EFFICACY OF ORGANIC ACID SALTS IN IMPROVING SOYBEAN MEAL AS FISHMEAL REPLACEMENT IN THE DIET OF BLUE SWIMMING CRAB, *Portunus pelagicus* (Linnaeus, 1758) JUVENILES

By

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Abstract of 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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Due to the ever-rising cost of commercial feeds for aquaculture species, it is becoming increasingly necessary to find new ingredients that are economically viable, do not harm the environment, and can meet the metabolic needs of blue swimming crab juveniles. A series of experiments was conducted to determine the optimum level of soybean meal as a fishmeal replacement in the diet on blue swimming crab juveniles and the use of organic acid salts to enhance soybean utilization of the crabs. The organic acid salts were then tested against *Vibrio harveyi* to determine whether they could inhibit the pathogenic bacteria. The crabs were housed individually in separate containers and were fed with their respective experimental diets for four weeks. Crabs were fed with six experimental diets containing different levels of fishmeal replacement with soybean meal (0, 20, 40, 60, 80 and 100%). The crabs fed 20% soybean meal diet had the best performance for growth, feed utilization, body proximate composition, nutrient retention, and histopathology of hepatopancreas. Based on this finding, subsequent feeding trials were conducted in which sodium acetate was supplemented in the diets to determine if it could improve the utilization of 20% soybean meal. Five different concentrations of sodium acetate (0, 1, 2, 3 and 4%) tested. It was found that 2% sodium acetate resulted in improved survival, growth performance, feed utilization, lipid and protein composition of body tissue, and hepatopancreatic tubule structure. Concentrations lower or higher than 2% reduced the growth performance of the crabs. The optimal level of 2% was then used for the next feeding experiment with six different types of organic acid salts supplemented into the diets, including no organic acid salt, sodium acetate, sodium butyrate, sodium citrate, sodium formate and sodium propionate. This study was designed to determine the best type of organic acid salts that could enhance soybean meal utilization, which could lead to the most optimum growth performance of the crabs. The crabs fed sodium acetate had the best growth performance compared to those fed with other organic acid salts, which implied that the optimum concentration with the correct type of organic acid salts played a crucial role in the growth of aquaculture species. A total of 30 crabs from each treatment were then used in a follow-up investigation to test if the organic acid salts could inhibit *V. harveyi* at a concentration of 10^7 CFU mL^{-1}. LC_{50}

was first conducted to determine the lethal concentration of the *V. harveyi* to be used in the challenge test. The results showed that crabs fed dietary sodium acetate were able to resist pathogenic bacteria better than those fed other treatments, as evidenced by higher survival (83.33%), lower *Vibrio* count in the hepatopancreas (5.18 ± 0.06 CFU mL^{-1}) and culture water (1.38 ± 0.01 CFU mL^{-1}) and improved hepatopancreas and gill structure after a 7-day of bacterial challenge. This study found that 20% of fishmeal replacement with soybean meal was recommended for blue swimming crab juveniles and 2% sodium acetate was the best concentration and type of organic acid salts for enhancing the soybean meal utilization by the crabs. The potential of sodium acetate to inhibit *V. harveyi* has been demonstrated.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEBERKESANAN GARAM ASID ORGANIK DALAM MENAMBAHBAIK TEPUNG KACANG SOYA SEBAGAI PENGGANTI TEPUNG IKAN DALAM MAKANAN ANAK KETAM BIRU, *Portunus pelagicus* (Linnaeus, 1758)

