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EFFECTS OF PARTIAL REPLACEMENT OF FISH OIL WITH DIFFERENT VEGETABLE OILS ON GROWTH, FEED UTILISATION AND FATTY ACID PROFILE OF HYBRID GROUPER JUVENILE (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂)

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ABSTRACT

This study was done to investigate the effects of fish oil (FO) replacement with different types vegetable oils (VO) on the growth performance, feed utilisation and fatty acid profile of hybrid grouper juvenile (Ephinephelus fuscoguttatus, ♀) *x giant grouper* (Epinephelus lanceolatus, ♂)*. Six isoprotein (50%) and isolipid (12%) diets were formulated using 100% FO (as the control) and 50% of FO was replaced with crude palm oil (CPO), refined, bleached and deodourised (RBD) palm olein (POo), crude palm kernel oil (CPKO), corn oil (CO) and coconut oil (COCO). Triplicate groups of hybrid grouper juvenile (11.12±0.04 g) were fed with the experimental diets for a period of 12 weeks. Fish fed with RBD POo diet showed significantly higher growth and slightly better feed efficiency than other diets. The different VO affected the body indices, whole body proximate composition and fatty acid profile of the muscle and liver. However, the survival of fish was not affected by the different diets. Present study suggests that RBD POo is the most suitable VO for hybrid grouper.*

Keywords: hybrid grouper, fish oil, vegetable oil, growth, feed utilisation.

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INTRODUCTION

Hybrid grouper is a cross breeding between brown-marbled grouper (*Ephinephelus fuscoguttatus,* ♀) x giant grouper *(E. lanceolatus,* ♂*)* which was produced in 2006 at Borneo Marine Research Institute of Universiti Malaysia Sabah (Ch'ng and Senoo, 2008). The hybrid grouper had then become a popular aquaculture fish in Malaysia and other South-east Asian countries. It has high aquaculture demand due to its high egg hatching rate, faster growth, strong tolerance to the environmental factors and resilience to diseases than its parental species (Mustafa *et al.*, 2013; Othman *et al.*, 2015; Koh *et al.*, 2016; Bunlipatanon and U-taynapun, 2017; Arrokhman *et al.*, 2017).

Feed is one of the major factors that plays an important role on the success of the aquaculture sector as it represents 50% to 70% of the total production costs (FAO, 2009). In the production of aquaculture feed, fish oil (FO) is the most important lipid sources. It is commonly used as the main lipid source for marine fish feed due to its high digestibility, essential fatty acids (EFA), in particular long chain polyunsaturated fatty acid (PUFA) (Nasopouluo and Zabetakis, 2012) and fat soluble nutrients for normal growth and development of fish (Turchini *et al.*, 2009). However, the rising

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environmental and economic costs of the feedstuff have encouraged its sparing using alternative lipid sources (Olsen *et al.*, 2011; Chen *et al.*, 2017).

Over the past two decades, vegetable oil (VO) has emerged as the alternative source of lipid in aquaculture feed, due to the steadily increasing production, high availability and better economic value of VO (Turchini *et al*., 2011). However, several studies had reported various results on the use of VO on the health or growth of freshwater and marine fish species (Glencross *et al.*, 2003; Torstensen *et al.*, 2004; Izquierdo *et al.*, 2005; Mourente and Bell, 2006; Lin and Shiau, 2007; Piedecausa *et al.*, 2007; Shapawi *et al.*, 2008). Among the VO, palm oil is one of the major oils and fats produced and traded in the world today. In 2016, palm oil and palm kernel oil had jointly accounted for 31% of total output in global production of oils and fats (Oil World, 2017; MPOC, 2017) and Malaysia is the second largest producer of palm oil in the world. Apart from palm oil and palm kernel oil, coconut oil (COCO) is also one of the major oils which is also being locally produced and available in Malaysia and had also contributed to the world oils production (USDA, 2017).

The oil palm fruit produces two types of oils; crude palm oil (CPO) which is derived from the mesocarp and crude palm kernel oil (CPKO) from the kernel. Then the CPO is further processed into refined, bleached and deodourised (RBD) palm olein (POo). All these oils are physically and chemically different form each other and have different fatty acid profiles. CPO and RBD PO are rich in palmitic acid (C16:0) and oleic acid (C18:1n-9), while CPKO is high in saturated fatty acid, lauric acid (C12:0) and myristic acid (C14:0) (Christine *et al.*, 2014) which shows very similar fatty acid profile with COCO (Turchini *et al*., 2010). Besides, COCO is gaining much attention as it is highly digestible (Rosjo *et al*., 2000) and anti-parasite (Hirazawa *et al.*, 2001).

The uses of VO in aquaculture feed are well documented. In grouper, study on humpback grouper, *Cromileptis altivelis* (Shapawi *et al.*, 2008) showed that there was no significant difference on growth, survival, feed utilisation, hepatosomatic index and lipid levels in muscle and liver between the fish fed with CPO, RBD PO, soyabean oil and canola oil at 50% of FO replacement. Besides, better growth performance was also found in brown-marbled grouper, *E. fuscoguttatus* (Mohd Faudzi, 2013) fed with RBD PO, soyabean oil and canola oil at 50% of FO replacement than fish fed with FO. The feed conversion ratio (FCR) was not affected by the experimental treatments.

Various studies also reported on the successful use of corn oil (CO) for several aquaculture species in fish feed (Lin *et al.*, 2007; Lin and Shiau, 2007; Al-Souti *et al.*, 2012; Pereira *et al.*, 2018). At 50% replacement of FO with CO, no significant difference in survival, growth performance and hepatosomatic index was found between the FO and CO treatments in orange spotted grouper, *E. coloides* (Lin *et al.*, 2007). While on COCO, there is limited study on grouper. Study on the hybrid grouper (*E. fuscoguttatus*) x giant grouper *(E. lanceolatus)* using COCO, CO and squid oil showed that COCO was not the best performing diet compared to fish fed on CO and squid oil although no significant effect on the growth was observed (Fitriyani *et al.*, 2015).

