



***ENCAPSULATION OF Lactobacillus plantarum ISO14 FOR SYNBIOTIC
TILAPIA FEED FORMULATION***

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TILAPIA FEED FORMULATION**

By

MUHAMAD AMIN BIN JAHARI

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

January 2019

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

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January 2019

Chairman : Shuhaimi Mustafa, PhD
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The increase cost of producing tilapia coupled with vigorous expansion of the industry pose a serious problem to the performance of the fish that lead to farmers loss. There is a need to develop feed additives incorporated in feed formulations to improve fish growth. The purpose of this study is to formulate a fish feed with synbiotic through encapsulation of *Lactobacillus plantarum* ISO14 with mannan to improve tilapia growth performance. The mannan was derived from palm kernel cake (PKC) and has the property of prebiotic. Since PKC are abundant and sustainable in Malaysia, its price is relatively low compared to imported ingredients and its supply for continuous fish feed production is promising. Encapsulation matrices optimization by response surface methodology (RSM) showed 1.66 % (w/v) of sodium alginate and 24.73 % (w/v) of mannan as coating materials would produce the highest survival of the probiotic. The verification experiment yielded a result close to the expected values, with no significant difference ($P > 0.05$) suggesting the model is adequate to be used as predictive model. The feed were formulated according to National Research Council (NRC) of standard tilapia nutritional requirement and added with encapsulated bacteria. Feeding trials in laboratory scale and industrial scale were conducted on growth performance of the tilapia. The fish were randomly assigned to two dietary treatments; control diet (without synbiotics) and synbiotic diet. Laboratory scale feeding demonstrated that synbiotic fed fish had a better growth rate with 12.6 ± 0.11 g fish $^{-1}$ of weight gain compared to control fed fish 9.7 ± 0.04 g fish $^{-1}$. Feed conversion ratio (FCR) was also significantly lower in the synbiotic fed fish, 1.53 gg $^{-1}$, compared to the control fed fish 1.85 gg $^{-1}$ suggesting a higher growth performance in synbiotic fed fish. Similar results were observed in the industrial scale feeding with synbiotic fed fish showed a better feed efficiency ($P < 0.05$) with the FCR of 2.12 gg $^{-1}$ compared to control fed fish 2.30 gg $^{-1}$. This study has contributes to a development of fish feed supplemented with synbiotics that able to increase tilapia growth performance in a sustainable way.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