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Oleh kerana kos makanan komersial untuk spesies akuakultur semakin meningkat, pencarian bahan makanan alternatif baharu yang berpatutan, tidak membahayakan alam sekitar, dan berupaya memenuhi keperluan metabolik anak ketam biru perlu dilaksanakan. Beberapa eksperimen dilakukan untuk menentukan tahap optimum tepung kacang soya sebagai pengganti tepung ikan dalam diet anak ketam biru dan penggunaan garam asid organik untuk meningkatkan kegunaan tepung kacang soya oleh ketam tersebut. Garam asid organik kemudian diuji terhadap *Vibrio harveyi* untuk menentukan sama ada ia boleh menghalang bakteria patogen ini. Ketam diletakkan secara individu dalam bekas yang berasingan dan diberi makanan ujian selama empat minggu. Anak ketam diberi makan dengan enam diet ujian yang mengandungi tahap penggantian tepung ikan dengan tepung kacang soya yang berbeza (0, 20, 40, 60, 80 dan 100%). Ketam yang diberi makan diet 20% tepung kacang soya mempunyai prestasi terbaik untuk pertumbuhan, penggunaan makanan, komposisi proksimat badan, pengekalan nutrien, dan histopatologi hepatopankreas. Berdasarkan penemuan ini, percubaan pemberian makanan berikutnya dilakukan di mana natrium asetat ditambah di dalam diet untuk menentukan apakah ia dapat meningkatkan kegunaan 20% tepung kacang soya. Lima kepekatan natrium asetat yang berbeza (0, 1, 2, 3 dan 4%) telah diuji. Didapati bahawa 2% natrium asetat meningkatkan kemandirian prestasi pertumbuhan, penggunaan makanan, komposisi lipid dan protein tisu badan, dan struktur tubulus hepatopankreas. Kepekatan yang lebih rendah atau lebih tinggi daripada 2% mengurangkan prestasi pertumbuhan ketam. Tahap optimum 2% kemudian digunakan untuk eksperimen pemberian makanan seterusnya dengan enam jenis garam asid organik yang ditambah ke dalam diet, termasuk tanpa garam asid organik, natrium asetat, natrium butirat, natrium sitrat, natrium format dan natrium propionat. Kajian ini dirancang untuk menentukan jenis garam asid organik terbaik yang dapat meningkatkan kegunaan tepung kacang soya, yang dapat menghasilkan prestasi pertumbuhan anak ketam yang paling optimum. Ketam yang diberi natrium asetat mempunyai prestasi pertumbuhan terbaik berbanding dengan yang diberi garam asid organik lain, yang menunjukkan bahawa kepekatan optimum dan jenis garam asid organik yang betul memainkan peranan penting

dalam pertumbuhan spesies akuakultur. Sebanyak 30 anak ketam dari setiap rawatan kemudian digunakan dalam penyelidikan lanjutan untuk menguji apakah garam asid organik dapat menghalang *V. harveyi* pada kepekatan 10^7 CFU mL^{-1}. Kepekatan $maut_{50}$ terlebih dahulu dilakukan untuk menentukan kepekatan maut *V. harveyi* yang akan digunakan dalam ujian cabaran. Hasil kajian menunjukkan bahawa ketam yang diberi makan natrium asetat dapat menghalang bakteria patogen lebih baik daripada yang diberi rawatan lain, seperti yang dibuktikan oleh kelangsungan hidup yang lebih tinggi (83.33%), jumlah *Vibrio* yang lebih rendah di dalam hepatopankreas (5.18 ± 0.06 CFU mL^{-1}) dan air kultur (1.38 ± 0.01 CFU mL^{-1}), dan peningkatan struktur hepatopancreas dan insang setelah 7 hari cabaran bakteria. Kajian ini mendapati bahawa penggantian 20% tepung ikan dengan tepung kacang soya disyorkan untuk anak ketam biru dan 2% natrium asetat adalah kepekatan dan jenis garam asid organik yang terbaik untuk meningkatkan penggunaan tepung kacang soya oleh ketam. Potensi natrium asetat untuk menghalang *V. harveyi* telah dibuktikan.

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Verily with the hardship, there is relief.
Verily, with the hardship, there is relief.

Al-Insyirah: 5-6

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Degree of Doctor Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

TSB	tryptic soy broth
TSA	tryptic soy agar
TCBS	thiosulfate citrate bile salt sucrose
EDTA	ethylenediaminetetraacetic acid
ATP	adenosine triphosphate
µm	micrometer
µg	microgram
µl	microliter
ppt	parts per thousand
mm	millimeter
nm	nanometer
^{o}C	degree celcius
rpm	revolution per minute
s	second
hr	hour
spp.	species
SGR	specific growth rate
SBM	soybean meal
FM	fishmeal
NaCl	sodium chloride
KOH	potassium hydroxide
CFU mL^{-1}	colony forming unit per milimeter

CHAPTER 1

INTRODUCTION

1.1 Background of study

Aquaculture industry is important to reduce gap between supply and demand of food fish worldwide as well as to lessen the pressure on the capture fisheries. It is one of the fastest growing food production sector in the world due to the intensification of culture system and advanced in feed formulation. Its global production overtook the capture fisheries production since 2000 (Tacon and Metian, 2015). In 2019, the global aquaculture production was 120.1 million tonnes while global total capture fisheries production was 93.6 million tonnes (FAO, 2020).

