UNIVERSITI PUTRA MALAYSIA

PRACTICAL USE OF SOYBEAN MEAL AND ITS SUPPLEMENTATION WITH AMINO ACIDS IN DIET OF MYSTUS NEMURVS (CUVIER AND VALENCIENNES) FINGERLINGS

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IB 2000 1

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BY

MD. ASADUZZAMAN

Thesis Submitted in Fulfilment of the Requirements for the Degree of
Master of Science in the Institute of Bioscience
Universiti Putra Malaysia

October 2000

DEDICATION

To the loving and sacred memory of my father

ALHAJ MD. NAZMUL HOQ

Who left me forever on 13th December 1997

AND

Ever loving my Mother, Bilkis Begum Hiron for her true love and affection

Those give me a true light that leads every success in my life.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

PRACTICAL USE OF SOYBEAN MEAL AND ITS SUPPLEMENTATION WITH AMINO ACIDS IN DIET OF *MYSTUS NEMURUS* (CUVIER AND VALENCIENNES) FINGERLINGS

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Chairman: Dr. Che Roos Saad

Faculty: Institute of Bioscience

Two experiments using different formulation of diets to determine the suitability of soybean meal in diet for *Mystus nemurus* fingerlings were carried out. In the first experiment, seven types of formulated diets were used to evaluate the effect of partial or complete replacement of fishmeal (FM) with solvent-extracted soybean meal (SSBM) and full-fat soybean meal (FFSBM) for the river catfish, *Mystus nemurus* fingerlings. Six isonitrogenous diets (42% crude protein) containing different content of FM:SSBM were D_1(61.29:0.0%), D_2(39.78:30%), D_3(32.6:40%), D_4(25.44:50%), D_5(18.37:60%), and D_6(0.0:85.84%) were randomly fed to catfish in triplicate tanks. The seventh diet (30% crude protein) containing 0% FM and 85.42% FFSBM was also used in the same manner. Average individual weight gain, feed conversion ratio, specific growth rate, protein efficiency ratio, and yield were not differ significantly ($p>0.05$) among the two comparable diets (D_1 and

D_4) where the FM and SSBM percentage were 61.29, 25.44 and 0, 50%, respectively. But apparent net protein utilization (between D_1 and D_4) was differing significantly ($p<0.05$). Survivability of the fish fed diet D_1 and diet D_4 with the value of 81.90 and 80.95%, respectively. This study indicate that 58.49% FM can be replaced by SSBM in the diets for better growth for intensive culture.

In the second experiment, seven types of isonitrogenous (42% protein) diets having fish meal with soybean meal (Control diet) and different proportion of soybean meal supplemented with three selected indispensable crystalline amino acids were used to determine their suitability as feed for *Mystus nemurus* fingerlings under close water recirculating system. Casein was added to make up 42% protein. The study showed that *M. nemurus* fed the control diet (D_1) with 25.44% FM and 50% SSBM (same as in experiment 1) had highest growth performances. The species fed the diet with 57% SSBM (D_3) with the selected amino acids had the second highest growth performances. But between the two treatments (D_1 and D_3) had no significant differences ($P>0.05$) on the basis of weight gain (%), FCR, SGR, PER, yield and survival.

Amino acid profiles of whole-body *M. nemurus* fingerlings at different feeding trials were determined to provide baseline information on the amino acid requirements of the fresh water catfish. The essential amino acid (A/E) ratio were calculated and found to be a possible method for evaluating the *M. nemurus* diet, as a superior ratio indicates superior production.

Abstrak tesis dipersembahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PENGGUNAAN PRAKTIKAL TEPUNG KACANG SOYA DAN SUPPLEMENTASINYA DENGAN ASID AMINO DI DALAM DIET JEJARI *MYSTUS NEMURUS* (CUVIER DAN VALENCIENNES)

Oleh

MD. ASADUZZAMAN

Oktober 2000

Pengerusi: Dr. Che Roos Saad

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Dua eksperimen menggunakan formulasi diet yang berbeza untuk menentukan kesesuaian tepung kacang soya di dalam diet jejari *Mystus nemurus* telah dijalankan: (i) Di dalam eksperimen pertama, tujuh jenis diet yang dirumus digunakan untuk menilai kesan penggantian separa atau sepenuh tepung ikan (FM) dengan tepung kacang soya yang diekstrak dengan pelarut (SSBM) dan tepung kacang soya mengandungi lemak penuh (FFSBM) untuk jejari baung, *M. nemurus*. Enam diet berisonitrogenus (42% protein kasar) mengandungi kandungan FM:SSBM yang berbeza adalah D_1 (61.29:0.0%), D_2 (39.78:30%), D_3 (32.6:40%), D_4 (25.44:50%), D_5 (18.37:60%), dan D_6 (0.0:85.84%) secara rawaknya diberikan makan kepada ikan baung dalam tangki dengan 3 replikasi bagi setiap satunya. Diet ketujuh (30% protein kasar) mengandungi 0% FM dan 85.42% FFSBM telah digunakan dengan kaedah yang sama. Purata pertambahan berat individu, nisbah pertukaran makanan, kadar tumbesaran spesifik, nisbah kecekapan protein, dan hasil adalah tidak berbeza dengan ketara ($p>0.05$) di antara kedua-dua diet yang