Therefore, this study was conducted to investigate the suitable type of VO, namely the CPO, RBD POo, CPKO, CO and COCO to partially replace FO in feed for hybrid grouper juvenile, (*E. fuscoguttatus*) x giant grouper *(E. lanceolatus*).

MATERIALS AND METHOD

Experimental Diets and Diet Preparation

Six isoproteic (50%) and isolipid (12%) experimental diets were formulated according to the requirement of hybrid grouper juvenile (Mohd Faudzi *et al*., 2017). Diet with 100% FO was served as control diet. Other diets were prepared by substituting 50% of the FO with different VO; diet with CPO, RBD POo, CPKO, CO and COCO (*Table 1*). The various types of palm oils were obtained from Sawit Kinabalu, Sabah, Malaysia while the COCO and CO were purchased from local market.

The experimental diets were prepared by mixing all the ingredients until a homogenous mixture was obtained, then 40% water was added to form a moist dough before passing the dough through a 3 mm dice kitchen mincer. The resultant strands of feed were dried in an oven at 40°C for 5-6 hr. All the experimental diets were stored in air-tight plastic containers and refrigerated at 4°C until used. All the diets were subjected to proximate analysis (AOAC, 1999) and fatty acid analysis before the feeding trial.

All diets contained approximately 50% crude protein and 12% crude lipid (*Table 1*). The fatty acid composition of FO and VO used were different among the different types of oils. FO contains mainly n-3 polyunsaturated fatty acid (PUFA) C20:5n-3 (EPA) and C22:6n-3 (DHA), CPO and RBD POo contains mainly C16:0 and C18:1n-9, CPKO contains mainly C12:0, C14:0 and C18:1n-9, CO contains mainly of C18:1n-9 and C18:2n-6 while COCO is abundant with the monosaturated fatty acid C12:0 (*Table 2*). The fatty acid profile of the oil was well reflected in the fatty acid profiles in the experimental diets (*Table 3*). The PUFA (C20:4n-6 - C22:6n-3) were detected in all of the VO experimental diets due to the use of FM and 50% FO in each diet.

TABLE 1. INGREDIENT COMPOSITION OF THE EXPERIMENTAL DIET AND PROXIMATE COMPOSITION (% of dry matter)

Ingredients	Experimental diet					
	FO	CPO	RBD POo	CPKO	CO	COCO
[a] Fish meal	65.98	65.98	65.98	65.98	65.98	65.98
[b] Tapioca starch	14.75	14.75	14.75	14.75	14.75	14.75
Alfa- cellulose	4.50	4.50	4.50	4.50	4.50	4.50
[c] Carboxymethyl cellulose (CMC)	1.50	1.50	1.50	1.50	1.50	1.50
[d] Vitamin premix	3.00	3.00	3.00	3.00	3.00	3.00
[e] Mineral premix	2.00	2.00	2.00	2.00	2.00	2.00
[f] Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00
[g] Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Fish oil (FO)	6.77	0.77	0.77	0.77	0.77	0.77
[h]Crude palm oil (CPO)	-	6.00	-	-	-	-
[i]Refined, bleached and deodourised palm olein (RBD POo)	-	-	6.00	-	-	-
[j]Crude palm kernel oil (CPKO)	-	-	-	6.00	-	-
[k]Corn oil (CO)	-	-	-	-	6.00	-
[l] Coconut oil (COCO)	-	-	-	-	-	6.00
	Proximate composition (% of dry matter basis)					
Moisture	10.09	11.45	10.02	10.28	10.17	10.33
Crude protein	52.94	52.99	51.62	51.91	51.76	51.65
Crude lipid	12.18	12.75	12.36	12.87	12.14	12.36
Crude ash	15.76	15.89	15.78	15.77	15.80	15.85

Note: [a]Fish meal - Danish fish meal, Denmark (75.78% crude protein). [b]Tapioca starch - Tapioca AAA brand, Bake With Me Sdn Bhd. [c]Carboxymethyl cellulose (CMC) - Sigma. [d]Vitamin premix - (g kg^{-1} premix): ascorbic acid, 45.0; Inosito, 5.0; choline chloride, 75.0; niacin, 4.5; riboflavin, 1.0; pyridoxide HCl, 1.0; thiamine HCl, 0.92; D - calcium panothenate, 3.0; retinyl acetate, 0.60. Vitamin D3, 0.083; menadione, 1.67; DL alpha tocopherol acetate, 8.0; D - biotin, 0.02; folic acid, 0.09; Vitamin B12, 0.00135. All ingredients were diluted with alpha cellulose to 1 kg. [e]Mineral premix - (g kg^{-1} premix): calcium phosphate monobasic, 270.98; calcium lactate, 327.0; ferrous sulphate, 25.0; magnesium sulphate, 132.0; potassium chloride, 50.0; sodium chloride, 60.0; potassium iodide, 0.15; copper sulphate, 0.785; manganese oxide, 0.8; cobalt carbonate, 1.0; zinc oxide, 3.0; sodium salenite, 0.011; calcium carbonate, 129.274. [f] Dicalcium phosphate - R&M chemical. [g]Chromic oxide - Sigma. [h]Crude palm oil (CPO) - supplied by Sawit Kinabalu. [I]Refined, bleached and deodourised palm olein (RPOo) - supplied by Sawit Kinabalu. [J]Crude palm kernel oil (CPKO) - supplied by Sawit Kinabalu. [k]Corn oil (CO) - cooking oil. [l]Coconut oil (COCO) - cooking oil.