Portunus pelagicus has the potential in becoming one of the alternative resources of crustaceans for human consumption. *P. pelagicus* also known as blue swimming crab is known to be the most commercially important crab species in the Indo-Pacific region (FAO, 2013). Ikhwanuddin *et al.* (2018) stated that this species could be a good entrants for both fisheries and aquaculture production. The world aquaculture production of this species is not consistent as there was an increase production from 2010 (25 tonnes) to 2011 (31 tonnes), decreased in 2012 (19 tonnes), increased in 2013 (27 tonnes) to 2014 (41 tonnes) and decreased again in 2015 (30 tonnes). Its production in 2019 was lower at about 23 tonnes (FAO, 2020). The inconsistency of aquaculture production for this species was due to low survival rate, with reduction in intermolt period and cannibalism (Azra *et al.*, 2019).

According to Bulbul *et al.* (2016), formulated feed cost that plays a crucial role for growth and development of aquatic species in aquaculture as it represents up to 60% of total farm production costs. The feed cost continues to rise owing to the increasing price and unsustainable fishmeal supply following the irregular landing of Peruvian fish for fishmeal production. Due to the escalating price and inconsistency of fishmeal supply, many studies have been conducted in order to find sustainable alternative protein sources that are readily available and cheaper than fishmeal.

With the fastest growing aquaculture industry, there will be a surge in demand of aquafeeds as the aquatic animals are now relying on the formulated diets (Hua *et al.*, 2019). Worldwide production of aquafeeds is expected to range between 41 and 58 million tonnes annually and by 2025 and it is expected to increase by 15-22 million tonnes annually (Tacon, 2020). According to Tacon *et al.* (2012), major protein ingredients used in commercial aquafeeds originated from fishmeal and soybean meal. The global market price for fishmeal and soybean meal are USD 1505.4 and USD 470.1 per metric ton, respectively. Soybean meal is the most abundant plant protein source used in aquafeed that comprise of 70% of seed meal production worldwide with a total production of 236 million metric tons per year (AAS, 2020).

The optimum protein required by mud crab, *Scylla paramamosain* juvenile in order to obtain maximum growth rate is 47.06% (Zheng *et al*., 2020). Meanwhile, the optimal dietary protein requirement for juvenile *Portunus trituberculatus* is 51.5% (Jin *et al*., 2013). Unnikrishnan and Paulraj (2010) reported that the optimal dietary protein requirement for juvenile *Scylla serrata* is 45%. However, the optimal dietary protein requirement for *Portunus pelagicus* has not been well documented and up to this date, there is limited formulated diet for *P. pelagicus* juveniles.

1.2 Problem statement

Soybean meal is deficient in methionine, lysine and threonine that become the limiting factor to be included in aquafeed at high level (Gatlin *et al.*, 2007). Besides, soybean meal also contains anti-nutritional factors (ANFs) like trypsin inhibitor, saponin, oligosaccharides, soy antigens and phytic acid that may impair the growth of aquaculture species if incorporated at high level in feed (Dersjant-li, 2002; Hardy, 2010). Replacement of fishmeal by soybean meal in crustaceans has been studied by many authors with only a successful partial replacement.

Even though intensification of culture system and advanced in feed formulation contribute to the rapid development of aquaculture, disease outbreak has become the limiting factor in aquaculture that causes a serious economic loss (Frans *et al.*, 2011). Pathogens can enter gastrointestinal tract of aquatic animals which is exposed continuously to opportunistic pathogens in the culture water (Salinas and Parra, 2015). Aquatic animals can be more vulnerable if they come into contact with the combination of pathogen invasion and the adverse effects of dietary soybean meal in aquafeeds (Zhang *et al.*, 2020). Thus, it is crucial to develop nutritional approaches in order to promote aquatic animal health and intestinal homeostatis.