**ENKAPSULASI *Lactobacillus plantarum* ISO14 UNTUK PENCPTAAN
MAKANAN IKAN TILAPIA YANG DILENGKAPI SINBIOTIK**

Oleh

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Peningkatan kos dalam menghasilkan tilapia ditambah dengan perkembangan pesat industri membawa masalah serius kepada tumbesaran ikan seterusnya menyebabkan kerugian kepada penternak. Terdapat keperluan untuk mencipta makanan tambahan yang dimasukkan dalam formulasi makanan bagi menambahbaik tumbesaran ikan. Tujuan kajian ini adalah untuk mencipta makanan ikan yang dilengkapi sinbiotik melalui enkapsulasi *Lactobacillus plantarum* ISO14 dengan mannan bagi meningkatkan prestasi ikan tilapia. Mannan diperolehi daripada kek isirung kelapa sawit (PKC) dan mempunyai sifat prebiotik. Memandangkan sumber PKC adalah banyak dan mampan di Malaysia, harganya adalah rendah berbanding bahan import dan bekalannya untuk pembangunan makanan ikan secara berterusan adalah menjanjikan. Pengoptimunan matriks enkapsulasi melalui metodologi permukaan tindak balas (RSM) menunjukkan 1.66 % (w/v) natrium alginate dan 24.73 % (w/v) mannan sebagai bahan salutan menghasilkan bilangan probiotik yang paling tinggi. Keputusan eksperimen pengesahan adalah hampir kepada nilai yang dijangka, dengan tiada perbezaan yang ketara ($P > 0.05$), menunjukkan model tersebut mencukupi untuk dijadikan sebagai model ramalan. Makanan ikan diformulasi berdasarkan nutrien asas keperluan tilapia Majlis Penyelidikan Kebangsaan (NRC) dan dicampur sinbiotik. Ujian haiwan pada skala makmal dan skala industri dijalankan ke atas tumbesaran ikan. Ikan dibahagikan secara rawak kepada dua kumpulan rawatan pemakanan; makanan kawalan (tanpa sinbiotik) dan makanan sinbiotik. Ujian haiwan skala makmal menunjukkan kumpulan sinbiotik mencatatkan prestasi pertumbuhan yang lebih bagus dengan 12.6 ± 0.11 g ikan $^{-1}$ pertambahan berat berbanding kumpulan kawalan yang merekodkan 9.7 ± 0.04 g ikan $^{-1}$. Nisbah penukaran makanan (FCR) juga rendah secara ketara dalam kumpulan sinbiotik, 1.53 gg $^{-1}$, berbanding kumpulan kawalan 1.85 gg $^{-1}$, mencadangkan prestasi pertumbuhan yang lebih baik bagi kumpulan sinbiotik. Keputusan yang sama diperhatikan di ujian haiwan skala industri dengan kumpulan sinbiotik menunjukkan penyerapan nutrien yang lebih efisien ($P < 0.05$) pertumbuhan prestasi ikan kumpulan dengan sinbiotik lebih baik ($P < 0.05$) dengan FCR 2.12 gg $^{-1}$ berbanding kumpulan kawalan 2.30 gg $^{-1}$. Kajian ini telah menyumbang kepada penciptaan makanan ikan yang dilengkapi sinbiotik yang mampu meningkatkan tumbesaran ikan tilapia dalam penternakan ikan yang mampan.

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

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LIST OF ABBREVIATIONS

AA	Amino acids
ANOVA	Analysis of variance
BD	Bulk density
BW	Body weight
CF	Crude fiber
CFU	Colony forming unit
Cm	Centimeter
CP	Crude protein
DOF	Department of Fisheries
ER	Expansion ratio
F	Floatability
FAO	Food and Agricultural Organization
FOS	Fructooligosaccharides
FI	Feed intake
FCR	Feed conversion ratio
G	Gram
h	Hour
IFFO	International Fishmeal and Fish Oil
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
<i>L. plantarum</i>	<i>Lactobacillus plantarum</i>
MJ/kg	Megajoules per kilogram
Mg	Milligram
Min	Minutes
ml	Millilitre
mM	Millimolar
MOS	Mannanoligosaccharide
NRC	National Research Council
°C	Degree celcius
PKC	Palm kernel cake
pH	Hydrogen ion concentration
Rpm	Revolutions per minute
RSM	Response surface methodology
SEM	Standard error of mean
SSF	Solid state fermentation
µg	Microgram
µl	Microlitre

CHAPTER 1

INTRODUCTION

Aquaculture is a multibillion dollar industry that continues to grow in a rapid pace due to increasing human consumption demand for protein sources as the world population continue to rise. As per stated by Food and Agricultural Organization of United Nations (FAO) (2016), by 2050, the world population is foreseen to intensify to higher than nine billion people as compared to seven billion people presently. To cater this overpopulation, aquaculture, whereby equipped with high end technology is shown to be a prominent and emerging field to meet the increasing demands of fish as food, commercial products or rehabilitate endangered species. As a potential solution for the crisis, Tilapia is probably one of the most significant fish species to satisfy the demand due to their growing popularity among consumers. Tilapia is regarded as second most cultured fish in the world with global production of more than 4.5 million tons and is anticipated to escalate to 7.3 million tons by 2030 (FAO, 2014; Wang & Lu, 2016). The tilapia production increased over the past decade due to marketability, price stability and ease of aquaculture (Wang & Lu, 2016). However, increasing cost of producing tilapia coupled with vigorous expansion of the industry limiting its economic profitability, and the fish growth should be improved to help farmers to reduce costs and increase profits. According to Huynh *et al.* (2017), major expenditure of modern tilapia farm were mainly from the production of feed, covering about 66% to 84% of the total farming cost. This emphasizes the significance of using high quality feed with high protein components containing basic nutrients and also additional additives in which indirectly will keep the animal healthy and promote their growth (El- Haroun *et al.*, 2006).

