FISH OIL REPLACEMENT WITH VEGETABLE OILS USING DIFFERENT SOURCES OF FISH MEAL IN PELLETED FEEDS FOR JUVENILE TIGER GROUPER, *Epinephelus fuscoguttatus*

NORFAZREENA BINTI MOHD FAUDZI

UMS
UNIVERSITI MALAYSIA SABAH

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BORNEO MARINE RESEARCH INSTITUTE
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JUDUL: FISH OIL REPLACEMENT WITH VEGETABLE OILS USING DIFFERENT SOURCES OF FISH MEAL IN PELLETED FEEDS FOR JUVENILE TIGER GROUPER, *Epinephelus fuscoguttatus*

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_____
Norfazreena Mohd Faudzi
PO2007-8717

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NAME:	**NORFAZREENA MOHD FAUDZI**
MATRIC. NO.:	**PO2007-8717**
TITLE:	**FISH OIL REPLACEMENT WITH VEGETABLE OILS USING DIFFERENT SOURCES OF FISH MEAL IN PELLETED FEEDS FOR JUVENILE TIGER GROUPER, *Epinephelus fuscoguttatus***
DEGREE:	**MASTER OF SCIENCE**
VIVA DATE:	**22 JANUARY 2013**

DECLARED BY

1. SUPERVISOR
Prof. Dr. Shigeharu Senoo **Signature**

2. CO-SUPERVISOR
Assoc. Prof. Dr. Rossita Shapawi

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Norfazreena Mohd Faudzi
16th April 2013

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ABSTRACT

FISH OIL REPLACEMENT WITH VEGETABLE OILS USING DIFFERENT SOURCES OF FISH MEAL IN PELLETED FEEDS FOR JUVENILE TIGER GROUPER, *EPINEPHELUS FUSCOGUTTATUS*

Tiger grouper, *Epinephelus fuscoguttatus*, is a high value marine fish, which belongs to Serranidae family. Rapid development of marine fish farming industry has increased the demand of this species. In order to reduce the dependence on wild fish stock and to develop cost–effective feed for grouper culture, three experiments were carried out to replace fish oil with vegetable oils using different sources of fish meal in the feeds of juvenile tiger grouper. Experiment 1 was conducted to screen the performance of different types of vegetable oils in the feeds for juvenile tiger grouper. Four experimental feeds (50% crude protein and 13% crude lipid; 57% replacement level of lipid) with different type of oil sources (Fish oil, FO; Canola oil, CNO; Refined, bleached, deodorized palm olein, RBDPO; Soybean oil, SBO) were formulated using local fish meal as a protein source. Triplicate group of twenty individual juvenile tiger grouper with mean body weight 42.8±0.6 g were stocked in 100 L fiberglass tanks and fed the experimental feeds at apparent satiation level twice a day for 6 weeks duration. The results showed that vegetable oils were able to replace fish oil without adverse effect on survival, growth and feed conversion ratio (FCR) of fish. Experiment 2 was conducted to further investigate the performance of vegetable oils in juvenile tiger grouper using laboratory-made fish meal as a protein source. Apart from growth performance and feed utilization, body proximate composition, hematological parameter and cost-benefit analysis were also determined in this experiment. Similar to the Experiment 1, 5 experimental feeds were formulated with 50% crude protein and 10% crude lipid at 50% replacement level. CNO, RBDPO and SBO treatments and a mixture of CNO, RBDPO and SBO with ratio 1:1:1 were tested. In general, the result showed an improvement on fish performance after changing the protein source. Survival rates in this experiment were above 86% in all dietary treatments. Weight gain (WG) was highest in CNO treatment followed by MIX, SBO, RBDPO and FO. No significant difference ($P>0.05$) was observed in FCR among the dietary treatments. Except for viscerosomatic index, body indices were independent of feeds. Protein and lipid contents of fish carcass were lowest in the FO treatment. Except for triglycerides content, no significant difference ($P>0.05$) was observed in the hematological parameter of FO and vegetable oil-based treatments. Feed cost calculation shows replacement of fish oil with vegetable oil is able to reduce the feed cost to culture juvenile tiger grouper. Experiment 3 was conducted to compare the performance of fish when fish oil was replaced with vegetable oils in the feeds based on high quality fish meal in combination of 15% defatted soybean meal. Even though the protein source was changed, vegetable oil–based feeds did not affect the performance of juvenile tiger grouper. In conclusion, at least 50% of fish oil can be replaced with vegetable oils in the feeds for juvenile tiger grouper regardless the source of fish meal. These findings are considered important towards reducing dependency of grouper culture industry on fish-based ingredients.

