on the fish feed only focusing on the optimum protein composition without diminish their scope into the function of limiting amino acids (i.e. methionine and lysine) that contribute for the growth of catfish fingerlings. Limiting amino acid methionine and lysine are critical elements to assist growth of catfish fingerlings and high levels of these amino acids are usually required in fish feeds.

Nevertheless, it is still unclear whether high level of methionine and lysine really affect the growth performance of African catfish fingerlings. Further study will need to be performed in order to clarify the amount of protein-bound methionine and lysine needed for the growth of African catfish fingerlings.

By incorporating several feedstuff ingredients in fish feed, it is ambiguous about the digestibility of protein-bound methionine and lysine in African catfish fingerlings. Digestibility study is required to determine the acceptability and quality of the formulated fish feed.

1.3 Research Objectives

The overall objective is to develop fish feed formulation with high level of protein-bound methionine and lysine for African catfish fingerlings utilization. The specific objectives of the studies are:

1. To characterize nutritional content in earthworm powder, fish meal, soybean waste, *Leucaena leucocephala* (petai belalang) leaves, and rice bran for fish feed formulation.
2. To formulate fish feeds with high level of protein-bound methionine and lysine for African catfish (*Clarias gariepinus*) fingerlings.
3. To investigate the effects of different protein-bound methionine and lysine level on the specific growth rate, feed conversion ratio and survival rate of African catfish fingerlings.
4. To analyze methionine and lysine digestibility of African catfish fingerlings.

1.4 Research Hypothesis

The hypothesis of this study is the formulation of fish feed with high level of protein bound methionine and lysine for African catfish using local agricultural wastes (earthworm powder, fish meal, soybean wastes, *L. leucocephala* leaves and rice bran) will give the better growth performance, feed conversion ratio, survival rate and methionine and lysine digestibility.