UNIVERSITI PUTRA MALAYSIA

UTILIZATION OF CAROB SEED GERM MEAL IN TILAPIA DIET

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By

ABDALBAST H. I. FADEL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to the spirit of my beloved father who taught me nobility and honesty, my affectionate mother who taught me patience and strength, my dear wife who has been my support from the beginning, and to all my dear sons, daughters, brothers and sisters.

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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Chairman : Professor Mohd Salleh Kamarudin, PhD
Faculty : Agriculture

The cost of commercial feeds for farmed fish continues to soar leading to the need to explore new alternative ingredients that can economically meet the metabolic needs of cultured tilapia. In this study, a series of experiments was conducted to investigate the nutritional value and the optimum inclusion level of carob seed germ meal (CSGM) as a substitute for soybean meal (SBM) in red tilapia feed.

In Experiment I, five isonitrogenous diets with increasing inclusion levels of untreated CSGM from 0-40% were tested. An inclusion of up to 30% untreated CSGM did not affect the growth and survival of red tilapia. However, the body biochemical composition and histopathological parameters indicated that fish health was compromised. Therefore, a maximum dietary inclusion of 20% untreated CSGM was recommended as a replacement of SBM for red tilapia.

In Experiment II, the efficiency of roasting, soaking, and autoclaving in reducing anti-nutritional factors (ANFs) in CSGM was evaluated. ANFs and functional properties of CSGM significantly decreased after all pre-treatment processes particularly the 30-min autoclaving. However, soaking appeared to be the most optimum and straight forward solution for decreasing ANFs while simultaneously maintaining nutrient value and functionality of CSGM.

Six experimental diets were formulated in Experiment III to contain 30 and 40% soaked CSGM, and 30 and 40% autoclaved CSGM with 0% and 40% untreated CSGM as negative and positive controls, respectively. Soaked or autoclaved CSGM at the 30% dietary inclusion level led to enhanced performance of CSGM in red tilapia

compared untreated CSGM although not to the level of performance obtained with the CSGM-free diet.

In Experiment IV, apparent digestibility coefficients (ADC) of untreated, soaked and autoclaved CSGM were determined. ADCs of nutrients and energy in untreated CSGM were significantly lower than those of soaked and autoclaved CSGM. Soaked CSGM had substantially better ADCs of nutrients and energy than autoclaved CSGM.

Experiment V was aimed to increase the digestibility of CGSM by *in vitro* and *in vivo* application of β-mannanase and α-galactosidase mixture. *In vitro* results demonstrated that smaller particle size of CSGM yielded a higher mannose at 0.1% of enzyme hydrolysis with lower fibre content than other treatments. Whereas *in vivo* results showed that the growth performance and feed utilization of fish fed enzyme pre-treated CGSM were not significantly different from those of fish fed CGSM-free diet. Fish fed a diet without enzyme pre-treated CSGM had significantly higher intestinal short chain fatty acids (SCFA) whereas body protein and lipid, muscle cholesterol, and plasma cholesterol and glucose were significantly lower than those in fish fed other pre-pretreated CSGM.

In conclusion, untreated CSGM could be used as a partial substitute of soybean meal in the red tilapia diet up to 20% dietary inclusion. Pre-treating CSGM by soaking for 24h and followed by a carbohydrase treatment significantly improved its nutritional quality and increased its optimal inclusion level to 30% of diet.

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

PENGGUNAAN TEPUNG GERMA BIJI KAROB DALAM MAKANAN TILAPIA

Oleh

ABDALBAST H. I. FADEL

Januari 2018

Pengerusi : Profesor Mohd Salleh Kamarudin, PhD
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Kos makanan komersil untuk ikan ternakan terus meningkat menyebabkan perlunya dilakukan pencarian bahan makanan alternatif baharu yang secara ekonomik dapat memenuhi keperluan metabolik tilapia ternakan. Dalam kajian ini, satu siri eksperimen telah dijalankan untuk menentukan nilai pemakanan dan paras optima penggunaan tepung germa biji karob (CSGM) sebagai pengganti tepung kacang soya (SBM) dalam makanan tilapia merah.