ABSTRAK

Kerapu harimau, Epinephelus fuscoguttatus, adalah ikan laut bernilai tinggi yang tergolong dalam Famili Serranidae. Pembangunan pesat dalam industri akuakultur telah meningkatkan permintaan spesies ini. Dalam usaha untuk mengurangkan kebergantungan terhadap stok ikan liar dan membangunkan diet ikan yang lebih kos efektif dalam penternakan kerapu harimau, tiga eksperimen telah dijalankan untuk menggantikan minyak ikan dengan minyak sayuran menggunakan sumber tepung ikan berbeza dalam diet kerapu harimau. Eksperimen 1 adalah saringan untuk mengenal pasti prestasi minyak sayuran dalam diet ikan untuk juvenil kerapu harimau. Empat diet ikan (50% protein dan 13% lipid; 57% tahap penggantian lipid) dengan jenis sumber minyak berbeza (minyak ikan, FO; minyak canola, CNO; minyak sawit yang ditapis, diluntur dan dinyahbaukan, RBDPO; minyak kacang soya, SBO) telah dihasilkan menggunakan tepung ikan tempatan sebagai sumber protein. Kumpulan replikat 20 individu juvenil kerapu harimau dengan purata berat badan 42.8±0.6 g dikultur dalam 100 L tangki gentian kaca dan diberi makan sehingga tahap kenyang dua kali sehari dalam tempoh masa 6 minggu. Hasil kajian menunjukkan bahawa minyak sayuran mampu untuk menggantikan minyak ikan tanpa kesan negatif kepada kemandirian, pertumbuhan dan nisbah penukaran diet (FCR) ikan. Eksperimen 2 telah dijalankan untuk mengkaji lebih lanjut prestasi minyak sayuran terhadap juvenil kerapu harimau dengan menggunakan tepung ikan buatan makmal sebagai sumber protein. Selain daripada prestasi pertumbuhan dan nisbah penggunaan diet, komposisi badan, parameter hematologi dan analisis kos faedah juga ditentukan dalam eksperimen ini. Seperti dalam eksperimen 1, 5 diet dihasilkan dengan kandungan protein 50% dan lipid 10% dengan tahap penggantian 50%. Rawatan CNO, RBDPO, SBO dan MIX yang merupakan gabungan CNO, RBDPO dan SBO dengan nisbah 1:1:1 telah diuji. Secara umum, keputusan menunjukkan peningkatan prestasi ikan selepas perubahan sumber protein. Kadar kemandirian dalam eksperimen ini adalah melebihi 86% dalam semua rawatan pemakanan. Penambahan berat badan adalah yang tertinggi dalam ikan rawatan CNO diikuti oleh MIX, SBO, RBDPO dan FO. Tiada perbezaan bererti (P>0.05) diperhatikan pada FCR di antara semua rawatan pemakanan. Kecuali bagi indeks visera, indeks badan tidak dipengaruhi oleh diet ikan. Kandungan protein dan lipid ikan yang diuji adalah terendah dalam rawatan FO. Kecuali untuk kandungan trigliserida, tiada perbezaan bererti (P>0.05) diperhatikan dalam parameter hematologi rawatan FO dan rawatan yang berasaskan minyak sayuran. Kajian ini dapat mengurangkan kos diet dalam penternakan kerapu harimau. Eksperimen 3 telah dijalankan untuk membandingkan prestasi pertumbuhan ikan apabila minyak ikan digantikan dengan minyak sayuran dalam diet ikan menggunakan tepung ikan yang berkualiti tinggi digabungkan dengan 15% tepung kacang soya yang diekstrak lemak. Walaupun sumber protein telah diubah, diet ikan berasaskan minyak sayuran tidak menjejaskan prestasi juvenil kerapu harimau. Kesimpulannya, sekurang-kurangnya 50% daripada minyak ikan boleh digantikan dengan minyak sayuran dalam diet juvenil kerapu harimau tanpa mengira sumber tepung ikan. Penemuan ini dianggap penting ke arah mengurangkan kebergantungan industri pengkulturan ikan kerapu kepada bahan-bahan berasaskan ikan.

