Journal of Sustainability Science and Management
Volume 16 Number 8, December 2021: 28-43
eISSN: 2672-7226
© Penerbit UMT

FEED VALUE OF FERMENTED COPRA MEAL AS A SUSTAINABLE FEED INGREDIENT IN THE DIET OF SALINE-TOLERANT NILE TILAPIA *OREOCHROMIS NILOTICUS* (LINNAEUS 1758)

INTOY, MODESTO MELDEN B.[1,2] AND TRAIFALGAR, REX FERDINAND M[1]*

[1]*Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines.* [2]*College of Fisheries and Marine Sciences, Aklan State University, New Washington, Aklan, Philippines.*

**Corresponding author: rmtraifalgar@up.edu.ph*
Submitted final draft: 8 February 2021 *Accepted: 18 February 2021* http://doi.org/10.46754/jssm.2021.12.003

Abstract: Tilapia cultures in seawater environment are currently experiencing an expansion, but their sustainability is primarily dependent on feed costs. Fermented copra meal is a potential source of sustainable feed ingredient, but its use in aquaculture feeds has not been fully investigated. This study evaluated the feed value of fermented copra meal (FCM) as a replacement of soybean meal (SBM) in the diet of juvenile Nile tilapia reared in seawater conditions. Four experimental diets containing graded levels of FCM were formulated replacing SBM at 0%, 50%, 75%, and 100%. Juvenile tilapia (size: 0.77 ± 0.04g) were divided into four treatment groups, ran in triplicates, with 10 fish per tank, and fed the experimental diets for 50 days. The results indicate that 75% of SBM could be replaced with FCM or at a dietary inclusion level of 285g of FCM per 1kg of diet. This high dietary inclusion level has no negative effect on nutrient retention, liver metabolic enzymes, digestive enzyme activities, and intestinal morphology of the studied fish. The use of FCM as a feed ingredient is a sustainable approach to satisfy the feed requirements of the expansion of tilapia aquaculture in seawater environment.

Keywords: Tilapia, fermented feed, copra meal, plant protein, saline-tolerant.

Introduction

The Nile tilapia (*Oreochromis niloticus*) is widely cultivated and an economically important fish species in the Philippines (Guerrero, 2019). However, the expansion and sustainability of Nile tilapia aquacultures are hindered by the limited freshwater culture area, rising costs of feeds, and erratic supply of feed ingredients (Guerrero, 2019). In the last decade, the progress in selective breeding has resulted in the development of tilapia strains with better growth performance, market acceptability, and adaptability to brackish and seawater culture systems (De Verdal *et al.,* 2014; El-Dakar *et al.,* 2015; Guerrero, 2019). The development of saline-tolerant Nile tilapia strains for cultures in seawater environment is a significant progress that offsets the problem of limited culture areas for the expansion of tilapia aquacultures (Jumah *et al*., 2016). It is projected that as tilapia aquacultures in coastal and marine ecosystem expand, the rising needs and increasing costs of feed ingredients would be the limiting factor in the growth and sustainability of the industry (Khan *et al.,* 2013; Ngugi *et al.,* 2017; Younis *et al.,* 2018).

With the current global decline and increase in the prices of fish meal, soybean meal (SBM) has become the primary source of feed-protein for formulated fish feed (Kokou *et al.,* 2016). However, soybean utilisation in aquaculture feed is limited by the high price and erratic supply due to competition with the livestock and poultry industries, human consumption and high importation costs (Plaipetch & Yakupitiyage, 2013; Boer *et al.,* 2014; Pach & Nagel, 2017).

The utilisation of fermented copra meal (FCM) has been proposed as a cheap alternative to the use of SBM in aquaculture feed. Copra meal is abundant in the Philippines as a by-product of the coconut oil production industry. In addition, fermentation of copra meal has been reported to improve its feed value through the reduction of insoluble fibres, partial hydrolysis of complex carbohydrates, and significant increase of soluble proteins and peptides (Pham *et al.,* 2017).

Although information regarding the use of fermented ingredients in diets of cultured aquatic animals has been scarce, early reports on the use of FCM as an alternative protein source in feed showed encouraging results. A 21% dietary inclusion of FCM was found to support normal growth of cultured black tiger shrimp (*Penaeus monodon*) (Apines-Amar *et al.,* 2016). Also, Laining *et al.* (2017) showed that FCM dietary inclusion of 13% was found to promote optimum growth performance of cultured rabbitfish (*Siganus guttatus*). In groupers (*Epinephelus fuscoguttatus*) FCM could be included in the diet up to 16% without affecting carcass composition, hepatic functionality and intestinal morphology (Mamaug *et al.,* 2019).

These earlier works suggest that the optimum dietary inclusion level of FCM may vary depending on the species being cultured. To date, information on the use of fermented feed ingredients in tilapia diets are few. Moreover, aspects regarding the nutrient utilisation and physiological responses of saline-tolerant tilapia when fed diets containing fermented feed ingredients have not been fully evaluated. The present study aims to evaluate the optimum replacement of SBM with FCM on growth performance, nutrient utilisation, carcass composition, liver metabolic enzymes, and intestinal morphology of juvenile Nile tilapia reared in seawater.

Materials and Methods

Experimental Diets

Four experimental diets were formulated with increasing inclusion levels of FCM as a replacement of SBM by weight, at 0% (Control), 50% (Trt50), 75% (Trt75), and 100% (Table 1). The FCM used in thisstudy is a product registered as PECM®, commercialised by the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, the Philippines. This feed ingredient is produced through the solid-state fermentation of copra cake with the fungi *Aspergillus niger*. The fermentation process enhances the degradation of the cellulosic components of the copra meal and increases the build-up of high-protein fungal biomass. Details of the fermentation process are described in the works of Hatta and Sundu (2009) and Pham *et al.* (2017). The experimental diets were prepared by mixing the dry ingredients separately before the addition of the feed oil and water. The resulting dough was cold-pelleted with a laboratory pelletiser and oven-dried at 60^{o}C until the moisture content of 10% - 12% is attained. The dried feed was then stored in a polyethylene bag at -20^{o}C until use. Prior to feeding, the feed was ground and sieved to the appropriate sizes. At the start of feeding, the experimental animals were fed diets with a particle size of 0.8 mm. When the experimental animals reached the size of 3g, the feed particle size was increased to 1.3 mm. Finally, when the animals reached 10g in size, they were fed diets with a particle size of 1.7 mm until the termination of the experiment. The proximate composition of the experimental diets is presented in Table 1.