berbanding (D_1 dan D_4) dimana peratusan FM dan SSBM masing-masing adalah 61.29, 25.44 dan 0, 50%. Tetapi penggunaan jelas protein bersih (di antara D_1 dan D_4) adalah berbeza dengan ketara ($p<0.05$). Kemandirian ikan yang diberi diet D_1 dan D_4, masing-masing dengan nilai 81.90 dan 80.95%. Kajian ini mencadangkan bahawa 58.49% FM boleh diganti dengan SSBM di dalam diet untuk tumbesaran yang lebih baik di dalm kultur intensif.

(ii) Di dalam eksperimen kedua, tujuh jenis diet isonitrogenus (42% protein) mengandungi tepung ikan dengan tepung kacang soya (diet kawalan) dan nisbah tepung kacang soya yang berbeza disupplementasi dengan tiga asid amino terpilih yang perlu telah digunakan untuk menentukan kesesuaiannya sebagai makanan untuk jejari *M. nemurus* di dalam sistem kitar semula air tertutup. Kasein telah ditambah untuk melengkapkan 42% protein. Kajian ini menunjukkan bahawa *M. nemurus* yang diberi diet kawalan (D_1) dengan 25.44% FM dan 50% SSBM (sama seperti eksperimen 1 mempunyai persembahan tumbesaran yang tertinggi. Spesis yang diberi diet 57% SSBM (D_3) dengan asid amino terpilih mempunyai persembahan tumbesaran kedua tertinggi. Tetapi di antara kedua-dua rawatan (D_1 dan D_3) tidak terdapat perbezaan ketara ($p<0.05$) yang berdasarkan kepada pertambahan berat (%), FCR, SGR, PER, hasil dan kemandirian.

Profil asid amino untuk keseluruhan badan jejari *M. nemurus* pada ujian makanan yang berbeza telah ditentukan untuk memberi maklumat asas ke atas keperluan asid amino ikan baung tersebut. Nisbah asid amino perlu (A/E) telah dihitung dan didapati sebagai kaedah yang mungkin untuk menilai diet *M. nemurus*, iaitu nisbah yang lebih baik menunjukkan penghasilan yang lebih baik.

ACKNOWLEDGEMENTS

I would like to extend my most sincere gratitude and deep appreciation to Dr. Che Roos Saad who provided me with invaluable guidance, objective discussions and criticisms, encouragement and support throughout the course of the study. I am also indebted to the members of my supervisory committee, Dr. Mohd Salleh Kamarudin, Assoc. Prof. Dr. Abdul Razak Alimon and Dr. Adam Puteh for providing laboratory facilities and their constructive suggetions.

I would like to acknowledge Universiti Putra Malaysia for the "Graduate Assistantship" under Intensification of Research in Priority Areas (IRPA) of the Ministry of Science Environment and Technology, Malaysian Government through Dr. Che Roos Saad which was kindly awarded to me during the tenure of my candidature as a M.S. student. This kind of generosity, no doubt, heralded in a new era in my life and would continue to remain as a perennial source of inspiration.

Thanks are extended to friends and colleagues; Dr. Paymon Roustaian for teaching me the operation of HPLC, Annie Christianus and the staff of Experiment Station under Institute of Bioscience, UPM, Mr. Zaidi, Mr. Jasni, Mr. Nordin and Mr. Krishnan for their valuable practical assistance. My appreciation is also extended to Madam Faridah, Graduate School, UPM, for her hospitality and support during my early days in Malaysia.

Words are not enough to express my heartfelt feelings to my parents for providing me with their untiring guidance and supports since my childhood. All of my brothers (Zaman, Atik, and Arif) and sisters (Lucky, and Alo) have extended their true love, moral support and encouragement which serves as my inspiration to perform this achievement.

And above all, to **Allah Almighty** for making all this possible.

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CHAPTER 1

INTRODUCTION

Background of the Study

Mystus nemurus has a great potential for culture in Malaysia that could and should make a major contribution to the provision of protein for food and job creation for society. These aims can realistically only be achieved by finding out the nutritional requirements of the species and develop the aquacultural system that can improve the performances of growth and survival.