Feeding trial

The feeding trial was conducted at the Fish Hatchery, Borneo Marine Research Institute of Universiti Malaysia Sabah, Malaysia. The fish was obtained from a local fish farm at Tawau, Sabah, Malaysia. The fish were acclimatised to experimental condition for a week while feeding with commercial diet (Leong Hup Feedmill, Malaysia; crude protein 44%, crude lipid 8%) before the feeding trial. Triplicate groups of hybrid grouper juvenile of initial body weight (BW) 11.12±0.04 g were each stocked into 100 litres fibre glass tank at 20 individuals/tank for each dietary treatment. All of the tanks were connected to a flow through water system with water flow rate at 5 litres min^{-1}. The tanks were placed under roofed hatchery which was exposed to natural photoperiods of almost 12 hr light and 12 hr dark. During the feeding trial, the fish were hand-fed up to apparent satiation twice daily at 0800 hr and 1500 hr, for a total of 12 weeks. The daily feed intake was recorded to estimate the feed utilisation.

The BW and total length (TL) of fish were measured individually at the beginning and end of the feeding trial, whereas bulk BW was weighed biweekly. Prior to every measurement, the fish were starved for 24 hr to ensure that there was no food in the digestive tract. A commercial anaesthetic (Transmore, NIKA brand) was used to anaesthetise the fish before taking the weight and length measurements to minimise the handling stress. At the end of feeding trials, fish from each treatment were sacrificed for biochemical analysis and estimation of body indices.

Biochemical Analysis

Proximate composition analyses. Experimental diet and fish whole body proximate composition analyses were done according to the standard method AOAC (1999). The moisture content was determined by drying the sample in oven at 105°C for 24 hr; ash content was determined by incinerating the dry sample in muffle furnace at 550°C for 5 hr. Crude protein (Nx6.25) was determined by digesting

TABLE 2. FATTY ACID COMPOSITIONS (% of total fatty acids) OF DIFFERENT LIPID SOURCES

Fatty acids	Type of lipid source					
	FO	CPO	RBD POo	CPKO	CO	COCO
C 8:0	-	-	-	3.28	-	6.13
C10:0	-	-	-	2.62	-	5.12
C12:0	0.13	0.12	0.14	48.77	-	51.11
C14:0	7.69	0.83	0.79	15.54	0.03	18.66
C16:0	22.80	44.63	42.33	8.01	10.78	8.74
C16:1	7.41	0.13	0.14	0.03	0.07	0.01
C18:0	3.38	3.59	3.84	1.91	1.63	2.73
C18:1n-9	15.20	41.13	43.04	17.39	28.58	5.61
C18:2n-6	1.05	8.75	9.35	2.53	57.31	1.31
C18:3n-3	0.80	0.21	0.12	-	0.77	-
C20:0	0.32	0.28	0.28	0.09	0.32	0.07
C20:1	6.69	0.11	0.11	0.08	0.18	0.03
C20:4n-6	1.23	-	-	-	-	-
C20:5n-3	17.75	-	-	-	-	-
C22:1n-9	3.41	-	-	-	-	-
C22:6n-3	9.19	-	-	-	-	-
Total saturates	35.07	49.45	47.38	80.22	12.76	92.56
Total monoenes	32.71	41.37	43.29	17.50	28.83	5.65
[1]Total PUFA	30.08	8.96	9.47	2.53	58.08	1.31
Total n-3	27.80	0.21	0.12	0.00	0.77	0.00
Total n-6	2.28	8.75	9.35	2.53	57.31	1.31
n-3/n-6	12.20	0.03	0.02	0.00	0.02	0.00

Note: Minor fatty acids (<1%) not listed was: C4:0, C6:0, C11:0 C13:0, C14:1, C15:0, C17:0, C18:2n9, C18:3n-6, C20:3n6, C22:0, C23:0, C24:0, C24:1. [1] Total PUFA (polyunsaturated fatty acid) - sum of total n-3 fatty acids and total n-6 fatty acids.

FO - fish oil.
CPO - crude palm oil.
RBD POo - refined, bleached and deodourised palm olein.
CPKO - crude palm kernel oil.
CO - corn oil.
COCO - coconut oil.

the sample in concentrated sulphuric acid with two tablet of catalyst (Kjeltabs, FOSS Tecator, Sweden) following Kjeldahl method using automatic system model Kjeltec™ 2300, Foss, Germany. Crude lipid was extracted using petroleum benzene (boiling point 40°C-60°C) with Soxtec™ 2043, Foss, Germany.

Fatty acid analysis. Fatty acid analysis was performed on the FO, VO, experimental diets, muscle and liver of the fish after the feeding trial. The crude lipid was extracted using chloroform: methanol (2:1,v/v) following Folch *et al.* (1957) and fatty acid methyl esters (FAME) was obtained after the *trans*-methylation process using sodium hydroxide in methanol (NaOH-methanol) and hydrogen chloride in methanol (HCl-methanol) (Yoshikara and Satoh, 1989). The FAME was analysed using gas chromatography (Shimadzu GC-2010, Shimadzu Corporation, Kyoto, Japan) with helium as carrier gas and nitrogen (N_2) as make up gas. The FAME was separated using a capillary column (60 m x 0.25 mm ID; BPX70 column, SGE Australia Pty Ltd, Ringwood, Victoria, Australia). The temperature programme ranged from 80°C-230°C (4°C min^{-1} from 80°C to 180°C and 2°C min^{-1} from 181°C to 230°C). The peaks obtained were identified by comparing the retention times of each peak with known standards (Supelco™37 Component FAME mix, Supleco Inc. Bellefonte, USA).

Statistical Analysis

Data on growth performance, survival, feed utilisation, body indices and body composition of hybrid grouper juveniles were analysed using one way analysis of variance (ANOVA) in the Statistical Package for the Social Science, SPSS 20.0. Tukey's multiple range tests were used to determine significant differences between all of the treatment means at P values is < 0.05.