Feeding Trial

The study was conducted at the Aquaculture Research Facility Complex of the university. The RRR strain of juvenile saline-tolerant Nile tilapia (*O. niloticus*) were collected from the production tanks, acclimatised to laboratory conditions, and fed a commercial diet (35% crude protein) for two weeks. The experimental fish (mean size: 0.77 ± 0.04) were then randomly allotted to 12 culture tanks with a capacity of 40 L, with 10 fish per tank. The experimental fish were reared in a flow-through seawater system with a flow rate of 50 ml/min. Feed was given to apparent satiation, twice daily at six a.m. and five p.m. for 50 days. Biomass sampling was conducted every 10 days. Adequate aeration was provided and water quality was maintained at the optimum range (water temperature: 25-28°C; dissolved oxygen: 5-8 mg/L; pH: 7.08-8.25; salinity: 31-34g/L).

Table 1: Feed formulation and proximate composition of the experimental diets with fermented copra meal (FCM) as soybean meal (SBM) replacement (g 100g $^{-1}$)

Ingredients	Control	Trt50	Trt75	Trt100
Fermented copra meal[1]	0	19.5	28.5	38
Soybean meal[2]	38	19.5	9.5	0
Defatted rice bran	14	14	14	14
Fish meal	13	13	13	13
Acetes meal	10	10	10	10
Bread flour	10	10	10	10
Soybean oil	6	6	6	6
Gluten	5	5	5	5
Trace mineral premix[3]	2	2	2	2
Vitamin premix[4]	2	2	2	2
Proximate composition (% dry matter)				
Crude protein	44.64	43.76	43.01	42.00
Crude lipid	9.51	9.44	9.50	9.76
Ash	9.62	9.65	9.56	9.82
Crude fibre	3.26	5.08	5.91	6.91

[1]Composition (% dry matter): protein, 44.40; lipid 3.9, fibre, 13.3, ash, 8.8, NFE, 29.60
[2]Composition (% dry matter): protein, 44.2; lipid 4.5, fibre, 9.6, ash, 8.9, NFE, 32.8
[3]Trace mineral premix (mg/kg diet): iron (800), manganese (200), zinc (800), copper (80), iodine (36), cobalt (0.4 mg), and selenium (4). All chemicals used were obtained from Merck-Sigma Aldrich Inc., Darmstadt, Germany.
[4]Vitamin premix (mg/kg Diet): Vitamin A (7.2), D3 (2.68), E (16), B1 (160), B2 (160), B6 (100), B12 (40), niacin (800), calcium pantothenate (400), biotin (0.8), folic acid (36), and ethoxyquin (10). All chemicals used were obtained from Merck-Sigma Aldrich Inc., Darmstadt, Germany.

A day following the termination of the experiment (51st day), the juvenile tilapia were collected and weighed. Fish from each treatment were immediately processed for the collection of samples for blood biochemistry and histological analyses. Blood biochemistry analyses were conducted immediately after the samples were collected. The intestines and intraperitoneal fat tissues were collected, fixed, and stored in preparation for the histological analysis. On the same day, fish samples to be used for the digestive enzyme assays and the carcass composition analyses were also collected and stored at -80°C until analysis. Assessments of growth performance, biological and somatic indices in response to the experimental diets were calculated as follows:

$$Percent\ Survival\ (S\ \%) = \left(\frac{final\ number\ of\ fish}{initial\ number\ of\ fish}\right) \times 100$$

$$Percent\ Weight\ Gain\ (WG\ \%) = \left(\frac{final\ weight\ (g) - initial\ weight\ (g)}{initial\ weight\ (g)}\right) \times 100$$

$$Specific\ Growth\ Rate\ (SGR) = \left(\frac{ln\ (weight\ gain\ (g))}{culture\ period\ (days)}\right) \times 100$$

$$Feed\ Conversion\ Ratio\ (FCR) = \left(\frac{total\ feed\ intake(g))}{weight\ gain\ (g)}\right) \times 100$$

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{weight\ gain\ (g)}{total\ protein\ intake\ (g)}$$

$$Protein\ Retention\ (PR) = \left(\frac{final\ body\ protein\ (g) - initial\ body\ protein\ (g)}{total\ protein\ intaken\ (g)}\right) \times 100$$

$$Lipid\ Retention\ (LR) = \left(\frac{final\ body\ lipid\ (g) - initial\ body\ lipid\ (g)}{total\ lipid\ intake\ (g)}\right) \times 100$$

$$Intraperitoneal\ Fat\ Ratio\ (IPF\ \%) = \left(\frac{intraperitoneal\ fat(g))}{whole\ fish\ weight\ \ (g)}\right) \times 100$$

Biochemical Analysis

The proximate composition analyses of the experimental diets and the animal carcass (initial and final carcass) were conducted to evaluate the influence of the dietary treatments on the carcass composition. Crude protein was determined using the Kjeldahl total protein nitrogen analysis (Foss Tecator™ Digestion and Foss Kjeltec™ 8200 Auto Distillation), while crude lipid was quantified following the method of Bligh and Dyer (1959) using the Foss Soxtec™ 2050 Automatic System. Crude fibre was determined using the ceramic fibre filter method (AOAC, 1996). Ash content was determined gravimetrically by combusting the sample in a muffle furnace at 600°C and dry matter content was measured using the Mettler Toledo Halogen Moisture Analyser (AOAC, 1996). Triplicate runs per sample were conducted in all analyses.

Gut tissue samples were collected from six fish from each treatment group and were prepared for the enzyme assays following the method of Lojda *et al.* (1979) and Pan *et al.* (2015). Enzyme assays were conducted in triplicate per enzyme and tissue sample. The collected gut tissue samples were weighed and homogenised in 50 mM citrate phosphate buffer (pH 7.0) for the amylase and potassium phosphate buffer for the protease and lipase assays. The homogenates were centrifuged at 4000 rpm for 15 minutes; the supernatants were collected and used for the analyses. Protein determination followed the method by Bradford (1976) using bovine serum albumin as the standard. The total protease was assayed following the method described by Buroker-Kilgore and Wang (1993) using casein as the substrate. The α-Amylase activity assay was based on the method by Bernfield (1951) using the buffered starch solution as a substrate. The lipase activity assay followed the copper-soap method by Lowry and Tinsley (1976), using undecanoic acid as the standard. Enzyme activities are expressed as units/mg protein.

Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were determined using an ILab 300 Plus System. In this analysis, six fish were also used from each treatment group. Blood samples were collected from the caudal vein of individual fish using a 1 ml syringe and were processed to obtain the serum. The serum was analysed using the ILab 300 Plus blood analysis system. Triplicate analysis runs were done for each fish from each treatment group.

Intestinal Morphology Assessment

To evaluate the influence of the dietary treatments on the intestinal and intraperitoneal fat tissues, samples were collected from six fish from each experimental treatment group. Collected tissues were processed and fixed immediately after collection for histological examination. The samples were fixed in Bouin's solution, dehydrated with isopropanol, cleared in xylene,

infiltrated in paraffin, sectioned to a thickness of 5 µm, mounted on a glass slide, and stained with haematoxylin and eosin. Histological images of the tissues were generated using Moticam® (Motic®, Hong Kong) connected to a compound microscope (Motic®, Hong Kong). The images were processed and analysed using Motic Images Plus 2.0 (Motic®, Hong Kong). Measurements and analyses of the histological images were done using Image J 1.52i (National Institute of Health, USA) as described by Tuller *et al*. (2012) and Abdel-Moneim *et al*. (2012).

