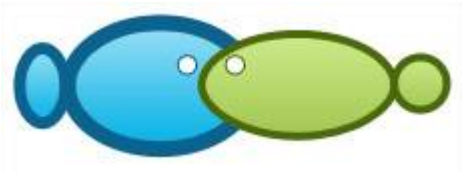

Effects of the replacement of dietary fish oil by soybean oil on growth performance, body composition, and fatty acid profiles of rabbitfish (*Siganus guttatus*) under mono- and polyculture systems

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Abstract. To evaluate the possible use of soybean oil (SO) as a dietary lipid in the herbivorous teleost rabbitfish *Siganus guttatus*, fingerlings were cultured under two different types of cultivation (mono- and polyculture with white shrimp *Litopenaeus vannamei* and fed with three practical diets including six treatments: rabbitfish monoculture fed with a full fish oil (FO) diet (RM-FO6); rabbitfish monoculture fed with the partial replacement of 50% FO with 50% SO (RM-FO3); and rabbitfish monoculture fed with the partial replacement of 85% FO with SO (RM-FO1). Similarly, in rabbitfish polyculture fed with a full FO diet (RP-FO6); rabbitfish polyculture fed with the partial replacement of 50% FO with 50% SO (RP-FO3); and rabbitfish polyculture fed with the partial replacement of 85% FO with SO (RP-FO1). Each treatment was performed in triplicate. After a ten-week feeding trial, the best growth performance of the cultured animal including final weight, total biomass, total weight gain, and feed conversion ratio were found in the RM-FO6 and RP-FO6 treatments ($p = 0.01$). Body composition parameters remained the same among treatments ($p = 0.06$). There was a linear relationship between the fatty acid composition of the fish lipid and the dietary fatty acid composition. Whole-body fish fatty acid composition, such as total saturated fatty acids and total monounsaturated fatty acids increased sharply with increasing dietary SO. However, concentrations of arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) were reduced significantly with increasing levels of dietary SO. The results suggest that under the current experimental conditions, FO in feed could be replaced by SO by at least 50% without important adverse effects on the performance or the body composition and only a minor effect on the fatty acid composition in the rabbitfish (e.g. 5% decline in docosahexaenoic acid). Increased levels of dietary SO (≥ 85%) affected the fatty acid profile of the fish, reducing the levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA).
Key Words: dietary lipid, fatty acids, *Siganus guttatus*, soybean oil, vegetable oils, white shrimp.

Introduction. Aquaculture is now the largest user of fish meal (FM) and fish oil (FO) (FAO 2020). Three-fourths of the world's FM and FO, extracted from ocean-caught small fish, are currently used in aquaculture feeds (Sarker et al 2016), resulting in FM and FO becoming scarce, and expensive. In the future, global fish oil production, with the rapid growth of aquaculture, may not be enough to cover the need for aquafeeds. The demand for fish and shellfish, high in essential amino acids and fatty acids, especially long-chain omega-3 fatty acids (n-3) (Naylor et al 2009), is leading to an increasing interest in the search for alternative ingredients for the formulated diets.

Among potentially suitable candidates for the replacement of FM and FO, vegetable oils have been considered to be more sustainable and even less expensive than fish oil (Montero et al 2008). Successful replacement of fish oil with vegetable oils would reduce both the absolute dependence on marine fish oils and the production costs (Piedecausa et al 2007). Some vegetable oils such as soybean, linseed, sunflower, corn, and olive oils, have been reported to be good alternative lipid ingredients for a full or

partial replacement of FO in the diets of white shrimp *Litopenaeus vannamei* (González-Félix et al 2010; Kumar et al 2018), sharpsnout seabream *Diplodus puntazzo* (Piedecausa et al 2007), seabass *Dicentrarchus labrax* (Yildiz & Sener 2004), Nile tilapia *Oreochromis niloticus* (Yones et al 2013), gilthead sea bream *Sparus aurata* (Montero et al 2008) and rabbitfish *Siganus canaliculatus* (Saoud et al 2010; Monzer et al 2017). Generally, freshwater fish are considered to have the capacity to convert C18 PUFA to the longer C20 and C22 homologues (Al-Souti et al 2012). In contrast, marine fish species are not or weakly able to synthesize long-chain polyunsaturated fatty acids (LC-PUFA) from linoleic and linolenic acids (abundant in many vegetable oils) (Tocher 2003) and thus, FO rich in LC-PUFA must be provided in the diet (Montero et al 2008). The use of vegetable oils as the only lipid source in marine fish diets is often limited by insufficient levels of essential fatty acids such as arachidonic (ARA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids, by anti-nutritional factors and poor palatability (Francis et al 2001).