Usually, farmers utilized growth-promoting additives such as antibiotics to increase weight gain, reduce mortality and improve feed utilization (Shalaby *et al.*, 2006). Yet, their inappropriate use can result in detrimental consequence to the animal and consumer (Defoirdt *et al.*, 2011; Levy & Marshall, 2004). For instance, numbers of antibiotic resistance microorganisms have been detected in Malaysia such as multi-drug resistant *Salmonella* spp. found in tilapia (*Tilapia mossambica*) and catfish (*Clarias gariepinus*) acquired from ponds and wet markets in Malaysia (Budiaty *et al.*, 2013) and multidrug-resistant *Vibrio* spp. found in aquatic environments of west coast of Peninsular Malaysia (You *et al.*, 2016). The development of antibiotic resistance in microorganisms due to improper use of antibiotics has led to an intensive research conducted on natural, alternative growth promoters such as probiotics, prebiotics and synbiotics to use in fish feeds (Abid *et al.*, 2013; Ebrahimi *et al.*, 2012; Gatesoupe, 1999; Irianto & Austin, 2002; Jahari *et al.*, 2018; Mohanty *et al.*, 1993; Verschueren *et al.*, 2000).

Probiotic has been established for the first time in 1974 and is defined as ‘organisms and substances that support intestinal microbial balance’ (Parker, 1974). In an empirical way, probiotics composed of live microorganisms used as dietary additives in aquaculture and as a mean to promote health and growth of the host (Gatesoupe, 1999; Kesarcodi-Watson *et al.*, 2008). On the other hand, Gibson and Roberfroid (1995) proposed the term prebiotic which defined as ‘non-digestible food ingredients that selectively stimulating growth and/or activity of the microorganisms in the colon and give positive impact to the

host'. The synbiotics are combination of prebiotics and probiotics. Gibson and Roberfroid (1995) asserts the application of synbiotics concept may confer benefits of both pro- and prebiotics on fish growth largely because of the synergistic effect. The reason for their combined use is due to the prebiotic usefulness as substrate for fermentation to promote viability of the probiotic organisms, hence, would recompense host in a better manner (Collins & Gibson, 1999).

However, Biradar *et al.* (2005) has highlighted the condition for probiotic to be effective, the bacteria must possess some attributes such as resistance towards bile environment, gastrointestinal acidic, and easily reproduced. To address those challenges, bacteria must be encapsulated with protective substances as encapsulation matrix. According to Sohail *et al.* (2011), encapsulation not only acting as a shelter against harsh condition, but also can increase the viability and stability of alive probiotic culture and make it easy to mass produce. Most of the prior studies uses alginate and prebiotic such as aloe-vera, psyllium, and fenugreek as a coating materials (Guo *et al.*, 2008; Haghshenas *et al.*, 2015; Lotfipour *et al.*, 2012; Syahirah *et al.*, 2013), however, in this study we uses prebiotic mannan-oligosaccharides (MOS) derived from palm kernel cake (PKC) as it is cheaper and its availability throughout the year are guaranteed (Sundu *et al.*, 2006).

Currently, there are a lot of studies that were carried out in fish feed formulation to aid the tilapia production by manipulating the nutritional contents (El-Haroun, Goda, & Chowdhury, 2006; Essa *et al.*, 2010; Soltan & El-Laithy, 2008). However, the study regarding fish feed additive using synbiotic that has been produced from encapsulation of probiotic with prebiotic is still limited. Therefore, the general objective of the present study was to formulate a fish feed with synbiotic through encapsulation of probiotic *Lactobacillus plantarum* ISO14 with prebiotic mannan for improving tilapia growth performance. While, the specific objectives of this research were:

- i. To encapsulates *L. plantarum* ISO14 using mannan and sodium alginate as coating materials for synbiotic feed formulation;
- ii. To formulate synbiotic tilapia feed containing encapsulated bacteria;
- iii. To investigate effects of feeding synbiotic tilapia feed on the growth performance and feed efficiency in red hybrid tilapia.

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