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LIST OF ABBREVIATION

AA	arachidonic acid
ALA	alpha linolenic acid
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ATP	adenosine-5-triphosphate-oxidase
BMRI	Borneo Marine Research Institute
CE	cholesterol esterase
CF	condition factor
cm	centimeter
CMC	carboxyl methyl cellulose
CNO	canola oil
CO	cholesterol oxidase
CCO	coconut oil
CP	crude protein
CPAD	crude protein apparent digestibility
CPO	crude palm oil
CTO	cottonseed oil
DHA	docosahexanoic acid
DMAD	dry matter apparent digestibility
ECR	economic conversion ratio
EDTA	ethylenediaminetetraacetic acid
EFA	essential fatty acids
EPA	eicosapentaenoic acid
FAO	Food and Agriculture Organization
FBW	final body weight
FCR	feed conversion ratio
FID	flame ionization detector
FO	fish oil
g	gram
GK	glycerol kinase

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HCl	hydrochloric acid
HPO	horseradish peroxidase
hr	hour
HSI	hepatosomatic index
HUFA	highly unsaturated fatty acids
IBW	initial body weight
IFFO	International Fishmeal and Fish Oil Organization
IUCN	*International Union for Conservation of Nature*
kg	kilogram
L	liter
LA	linoleic acid
LPL	lipoprotein lipase
M	million
mg	milligram
min	minute
MIX	mixture of oils
mL	milliliter
mm	millimeter
mmol	millimoles
MPOB	Malaysia Palm Oil Board
MUFA	monounsaturated fatty acid
nm	nanometer
NRC	National Research Council
n-3	omega-3
n-6	omega-6
n-9	omega-9
OA	oleic acid
OO	olive oil
PCV	packed cell volume
PO	peanut oil
POD	peroxidase
PUFA	polyunsaturated fatty acids

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RBDPO	refined, bleached and deodorized palm olein
RGR	relative growth rate
RM	Ringgit Malaysia
rpm	revolution per minute
s	second
SBO	soybean oil
SE	standard error
SFA	saturated fatty acids
SFO	sunflower oil
SGR	specific growth rate
sp.	species
SPSS	Statistical Package of Social Sciences
t	tonnes
Tal	tallow
TP	total protein
UMS	Universiti Malaysia Sabah
USD	United States Dollar
VO	vegetable oil
VSI	viscerosomatic index

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LIST OF SYMBOLS

α	alpha
~	approximately
º C	degree centigrade
$	dollar
>	greater than
<	less than
%	percent
µ	micro

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APPENDIX

PAPER AND POSTER PRESENTED IN CONFERENCES/ SEMINARS

Paper presentations:

1. Norfazreena M. F., Shapawi R. and Senoo S., 2009. Effects of Fish Oil Replacement with Vegetable Oils in Pelleted Feeds for Juvenile Tiger Grouper, *Epinephelus fuscoguttatus.* Oral presentation presented at the "8th Annual Seminar on Science and Technology, "Science and Technology Advancement Towards 1 UMS", December 18 - 19, 2009, Kota Kinabalu, Sabah".

2. International Seminar on Marine Science and Aquaculture, "Sustainable Development and Management of Aquatic Resources in a Changing Climate", March 13 - 15, 2012, Kota Kinabalu, Sabah.

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Poster presentations:

1. Norfazreena M. F., Shapawi R. and Senoo S., 2010. Substitution of Fish Oil with Vegetable Oil – Based Support Good Growth and Survival of Tiger Grouper, *Epinephelus fuscoguttatus* under Flow – Through Culture Condition. Won silver medal for poster presented at the "Pertandingan Penyelidikan dan Rekacipta 2010, Universiti Malaysia Sabah, Kota Kinabalu".

2. Norfazreena M. F., Shapawi R. and Senoo S., 2010. Substitution of Fish Oil with Vegetable Oils in the Diets for Tiger Grouper, *Epinephelus fuscoguttatus.* Poster presented at the "9th International Annual Symposium on Sustainability Science and Management, "Towards a Healthier and Sustainable Future", May 8 - 11, 2010, Kuala Terengganu, Terengganu".

3. Won 1st prize for poster presented at the "9th Annual Seminar on Marine Science and Aquaculture, "Indicators for Sustainability of Fisheries and Aquaculture", March 10 - 12, 2010, Kota Kinabalu, Sabah".

4. 3rd International Symposium on Cage Aquaculture in Asia 2011, "Securing the Future", November 17-20, 2011, Kuala Lumpur, Malaysia.

