UNIVERSITI PUTRA MALAYSIA

EVALUATION OF FISH SILAGE AS A PROTEIN SOURCE IN THE DIET FOR RIVER CATFISH MYSTUS NEMURUS (C. &V.)

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By

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A Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Institute of Bioscience Universiti Putra Malaysia

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DEDICATION

To my parents, H. Hasan and Hj. Nurimah; my wife Fahria Hadari;
my children, Rizka Habiba Bustari and Muhamad Rizki Bustari who gave me
supports and understandings during my study

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Che Roos Saad, Ph.D

Faculty : Institute of Bioscience

A series of experiments was conducted to determine: 1) optimal dietary energy and protein levels for maximum growth of *Mystus nemurus* (C. & V.), 2) preparation and nutritional quality of fish silage, 3) optimal inclusion levels of fish silage replacing fishmeal in the fish diets, 4) digestibility values of dietary fish silage by fish. Eight experimental diets were formulated to contain four energy levels (2.75, 3.00, 3.25 and 3.50 kcal DE/g) at each of the two protein levels (38% and 42%), and fed to groups of fingerlings (1.88-213 g) for 10 weeks). It was found that the diet containing 3.25 kcal DE/g and 42% protein (7.74 kcal DE/g protein) produced the best growth and food utilization.

Five fermented silage and one acid silage were made from short-bodied mackerel (*Rastrelinger brachysoma*). The fermented silage were prepared by the

addition of molasses with different fermentation starters to fish mince, namely: LBPN (80% fish + 15% molasses + 5% *Lactobacillus pentosus* pure culture), LBPL (80% fish + 15% molasses + 5% *Lactobacillus plantarum* pure culture), LFBS (70% fish + 15% molasses + 15% liquid fermented bamboo shot), ALPN (70% fish + 15% molasses + 15% aged *Lactobacillus pentosus* silage), ALPL (70% fish + 15% molasses + 15% aged *Lactobacillus plantarum* silage). Acid silage (FAS) was made by addition of 3% (w/w) of formic acid to fish mince. These silage were stored at room temperature for 60-180 days. All silage were stable in pH (< 4.5), and no spoilage odor was detected through out the storage duration. Nutritional quality of the silage varied with preparation methods. Fermented silage, especially LBPN was more desirable due to less non-protein nitrogen production than acid silage.

Fermented and acid fish silage were co-dried with soybean meal (1:1 dry weight basis) and the mixtures were incorporated in fish diets at various levels to substitute fishmeal. Eight test diets (42% protein and 4.20 kcal GE/g) were formulated to contain co-dried fermented fish silage (CFS) and co-dried acid fish silage (CAS) as a replacement for fishmeal. Four of the test diets were composed of CFS at the inclusion levels of 15% (CFS-1), 30% (CFS-2), 45% (CFS-3), and 60% (CFS-4); and the other four diets were composed of CAS at the inclusion levels of 15% (CAS-1), 30% (CAS-2), 45% (CAS-3), and 60% (CAS-4). A control diet (C) was prepared with 60% fishmeal without fish silage. The diets were fed to *Mystus nemurus* fingerlings for 10 weeks. One percent of chromium

oxide (Cr_2O_3) was included in the diets for diet digestibility determination. The losses of dry matter (LDM) of the diets after 10 and 30 minutes in the water (LDM-10 min and LDM-30 min) were 2.31-4.47% and 8.19-18.58%, respectively for CFS diets, 2.40-4.51% and 9.32-18.26%, respectively for CAS diets and 2.12 and 6.43%, respectively for control diet. The pH values were 6.57-5.78 for CFS diets, 5.64-4.96 for CAS diets and 6.78 for control diet. The essential amino acid profiles (A/E ratio) of both CFS and CAS diets were comparable to A/E ratio of *Mystus nemurus*. Apparent dry matter, protein and energy digestibility values for both CFS and CAS diets were similar to the control diet. Inclusion of CFS in fish diets up to 45% did not affect growth performance as compared to control diet. However inclusion of more than 15% CAS in fish diets reduced growth performance.