Statistical Analysis

All data gathered were subjected to one-way analysis of variance (ANOVA). Tukey's post-hoc test was used to resolve the differences among the experimental groups. Probability values are set at a significance level of 0.05. Statistical analysis was performed using the SPSS statistical package for Windows version 18.

Results

Survival, Growth Performance, Feed, and Nutrient Utilisation

The survival, growth performance, feed efficiency, and nutrient retention of juvenile saline-tolerant Nile tilapia fed diets containing FCM as replacement of SBM are shown in Table 2. Survival was high in all the treatments and the increasing replacement of SBM with FCM had no negative effects on the survival of the experimental fish. The growth performance of the fish was found to be influenced by the dietary treatments. Weight Gain (WG) was observed to increase as the FCM inclusion level increases, exhibiting the highest value at Trt75 and declined at Trt100 (Figure 1). However, the WG value at Trt75 is not different from Trt50 and the control. The final weight of the experimental fish exhibited a similar trend with that of the WG value. In terms of specific growth rate (SGR), Trt50, Trt75, and control treatment exhibited similar growth rates. Trt100 exhibited the lowest WG and SGR compared with other treatments.

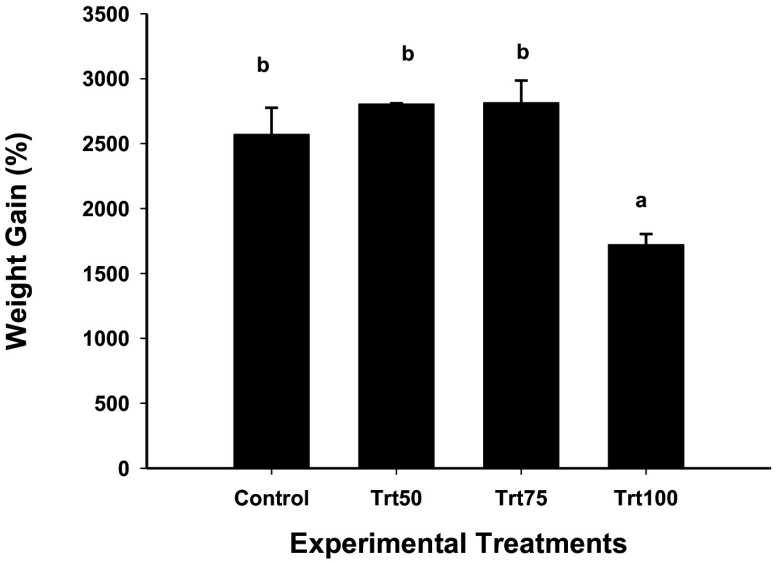

Figure 1: Weight gain of juvenile saline-tolerant Nile tilapia fed diets containing increasing levels of fermented copra meal as a replacement of soybean meal. Values bearing similar superscripts are not significantly different (α= 0.05)

The feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), and lipid retention (LR) of the juvenile saline-tolerant Nile tilapia were similar in all treatments and were not affected by the varying levels of FCM as replacement of SBM. The FCR values ranged from 0.97 to 1.23 and were statistically similar among all treatments. Also, total feed intake appears to decline as the substitution level of SBM with FCM increases, but the values were not statistically different between treatments.

Carcass Composition

Carcass crude protein content was significantly lower in Trt100 when compared with the positive control ($p = 0.03$). Trt50 and Trt75 did not differ significantly with both the positive control and Trt100 (Table 3). Carcass total lipid content was not influenced by FCM inclusion levels. Ash content of the carcass was significantly increased with elevated FCM inclusion.

Table 2: Survival, growth performance, feed efficiency, and nutrient retention indices of juvenile saline-tolerant Nile tilapia fed varying levels of fermented copra meal

Growth Indices	Experimental Treatments				
	Control	Trt50	Trt75	Trt100	P value
S	95 ± 4.08	100 ± 0	100 ± 0	95 ± 4.08	0.615
FBW	22.04 ± 0.11^{b}	22.51 ± 0.09^{ab}	23.95 ± 0.18^{a}	19.23 ± 0.58^{c}	0.002
SGR	6.56 ± 0.22^{b}	6.74 ± 0^{b}	6.74 ± 0.12^{b}	5.8 ± 0.09^{a}	0.009
TFI	24.32 ± 2.79	21.74±0.30	22.51± 0.64	20.51± 1.21	0.012
FCR	1.13 ± 0.10	1.01 ± 0.01	0.97 ± 0.02	1.14 ± 0.03	0.125
PER	2.00 ± 0.18	2.26 ± 0.02	2.36 ± 0.06	2.09 ± 0.06	0.784
PR	20.27 ± 2.72	22.34 ± 2.03	25.47 ± 2.56	24.31 ± 1.05	0.873
LR	48.06 ± 5.28	43.53 ± 3.41	51.89 ± 4.93	53.69 ± 0.11	0.490

S (%): survival rate; FBW: final body weight in grams; WG (%): percent weight gain; SGR: specific growth rate; TFI: Total feed intake in grams: FCR: feed conversion ratio; PER: protein efficiency ratio; PR: protein retention; LR: lipid retention. Values are mean ± SEM. Mean values with different superscripts in each parameter indicate significant differences ($p<0.05$).

Table 3: Carcass composition of juvenile saline-tolerant Nile tilapia fed varying levels of fermented copra meal

Carcass Composition	Experimental Treatments				
	Positive Control	Trt50	Trt75	Trt100	P-value
Crude Protein (% dry matter)	63.17 ± 0.99^{b}	61.87 ± 0.27^{ab}	62.44 ± 0.43^{ab}	59.31 ± 0.25^{a}	0.03
Crude Lipid (% dry matter)	25.91 ± 1.83	24.78 ± 0.62	21.5 ± 0.59	23.95 ± 0.91	0.17
Ash (% dry matter)	11.82 ± 0.04^{a}	17.17 ± 0.09^{b}	18.63 ± 0.17^{c}	18.56 ± 0.10^{c}	0.00

Values are mean ± SEM. Mean values with different superscripts in each parameter indicate significant differences ($p<0.05$).

Digestive Enzymes

The activities of digestive lipase and proteases of the experimental fish were influenced by FCM dietary inclusion levels (Table 4). The amylase enzyme activities were similar in all treatments ($p>0.05$) while protease enzyme activities in Trt50 and Trt75 were lower when compared with the control and Trt100. Lipase enzyme activities increased with increasing FCM dietary inclusion.