Dalam Eksperimen I, lima diet isonitrogen dengan peningkatan paras penggunaan CSGM dari 0-40% telah diuji. Penggunaan sehingga 30% CSGM yang tidak dirawat tidak menjejaskan pertumbuhan dan kemandirian tilapia merah. Walau bagaimanapun, komposisi biokimia badan dan parameter histopatologi menunjukkan bahawa kesihatan ikan telah dikompromi. Oleh itu, penggunaan maksimum CSGM yang tidak dirawat sebanyak 20% dicadangkan sebagai pengganti SBM untuk tilapia merah.

Dalam Eksperimen II, kecekapan pra-rawatan panggang, perendaman dan autoklaf dalam mengurangkan faktor anti-pemakanan (ANFs) dalam CSGM telah dinilai. ANFs dan sifat fungsian CSGM berkurangan dengan ketara selepas kesemua proses pra-rawatan terutamanya melalui autoklaf selama 30 min. Walau bagaimanapun, rendaman didapati merupakan penyelesaian yang paling optimum dan termudah untuk menurunkan ANFs dan pada masa yang sama mengekalkan nilai nutrien dan kefungsian CSGM.

Enam diet ujian telah dirumuskan dalam Eksperimen III untuk mengandungi 30 dan 40% CSGM pasca-rendam, dan 30 dan 40% CSGM pasca-autoklaf dengan diet 0 dan 40% CSGM yang tidak dirawat, masing-masing, sebagai kawalan negatif dan positif. CSGM pasca-rendam atau pasca-autoklaf pada paras penggunaan 30% menyebabkan peningkatan prestasi CSGM dalam tilapia merah berbanding CSGM yang tidak dirawat walaupun tidak mencapai tahap prestasi yang diperolehi dengan diet bebas-CSGM.

Dalam Eksperimen IV, pekali kebolehcernaan ketara (ADC) CSGM yang tidak dirawat, pasca-rendam dan pasca-autoklaf telah ditentukan. ADC nutrien dan tenaga dalam CSGM yang tidak dirawat adalah ketara lebih rendah daripada CSGM pasca-rendam dan pasca-autoklaf. Walau bagaimanapun, CSGM pasca-rendam mempunyai nilai ADC nutrien dan tenaga yang ketara lebih baik daripada CSGM pasca-autoklaf.

Eksperimen V bertujuan untuk meningkatkan kebolehcernaan CSGM menerusi penggunaan campuran β-mannanase dan α-galactosidase secara *in vitro* dan *in vivo*. Keputusan rawatan *in vitro* menunjukkan bahawa saiz partikel CSGM yang kecil menghasilkan mannose yang lebih tinggi pada 0.1% enzim hidrolisis dengan kandungan serat yang lebih rendah daripada rawatan lain. Sementara itu, keputusan *in vivo* menunjukkan prestasi pertumbuhan dan kecekapan pemakanan adalah tidak ketara berbeda antara ikan yang diberi makan CGSM terawat enzim dengan ikan yang diberi makan bebas-CSGM). Ikan yang diberi makan diet CGSM tanpa rawatan enzim mempunyai asid lemak rantaian pendek (SCFA) yang ketara lebih tinggi di samping kandungan protein dan lipid badan, kolesterol otot, serta kolesterol dan glukosa plasma yang ketara lebih rendah dari ikan yang diberi diet CGSM terawat lain.

Kesimpulannya, CSGM tanpa rawatan boleh digunakan sebagai pengganti tepung kacang soya dalam diet tilapia merah sehingga 20% diet. Perendaman selama 24 jam dan diikuti rawatan enzyme karbohidrase ketara meningkatkan mutu pemakanan CSGM dan paras penggunaan optimumnya kepada 30% diet.