Rabbitfish *Siganus guttatus*, belonging to the family Siganidae (Lam 1974), is a native species in Southeast Asia including Vietnam (Ayson et al 2014). It is an important economic and commercial fish with tasty white meat (Syah et al 2020). Because of its osmoregulation capacity, rabbitfish inhabits coastal areas, including estuaries and the sea, it adapts to both brackish water and saltwater (Lam 1974; Ayson et al 2014). Furthermore, rabbitfish, a non-aggressive and tolerant species to changing conditions (e.g., temperature and salinity in water), can be cultured in monoculture conditions with high stocking density (Saoud et al 2008; Syah et al 2020) as well as polyculture conditions with shrimp (Luong et al 2014). They are herbivores, and responsive to artificial food (Syah et al 2020). As herbivores, their formulated diets can contain less FO and more vegetable oils, contributing to sustainability and lowering costs (Montero et al 2008). Moreover, and this for the first time in a euryhaline herbivorous fish, rabbitfish was reported to have the ability to convert linoleic and linolenic acids to corresponding LC-PUFA (Li et al 2008; Xu et al 2012; Xie et al 2015), as this fish possess the key genes (Δ5/Δ6 fad, Δ4 fad, elovl5, and elovl4) encoding the enzymes required for LC-PUFA biosynthesis (Li et al 2008; Li et al 2010; Monroig et al 2012). Le Van Bao (2017) also detected that there is no significant difference in the levels of ARA in rabbitfish larvae collected from the wild and hatchery, indicating that rabbitfish may also have the ability to convert LA and LNA to LC- PUFA. The present study aims to evaluate the effect of partial replacement of FO by SO on growth performance, body composition, and fatty acids composition of rabbitfish under two different types of cultivation (mono- and polyculture).

Material and Method

Experimental diets. Three experimental diets were formulated to be iso-nitrogenous (360 g kg^{-1} crude protein), iso-energetic (≥ 360 Kcal kg^{-1}), and iso-lipidic (100 g kg^{-1} lipid) based on the suitable nutrient values for rabbitfish (Parazo 1990; Ghanawi et al (2011) and for white shrimp (National Research Council 2011). In the experimental diets, FO was partially replaced by SO. The control diet (FO6) was formulated to be similar to nutritionally complete, commercially available diets with 60 g kg^{-1} FO as the lipid source. The experimental diet 1 (FO3) had a partial replacement of 50% FO with 50% SO (30 g kg^{-1} FO, 30 g kg^{-1} SO) and in experimental diet 2 (FO1), nearly 85% FO was replaced by SO (10 g kg^{-1} FO, 50 g kg^{-1} SO) (Table 1).

Table 1

Ingredients and proximate composition of the experimental diets

Ingredients (g)	*Diets*		
	FO6	*FO3*	*FO1*
Fish meal	340	340	340
Soybean meal	260	260	260
Corn starch	160	160	160

Rice bran	150	150	150
Vitamin and mineral premix	2.40	2.40	2.40
Fish oil (cod liver oil)	60	30	10
Soybean oil	0	30	50
Binder	6	6	6
Proximate composition (g kg^{-1})			
Dry matter	869	875	874
Crude protein	358	354	355
Crude lipid	101	105	104
Crude fiber	32	31	34
Ash	77	75	78
Cross energy (Kcal/100g)	3624	3619	3620
Main fatty acid (% total fatty acids)			
C14:0	4.86	3.97	3.15
C16:0	17.34	18.83	20.58
C18:0	11.56	12.77	13.26
16:1	2.74	2.43	2.25
18:1	12.45	14.05	16.31
18:2n-6	14.57	16.98	18.99
18:3n-6	0.38	0.33	0.22
18:3n-3	3.98	4.38	4.97
20:3n-6	1.12	1.01	0.86
20:4n-6 (ARA)	0.89	0.86	0.45
20:4n-3	1.57	1.61	1.45
20:5n-3 (EPA)	7.58	4.98	3.46
22:6n-3 (DHA)	11.89	7.68	4.15
ΣSFA	35.18	37.31	38.29
ΣMUFA	18.75	19.92	21.71
ΣPUFA	42.22	39.08	36.38
Σn-3 PUFA	25.02	19.49	14.99
Σn-6 PUFA	17.20	19.59	21.39
n-3/n-6 PUFA	1.45	0.99	0.70
EPA/DHA	0.64	0.65	0.83

Note: SFA = saturated fatty acids; MUFA = plant-based monounsaturated fatty acids; PUFA = polyunsaturated fatty acid double bonded; ARA = arachidonic acid (20:4n-6); EPA = eicosapentaenoic acid (20:5n-3); DHA = docosahexaenoic acid (22:6n-3).