RESULTS

Growth Performance, Survival and Feed Utilisation

At the end of the feeding trial, fish fed with RBD POo achieved the highest growth and it was significantly higher (P<0.05) than fish fed with other diets in terms of weight gain (WG) and specific

TABLE 3. FATTY ACID COMPOSITIONS (% of total fatty acids) OF EXPERIMENTAL DIETS

Fatty acids	Experimental diets					
	FO	CPO	RBD POo	CPKO	CO	COCO
C12:0	0.12	0.10	0.12	26.81	0.04	26.48
C14:0	7.08	3.60	3.67	12.73	3.25	15.15
C14:1	0.22	0.12	0.12	0.14	0.13	0.13
C15:0	0.44	0.22	0.23	0.25	0.22	0.24
C16:0	19.89	34.95	33.44	14.50	15.74	16.73
C16:1	6.28	2.35	2.64	2.52	2.36	2.69
C17:0	0.32	0.18	0.19	0.15	0.17	0.15
C18:0	2.94	3.44	3.47	2.58	2.33	3.26
C18:1n-9	13.88	31.16	32.76	16.11	23.64	10.83
C18:2n-6	2.11	6.44	6.57	2.71	34.28	2.65
C18:3n-3	1.02	0.67	0.57	0.58	1.04	0.57
C20:0	0.29	0.31	0.29	0.18	0.33	0.17
C20:1	9.09	5.40	5.24	5.86	5.47	4.97
C20:4n-6	0.99	0.31	0.31	0.32	0.30	0.33
C20:5n-3	13.04	1.50	1.06	1.50	0.88	1.58
C22:1n-9	9.34	1.08	1.00	0.07	0.07	0.16
C22:6n-3	9.82	5.20	5.05	5.59	5.20	5.97
Total saturates	31.09	42.80	41.42	60.06	22.03	66.39
Total monoenes	38.81	40.11	41.76	24.70	31.67	18.78
[1]Total PUFA	26.97	32.82	32.77	22.74	45.14	21.70
Total n-3	23.88	7.37	6.68	7.69	7.12	8.12
Total n-6	3.10	6.75	6.88	3.03	34.58	2.98
n-3/n-6	7.70	1.09	0.97	2.54	0.21	2.72

Note: Minor fatty acids (<1%) not listed was: C4:0, C11:0, C13:0, C18:2n9, C18:3n-6, C20:3n6,C22:0, C23:0, C24:0, C24:1. [1]Total PUFA (polyunsaturated fatty acid) - sum of total n-3 fatty acids and total n-6 fatty acids.

FO - fish oil.
CPO - crude palm oil.
RBD POo - refined, bleached and deodourised palm olein.
CPKO - crude palm kernel oil.
CO - corn oil.
COCO - coconut oil.

growth rate (SGR) (*Table 4*). On the other hand, the lowest growth was observed in fish fed with CO diet. Besides, the growth performance of fish fed with FO, CPO, CPKO, CO and COCO diets showed no significant differences among the treatments. The experimental diets did not influence the survival of the fish as high survival rate of 98.81%-100% was observed among the treatments.

For feed utilisation, significantly higher total feed intake was found in fish fed with RBD POo diet compared to fish fed with CO diet ($P<0.05$). No significant difference was found among fish fed with CPO, CPKO, CO and COCO diets with FO diet. For food conversion ratio (FCR), no significant effect was found on fish fed the different experimental diets. However, overall, the fish fed with FO, CPO, RBD POo and CO demonstrated better FCR in the range of 1.26-1.34, while fish fed with CPKO was slightly higher at 1.47 and the highest was in the fish fed with COCO at 1.62. The protein efficiency ratio (PER) was not significantly different among the treatments. However, higher efficiency was found in fish fed with FO, CPO and RBD POo. Similarly, no significant differences were observed on the net protein utilisation (NPU), although fish fed with RBD POo and CO showed higher NPU than other diets.

Body Indices and Whole Body Proximate Composition

Results on the body indices and the whole body proximate composition of hybrid grouper juvenile at the end of the feeding trial are presented in *Table 5.* The condition factor (CF) was not significantly different among the treatments ($P>0.05$). The hepato-somatic index (HSI) in fish fed with FO, CPO and RBD POo was significantly higher than fish fed with CPKO and COCO. On the other hand, the viscerosomatic index (VSI) was significantly higher in fish fed with CO and COCO compared to fish fed with CPKO while no significant difference was observed among other treatments. For intraperitoneal fat (IPF), an almost similar pattern with HSI was observed.

The whole body proximate composition showed that the body protein of fish fed with CPO and RBD POo was almost similar with the fish fed with FO without significant difference ($P>0.05$). While significantly higher body protein content was observed in fish fed with CPKO, CO and COCO

TABLE 4. GROWTH PERFORMANCES, SURVIVAL AND FEED UTILISATION OF HYBRID GROUPER JUVENILE IN 12 WEEKS OF FEEDING TRIAL

Variable	Experimental diet					
	FO	CPO	RBD POo	CPKO	CO	COCO
[1]IBW (g/fish)	11.13±0.88	11.15±0.03	11.15±0.04	11.15±0.11	11.12±0.06	11.12±0.1
[2]FBW (g/fish)	121.93±2.82[a]	122.34±3.60[a]	148.57±9.0[b]	122.16±1.24[a]	109.95±4.23[a]	121.15±4.48[a]
[3]WG (%)	995.76±2.85[a]	997.13±3.61[a]	1232.75±9.02[b]	995.58±1.18[a]	888.82±4.22[a]	989.65±8.9[a]
[4]SGR (%)	4.05±1.17[a]	4.12±1.20[a]	4.32±1.29[b]	4.17±1.25a	3.99±1.21[a]	4.00±1.14[a]
Survival (%)	99.52±0.81	100.00	99.05±2.52	98.81±2.50	99.52±0.82	98.81±2.50
[5]FI (g/fish)	153.52±2.70[a]	162.44±2.37[ab]	176.06±5.31[b]	145.75±5.31[ab]	132.38±2.76[a]	156.39±5.67[ab]
[6]FCR	1.26±0.07	1.32±0.16	1.29±0.11	1.47±0.14	1.34±0.08	1.62±0.43
[7]PER	1.36±0.80	1.32±0.15	1.36±0.12	1.19±0.11	1.250.07	1.20±0.14
[8]NPU	22.75±1.38	23.12±2.54	24.28±2.08	21.90±2.05	26.67±1.47	23.22±2.68