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I certify that a Thesis Examination Committee has met on 22 January 2018 to conduct the final examination of Abdalbast.H.I.Fadel on his thesis entitled "Utilization of Carob Seed Germ Meal in Tilapia Diet" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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

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LIST OF ABBREVIATIONS

ANFs	Anti-nutritional factors
ANOVA	Analysis of Variance
CF	Condition factor
CSGM	Carob seed germ meal
FM	Fishmeal
DFI	Daily Feed Intake
DO	Dissolved Oxygen
EAA	Essential Amino Acid
FA	Fatty Acid
FC	Foaming Capacity
FCR	Feed Conversion Ratio
FS	Foam Stability
HSI	Hepato-somatic Index
MUFA	Monounsaturated Fatty Acid
n-3	Omega 3
n-6	Omega 6
PER	Protein Efficiency Ratio
PUFA	Polyunsaturated Fatty Acid
OAC	Oil Absorption Capacity
SBM	Soybean meal
SD	Standard Deviation
SCFAs	Intestinal Short-Chain Fatty Acids
SFA	Saturated Fatty Acids
VSI	Viscera-somatic Index
WAC	Water Absorption Capacity
WG	Weight Gain

CHAPTER 1

INTRODUCTION

The global total consumption of seafood per capita has increased from an average of 5.2 kg in 1961 to 20 kg in 2014 varying according to region and type of fish (FAO, 2016b; Tidwell, 2012). In 2014, the total fisheries and aquaculture production reached 160 million tonnes of which 146.3 million tonnes was for human consumption (FAO, 2016b). The Food and Agriculture Organisation (FAO) predicted that the global production of the fisheries and aquaculture sector would reach 172 million tonnes in 2021, primarily because of higher demand for fish and continuous growth of human population (FAO, 2012). Further expansion of capture fisheries is unlikely in the future as most fishing grounds have been subjected to over-fishing and improper stock management. Therefore, aquaculture must play a more important and dominant role in the future seafood production and its production need to be increased by 33% between 2012 and 2021 to meet the demand (FAO, 2012).

The global aquaculture production has increased by 80% in the last two decades (Fig. 1.1, (FAO, 2016b). Asia has accounted for about 88.91% (6.56 million tonnes) of the world aquaculture production in 2014. America, Europe and Africa improved their respective shares by 4.54% (3.35 million tonnes), 3.97% (2.93 million tonnes) and 2.32% (1.71 million tonnes), respectively. In comparison, the total production of Oceania only improved by only 0.26% (189.2 tonnes). Aquaculture is now an important part of the economy in several countries creating many jobs. An estimated 16.7 million people were employed in aquaculture in 2014 (FAO, 2016b).

