EFFECTS OF DIFFERENT INCLUSION OF SIRULINA MEAL ON GROWTH, SURVIVAL AND FEED UTILIZATION OF JUVENILE ASIAN SEABASS (*Lates calcarifer*) REARED IN FRESHWATER

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THIS DISSERTATION IS SUBMITTED AS A PARTIAL FULFILLMENT OF THE REQUIREMENTS TO GRADUATE AS A BACHELOR OF SCIENCE WITH HONOURS

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25 JUNE 2015
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ABSTRACT

EFFECTS OF DIFFERENT INCLUSION OF SPIRULINA MEAL ON GROWTH, SURVIVAL AND FEED UTILIZATION OF JUVENILE ASIAN SEABASS (*Lates calcarifer*) REARED IN FRESHWATER

The objective of this study is to evaluate the possibility of Spirulina meal (SP) to replace fish meal in the diet of Asian seabass, *Lates calcarifer*. Five isoproteic (45% crude protein) and isolipid (12% crude lipid) diets were formulated to replace FM with 0 (control), 5, 10, 20 and 30% Spirulina (as SP0, SP5, SP10, SP20 and SP30 respectively) and fed to fish (initial body weight, 6.00±0.02g) for eight weeks. Triplicate group of 20 tails juvenile Asian seabass were stocked in recirculating aquaculture system. At the end of feeding trial, there was no significance difference (*p*>0.05) in term of weight gain, specific growth rate, feed intake, protein efficiency ratio, feed conversion ratio, viscerosomatic index and condition factor. Fish fed SP0 showed significantly higher (*p*<0.05) survival compared to SP20 and SP30 but no significance difference from fish fed SP5 and SP10. The weight gain of juvenile Asian seabass ranged from 109.03% to 198.35% whereby SP10 showed the highest compared to SP0 and SP30 was the lowest. The highest feed intake was from fish fed SP10 and was statistically proven (*p*<0.05). There was significance difference in hepatosomatic index (HSI) whereby HSI increased with higher inclusion level of SP ranged from 0.81 to 1.51. Whole body-proximate composition were significantly affected by the inclusion of SP in diet of Asian seabass. The present study indicate replacement level 5-10% inclusion are recommend for a long term use.
ABSTRAK

Objektif kajian ini adalah untuk menilai potensi Spirulina (SP) menggantikan tepung ikan (FM) dalam makanan ikan untuk diet juvena ikan siakap, Lates calcarifer. Lima isoproteic (45% protein) dan isolipid (12% lipid) diet diformulasi bagi menggantikan FM dengan 0 (kawalan), 5, 10, 20 dan 30% SP (masing-masing sebagai SPO, SP5, SP10, SP20 dan SP30) dan diberi makan kepada ikan siakap (berat badan, 6.00 ± 0.02g) selama lapan minggu. Sebanyak 20 ekor ikan siakap (triplikasi) ditemak dalam sistem akuakultur resirkulasi. Pada akhir ekperimen ini, dilaporkan tiada perbezaan signifikan (p>0.05) dari segi pertambahan berat badan, kadar pertumbuhan spesifik, pengambilan makanan, kadar kecekapan protein, nisbah penukaran makanan, indeks viseralsomatik (VSI) dan faktor kondisi antara semua rawatan. Ikan makan diet SPO menunjukkan perbezaan signifikan (p<0.05) yang tinggi dalam kemandiran berbanding dengan SP20 dan SP30 tetapi tiada perbezaan yang signifikan dengan SP5 dan SP10. Pertambahan berat badan juvena siakap berada di antara 109.03% dan 198.35% dimana SP10 adalah tertinggi berbanding SP0 manakala SP30 adalah terendah. Pengambilan makanan tertinggi adalah dari SP10 dan ini dibuktikan secara statistik (p<0.05). Didapati Indeks hepatosomatik (HSI) mempunyai perbezaan signifikan (p<0.05) dimana HSI meningkat dengan penambahan SP di antara 0.81 dan 1.51. Penambahan SP dalam diet ikan siakap memberi kesan kepada komposisi proksimat ikan siakap dengan perbezaan signifikan. Kajian ini menunjukkan pergantian sebanyak 5-10% boleh digunakan untuk penggunaan jangka panjang.
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LIST OF SYMBOLS AND ABBREVIATION