Among the feed additives, organic acid salts are potentially able to act as dietary supplements in aquafeeds that can enhance nutrient utilization, growth and development as well as resistance to disease (Lim *et al.*, 2015). Zhu *et al.* (2015) stated that the chelating mechanism of citric acid may chelate the complex formation of minerals that increases the bioavailability of dietary minerals to aquaculture species. In fact, effects of citric acid on growth, immune response and utilization of minerals in aquaculture species have been demonstrated by many authors (Ng and Koh, 2011). Talpur *et al.* (2011) observed that a high mortality due to bacterial infection particularly *Vibrio harveyi* during larval culture of *P. pelagicus* which leads to a low to zero survival of the larvae. Meanwhile, dietary sodium propionate is able to enhance survivability of silver catfish, *Rhamdia quelen* when challenged with *Aeromonas hydrophilla* (Pereira *et al.*, 2018).

Use of live food in aquaculture hatcheries have become the major problem for expansion of the crab farming industry (Genodepa *et al.*, 2004). Jin *et al.* (2013) stated that use of live food can easily spread disease and deteriorate the water quality of culture system. Jiang *et al.* (2005) stated that replacement of live food with formulated diets for larvae and juvenile stage of non-penaied crustaceans species has only been conducted in laboratory condition. Hence, use of formulated feed in crab hatcheries are necessary to

improve the hatchery protocols. Up to this date, the development of formulated feed for *P. pelagicus* juvenile is still in its infancy. Therefore, it is significant to develop a formulated diet that is cost effective, produce better aquaculture production as well as environmental friendly.

1.3 Significant of study

Finding a cheaper and widely available alternative ingredient with good protein content and comparable amino acid profile is the aim by many researchers to replace fishmeal in aquafeeds. Soybean meal is one of the potential ingredients. However, the inclusion of soybean meal in high amount in aquafeeds will affect the growth of aquaculture species due to the anti-nutritional factors (ANFs) and deficiency in some amino acid profile present in the soybean meal (Shiu *et al.,* 2015).

Organic acid salts are potentially able to act as dietary supplements in aquafeeds that may help in enhancing soybean meal utilization by aquatic animals. However, different concentrations and types of dietary organic acids and their salts in aquafeeds and different aquaculture species give different results. Furthermore, dietary organic acid salts are able to reduce or inhibit pathogenic bacteria through its prophylactic ability.

The mass culture of blue swimming crab, *P. pelagicus* during larvae or juvenile stage is still under development phase (Ikhwanuddin *et al.,* 2012a). In fact, study on feed formulation for this species is still limited. Study concerning the essential nutrient requirements for this species through feed formulation offers a valuable information to influence the growth performance of this species without depending on the live feed.

1.4 Objectives of study

There is an urgent need to replace fishmeal either fully or partially with other alternative and renewable protein sources due to its expensive price and unsustainable fishmeal supply. Incorporation soybean meal in aquafeeds is an attempt to produce reasonable aquafeeds without impairing the growth and development of *P. pelagicus* juveniles. However, soybean meal has anti-nutritonal factors (ANFs) and limitation of some amino acid profile that may hold back the growth of the crabs and reduce nutrient utilization of the crabs. Hence, organic acid salts were used in the study to enhance the performance of soybean meal as organic acids and their salts are capable to improve digestion and minerals absorption in fish by chelating minerals from the phytic acid in soybean meal-based diet (Baruah *et al.,* 2007). The specific objectives of this research were:

1. To determine the feasibility of fishmeal replacement with soybean meal to blue swimming crab, *P. pelagicus* juveniles.
2. To elucidate the effect of different concentration of sodium acetate in improving soybean meal utilization on survival, growth performance, body proximate composition, nutrient retention and hepatopancreatic histopathology of blue swimming crab, *P. pelagicus* juveniles.

3. To investigate the effect of different types of organic acid salts (sodium acetate, sodium butyrate, sodium citrate, sodium formate and sodium propionate) on survival, growth performance, body proximate composition, nutrient retention and hepatopancreatic histopathology of blue swimming crab, *P. pelagicus* juveniles.
4. To examine the potential prophylactic of organic acid salts to blue swimming crab, *P. pelagicus* juveniles against pathogenic bacteria, *V. harveyi.*

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