Liver Metabolic Enzymes

Examination of serum glutamic oxalo-acetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels indicates that these liver metabolic enzymes are not affected by SBM replacement with FCM in the formulated diets. No significant differences were observed in the values of SGPT and SGOT in fish receiving the different dietary treatments ($p>0.05$) (Table 5).

Intestinal Morphology

Examination of the tissue sections of the villi and enterocytes of the experimental fish showed no negative influence of FCM on intestinal morphology and structures. No significant differences were observed in the intestinal villi length in all treatments. The enterocyte heights of fish in all the experimental treatments were statistically similar (Table 6). No morphological abnormalities and tissue damages were observed in the intestinal tissue of fishes receiving the dietary treatments (Figure 2A-C).

Moreover, the intraperitoneal fat size was found to be similar among the treatments. No steatosis was detected in the intestines of the fish fed any of the dietary treatment, including the control.

Table 4: Activities of digestive enzymes of juvenile saline-tolerant Nile tilapia fed diets containing fermented copra meal as replacements of soybean meal

Digestive Enzymes	Experimental Treatments				
	Control	Trt50	Trt75	Trt100	P-value
Amylase (Unit)	384.33 ± 133.22	567.11 ± 185.64	307.2 ± 59.77	602.75 ± 148.47	0.46
Protease (Unit)	1.8 ± 0.04^{b}	0.69 ± 0.04^{a}	1.06 ± 0.05^{a}	1.65 ± 0.13^{b}	0.001
Lipase (Unit)	1620.48 ± 15.62^{a}	2925 ± 31.94^{b}	3420.19 ± 1.5^{c}	4441.29 ± 5.51^{d}	0.00

[a] Amylase unit = ug glucose/min/mg protein, [b] Protease unit = umole tyrosine/min/mg protein, [c] Lipase unit = µg methyl undeacanoate/min/mg protein. Values are mean ± SEM. The mean values with different superscripts indicate significant differences ($p<0.05$).

Table 5: Serum aminotransferase levels of the juvenile saline-tolerant Nile tilapia fed diets containing varying levels of fermented copra meal as a soybean meal replacement

Liver Metabolic Enzymes	Experimental Treatments				
	Control	Trt50	Trt75	Trt100	P-value
SGOT, (Units /L)	12.0 ± 2.0	13.5 ± 0.50	12.5 ± 0.50	17.0 ± 1.00	0.12
SGPT, (Units /L)	33.5 ± 3.5	16.0 ± 2.00	30.5 ± 17.5	19.5 ± 4.50	0.54

SGOT, serum glutamic oxalo-acetic transaminase; SGPT, serum glutamic pyruvic transaminase. Values are mean ± SEM, n = 3. Mean values with different superscripts indicate significant differences ($p<0.05$)

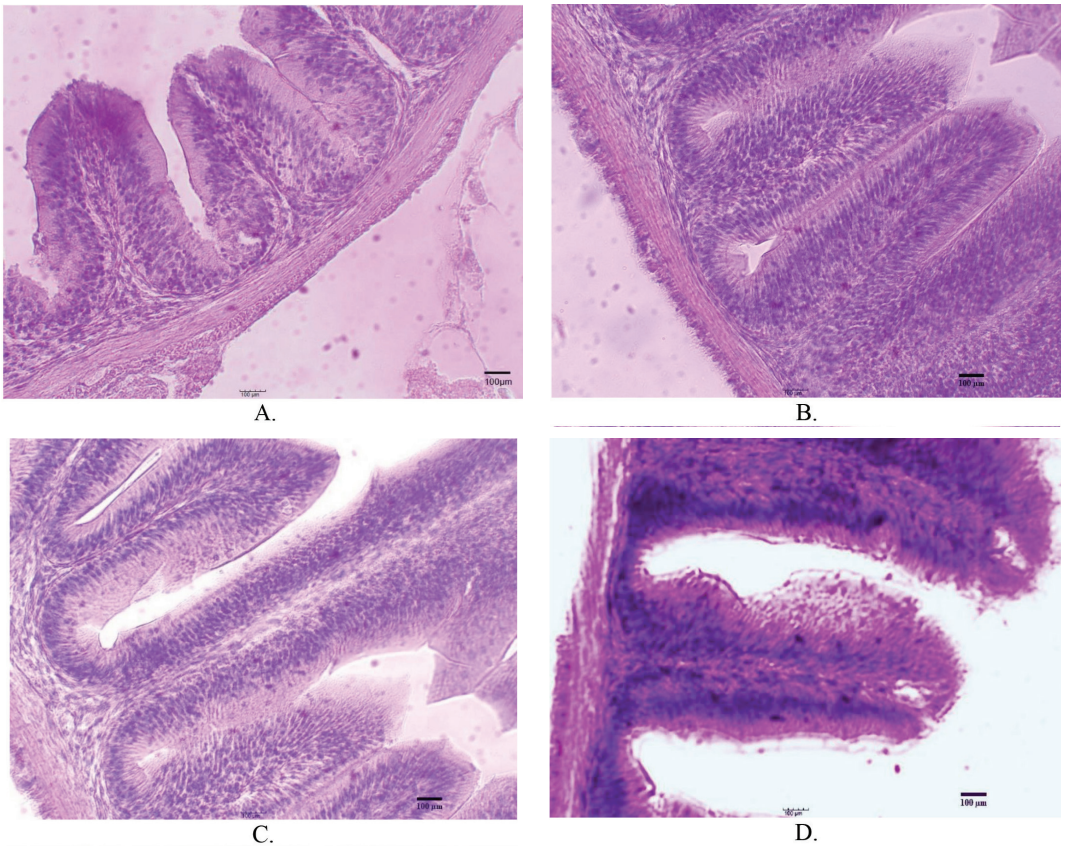

Figure 2: Intestinal histological morphology of the juvenile saline-tolerant Nile tilapia fed diets containing increasing levels of fermented copra meal as a replacement of soybean meal. A. Control, B. Trt50, C. Trt70, D. Trt100

Table 6: Intestinal histology of the juvenile saline-tolerant Nile tilapia fed diets containing varying levels of fermented copra meal as replacement of soybean meal

Intestine Morphology	**Experimental Treatments**				
	Control	**Trt50**	**Trt75**	**Trt100**	**P-value**
Enterocyte height (µm)	85.12 ± 4.34	93.62 ± 5.74	83.19 ± 52.73	83.21 ± 2.28	0.27
Villi length (µm)	155.49 ± 3.21	230.51 ± 9.38	286.54 ± 1.84	148.95 ± 2.16	0.31
IPF (µm)	0.45 ± 0.06	0.23 ± 0.04	0.29 ± 0.04	0.31 ± 0.04	0.11

Values are mean ± SEM. Mean values with different superscripts indicate significant differences ($p<0.05$). IPF, Intraperitoneal fat, measured as the diameter of the fat cell.