Note: Mean ± standard deviation (n=3); within the same row with different letters are significantly different at P< 0.05.[1]IBW - initial body weight. [2]FBW - final body weight. [3]WG - weight gain = 100 × [final body weight (g) - initial body weight(g)]/initial body weight (g). [4]SGR - specific growth rate = 100 × [Ln final body weight (g) - Ln initial body weight (g)]/(days of feeding trial). [5]FI - feed intake = (total feed intake (g)/number of fishes) in 12 weeks of feeding trial. [6]FCR - feed conversion ratio = dry feed consumed (g)/wet weight gain (g). [7]PER - protein efficiency ratio = wet weight gain (g)/total protein intake (g). [8]NPU - net protein utilisation = 100 × (final – initial fish body protein)/total protein intake.

FO - fish oil.
CPO - crude palm oil.
RBD POo - refined, bleached and deodourised palm olein.
CPKO - crude palm kernel oil.
CO - corn oil.
COCO - coconut oil.

TABLE 5. BODY INDICES AND WHOLE BODY PROXIMATE COMPOSITION OF HYBRID GROUPER JUVENILE AT THE END OF THE 12 WEEKS OF FEEDING TRIAL

Variable	Experimental feed					
	FO	CPO	RBD POo	CPKO	CO	COCO
[1]CF	3.16±0.94	3.09±0.28	3.35±0.44	3.04±0.13	2.99±0.25	2.93±0.22
[2]HSI (%)	1.38±0.28[b]	1.51±0.18[b]	1.52±0.08[b]	1.15±0.19[a]	1.31±0.20[ab]	1.16±0.07[a]
[3]VSI (%)	10.31±0.3[ab]	10.39±0.60[ab]	10.40±0.67[ab]	9.23±1.32[a]	11.31±0.86[b]	11.59±1.19[b]
[4]IPF (%)	2.44±0.16[ab]	2.50±0.30[ab]	2.88±0.29[b]	2.32±0.60[ab]	2.10±0.10[a]	2.34±0.12[ab]
Moisture	70.68±0.39	68.14±1.34	70.28±0.10	69.23±1.72	70.72±1.65	69.86±0.63
Protein	16.42±0.6[a]	16.99±0.40[a]	17.40±0.22[a]	18.66±0.4b	18.44±0.1[b]	18.48±0.01[b]
Whole body lipid	4.43±0.16[a]	4.54±0.29[a]	4.47±0.47[a]	4.44±0.10[a]	5.28±0.08[b]	5.42±0.20[b]
Ash	5.68± 0.10	5.98±0.29	5.77±0.02	6.37±0.73	5.56±0.22	5.89±0.20
Liver lipid (dry weight)	25.29±1.2[b]	29.88±1.33[c]	30.67±0.75[c]	21.37±0.51[a]	26.42±0.4[b]	21.31±1.05[a]
Muscle lipid (dry weight)	7.62±1.82[a]	7.83±1.06[a]	7.93±0.57[ab]	6.59±0.84[a]	9.20±1.19[ab]	10.91±0.72[b]

Note: Mean ± standard deviation (n=3); within the same row with different letters are significantly different at P< 0.05. [1]CF-condition factor = [fish body weight(g)/(total length(cm))^3] × 100. [2]HSI - hepatosomatic index = [liver weight(g)/ fish body weight(g)] × 100, [3]VSI - viscerosomatic index = [viscera organ weight(g)/fish body weight (g)] × 100, [4]IPF- intraperitoneal fat = [Intraperitoneal fat weight (g)/ fish body wet weight(g)] × 100.

FO - fish oil.
CPO - crude palm oil.
RBD POo - refined, bleached and deodourised palm olein.
CPKO - crude palm kernel olein.
CO - corn oil.
COCO - coconut oil.

than fish fed with FO (P<0.05). The body lipid content showed that the fish fed with FO, CPO, RBD PO and CPKO was significantly lower than the fish fed with CO and COCO. While the body moisture and ash content was not affected by the treatments.

Fatty Acid Profile

Generally, the major fatty acid profile of the fish muscle (*Table 6*) and liver (*Table 7*) reflected the fatty acid profile of respective experimental diets (*Table 3*).

TABLE 6. FATTY ACID COMPOSITIONS (% of total fatty acids) AND LIPID CONTENT OF FISH MUSCLE TISSUE AT THE END OF THE FEEDING TRIAL