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CHAPTER 1

GENERAL INTRODUCTION

Marine fish culture started in Malaysia since 1973 in Penang (Chuah and Teng, 1987) and since then, it has gradually developed throughout Peninsular and East Malaysia. Net cages are the most popular culture system due to the lack of information on culture techniques and a relatively cheaper option compared to land-based culture systems. Several important species such as sea bass (*Lates calcarifer*), snappers (*Lutjanus rivulatus* and *L. argentimaculatus*) and groupers (*Epinephelus fuscoguttatus, E. lanceolatus, E. coiodes, E. malabaricus* and *Cromileptes altivelis*) are cultured using net cages in the coastal area (FAO, 1991).

Due to the demand in the live fish trade for high commercial value fishes, grouper is one of the most sought after fish species by many marine fish farmers or entrepreneurs. Globally, Asia is the major producer of farmed grouper with Taiwan and Indonesia as the biggest producers (Rimmer, 2004). In Malaysia, the grouper production from ponds is 47.6 tonne (0.05%) from total ponds production which the wholesale value is RM 1,373,310. The grouper production from cages contributes about 4,521.63 tonne (18.59%), wholesale value of RM 188,320,930 in year 2010 (DoF, 2011). The grouper production has increased to 0.06% (ponds) and 24.68% (cages) with a total wholesale value of RM 250,962,930 in the year 2011 (DoF, 2012). Among other groupers, the tiger grouper is the most targeted species due to high demand in the market (Subramaniam, 1999). The price at seafood restaurants in Kota Kinabalu, Sabah can reach up to USD$ 20-26 per kilogram (kg).

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Figure 1.1: Tiger grouper, *Epinephelus fuscoguttatus*, 5 kg in body weight reared in the hatchery of Universiti Malaysia Sabah (UMS)

Feeds for marine fish especially during grow out culture are one of the biggest components in the operation cost (Craig, 2009). Most of the fish farmers depend on raw fish as feeding material. However, dependency on raw fish caused several disadvantages such as variable nutritional composition and poor feed conversion ratio (Ackefors and Enell, 1994; Cho *et al.*, 1994; Nijhof, 1994; Talbot and Hole, 1994; Leung, 1996). Thus, raw fish is not cost-effective to be used for feeding purposes. In addition, the availability of raw fish depends on the weather and season. Even though the price per kilogram is relatively cheaper than formulated feed, the price often fluctuates due to the availability (FAO, 2005). Besides that, the usage of raw fish for marine fish feeding during culture period can promote disease outbreak and increased competition between aquaculture industry and human especially in rural area where raw fish is most important source of protein. Inappropriate handling practices of raw fish also can cause spoilage which polluted the environment (Tacon *et al.*, 1995; Yashiro *et al.*, 1999; Ottolenghi *et al.*, 2004; Rachmansyah *et al.*, 2009).

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Due to the disadvantages of raw fish coupled with the limited supply of conventional feed ingredients, a development of cost-effective fish feed is critical. To date, several studies have been conducted on the nutrition and feeding aspects of grouper species (Laining *et al.*, 2004; Giri *et al.*, 2004; Rachmansyah *et al.*, 2009). These studies have demonstrated that tiger grouper fed pelleted feeds grew as well as those fed by raw fish. In general, grouper requires high protein and moderate lipid levels in the pelleted feeds (Chen and Tsai, 1994; Giri *et al.*, 2004).

Due to the rapid expansion on grouper culture, the fish farmers are encouraged to use pelleted feeds to sustain the development of this industry (Boonyaratpalin, 1997; Rimmer, 2004; Williams and Rimmer, 2005). Major ingredients used to formulate the pelleted feed are fish meal and fish oil (Pike, 2005). The development of the pelleted feed industry increased the demand of fish meal and fish oil rapidly. Barlow (2000) reported that the supply of major ingredients especially fish oil was limited because they are used not only for aquaculture industry but also for other industries and also as human consumption (Pike, 2005). The demand of fish oil is expected to exceed supply in the next few years.

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Even though fish oil is very important for fish to provide energy and essential fatty acids in fish, there is a concern on the sustainability of wild stock used for fish oil production (Milewski, 2002; New, 2002; Staniford, 2002; Allan, 2004). Besides, the price of fish oil is more expensive compared to alternative oils such as vegetable oils (Tacon *et al.*, 2006). Production of vegetable oil has steadily increased in recent years, reaching a volume of 100 times more than fish oil (Bimbo, 1990). Therefore, replacement of fish oil with vegetable oils appears to be a viable option considering their availability, competitive price and absence of dioxins and pollutants (Cabalerro *et al.,* 2002; Izquierdo *et al.,* 2003).