Discussion

Copra meal, a by-product of the coconut oil industry, is an abundant biomass resource with high potential as a feed ingredient for aquaculture use. Unprocessed copra meal has poor feed value, but improvements in terms of nutrient quality, bioavailability, and enhancement of amino acid profile through fermentation have been documented (Sundu *et al.,* 2009, Laining *et al.,* 2017). However, the feed value and potential of this fermented biomass as a feed ingredient have not been fully evaluated in aquatic animals. To the best of our knowledge, the present study is the first to report the successful replacement of SBM with FCM in the diet of juvenile Nile tilapia reared in seawater.

The high survival rate of the experimental fish in this study indicates the adaptability of this tilapia strain to full-strength seawater culture conditions. Moreover, their survival was found to be not affected by the dietary treatments in the present study. Experimental fish exhibiting the highest WG, final weight, and SGR were recorded in treatment Trt75 (28.5% FCM dietary inclusion), indicating that 75% of dietary SBM could be replaced with FCM. However, increasing SBM replacement to 100% (Trt100) decreased growth performance, suggesting that full SBM replacement with FCM is not feasible. Growth inhibition at a higher dietary inclusion level may indicate the presence of anti-nutritional factors and unavailability of nutrients that can limit assimilation to attain maximum growth. Similar results were also observed by Laining *et al.* (2017), who indicated that total replacement of SBM with FCM is detrimental to the growth of rabbitfish, They also and only 13.7% g of this ingredient was recommended for inclusion in the diets of siganids. The limitation of FCM dietary inclusion in the fish feed may be attributed to its content of non-starch polysaccharides, which affects the digestive physiology of fish. Although major anti-nutrients present in copra meal, such as tannins and phytic acids, can be removed through fermentation (Mukhopadhyay, 2000), other components, including non-starch polysaccharides (NSP), may still be present and decrease the feed value of the ingredient (Sundu *et al.,* 2009). Furthermore, raw copra meal has been reported to contain 42.2% NSP, comprising water-soluble galactomannans and water-insoluble mannan oligosaccharides (Knudsen, 1997). Galactomannans have been documented in rats to reduce the availability of dietary cholesterol by binding with the bile salts (Moundras *et al.,* 1997). Additionally, it has been shown that dietary galactomannan increases gut content viscosity that inhibits efficient nutrient absorption in carp (Hossain *et al.,* 2001). Decreased feed utilisation efficiency, resulting in growth retardation in tilapia, was also linked to the presence of galactomannan in the feeds (Hossain *et al.,* 2003). Similarly, in rainbow trout, reduced dietary glucose availability has been associated with high dietary inclusion of guar gum galactomannan oligosaccharides (Storebakken, 1985). Though the NSP content of FCM was not measured in the present study, it is tempting to speculate that the poor growth observed in treatments with higher dietary inclusion of FCM as a SBM replacement (higher than 75%) can be attributed to this anti-nutrient.

In contrast to the present findings, Mamaug *et al.* (2019), showed that 100% FCM was able to replace SBM meal (16% dietary inclusion) and was found not to affect the growth and feed utilisation of the grouper (*Epinephelus fuscoguttatus*). However, in this study, the diet is a fish meal-based formulation containing 45% (40% fish meal and 5% squid meal) marine protein meals, and only 16% SBM is included. The experimental diet used in the present study is a SBM–based diet containing only 13% fish meal and 38% SBM. These contrasting results may have been due to the differences in marine protein meals included in the experimental diets that can mask the negative effects of plant-based protein meals on the growth performance of the experimental animals.

Our present results suggest that FCM could replace SBM in the diet of the saline-tolerant strain of Nile tilapia to about 75% by weight (inclusion level of 28.5% of the diet). In the present study, the growth performance

of the experimental fish in terms of WG ranged from 1,720.35% to 2,814.46%, which is comparatively higher than the previously reported growth of juvenile tilapia (size, 0.83g), cultured in freshwater with a WG of 1,132% to 2,410.7% (Siddiqui *et al.,* 1988). Also, the SGR values obtained in the present study (SGR of 5.8-6.74) is comparably higher than those reported in early juvenile Nile tilapia (0.25g) reared in different salinities (SGR of 1.85-2.38), including those that are reared in full-strength seawater (Larumbe-Morán *et al.,* 2010). The SGR obtained in the present study is also higher than those reported in early juvenile Nile tilapia (0.98g) fed diets containing feeding attractants and animal by-product meals (Montoya-Mejía *et al.,* 2017) and those fed with sodium butyrate as growth stimulants (El-Naby *et al.,* 2019). The higher growth rate data obtained in the present study indicates that FCM is safe to be utilised as a dietary feed ingredient even at relatively high substitution levels in early juvenile saline-tolerant Nile tilapia. The use of raw copra meal as a feed ingredient for the animal-growing industries has been a subject of intensive research during the last decades. However, the feed value of this material has been attributed to the presence of anti-nutrients, including tannins, galactomannan oligosaccharides, and Maillard reaction products formed during the oil extraction process. The presence of these anti-nutrients is considered as a limiting factor in the full utilisation of this biomass for animal feed (Sundu *et al.,* 2009). Fermentation has been proven to improve the feed value of copra meal. Copra meal fermented with *Aspergillus* and *Trichoderma* has been found to have lower phytic acid and tannin contents. Application of this fermented ingredient as feed significantly increased digestibility and improved growth rates of broiler chicken compared with those fed with diets containing raw copra meal (Hatta & Sundu, 2009). The protein content of copra meal has been known to be of poor feed value since it is water-insoluble and has poor availability, but fermentation with yeast in tandem with mannanase enzyme addition showed that available proteins in the form of peptides and amino acid were increased, while galactomannan levels were decreased (Kraikaew *et al.,* 2020). In the present study, the high growth rate of tilapia at high FCM (75%) substitution of SBM could be attributed to the improvement of nutrient quality of copra meal due to fermentation, similar to that observed in earlier works.

Also, the results of the present study indicate that the treatments did not affect the FCR of the experimental animals. The FCR in the present study is in the range of 0.97 to 1.23, which *is considered good by the standards described by De Silva and Anderson (1995) and Craig and* Helfrich (2002). In addition, the PER, PR, and LR were similar in all the treatments. In earlier *studies*, it has been shown that increasing replacement of fish meal with plant protein ingredients reduces protein content, nutritive value, and retention in aquatic animals (Chavez *et al.,* 2016). In tilapia larvae and early juveniles, it has been reported that growth is highly sensitive to the quality and quantity of dietary proteins. Excessive or inadequate dietary proteins decrease FCR, growth performance, and survival of the larvae (Siddiqui *et al*., 1988). Also, Linn *et al.* (2014) found that replacing fish meal with plant protein on red sea bream exhibited a decreased PER value as the amount of replacement increases. This decreasing nutrient retention has been linked to the low palatability of plant-based diet and the presence of anti-nutritional factors that can result in reduced feed intake, poor digestibility, and limited nutrient utilisation (Paranamana *et al.,* 2014). However, Espe *et al*. (2006) showed that in diets with higher plant meal inclusions, nutrient retention and availability are not affected if dietary amino acids are balanced and feed intake is not reduced. Our present findings indicate that feed intake was not affected by FCM inclusion levels, suggesting that this ingredient may contain balanced amino acids that promote positive gustatory response. The decrease in growth performance observed in treatments with complete replacement of SBM with FCM in the present study could not be attributed to feed palatability issues, but could be due to the

content of NSP that are known to inhibit nutrient absorption and utilisation (Hossain *et al.,* 2001).