Fatty acid	Experimental diets					
	FO	CPO	RBD POo	CPKO	CO	COCO
C12:0	2.04±0.50^{b}	-	-	12.56±0.15^{c}	-	17.45±0.30^{d}
C14:0	7.55±0.10^{b}	3.42±0.07^{a}	3.22±0.01^{a}	13.43±0.17^{c}	3.56±0.40^{a}	15.01±0.08^{d}
C16:0	25.58±0.06^{d}	34.06±0.12^{f}	32.37±0.08^{e}	18.89±0.14^{a}	20.34±0.34^{b}	22.10±0.07^{c}
C16:1	4.57±0.01^{e}	2.10±0.02^{ab}	2.36±0.01^{c}	1.98±0.06^{a}	2.27±0.08 b^{c}	2.64±0.02^{d}
C18:0	6.00±0.02^{d}	6.37±0.01^{cd}	5.68±0.03^{b}	5.47±0.09^{b}	4.69±0.12^{a}	6.22±0.07^{c}
C18:1n-9	16.35±0.07^{b}	29.18±0.07^{e}	30.84±0.08^{f}	19.60±0.20^{c}	22.07±0.41^{d}	14.35±0.01^{a}
C18:2n-6	2.30±0.01^{a}	6.28±0.03^{c}	6.18±0.01^{c}	3.85±0.03^{b}	24.38±0.26^{d}	2.44±0.01^{a}
C18:3n-3	0.66±0.07b^{c}	0.46±0.02^{ab}	0.45±0.01^{a}	0.77±0.04^{d}	0.66±0.05^{cd}	0.43±0.02^{a}
C20:1	4.32±0.03^{d}	3.04±0.04^{a}	2.95±0.01^{a}	3.97±0.06^{c}	3.52±0.04^{b}	3.94±0.02^{c}
C20:4n-6	1.29±0.02^{d}	0.59±0.01^{ab}	0.64±0.01b^{c}	0.73±0.01^{c}	0.53±0.04^{a}	0.59±0.01^{ab}
C20:5n-3	7.03±0.01^{f}	3.19±0.010^{b}	3.94±0.05^{e}	3.77±0.01^{ab}	2.53±0.04^{d}	3.50±0.01^{c}
C22:1n-9	0.82±0.01^{c}	0.36±0.01^{a}	0.34±0.02^{a}	0.48±0.04^{b}	0.36±0.03^{a}	0.54±0.01^{b}
C22:6n-3	18.45±0.10^{d}	9.74±0.07^{a}	9.35±0.06^{a}	12.20±0.17^{c}	10.35±0.24^{b}	9.40±0.07^{a}
Total saturates	41.17±0.17	43.85±0.06	41.27±0.04	50.35±0.14	28.59±0.30	60.78±0.13
Total monoenes	27.40±0.02	35.42±0.03	37.17±0.03	27.91±0.06	28.93±0.04	22.20±0.02
[1]Total PUFA	29.35±0.03	19.65±0.07	20.49±0.04	20.72±0.04	40.36±0.17	16.04±0.04
Total n-3 PUFA	26.08±0.07	13.39±0.23	13.74±0.08	16.73±0.05	13.53±0.45	13.34±0.10
Total n-6 PUFA	3.27±0.02	6.26±0.02	6.75±0.02	3.98±0.02	26.830±0.17	2.70±0.03
Muscle lipid content (%)	7.62±1.82^{a}	7.83±1.06^{a}	7.93±0.57^{ab}	6.59±0.84^{a}	9.20±1.19^{ab}	10.91±0.72^{b}

Note: Minor fatty acids that less than 1% were not listed. These included C4:0, C13:0, C15:0, C20:0, C22:0, C23:0, C24:0.
[1]Total PUFA (polyunsaturated fatty acid) - sum of total n-3 fatty acids and total n-6 fatty acids.
FO - fish oil.
CPO - crude palm oil.
RBD POo - refined, bleached and deodourised palm olein.
CPKO - crude palm kernel oil.
CO - corn oil.
COCO - coconut oil.

TABLE 7. FATTY ACID COMPOSITIONS (% of total fatty acids) OF LIVER TISSUE OF HYBRID GROUPER JUVENILE

Fatty acid	Experimental diets					
	FO	CPO	RBD POo	CPKO	CO	COCO
C12:0	-	-	-	5.32 ±0.06^{a}	-	9.17 ±0.14^{b}
C14:0	9.92±0.74^{c}	4.60±0.40^{a}	5.25 ±0.86^{ab}	17.18 ±3.20^{d}	5.77 ±0.43^{ab}	23.21 ±0.35^{d}
C16:0	39.48±1.01^{b}	45.57±0.26^{c}	45.23±0.58^{bc}	30.11±1.58b^{c}	33.57±4.00^{ab}	31.46 ±0.78^{ab}
C16:1	7.15±1.15^{b}	3.02±0.62^{a}	3.22±0.38^{a}	3.69 ±0.17^{a}	3.37 ±0. 50^{a}	3.69 ±0.17^{a}
C18:0	7.20±0.56^{c}	5.30±0.33^{a}	5.80±0.69^{ab}	6.65 ±0.09^{a}	5.26 ±0.19^{a}	6.65 ±0.07^{bc}
C18:1n-9	19.27±1.40^{ab}	29.67±0.78d^{e}	33.12±0.15^{e}	15.38±0.20b^{c}	25.26 ±0.21d^{c}	15.38 ±0.16^{a}
C18:2n-6	1.16±0.22^{a}	2.23±0.20^{a}	2.60±1.23^{a}	1.03 ±0.08^{a}	12.05 ±0.77^{b}	1.03 ±0.08^{a}
C20:1	5.69±0.68	4.12±1.44	4.80±0.43	5.75 ±1.00	5.96 ±0.16	5.75 ±1.01
C20:5n-3	1.79±0.24^{b}	0.35±0.02^{a}	0.72±0.05^{a}	0.31 ±0.02^{a}	0.30 ±0.70^{a}	0.31 ±0.02^{a}
C22:1n-9	0.67±0.05^{a}	0.37±0.02^{a}	0.35±0.05^{a}	0.47 ±0.09^{c}	4.80 ±0.82^{b}	0.38 ±0.08^{a}
C22:6n-3	14.23±0.69^{d}	6.39±0.22^{b}	7.57±0.22^{c}	2.87 ±0.10^{a}	3.37 ±0.34^{a}	2.97 ±0.06^{a}
Total saturates	56.60±0.77	55.47±0.33	56.28±0.71	59.26 ±1.24	44.60 ±1.54	61.32 ±0.40
Total monoenes	32.78±0.82	37.18±0.72	41.49±0.25	25.29 ±0.37	39.39 ±0.43	25.20 ±0.36
[1]Total PUFA	18.43±0.22	10.25±0.15	12.28±0.14	4.44 ±0.08	18.73 ±0.47	4.76 ±0.07
Total n-3 PUFA	16.02±0.47	6.75±0.12	8.29±0.14	3.18 ±0.06	3.67±0.52	3.28 ±0.04
Total n-6 PUFA	2.41±0.15	3.25±0.31	3.99±0.25	1.26 ±0.08	15.06 ±0.77	1.48 ±0.08
Liver lipid content (%)	25.29±1.2^{b}	29.88±1.33^{c}	30.67±0.75^{c}	21.37±0.51^{a}	26.42±0.4^{b}	21.31±1.05^{a}