Soybean meal has currently become the most important source of plant proteins in the diets of monogastric animals, especially for poultry in the U.S. The U.S.A is the largest producer of soybeans {49.2 million metric tones (MMT) estimated for 1993} followed by Brazil (24.4 MMT), Argentina (12.1 MMT), India (4.1 MMT) and China (12.3 MMT) (Swick, 1994) and American soybean meal, contents 44.6 CP, 3294 TME, 6.1 ash, 1.2 fat, 7.3 fiber and FCR 1.49. Most studies on protein requirement show that whole protein composites, such as fish meal, soybean meal and cod muscles are used as a standard reference diet (Jauncey, 1982; Siddique et al., 1988; Shiau and Huang, 1989; Santiago and Reyes, 1991).

Soybean meal is a potential protein source for fish which covers the essential amino acids that might replace the fish meal significantly. Presently, the question is arised is how much fish meal could be replaced by soybean meal and soybean meal

with amino acid to achieve the maximum growth of catfish *Mystus nemurus*. Thus, it is essential to know the growth performance of fish in soybean meal based diet with different proportion. It is also essential to know the nutrients status, efficiency of diets containing soybean meal.

Statement of the Problem

The Malaysian river catfish, *Mystus nemurus* is considered a "new" species in aquaculture in Malaysia and its production has only become significant in 1993 (Kamarudin, 1999). This species is one of the most demanded and popular fresh water catfish due to its delicacy, meat quality and high lean percentage (Khan et al., 1990; Kamarudin et al., 1987 and Thalathiah et al., 1988). As culture practices continue to intensify, *M. nemurus* will rely heavily on prepared feeds. However, its nutritional needs are largely unknown. This study is therefore designed to determine that nutritional needs in both controlled recirculatory and open water systems. This in turn, is ultimately linked to status of fingerling production which is the corner stone in culture of any aquatic species.

Protein is a very important constituent of diet, both qualitatively and quantitatively, as it is the building material for the growing animal organism. It is also important for the production of enzymes, among other things and is also the most expensive ingredient in animal feeds. Compared with other vertebrate animal, fish is characterized by a high protein requirement. Relevant studies have been performed on various different fish species and have given rich to substantially

consistent results. In recent years, the culture of many catfish species has been intensified by increasing nutrient density and feeding allowance (Li, 1989). Fish meal has traditionally been used as a major ingredients in the fish diet because of its high protein quality and palatability to the fish. However, the high cost and uncertain availability, it has been solicited to find out a suitable replacement of fish meal. Cho et al., (1983) reported that feed cost is the single largest expense (33-50 percent) of the total production cost for most fish farm operations. Although feed costs significantly affect production costs, relatively few studies have been conducted toward the development of high-quality yet, economical diets. Barbieri and Cuzon (1980) mentioned that economic interest of a diet depends on the low feed cost, feed conversion efficiency, and high performance. As culture practices continue to intensify, *M. nemurus* will rely heavily on prepared feeds; but its nutritional needs are largely unknown. It has been established that protein is required by all animal for body maintenance and growth, and that protein level needed for this function varies with the species size and culture environment (Munson et al., 1954; Phillips et al., 1957; Delong et al., 1958; Lovell, 1972).

Succesfull replacements by other protein feedstuffs have been reported, mostly of animal origin, such as milk or whey powder (Meske et al., 1977), feathermeal and meatmeal (Tiews et al., 1976), krillmeal (Pfeffer and Meske, 1978), single- cell protein (Atack et al., 1979), and algal meal (Sandbank and Hepher, 1978). Unfortunately, most of these materials are as scarce and/or expensive as fishmeal. Fishmeal is becoming increasingly expensive all over the world and this threatens the whole aquaculture industry. That is why many researchers started a

systematic study on the possibility of its partial or complete replacement by upgrading feeds containing solvent extracted soybean meal and full-fat soybean meal and soybean meal with amino acid supplementation. Problems associated with digestibility, acceptability, stability and nutritional status of the formulated diets may single-handedly or in combination contribute to lack of success in total or partial replacement with fishmeal. An easily available, cheap, and nutritionally sound artificial diet is therefore desired to reduce dependence of fishmeal in rearing of fish in developing nations.

Significance of the Study

Malaysian weather is suitable for culture this species. Artificial food or feeding is one of the principal methods of increasing production in fish cultivation. Its importance varies according to the intensity of cultivation. As it was indicated earlier, the major challenge in the development of culture system for *M. nemurus* is to reduce its dependency upon fishmeal which are both costly and nutritionally inconsistent.