Abstrak disertasi dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk mendapatkan Ijazah Doktor Falsafah

PENILAIAN SILAJ IKAN SEBAGAI SUMBER PROTEIN DALAM MAKANAN UNTUK IKAN BAUNG *MYSTUS NEMURUS* (C&V)

Oleh

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Satu siri penyelidikan telah dijalankan untuk mengetahui: 1) paras tenaga dan protein untuk pertumbuhan maksimum ikan baung (*Mystus nemurus,* C.&V.), 2) penyediaan dan mutu pemakanan silaj ikan, 3) paras kandungan silaj menggantikan tepung ikan dalam makanan ikan dan 4) nilai penghadaman silaj bagi ikan. Lapan diet ujian dirumuskan mengandungi empat paras tenaga (2.75, 3.00, 3.25 dan 3.50 kcal DE/g) pada setiap paras dari pada dua paras protein (38 dan 42%) dan diberikan kepada kumpulan anak ikan (1.88-2.13 g) selama 10 minggu. Didapati makanan yang mengandung 3.25 kcal DE/g dan 42% protein (7.74 kcal DE/g protein) menghasilkan pertumbuhan dan penggunaan makanan yang terbaik.

Lima silaj penapaian dan satu silaj asid dibuat dari ikan kembong kecil (*Rastrelinger branchysoma*). Silaj penapaian disediakan dengan penambahan

gula molas dengan starter penapaian yang berlainan kepada hancuran ikan: LBPN (80% ikan + 15% gula molas + 5% kultur murni *Lactobacillus pentosus*), LBPL (80% ikqn + 15% gula molas + 5% kultur murni *Lactobacillus plantarum*), LFBS (70% ikan + 15% gula molas + 15% air jeruk rebung), ALPN (70% ikan + 15% gula molas + 15% silaj *Lactobacillus pentosus* yang sudah jadi), ALPL (70% ikan + 15% gula molas + 15% silaj *Lactobacillus plantarum* yang sudah jadi). Silaj asid (FAS) dibuat dengan menambahkan asid formik 3% (berat/berat) kepada hancuran ikan. Keseluruhan silaj disimpan pada suhu bilik selama 60-180 hari. Keseluruhan silaj adalah stabil dalam pH dan tidak didapati bau busuk selama penyimpanan. Nilai mutu pemakanan silaj berbeza mengikut kaedah penyediaan. Silaj peragian, khususnya LBPN lebih dipilih kerana pengeluaran non-protein nitrogen yang lebih rendah dari pada silaj asid.

Silaj peragian dan silaj asid dikeringkan dengan tepung soya (1:1 berat kering) dan campuran dimasukan ke dalam makanan ikan pada pelbagai paras untuk menggantikan tepung ikan. Lapan diet ujian (42% protein dan 4.20 kcal GE/g) dirumuskan untuk mengandungi silaj penapaian campuran (CFS) dan silaj asid campuran (CAS). Empat dari pada diet ujian mengandungi CFS: 15% (CFS-1), 30% (CFS-2), 45% (CFS-3), dan 60% (CFS-4); dan empat diet lain mengandungi CAS: 15% (CAS-1), 30% (CAS-2), 45% (CAS-3), dan 60% (CAS-4). Diet kawalan (C) disediakan dengan 60% tepung ikan tanpa silaj. Kesemua diet diberikan kepada ikan selama 10 minggu. Satu peratus oksida kromium (Cr_2O_3) dimasukan ke dalam diet ikan untuk menentukan nilai