Note: Minor fatty acids that less than 1% were not listed. These included: C4:0, C13:0, C17:0, C20:0, C22:0, C23:0, C24:0, [1]Total PUFA (polyunsaturated fatty acid) - sum of total n-3 fatty acids and total n-6 fatty acids.
FO - fish oil.
CPO - crude palm oil.
RBD POo - refined, bleached and deodourised palm olein.
CPKO - crude palm kernel oil.
CO - corn oil.
COCO - coconut oil.

There were significant differences among all the fatty acids between the diets. Comparison between the fatty acid profile of the muscle and liver showed that in all of the treatments the fish muscle contained higher percentage of PUFA particularly C20:4n6, C20:5n-3(EPA) and C22:6n-3(DHA), while the liver contained higher percentage of saturated (C14:0, C16:0, C18:0) and monosaturated (C16:1, C18:1n-9) fatty acids.

DISCUSSION

The present study showed that all of the hybrid grouper juvenile responded well to the experimental diets which were 50% partially replaced with various VO. The different types of VO with different fatty acid profile affected the growth of the fish. Hybrid grouper juvenile fed with CPO, CPKO, CO and COCO showed almost similar growth performance with the control fish fed with FO. Fish fed with RBD POo showed significantly higher body weight gain (WG), and SGR than other diets ($P<0.05$). Growth of the hybrid grouper brown-marbled grouper x giant grouper in the present study was comparable to those reported in previous studies (Firdaus *et al.*, 2016; Mohd Faudzi *et al*., 2017; Ismail *et al.*, 2018). Besides, the survival of the hybrid grouper in the present study was not affected by the diets. Similar results have also been reported in studies on hybrid grouper (Firdaus *et al.*, 2016; Mohd Faudzi *et al*., 2017; Ismail *et al.*, 2018).

In the present study, hybrid grouper fed with RBD POo showed significantly better growth than fish fed with FO and almost higher feed efficiency compared to FO. This is different with the parental fish, brown-marbled grouper. At similar level of FO replacement with the present study, the brown-marbled grouper fed with RBD POo showed no significant difference on growth compared to fish fed with FO (Mohd Faudzi, 2013). Similarly, the study on humpback grouper, *C. altivelis* (Shapawi *et al*., 2008) reported at 50% replacement level of FO with CPO and RBD POo no significant different on the growth was observed compared to the control fish fed with FO diet. Among the VO tested, RBD POo is the most suitable VO for hybrid grouper. The highest growth performance and feed intake in fish fed with RBD POo showed that the hybrid grouper can utilise the feed well for growth.

In barramundi, *Lates calcarifer*, a complete replacement of FO with CPO and RBD POo in a poultry meal-based diets also demonstrated similar growth performance with fish fed the control diet FO (Wan Ahmad *et al*., 2013). Positive results on the use of PO products were also documented on freshwater fish (Kamarudin *et al.*, 2012; Ng *et al.*, 2013). Study on Malaysian mahseer, *Tor tambroides* highest growth performance was found in fish diet replaced with 50% and 100% of palm oil (Kamarudin *et al.*, 2012). In a study on red hybrid tilapia, *Oreochromis* sp., the growth performance and feeding efficiencies in fish fed with crude PO at 50% replacement level was not significantly affected compared to the control FO diet (Ng *et al.*, 2013). On the other hand, study on red sea bream, *Pagrus major* showed that the substitution of PO at 60% or higher affected the growth of the fish compared to control diet (Komilus *et al.*, 2008) and substitution of palm oil at 69% also affected the growth of gilthead sea bream, *Sparus aurata* (Fountoulaki *et al.*, 2009). These demonstrated that the potential use of PO products in aquaculture feed depends on the species and the level of oil used in the feed.

The present study showed that the growth performance and feed utilisation of hybrid grouper fed with COCO diet was comparable to the control diet FO although the FCR was slightly higher in fish fed with COCO. This is in agreement with previous study that the growth of fish was not affected by the COCO, CO, or squid oil. However, the feed efficiency and FCR were better in fish fed with CO compared to COCO and squid oil (Fitriyani *et al.,* 2015). Although limited study can be found on the use of COCO in marine fish, COCO is commonly reported to be a good replacement for FO in freshwater fish (Bahurmiz and Ng, 2007; Aderolu and Akinremi, 2009; Babalola and Adebayo, 2007; Apraku *et al.,* 2017). Studies on African catfish, *Clarias gariepinus* showed that better growth performance was found in fish fed with COCO when compared with control that fed with FO (Bahurmiz and Ng, 2007; Aderolu and Akinremi, 2009; Babalola and Adebayo, 2007). Study in Nile tilapia, *Oreochromis niloticus* showed that 100% replacement of FO with COCO is possible as it resulted in better growth performance and FCR with no significant difference with the FO diet (Apraku *et al.,* 2017).