Experimental animals. The study was carried out from June to August 2022 at the Aquaculture research Center of Hue University of Agriculture and Forestry, Hue University, Vietnam. Fingerlings of rabbitfish and disease-free post-larval Pacific white shrimp *Litopenaeus vannamei* were bought from a commercial hatchery, which was given a quarantine certificate, located in Thua Thien Hue province, Vietnam. Upon arrival at The Research Center (Hue University of Agriculture and Forestry, Hue University), the aquatic animals were acclimated to laboratory conditions for 3-4 weeks. Salinity was slowly adjusted to 15-20 ppt by adding additional freshwater over a period of two weeks. Fish and shrimp were hand-fed at a feeding rate of 5% body weight per day using 38% crude protein commercial pellets (GroBest Co. Ltd. Vietnam). Aquatic organisms remained in the nursery tanks until they reached the right size for the feeding trial (fish 4.50 g and shrimp 1.25 g).

Experimental design. A 2 x 3 factorial design, indoor experiment conducted in eighteen cement tanks (5 m^3) for ten weeks, was performed by analyzing factor 1 (monoculture/polyculture) and factor 2 (three different partial replacements of FO by SO) in the following treatments: rabbitfish monoculture fed with a full FO supplementation diets (RM-FO6), served as a control treatment; rabbitfish monoculture fed with the experimental diet 1, had partial replacement of 50% FO with 50% SO (RM-FO3); and rabbitfish monoculture fed with the experimental diet 2, had partial replacement of 85% FO with SO (RM-FO1). Similarly, in rabbitfish polyculture fed with a full FO

supplementation diet (RP-FO6); rabbitfish polyculture fed with the experimental diet 1, had partial replacement of 50% FO with 50% SO (RP-FO3); and rabbitfish polyculture fed with the experimental diet 2, had partial replacement of 85% FO with SO (RP-FO1). All experimental treatments were randomly distributed among the tanks with three replicates per treatment.

In the rabbitfish monoculture treatments, fish fingerlings (4.50±0.12 g) were stocked at a density of 30 fish m^{-3} (biomass of 675 g $tank^{-1}$). However, in the rabbitfish polyculture treatments, fish were stocked at a density of 15 fish m^{-3}, and white shrimp (1.25±0.14 g) were added into polyculture tanks at a density of 54 shrimp m^{-3} to get total biomass of 675 g $tank^{-1}$ (Table 2). Water from the sea was filtered through a sand filter and pumped into a container tank (50 m^3) where seawater was diluted with groundwater to produce the experimental seawater (15-20 ppt). Aeration was supplied constantly in experimental tanks by air stones throughout the experiment period to maintain dissolved oxygen (DO) concentrations at high levels (≥ 5 mg L^{-1}). The light intensity was kept at 600 lux using a fluorescent lamp with a 12 h light/dark regime.

Throughout the experiment, aquatic animals were hand-fed the experimental diets four times a day (06:00, 10:00, 14:00, and 18:00 h). Feeding rates started at 5% total biomass per day and was adjusted according to the satiation of the fish and shrimp. Feed amounts were calculated and adjusted once per half-month based on biomass estimates after bulk weight was taken. The total amount of feed given to the monoculture treatments (RM-FO6, RM-FO3, and RM-FO1) were 4043.3, 3982.3, and 3798.0, and the polyculture treatments (RP-FO6, RP-FO3, and RP-FO1) were 5568.7, 5542.3, and 5158.7, respectively. The tank bottoms were cleaned daily by siphoning and the water loses due to evaporation and siphoning was compensated (about 5% of the water in each tank every day). Water exchange was performed at a weekly rate of 30% in all of the tanks.