Knowledge on the nutritional requirements of the *M. nemurus* is essential prerequisite for development of a nutritionally adequate diet. Moreover, such information could be used in making a cost-effective diet by furnishing some flexibility in the choice of ingredients. Learning to accept artificial diet appears to be the dominant factor in food selectivity. However, it is important to use feeds with suitable dimension and texture to optimize consumption and help maintain good

water quality (Knights, 1983). The development of a high quality artificial diet could reduce the high cost of fishmeal dependent diet as well as ameliorate water quality and disease problems (Bengtson, 1993). Eventhough, the aquacultural system is an important factor that controls the much better environment for culture. However, the information about nutritional requirements of the river catfish *M. nemurus* is quite scarce, though the rearing techniques developed.

Objectives of the Research Work

The aim of this study are to identify the optimum feeding strategy that will ensure maximum utilization of a practical diet and to provide more insight on the biochemical composition in general and amino acid nutrition in fingerling stages of *M. nemurus*. As a new aquaculture species, no conclusive information concerning such scope is available for the fingerling stage of this economically important species. Such information would be valuable to shed more lights on developing artificial diets that can reduce the dependence on expensive fishmeal dominant diet. In this context, the specific objectives of the research are as follows:

1. Formulation of diets with proximate analysis of experimental feed and its ingredients and those of *M. nemurus* including their amino acid compositions,
2. To examine the effects of gradual replacement of fishmeal with soybean meal on fingerlings survival and production of *M. nemurus*,
3. To examine the effects of soybean meal with casein and supplemented amino acid on fingerlings survival and production of *M. nemurus*, and

4. To examine the dietary amino acid needs of *M. nemurus* could be estimated from the amino acid composition of carcass tissue. The quantitative amino acid profile of a particular protein diet contributing highest growth in *M. nemurus* should serve as indices in formulating or predicting the requirement of the species.

CHAPTER 2

LITERATURE REVIEW

Nutritional Requirements

The ultimate objective of virtually all nutritional experiments with any aquaculture species is the isolation of those dietary or nutritional parameters whose interactive effects will enhance growth rate in the animal. A knowledge of the developmental changes associated with the process of food ingestion, digestion and assimilation is essential to any understanding of the nutritional physiology of fish. There have been extensive studies of digestibility of channel catfish, *Ictalurus punctatus* and many finfishes with different feed ingredients and rations (Smith and Lovell, 1971, 1973; Page and Andrews, 1973; De Silva, 1985; Wilson and Poe, 1981, 1985b).

Protein Requirement in Fish

Proteins are the major organic component in fish tissue, making up roughly 65-75% of its total dry weight. Protein is usually given more attention in any aquatic diet formulation as it represents the major and most expensive component of feeds (Santiago and Reyes, 1991; Tidwell et al., 1992; Murai, 1992; Van Der Meer et al., 1995; Catacutan and Coloso, 1995). Fish utilizes protein to obtain amino acids that are absorbed into the organ tissues through the intestinal tract (Wilson, 1989). Catfish generally do not need protein but they absolutely require amino acids for

metabolism (Robinson and Wilson, 1985). Hence, information regarding protein requirement is essential in the formulation of well-balanced low cost artificial diets (Stottrup et al., 1986). Wilson (1989) found that the dietary protein requirement is affected by the quality of protein found in the test diets. Casein, for example, is known to be lacking in arginine for most fishes.

For European catfish growth and feed conversion ratio are shown to improve as the protein content was raised from 30%-50% (Hilge and Gropp, 1985). For African catfish *Clarias geriepinus*, (40-120g) the rate of weight gain improved as the protein content of the diet was raised from 20% to 40% (Machiels and Henken, 1985), depending on the energy content of the feed, the fastest growth rate could be obtained at 49% dietary protein (dry weight) at 24^{O}C and 65% at 29^{O}C (Henken et al. 1986), corresponding to daily intakes of 4.0g and 7.3g crude protein / $kg^{0.8}$ respectively. These values correspond to daily intakes of 3.8g and 5.7g crude protein / $kg^{0.8}$. At lower protein contents (and lower energy content in the feed), both growth and feed utilization were better at 24^{O}C then 29^{O}C, while for higher protein contents the converse applied. In further experiments (27.5^{O}C) the highest gain was recorded at a dietary protein content of 60% and fat contents ranging from 10% to 30% (Machiels and Van Dam, 1987). Khan et al. (1993) reported that 42% dieting protein is the optimal level for the maximum growth of young *Mystus nemurus*. They also observed a significantly ($p<0.05$) higher gain in the diet containing 42% crude protein over the 27%, 32%, 37%, and 47%.