In the present study, carcass content analyses showed that the experimental treatment does not significantly influence carcass composition, but the highest replacement level resulted in decreased carcass protein content. Decreasing protein deposition in fish carcass has been commonly observed in fish maintained with a higher level of dietary plant protein meals. In juvenile *P. vannamei*, it has been shown that a significant reduction in carcass protein was observed in higher replacement levels of soybean concentrates with fermented agricultural biomass (Qiu & Davis, 2017). Decreased growth, PER and carcass protein content were also observed in red drum maintained with diets containing yeast fermented biomass (> 50%) as a replacement of fishmeal (Rosales *et al.,* 2017). Similar to our present findings, the decreasing carcass protein content in Nile tilapia early juvenile was linked with a high inclusion level of yeast or bacteria-fermented sunflower meal (Hassaan *et al.,* 2018). The decrease in growth performance and carcass protein content at higher inclusion levels of most plant protein ingredients in fish diets has been associated with the imbalance profile and unavailable essential amino acids that promote protein catabolism rather than anabolism (Lim & Lee, 2011).

No obvious effect of the treatments was observed on enterocyte height, villi length, and intraperitoneal fat size. Changes in the structure of the enterocyte, villi, and build-up of intraperitoneal fat have been associated with the presence of anti-nutritional factors found in fermented and plant-based feed ingredients (Berilis *et al*., 2017). Intestinal villi damage has been linked to the presence of alcohol-soluble anti-nutrient in most plant-based feed ingredients, but has been reported to be absent in FCM (Mamaug *et al.,* 2019). Our present result concurs with the findings of Aanyu *et al.* (2014), who indicated that tilapia enterocyte has a higher tolerance to damage attributed to diets containing high inclusion levels of plant-protein ingredients. Similar to our findings, it was observed that feeding grouper with formulated diets containing higher inclusion levels of FCM does not affect the intestinal morphology (Mamaug *et al.,* 2019). The higher replacement level of SBM with FCM in our study (75%) may have been attributed to the tolerable content of anti-nutritional factors present in FCM. Our results suggest that the use of FCM as a feed ingredient could not cause morphological changes and damage in the intestine of juvenile saline-tolerant Nile tilapia.

Digestive enzyme activities were suggested as predictors of potential feed utilisation and feed ingredient quality in fish (Rungruangsak-Torrissen, 2014). In the present study, activities of the digestive enzymes, including amylase protease and lipase, were high in all the treatment groups as compared with the control. It has been reported that early juvenile tilapia are already well equipped with the enzymatic capacity to digest complex feed materials and have wide adaptability in digesting different food items, including plant materials. Furthermore, it has been shown that enhanced protease activity has been correlated to diet nutrient availability, efficient feed-protein utilisation, and improved growth performance in early tilapia juvenile (Montoya-Mejía *et al.,* 2017, Santos *et al.,* 2013). Fermentation of feed ingredients has been previously reported to improve digestibility and documented to generate low molecular weight peptides and amino acids (Kraikaew *et al.,* 2020). The presence of pre-digested proteins and peptides in feeds have been known to activate digestive enzyme activities (Montoya-Mejía *et al.,* 2017; Santos *et al.,* 2013) and may explain the high digestive enzyme activities of tilapia fed diets containing FCM in the present study. Similarly, decreasing activity of digestive enzymes has also been reported in juvenile tilapia, *O. niloticus* x *O. aureus* fed with diets containing high levels of SBM. The presence of anti-nutritional factors in SBM was associated with the decrease of the digestive enzyme activities (Lin & Luo, 2011).

In addition, activities of liver enzymes, including SGPT and SGOT, have been used as

indices in fish nutrition to evaluate the influence of a feed ingredient on fish performance. Elevated values of SGPT and SGOT have been linked with liver damage caused by ingredient-associated anti-nutritional and toxic factors (Soltan *et al.,* 2008). Evaluation of serum SGPT and SGOT in the present study indicates that FCM replacement of SBM has no negative effect on these indices, indicating that liver functions are not affected by these treatments. The high activities of the digestive enzymes and the similar values of liver metabolic enzymes observed in the present study may indicate the absence of enzyme inhibitors in FCM and support the findings of a higher replacement level of SBM without negatively affecting the growth performance of saline-tolerant tilapia.

Conclusion

The present study concluded that dietary soybean meal could be replaced with fermented copra meal up to 75% by weight without effecting growth, nutrient utilisation, and carcass composition of saline-tolerant Nile tilapia (*Oreochromis niloticus*). Fermented copra meal is a sustainable source of feed protein ingredient that could satisfy the growing demands for low-cost feed ingredients for a sustainable tilapia aquaculture.

Acknowledgements

The authors acknowledge the Department of Science and Technology Student Research Support Fund and Accelerated Science and Technology Human Resource Development Programme of the Philippine government for the scholarship and graduate thesis grant necessary for the realisation of this study.

References

Aanyum, M., Ondhoro, C. C., Ganda, E., Kato, D. G., & Basiita, R. K. (2014). Intestine histology, nutrient digestibility and body composition of Nile tilapia (*Oreochromis niloticus*) fed on diets with both cotton and sunflower seed cakes. *African Journal of Biotechnology*, *13*(37), 3831-3839

Abdel-Moneim, A. M., Al-Kahtani, M. A., & Elmenshawy, O. M. (2012). Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia. *Chemosphere*, *88*(8), 1028-1035.

Apines-Amar, M. J., Andrino-Felarca, K. G., Cadiz, R., Corre Jr., V., & Calpe, A. (2016). Effects of partial replacement of fish meal by Fermented Copra Meal on the growth and feed efficiency in Black Tiger Shrimp, *Penaeus monodon*. *The Israeli Journal of Aquaculture - Bamidgeh*, *68*, 1244-1249.

Areekijseree, M., Engkagul, A., Kovitvadhi, U., Thongpan, A., Mingmuang, M., Pakkong, P. & Rungruangsak-Torrissen, K. (2004). Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel *Hyriopsis bialatus* Simpson 1900. *Aquaculture, 234*, 575-587.