°C  Degree celcius
<  Less than
%  Percentage
FAO  Food and Agriculture Organization
RM  Ringgit Malaysia
ppt  Part per thousand
Kg  kilogram
EAA  Essential amino acid
CMC  Carbomethylcellulose
AOAC  Association of Official Analytical Chemists
N  Normality
SP  Spirulina
RAS  Recirculating Aquaculture System
HP  Horsepower
CRD  Completely Randomized Design
DO  Dissolve Oxygen
HSI  Hepatosomatic Index
VSI  Visceralsomatic Index
WG  Weight Gain
SGR  Specific Growth Rate
FI  Feed Intake
FCR  Feed Conversion Ratio
PER  Protein Efficiency Ratio
CHAPTER 1

INTRODUCTION

Currently the world aquaculture production had increased tremendously since 1950 and in 2012, the production reached 66.6 million tonnes (FAO 2014). This figure will keep on increasing and predicted to be surpass production of capture fisheries in the future as the production is declining due to overfishing and slow recovery on wild population. As the aquaculture industry increase, the demand for fish meal and fish oil are also increased. This has resulted in increase of fish meal’s price (Tacon 1993). Therefore, many researcher try to find a solution to cope this problem through utilization of plant source and animal by-product as protein source (Higgs et al., 1995).

The most commonly alternative ingredient today are the animal by-product and plant based protein. The most promising and highly popular is soy bean meal due to its considerably high protein content of about 48% and acceptable amino acid profile (El-sayed, 1999). Due to its cost efficient compared to fish meal, many commercial pellet contain soy bean meal to partially replace fish meal. But the limitation of this soy bean meal is the presence of anti-nutritional factor that interfere the digestion process (Liener, 1994). Therefore, more effort should be carried out to evaluate other alternative ingredients, especially those with sustainable supply. Spirulina, *Arthrospira platensis* is blue-green microalgae from fresh water and have spirally coiled shape similar to cyanobacteria (Henrikson, 2009). Spirulina are rich is source of protein, vitamins, essential amino acids, essential fatty acids and antioxidant pigments (Belay et al., 1996). The protein content of Spirulina can reach up to 70%.
Microalgae also obtained attention as a protein source in aquafeed. Few algal species have been used in aquaculture feed (Muller-Fuega, 2000). Spirulina especially *Arthrospira platensis* have been widely tested in aquaculture species and the result is very convincing where most of the culture organism performed better in growth and protein assimilation when fed with algae-based diet. The studied done on silver seabream (*Rhabdosargus sarba*) where replacement of 50% fish meal with Spirulina gave better growth rate compare to control diet without adverse effect (El-sayed, 1994).

Asian seabass, *Lates calcarifer* is a very popular species aquaculture farmers among Southeast Asia due to its easy cultivation. Seabass is a hardy aquatic animal and adapt well in fresh water and brackish water (Tookwinas and Charearnrid 2008). Seabass has moderate market price range from RM 25 to RM 30 (Odhman, 2006). Unfortunately the industry is challenged by high cost of feed and disease outbreak. The use of properly formulated balanced diet is important to overcome these problems.

The objectives of the present study are:

- To evaluate the possibility of replacement fish meal with Spirulina meal on growth, survival and feed utilization of juvenile Asian seabass

- To determine the optimum inclusion of Spirulina meal of juvenile Asian seabass
CHAPTER 2

LITERATURE REVIEW

2.1 Potential of Asian Seabass in Aquaculture

Seabass is one of the most important aquaculture species that widely culture especially in Southeast Asia. This catadromous species inhabit the Indo-west Pacific Region. Seabass is well adapt in brackish water and freshwater where farmer tend to culture in both different saline water either in freshwater pond or marine cages (Cheong, 1989). The production of seabass increase steadily since 1998 and fetch up to around 75,000 tonnes in 2012 as shown in Figure 2.1. Seabass is good aquaculture candidate due to reasons such as:

   a) Seabass is hardy species animals that can be cultured in wide range of salinity which ranged from 10 to 31 ppt (Tookwinas & Charearnrid, 2008). Besides that, seabass is can be stocked at high density.
   b) Established seed production from artificial breeding in hatcheries where juvenile are easily available (Odman, 2006).
   c) Seabass exhibits higher growth rate where 3 to 5kg can be achieve within 2 years (Tookwinas & Charearnrid, 2008).
   d) It can adapt well on pelleted feed.
   e) It have moderate market price range from RM 25 to RM30 (Odman, 2006).
Figure 2.1 The annual aquaculture production of Asian Seabass (tonnes) since 1980 (FAO fisheries statistic, 2012)

2.2 Biology and Distribution of Asian Seabass, *Lates calcarifer*

Asian Seabass, scientifically known as *Lates calcarifer* and commonly called in Malaysia as siakap, kakap or seabass. The taxonomy of Asian seabass are shown in Table 2.1. The morphological of seabass it have elongated and compressed body. The mouth is large where upper jaw extend till behind eye. The dorsal fin contain 7 to 9 spines and 10 to 11 soft rays. It have short and rounded pectoral fin. The anal fin and caudal fin are rounded. The teeth of seabass is viliform and canine teeth absent. Strong spine present in lower edge of preoperculum (FAO 1974). Small spine are found on operculum and above lateral line, serrated flap are seen (Kungvankij et al, 1985). Commonly the seabass possess silver colour as show in Figure 2.2.