penghadaman diet. Kehilangan bahan kering di dalam air setelah 10 dan 30 minit (LDM-10 min and LDM-30 min) adalah 2.31-4.47% and 8.19-18.58% berturut-turut untuk diet CFS, 2.40-4.51% dan 9.32-18.26% berturut-turut untuk diet CAS dan 2.12 and 6.43% berturut-turut untuk diet kawalan. Nilai pH adalah 6.57-5.78 untuk diet CFS, 5.64-4.96 untuk diet CAS and 6.78 untuk diet kawalan. Profil asid amino perlu (A/E ratio) dari kedua diet CFS dan CAS adalah setara dengan A/E ratio bagi *Mystus nemurus*. Nilai penghadaman bahan kering, protein dan tenaga untuk kedua jenis diet adalah serupa dengan diet kawalan. Pemasukan CFS ke dalam diet sehingga 45% tidak memberi kesan negatif terhadap pertumbuhan ikan bila dibandingkan dengan diet kawalan. Namun pemasukan CAS dalam diet melebihi dari 15% akan mengurangi penampilan pertumbuhan ikan.

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LIST OF ABBREVIATIONS

AA= Amino acid

ADC= Apparent digestibility coefficient

A/E ratio= Amino acid/total essential amino acid (%)

ALPN= Aged-silage *Lactobacillus pentosus*

ALPL= Aged-silage *Lactobacillus plantarum*

ANOVA= Analysis of variance

AOAC= Association of official analytical chemists

BHT= Butylated hydroxy toluene

C= Control

CFS= Co-dried fermented Fish silage

CAS=Co-dried acid Fish silage

CP= Crude protein

DE= Digestible energy

DHA= Decosahexaenoic acid

EAA= Essential amino acid

EI= Energy intake

EPA- Eicosapentaenoic acid

FAS= Formic acid silage

FER= Food efficiency ratio

FG= Fat gain

FI= Food intake

FM= Fishmeal

FS= Fish size

GE= Gross energy

HPLC= High performance liquid chromatography

IDF= International dairy federation

Kcal= Kilo calorie

LAB= Lactic acid bacterial counts

LBPN= *Lactobacillus pentosus*

LBPL= *Lactobacillus plantarum*

LDM= Loss of dry matter (%)

LFBS= Liquid fermented bamboo shoot silage

ME= Metabolisable energy

Min. mix= Mineral mixture

MRS= De Man Rogosa Sharpe

NPN= Non-protein nitrogen

NPU= Net protein utilization

NRC= National research council

PE= Protein-energy

PER= Protein efficiency ratio

PG= Protein gain

PI= Protein intake

PKC= Palm kernel cake

PO= Palm oil

REF= Reference

RF= Raw fish

SAS= Statistical analysis system

SBM= Soybean meal

SD=Standard deviation

SEM= Standard error of means

SGR= Specific growth rate

SR= Survival rate

TA= Titratable acidity

TCA= Trichloroacetic acid

Vit. mix= Vitamin mixture

WG= Weight gain

CHAPTER 1

GENERAL INTRODUCTION

Aquaculture in Malaysia has rapidly expanded recently. Since 1990, the total area devoted has increased from 9,085.03 to 16,166.93 hectares in 1998; and aquaculture production increased from 14,788.77 tons in 1990 to 133,646.64 tons in 1998, with values reaching RM 654,294.77 millions in 1998 (Anon, 1990-1998).

One of the popular and highly demanded aquaculture species is the river catfish (*Mystus nemurus*). Naturally, the fish is a carnivorous species with stout, sturdy and scaleless body of 0.4-1.5 kg in weight, and of lake or river origin and bottom feeder (Khan, 1993). The fish is identified as a potential aquaculture species due to its successful artificial spawning, ability to withstand in relatively low pH and dissolved oxygen, high dress-out percentage as well as high economic value (Khan et al., 1993a and Khan, 1993).

Extensive culture of the species has been practiced in cages and reservoirs. Seed supply is routinely available from Freshwater Research Center in Malacca, Aquatic Resources Technology at Universiti Putra Malaysia and other private hatcheries around Peninsular Malaysia. The fish is cultured at a density rate of 2000 fish per hectare and fed with commercial fish pellets, trash