Association of Official Analytical Chemists (AOAC). (1996). In Cuniff, P. (Ed.), *Official methods of analysis of AOAC International* (16th edition Volume 1, 4th revision). Washington: Association of Office Analytical Chemists International Publisher.

Berillis, P., Martin, S., & Mente, E. (2017). Histological methods to assess the effect of diet and a single meal on the liver and intestine of rainbow trout: Fishmeal and fish oil replacement with plant protein and oil. In P. Berillis (Ed.), *Trends in fisheries and aquatic animal health* (1st ed., pp. 262-276). Sharjah, UAE: Bentham Science Publisher.

Bernfield, P. (1951). Enzymes of starch degradation and synthesis. *Advances in Enzymology and Related Areas of Molecular Biology*, *12*, 379-428.

Bligh, E. G., & Dyer, W. G. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry Physiology*, *37*, 911-917.

de Boer, H. C., van Krimpen, M. M., Blonk, H., & Tyszler, M. (2014). Replacement of soybean meal in compound feed by European protein sources- Effects on carbon footprint. Wageningen, Wageningen University Livestock Research. *Livestock Research Report*, *819*, 1- 46.

Boonanuntanasarn, S., Nakharuthai, C., Schrama, D., Duangkaew, R., & Rodrigues, P. (2018). Effects of dietary lipid sources on hepatic nutritive contents, fatty acid composition, and proteome of Nile tilapia *Oreochromis niloticus*. *Journal of Proteomics*, *192*, 208-222.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Buroker-Kilgore, M., & Wang, K. K. W. (1993). A coomassie brilliant blue G-250-based colorimetric assay for measuring activity of Calpain and other proteases. *Analytical Biochemistry*, *208*(2), 387-392.

Chavez, N. N. G., Ragaza, J. A., Corre Jr, V. L., Serrano, A. E., & Traifalgar, R. F. M. (2016). Effects of water hyacinth Eichhornia crassipes leaf protein concentrate as soybean protein replacement in white shrimp Litopenaeus vannamei (Boone) postlarvae diet. *Aquaculture Research*, *47*(8), 2642-2649.

Craig, S., & Helfrich, L. A. (2002). Understanding fish nutrition feeds and feeding. *Virginia Cooperative Extension*, *63*, 256-270.

De Silva, S. S., & Anderson, T. A. (1995). *Fish nutrition in aquaculture*. Netherland: Springer Science Publisher.

De Verdal, H., Rosario, W., Vandeputte, M., Muyalde, N., Morissens, P., Baroiller, J. F., & Chevassus, B. (2014). Response to selection for growth in an interspecific hybrid between *Oreochromis mossambicus* and Oreochromis *niloticus* in two distinct environments. *Aquaculture*, *430*, 159-165.

El-Dakar Y., Shalaby, A., & Abd, A. (2015). Growth performance and feed utilization of hybrid Red Tilapia, *Oreochromis niloticus* (Linnaeus) X *Oreochromis mosambicus* (Peters) fed different dietary protein and energy levels under rearing in seawater conditions. *Mediterranean Aquaculture Journal*, *7*, 12-21.

El-Naby, A., Khattaby, A. E. R., Samir, F., Awad, S., & Abdel-Tawwab, M. (2019). Stimulatory effect of dietary butyrate on growth, immune response, and resistance of Nile tilapia *Oreochromis niloticus* against *Aeromonas hydrophila* infection. *Animal Feed Science and Technology*, *254*, 114212. https://doi.org/10.1016/j.anifeedsci.2019.114212

Espe, M., Lemme, A., Petri, A., & El-Mowafi, A. (2006). Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? *Aquaculture*, *255*(4), 255-262.

Guerrero, R. D. (2019). Farmed tilapia production in the Philippines is declining: What has happened and what can be done. *Philippine Journal of Science*, *148*(2), 11-15.

Hassaan, M. S., Soltan, M. A., Mohammady, E. Y., Elashry, M. A., El-Haroun, E. R., & Davies, S. J. (2018). Growth and physiological responses of Nile tilapia *Oreochromis niloticus* fed dietary fermented sunflower meal inoculated with *Saccharomyces cerevisiae* and *Bacillus subtilis*. *Aquaculture*, *495*, 592-601.

Hatta, U., Sundou, B. (2009). Effects of copra meal fermented by Aspergillus niger and Trichoderma spp on performance of broiler. *Proceedings of the 1st International Seminar on Animal Industry 2009 Faculty of Animal Science, Bogor Agricultural University. Indonesia*. 332-335. http://repository.ipb.ac.id/handle/123456789/33962

Hossain, M. A., Focken, U., & Becker, K. (2003). Antinutritive effects of galactomannan-rich endosperm of Sesbania (*Sesbania aculeata*) seeds on growth and feed utilisation in

tilapia, *Oreochromis niloticus*. *Aquaculture Research*, *34*, 1171-1179.

Hossain, M. A., Focken, U., & Becker, K. (2001). Galactomannan-rich endosperm of Sesbania (*Sesbania aculeate*) seeds responsible for retardation of growth and feed utilisation in common carp, *Cyprinus carpio* L. *Aquaculture*, *203*, 121-132.

Hutagalung, R. I. (1981). The use of tree crops and their by-products for intensive animal production. *British Society of Animal Production Occasional Publication*, *4*, 151-184.

Jumah, Y. U., Traifalgar, R. F. M., Monteclaro, H. M., Sanares, R. C., Jumah, D. S. U., & Mero, F. F. C. (2016). Influence of hyperosmotic culture conditions on osmoregulatory ions, gill chloride cells and Na + / K + -ATPase activity of Nile tilapia *Oreochromis niloticus*. *Aquaculture, Aquarium, Conservation and Legislation - International Journal of the Bioflux Society*, *9*(3), 498-506.

Khan, M. S. K., Siddique, M. A. M., & Zamal, H. (2013). Replacement of fish meal by plant protein sources in Nile tilapia (*Oreochromis niloticus*) diet: Growth performance and utilization. *Iranian Journal of Fisheries Sciences*, *12*(4), 855-863.

Knudsen, K. E. B. (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology*, *67*, 319-338.

Kokou, F., Rigos, G., Kentouri, M., & Alexis, M. (2016). Effects of DL- methionine-supplemented dietary soy protein concentrate on growth performance and intestinal enzyme activity of gilthead sea bream (*Sparus aurata* L.). *Aquaculture International*, *24*, 257-271.

Kraikaew, J., Morakul, S., & Keawsompong, S. (2020). Nutritional improvement of copra meal using mannanase and *Saccharomyces cerevisiae*. *3 Biotech*, *10*, 274. https://doi.org/10.1007/s13205-020-02271-9

Laining, A., Usman, U., & Syah, R. (2017). Nutritive value of copra cake meal fermented with Rhizopus spp. and its use as a protein source in practical diets for rabbitfish *Siganus javus*. *Journal of Applied Aquaculture*, *29*(3-4), 307-321.