Figure 2.2 Juvenile Asian seabass, *Lates calcarifer* at juvenile stage
Table 2.1: The taxonomy of Asian seabass, *Lates calcarifer*

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<td>Chordata</td>
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<tr>
<td>Class</td>
<td>Pisces</td>
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<tr>
<td>Order</td>
<td>Percomorphi</td>
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<tr>
<td>Family</td>
<td>Centropomidae</td>
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<tr>
<td>Genus</td>
<td><em>Lates</em></td>
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<tr>
<td>Species</td>
<td><em>Lates calcarifer</em> (Bloch, 1970)</td>
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Seabass is available in tropical and sub-tropical areas of Western Pacific and Indian Ocean. It is commonly found along the northern part of Asia, stretch to Australia and west part of East Africa (FAO 1974) as shown in Figure 2.2. Seabass is euryhaline and catadromous species. This pattern shown where the adult seabass inhabit in fresh water area and migrate to more saline water for spawning. The newly-hatched are widely abundant in brakishwater estuaries. Seabass show protandrous hermaphrodite where fully mature male require at least 4 years and sex changing occur at seven years to become females (Moore, 1979). Though it is hardy species which can survive wide range of salinity.

Figure 2.3: Geographical distribution of *Lates calcarifer* (FAO 1974)
The major habitat spent during Asian seabass life time is in freshwater area like rivers and lakes where sea is connected seabass have higher growth rate which able to reach 3 kg within 2 years but very dependent on temperature (Tucker, 2001 & Russel and Rimmer, 2004). Spawning season normally occur during lunar cycle with onset of new moon and exact time is synchrony with incoming tide. The adult fish with at least 4 years age move to river mouth where salinity range between 30 to 35 ppt for gonadal maturation and spawning. Asian seabass is carnivorous species but this feeding habit during the juvenile stage which shown omnivorous behaviour. According to Kungvankij (1985), the juvenile seabass with length range from 1 to 10 cm indicate 20% of diatom and algae in stomach content while the rest are mainly small shrimp and fish. Moreover, seabass length that more than 20 cm contain only fauna organism.

2.3 Advantages of seabass culture in freshwater

Seawater resource is not easily access to fish famer that inhabit in inland and it is very far away from coastal area. While freshwater resource easily available locally especially in tropical country of Malaysia where it found abundantly elsewhere. This create convenience to fish farmer that intend to culture moderate market value Asian seabass in freshwater.

2.4 Nutrition Requirement of Asian Seabass

Like other aquatic animal, the nutritional requirement seabass is influenced by life stage and feeding habit that associated with morphology of digesting system. Determination of nutritional requirement of candidate species help create cost effective and eco-friendly feeds.

2.4.1 Protein and Essential Amino Acids (EAA)

Seabass require high dietary protein requirement as their feeding habit is toward carnivorous. The dietary protein requirement suggested by many researcher ranged between 45 to 55 % protein (Glencross, 2006). Seabass require ten essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine). Only the requirement for methionine (2.2%), lysine (4.9%) and arginine (3.8%) of dietary protein were determined so far (Millamena et al. 1994).
2.4.2 Lipids and Essential Fatty Acids

The optimum lipid content is closely related to protein content. Seabass indicate good growth performance at protein levels of 45% to 50% with 9% or 13% lipid (Sakaras et al, 1988; 1989). Moreover another studies showed the growth rate was highest with 15% lipid level at any three protein level (35%, 42.5% and 50%) that have done (Catacutan & Coloso, 1995). About 1.0 % of dietary n-3 fatty acids level is recommended for juvenile seabass (Wanakowat et al., 1993). While the optimum ratio of n-3 to n-6 suggested by Williams and Barlow (1999) is 1.5 to 1.8: 1. The source of lipid propose only restricted to fish oil but some study reported the vegetable oils can be used to partially replaced fish oil (Shapawi et al, 2011)

2.4.3 Carbohydrates

Though seabass is carnivorous species which have poor digestibility in utilize carbohydrate for energy source, partial amount of carbohydrate are required especially in juvenile stage as it have sparing effect for lipid (Catacutan and Coloso, 1997). About 20% dietary carbohydrate is recommended.