The growth performance was lowest in hybrid grouper fed with CO diet, however, it was not significantly different with fish fed with FO diet. Similarly, in orange spotted grouper, *E. coloides* at 50% replacement of FO, no significant difference in growth performance and survival was found between the fish fed with FO and CO treatments (Lin *et al.,* 2007). Study on malabar grouper, *E. malabaricus* also resulted in no significant difference in growth and feed utilisation, and improvement of the non-specific immune system in fish fed with feed containing 50% of CO compared to FO-based diet (Lin and Shiau, 2003; 2007). In freshwater fish, study on gibel carp, *Carassius auratus gibelio* had showed that CO can be replaced up to 50% with anchovy oil in the diet without negative effects on the growth performance (Chen *et al.*, 2011).Total replacement is possible in the red hybrid tilapia, *Oreochromis* sp. (Al-Souti *et al.*, 2012) without any significant effects on the growth performance, FCR, and whole body proximate composition. The present study showed that COCO or CO can be an alternative source of lipid for hybrid grouper without compromising their growth.

The present study showed that the different VO influenced the whole body composition of the hybrid grouper. The whole body proximate composition showed that hybrid grouper contained higher body protein level in fish fed with CPKO, CO and COCO compared to fish fed with FO, CPO and RBD POo. However, it showed contrasting result in the parental fish, brown-marbled grouper (Mohd Faudzi, 2013) and in humpback grouper (Shapawi *et al.*, 2008) as the whole body composition was not influenced by the VO replacement. Similar result was reported in common carp, *Cyprinus carpio* L. that 50% replacement using CO resulted in higher protein content in fish body than fish fed with FO diet and 100% replacement level also showed similar result in tambaqui, *Golossoma macropomum* (Pereira *et al.*, 2018). Similar result had been reported in different level of replacement of FO using palm oil (Komilus *et al*., 2008) and soyabean oil (Komilus *et al*., 2015) in *Pagrus major*.

In the present study, fish fed with CPO and RBD POo showed higher HSI value while CPKO and COCO showed significantly lower value ($P< 0.05$). Besides, a similar trend of the liver fat content with the HSI was also demonstrated significantly higher liver fat content was found in fish fed with CPO and RBD POo, and lowest in fish fed with CPKO and COCO. This suggests that the HSI in the present study is affected by the liver fat content among other factors. The experimental diets fatty acids profile showed that diets consisting of CPO and RBD POo contained high percentage of saturated palmitic (C16:0) and monounsaturated oleic acids (C18:1n-9) while CPKO and COCO showed high content of saturated fatty acid lauric (C12:0) and myristic acids (C14:0). The fatty acids may have influenced the deposition of fat in the liver. The present result is almost similar with the study on Japanese seabass that showed fish fed with diets added with C16:0, C18:0, C18:1n-9 or C18:3n-3 showed higher HSI and liver lipid content compared to fish fed with medium chain fatty acids C8:0 and C10:0 (Xu *et al*., 2016). In contrast with HSI and liver fat content, the VSI, whole body lipid content and muscle lipid content in the present study showed higher values in fish fed with CO (high level of C18:2n-6) and COCO (high level of C12:0 and C14:0). This showed that diets containing CPO and RBD POo prompted higher lipid accumulation in the liver that led to higher HSI, while diets containing CO and COCO induced higher values of VSI and muscle lipid deposition compared to other diets. This suggested oil with different fatty acid profile can affect the body indices, deposition of fat in the liver and muscle. However, studies on other groupers showed that no significant difference on the HSI was reported for humpback grouper (HSI 1.18-1.73), *C. altivelis* (Shapawi *et al.*, 2008), orange spotted grouper (HSI 1.6-2.2), *E. coioides* (Lin *et al.*, 2007) when fed the fish with feed partially replaced with VO.

It is well documented that the dietary fatty acid composition can directly influence the fish body fatty acid composition (Izquierdo *et al*., 2003; Al-Souti *et al*., 2012; Komilus *et al*., 2015; Xu *et al*., 2016; Monge-Ortiz *et al*., 2018) and similarly it was observed in the present study. At the end of the 12-week feeding trial, 50% of FO replacement with VO resulted in slightly lower level of n-3 PUFA and increased n-6 PUFA in fish muscle. Besides, considerable amount of DHA and EPA were accumulated in the muscle as shown in all the treatments. This is in agreement with a previous study which showed that malabar grouper, *E. malabaricus* was capable of directly incorporating dietary DHA and EPA into body tissue, in which diets low in DHA level tended to promote DHA deposition (Wu *et al.*, 2002). Comparison between the fatty acid profile of muscle and liver, it demonstrated that the muscle contained relatively higher percentage of most of the PUFA such as C18:2n-6, C20:4n-6, EPA, DHA while the liver contained relatively higher percentage of the saturated and monounsaturated fatty acids. This demonstrated different utilisation or deposition of fatty acid between the two tissues and this is in agreement with previous studies (Izquierdo *et al*., 2003). Studies on *P. major* (Komilus *et al.*, 2015) fed with different level of soyabean oil had resulted in high amount of EPA and DHA content in the muscle while high amount of total saturated fatty acid in the liver. Similar results was also found in greater amberjack, *Seriola dumerili* (Monge-Ortiz *et al*., 2018) fed with 50% and 100% replacement of fish oil using mixture of (4:1 v/v, palm oil:linseed oil). Besides, Turchini *et al.* (2009) reviewed that when fish were fed with diet containing high concentration of saturated fatty acid it resulted in significantly less muscle deposition. The difference in the fat deposition of fish could be due to species specific differences. Further investigation on organ histology, hematology and sensory assessment should be carried out to determine the full potential of RBD POo as the preferred lipid sources in hybrid grouper juvenile.

CONCLUSION

Overall, VO tested in the present study have potential to be used as an alternative to partially replace FO in hybrid grouper feed. The present study showed that at 50% replacement of FO with RBD POo is the most suitable VO for hybrid grouper as it promoted higher growth and feed utilisation. The different

VO contributed to the differences in body condition, whole body composition, lipid deposition and fatty acid profile in the muscle and liver.

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