Larumbe-Morán, E., Hernández, M., Olvera-Novoa, M., & Pérez-Rostro, C. (2010). Protein requirements of Nile tilapia (*Oreochromis niloticus*) fry cultured at different salinities. *Aquaculture Research*, *41*, 1150 - 1157.

Lim, S. J., & Lee, K. J. (2011). A microbial fermentation of soybean and cottonseed meal increases antioxidant activity and gossypol detoxification in diets for Nile tilapia, Oreochromis niloticus. *Journal of the World Aquaculture Society*, *42*(4), 494-503.

Lin, S., & Luo, L. (2011). Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes, and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *Oreochromis aureus*. *Animal Feed Science and Technology*, *168*(1-2), 80-87.

Linn, S. M., Ishikawa, M., Koshio, S., Yokoyama, S., Murata, T., Hamasaki, Y., & Nankervis, L. (2014). Effects of replacing fish meal with plant protein on growth performance, feed utilization and oxidative condition of Red Sea Bream, *Pagrus major*. *Aquaculture Science*, *62*(4), 341-352.

Lojda, Z., Gossrau, R., & Schiebler, T. H. (1979). *Enzyme histochemistry, a laboratory manual*. Berlin, Germany: Springer Publisher.

Lowry, R. R., & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society*, *53*(7), 470-472.

Mamaug, R. E. P., Ragaza, J. A., & Nacionales, T. (2019). Fish performance, nutrient digestibilities, and hepatic and intestinal morphologies in grouper *Epinephelus*

fuscoguttatus fed fermented copra meal. *Aquaculture Reports*, *14*, 2-7.

Montoya-Mejía, M., García-Ulloa, M., Hernández-Llamas, A., Nolasco-Soria, H., & Rodríguez-González, H. (2017). Digestibility, growth, blood chemistry, and enzyme activity of juvenile *Oreochromis niloticus* fed isocaloric diets containing animal and plant by-products. *Revista Brasileira de Zootecnia*, *46*(12), 873-882.

Moundras, C., Behr, S. R., Remesy, C., & Demigne, C. (1997). Faecal losses of sterols and bile acids induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. *The Journal of Nutrition*, *127*, 1068-1076.

Mukhopadhyay, N. (2000). Improvement of quality of copra dried kernel of *Cocos nucifera* seed meal protein with supplemental amino acids in feeds for rohu, *Labeo rohita* Hamilton fingerlings. *Acta Ichthyologiya et Piscatoria*, *30*, 21-34.

Ngugi, C. C., Oyoo-Okoth, E., Manyala, J. O., Fitzsimmons, K., & Kimotho, A. (2017). Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds. *Aquaculture Reports*, *5*, 62-69.

Pan, M. V., Traifalgar, R. F. M., Serrano, A. E., & Corre, V. L. (2015). Immunomodulatory and growth-promoting effects of peptidoglycan supplementation in Black Tiger Shrimp *Penaeus monodon* Fabricius 1798. *Asian Fisheries Science*, *28*, 60-71.

Pach, F., & Nagel, F. (2017). Replacing the substitute-Guar meal as an alternative for non-genetically modified soybean meal in the nutrition of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). *Aquaculture Nutrition*, *24*(1), 666-672.

Paranamana, N., Gamage, K., & Bulugahapitiya, V. (2014). Nutritional and anti-nutritional contents of alternative plant feed ingredients for fish feed formulation. *The Indian Journal of Animal Sciences*, *85*, 212-215.

Pham, L. J. (2017). A process of producing protein enriched copra meal as feed ingredient for the livestock and aquaculture industry. Patent Publication No. PH1/2017/000081(A1)

Plaipetch, P., & Yakupitiyage, A. (2013). Effect of replacing soybean meal with yeast-fermented canola meal on growth and nutrient retention of Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). *Aquaculture Research*, *45*, 1744-1753.

Qiu, X., & Davis, D. A. (2017). Evaluation of dried fermented biomass as a feed ingredient in plant-based practical diets for juvenile Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, *24*(1), 383-391.

Rosales, M., Castillo, S., Pohlenz, C., & Gatlin, D. M. (2017). Evaluation of dried yeast and threonine fermentation biomass as partial fish meal replacements in the diet of red drum *Sciaenops ocellatus*. *Animal Feed Science and Technology*, *232*, 190-197.

Rungruangsak-Torrissen, K. (2014). Atlantic Salmon, *Salmo salar* L.: Food utilization, protein growth efficiency and maturation. In P. T. L., Woo & D. J., Noakes (Eds.), *Salmon biology, ecological impacts and economic importance* (1st ed., pp. 121-154). New York: Nova Science Publishers Inc.

Santos, J., Castro, P., Leal, A., Júnior, A., Lemos, D., Carvalho, L., Bezerra, R. (2013). Digestive enzyme activity in juvenile Nile tilapia (*Oreochromis niloticus*, L) submitted to different dietary levels of shrimp protein hydrolysate. *Aquaculture International*. *21*, 563-577.

Siddiqui, A. Q., Mohammad, S., Howlader, A., & Adam, A. (1988). Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*, *Aquaculture*, *70*, 63-73.

Soltan, M., Hanafy, M. A., & Wafa, M. I. (2008). Effect of replacing fish meal by a mixture

of different plant protein sources in Nile Tilapia (*Oreochromis niloticus* L.) diets. *Global Veterinarian*, *2*, 157-164.

Storebakken, T. (1985). Binders in fish feeds: I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. *Aquaculture*, *47*, 11-26.

Sundu B., Kumar, A., & Dingle, J. (2009). Feeding value of copra meal for broilers. *World's Poultry Science Journal*, *65*, 481-489.

Tuller, J., Santis, C. D., & Jerry, D. R. (2012). Dietary influence of Fucoidan supplementation on growth of *Lates calcarifer* (Bloch). *Aquaculture Research*, *45*(4), 749-754.

Van Doan, H., Hoseinifar, S. H., Chitmanat, C., Jaturasitha, S., Paolucci, M., Ashouri, G., Dawood, M. A. O., & Esteban, M. A. (2019). The effects of Thai ginseng, Boesenbergia rotunda powder on mucosal and serum immunity, disease resistance, and growth performance of Nile tilapia *Oreochromis niloticus* fingerlings. *Aquaculture*, *513*, 734388. https://doi.org/10.1016/j.aquaculture.2019.734388

Younis, E. S. M., Al-Quffail, A., Al-Asgah, N. A., Abdel-Warith, A. W. A., & Al-Hafedh, Y. S. (2018). Effect of dietary fish meal replacement by red algae, *Gracilaria arcuata*, on growth performance and body composition of Nile tilapia *Oreochromis niloticus*. *Saudi Journal of Biological Sciences*, *25*(2), 198-203.