2.4.4 Vitamins and Minerals

According to Boonyaratpalin et al (1989), Vitamin C (Ascorbic Acid) content in diet should be about 500 to 700 mg/kg. The requirement of phosphorus is between 0.55% to 0.65%(Boonyaratpalin & Phongmaneerat, 1990). Apart from that, no information available on the specific vitamin and mineral requirement for this species.
2.5 Problems of Fish Meal in Aquaculture Feed

Feeds are the major constituent in operating cost with weightage up to 70% in shrimp and fish culture (Wee, 1992). The greatest price contribute in feed cost is fish meal which is the major protein source for any herbivorous, omnivorous or carnivorous species (Lovell 1989). About 60% fish meal occupy in formulated diets (Wee, 1992).

The aquaculture is expanding rapidly every year but the production of fish meal will not be able to catch up with the trend. The global production have increasing tremendously for aquaculture use as shown in Figure 2.4. Besides that, the production of fish meal was inconsistent from 1994 to 2007 as shown in Figure 2.5. This cause high demand on fish meal and price increase. The price of fish meal in 2011 was USD 1351 per tonnes and increase to USD 1775 in 2012 (FAO 2013).

![Figure 2.4 World inland aquaculture and mariculture production, 1980 to 2012 (FAO, 2014)](image-url)
2.6 Alternative Protein Source

The criteria for alternative protein source to replace fish meal have to look into lipids, anti-nutritional factors and essential amino acids for complete nutrition diet. The amount of nutrient required is species specific. Until now there are research carried on finding alternative protein source from fishery product, animal protein source and plant protein source. Fish waste obtained from discarded processed seafood and unintended by-catch such as non-target fish species that have low commercial value and not needed are suitable to be used as substitute for fish meal (Yano et al., 2008; Harrington et al., 2005). But the proximate composition and nutritional value are differ according to its physical nature. Fish waste that contain intestine has high lipid but low protein content (Yano et al., 2008).

Animal Protein source such as mean and bone meal is a promising protein source as meat industry produces abundant of organic waste (Banks and Wang, 2006). It is produced from animal waste tissue (trimming, bones, viscera, blood, heads, hooves and undigested feeds) that are not used for human consumption (Shirley and Parson, 2001). The crude protein content is around 47.3% to 54.3% and crude lipid is 7.2% to 10.3% (Guimaraes et al., 2008).

Plant protein source are the popular substituent in replace fish meal. The reason of utilize plant protein is lower price, greater availability and sustainable. Even though...
plant-derived protein have anti-nutritional factor acid, this problem can be solve through processing method such as heat treatment and dehulling (Francis et al., 2001; Bau et al., 1997). Example of commonly used plant-based protein source include soybean meal, corn gluten meal, canola meal and microalgae meal.

2.7 Replacement of Fish Meal for Marine Species

Studied of alternative protein source for Asian seabass mostly acquire from sustainable protein source that easily obtain terrestrially. With replacement up to 30% of soybean meal show no significance difference in term of growth performance, feed conversion ratio and survival rates with the control diet (Shapawi et al., 2013). Another studies that utilized green pea (Piscum stativum) at inclusion level 10% gave no adverse effect on growth of Asian seabass (Ganzon-Naret, 2013) in comparison with control diet. Moreover, other researched utilized yeast-fermented canola meal replace fish meal at ratios of 25, 50 and 75% in diet of Asian seabass (Pitchet & Amararatne, 2012). The results presented replacement of 25 and 50% of yeast-fermented canola meal showed no Significant difference (p>0.05) in term of growth and survival. The replacement of microalgae meal in diet of Asian seabass have not been studied.

2.8 Overview of Microalgae Industry in Aquaculture

Microalgae is microscopic unicellular algae that able to photosynthesis in present of light. The efficiency of microalgae to utilize sunlight energy better than higher plant allow production of wide range of metabolite such as proteins, lipids, carbohydrates, carotenoids, vitamins and minerals. Therefore, application of microalgae is widely seen such as human food, cosmetic, food colorant, biofuel, bio-fertilizer, pharmaceuticals and aquaculture feeds.

The main application of microalgae is for human and animal consumption due to reliable source of protein (Colla et al., 2007) and polyunsaturated fatty acids (Sajilata 2008). The common species of microalgae use for consumption are Chlorella and Spirulina. In aquaculture, microalgae usually used as feed for larvae of crustacean and fish where the pure culture microalgae are directly added into water column. Besides that, microalgae help increase the nutritional value of zooplankton to feed juvenile aquatic animal (Chen, 2003). It can be incorporated into pelleted feed for feeding of juvenile or adult aquatic animal.
REFERENCES