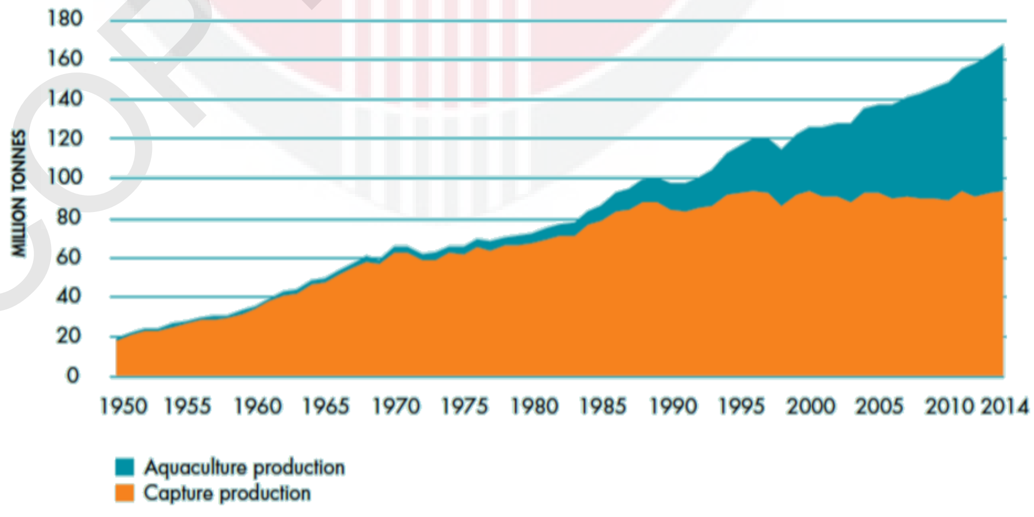

Figure 1.1 : World capture fisheries and aquaculture production
(Source: (FAO, 2016b)

Due to the increasing demand for farmed fish, the industry must produce large quantities of aquafeeds from readily accessible/available ingredients (Naylor et al., 2000). The major bulk of commercial aquafeeds, especially protein, originates from fishmeal and soybean meal (Tacon et al., 2012). It has been estimated that the aquaculture feed sector utilises over 73% of total global FM production or nearly 21% of total fish production (FAO, 2014). Given the current very rapid increase in the intensification of fish farming in Asia and globally, intense competition for limited global supplies of fishmeal and soybean meal is likely to arise in the near future. This strong demand for available feed resources is expected to have considerable impact on global commodity markets and fish feed prices. (FAO, 2016b).

Consequently, aquafeeds have become costly (Tacon et al., 2012). Aquafeeds for both carnivorous and omnivorous fish species contain fishmeal which has been the main source of protein in the past (Hardy, 2010). In general, 45% of the fishmeal is used in aquafeeds for carnivorous fish species such as salmon, trout, sea bass, sea bream, and yellowtail. Meanwhile, 21% of the fishmeal is used in aquafeeds for omnivorous species such as fry and fingerling carps, tilapia and catfish (Hardy, 2010). Due to the escalating price and lack of stability in the supply of fishmeal, many studies have been conducted to replace fishmeal with less expensive plant and animal protein sources.

Soybean is the second most important source of protein used by the aquafeed industry (El-Sayed, 2007). Soybean meal appears to be an excellent substitute of fishmeal and is being increasingly used in aquafeeds (Nguyen, 2008). However, the heavy dependence on soybean meal in aquafeeds has also increased its price (Akinleye et al., 2012). According to FAO (2016b), the price of soybean meal per tonne rose from US$343 in March 2004 to $506.9 in March 2014, representing an increase of 47%. In addition, soybeans are also used in the production of human foods. Hence, there is an urgent need to explore alternative inexpensive plant protein sources for use in fish diets. Current and future research need to be focused on introducing technologies for producing practical, cheap and readily available new protein sources (Nunes et al., 2014; Tiamiyu et al., 2016).

Carob seed germ meal is an example of a cheap dietary food source that does not compete with human needs. It is the by-product of the food grade gum industry and comparatively cheaper to fishmeal or soybean meal. The potential of carob seed germ meal as an alternative protein source in aquafeeds has attracted the intention of several researchers (Alexis, 1990; Couto et al., 2016; Martínez-Llorens et al., 2012).

1.1 Objectives of the study

The general objective of this study was to evaluate the nutritional value of carob seed germ meal as an alternative source of dietary protein for red tilapia. The specific objectives of this study were:

1. To investigate potential of carob seed germ meal as a partial soybean meal replacement in the diet of red hybrid tilapia
2. To evaluate the effects of several pre-treatment methods on the nutritional components, anti-nutritional components and functionality of carob seed germ meal
3. To determine the performance of pretreated carob seed germ meal as an alternative plant protein in the red tilapia diet
4. To study the effects of pretreatment on the digestibility of carob seed germ meal and plasma glucose, cholesterol and amylase in red tilapia, and
5. To assess the effects of multi-enzyme pre-treatment of carob seed germ meal and delivery method on growth and feed utilisation in red tilapia.

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