Sample collection and composition analysis. Aquatic organisms (fish and shrimp) were collected at the initial and the end of the experimental period of 70 days. Fish and shrimp from the individual tank were harvested, bulk weighed, and counted. Six shrimp and/or fish from each tank were anesthetized in an ice bath (0°C) and transported on ice (0°C) to the Faculty of Animal Science, Hue of Agriculture and Forestry, Hue University, Vietnam for proximate analysis. At the same time, six shrimp and/or fish from each tank were randomly selected and euthanized in an ice bath (0°C) and stored at -20°C until analysis. Samples of the whole body (shrimp and fish) and feed were collected for proximate composition: crude protein, crude lipid, moisture, and ash contents in triplicate using standard methods (AOAC 2005). In brief, crude protein was determined by measuring nitrogen using the Kjeldahl method; crude lipids were determined by ether extraction using Soxhlet, and ash was measured by oven incineration at 550°C. Moisture content was determined by oven drying at 105°C for 24 h and crude fiber was measured according to official methods, namely AOAC (2005). Lipid extraction and fatty acid proximate composition of those samples was analyzed at the National Institute of Livestock Production, Vietnam. Briefly, lipids were extracted by following the method of Folch et al (1957) and quantified gravimetrically after drying under nitrogen. Fatty acids were transesterified with boron trifluoride and fatty acid methyl esters (FAME) were analyzed with a Shimadzu GC-17A gas chromatograph (Shimadzu Scientific Instruments Inc., Portland, OR, USA) equipped with a capillary column (Omegawax 530, 30 m×0.53 mm×0.5 µm film thickness, Supelco 2-4019, Sigma-Aldrich, Oslo, Norway) using helium as the carrier gas and a flame-ionization detector as previously described by Quintero et al (2011) FA were identified by comparison of retention times to those of known standards and they were quantified by using an internal standard, nonadecanoic acid methyl ester (C 19:0, Sigma-Aldrich, St. Louis, MO, USA); they were expressed as percent of the total identified FAME.

Water quality analysis. Daily water temperature, dissolved oxygen (DO), pH, and salinity in all tanks were measured *in situ* using a portable DO meter (Hanna Model HI-9146, Rumani), pH meter (pH/temperature Hanna Model-HI98190, Rumani), and a refractometer (Atago Model 2491-master's, Japan), respectively. Water in the

experimental tanks was sampled once every week at 09:00h for analyses of total alkalinity, nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and phosphate (PO_4^3-P) using multi-spectrophotometers (Hanna Model-HI83099, Rumani). Total phosphate in water samples was measured following the procedures in the Standard Methods for the Examination of the Water and Wastewater (APHA 1998).

Calculations. The following calculations were used to determine the growth performance and feed utilization (Sandre et al 2017):

$$\text{Biomass (g)} = \text{sum of individual weight} \times \text{tank}^{-1}$$

$$\text{Total weight gain (g)} = \text{final total weight} - \text{initial total weight}$$

$$\text{Daily growth rate (g day}^{-1}\text{)} = (\text{final wet weight} - \text{initial wet weight}) \times \text{days of culture}^{-1}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake} \times \text{total wet weight gain}^{-1}$$

$$\text{Survival (\%)} = (\text{number at harvest} \times \text{number at stocking}^{-1}) \times 100$$

$$\text{Protein efficiency ratio (PER)} = \text{mean weight gain (g)/crude protein intake (g)}$$

Statistical analysis. Parametric assumptions (water quality parameters, animal production, proximate composition, fatty acid profile) were checked using Levene's test for homogeneity of variances and Shapiro-Wilk's test for normality. Survival of shrimp and fish were analyzed using arcsin 0.5 transformed data. Data were normally distributed, homoscedastic, and were compared by using two-way ANOVA, followed by Tukey's test. Significant differences between the treatments and their interactions were analyzed according to Zar (2010). All data were analyzed using the SPSS program version 20.0 for Windows.

Results

Environmental parameters. Water quality parameters such as salinity (18-21 ppt), temperature (26.3-33.2^{o}C), DO (4.65-6.50 mg L^{-1}), pH (7.2-8.0), alkalinity (80-110 mg $CaCO_3$ L^{-1}), phosphate (0.4-2.5 mg L^{-1}), and N-NO_2 (0.15-0.54 mg L^{-1}) tended to be similar among treatments. These water parameters were not affected by either polyculture/FO replacement or the interaction between polyculture and FO replacement ($p > 0.05$). Except for NH_4^+-N (0.22-0.94 mg L^{-1}) and NO_3 (0.75-5.71 mg L^{-1}), these water parameters were significantly lower in polyculture tanks as compared to those in monoculture tanks ($p = 0.01$ and 0.03, respectively). In general, water parameter values in the experimental tanks seemed to be stable throughout the experimental period and were within the optimum ranges for growing rabbitfish and white shrimp according to Lam (1974) and Van Wyk et al (1999).

Growth performance. At the end of the feeding trial, fish and shrimp survival varied from 85 to 88% and 88 to 89%, respectively, and there were no significant differences among treatments ($p > 0.05$), indicating that all diets were well accepted by the animal (Table 2). The final weight (FW) and daily growth rate (DGR) of animals (fish and shrimp) were not affected by polyculture but influenced by FO replacement. These growth parameter values in RM-FO6 and RP-FO6, and RM-FO3 and RP-FO3 treatments were higher than those in RM-FO1 and RP-FO1 treatments ($p < 0.001$), indicating that SO at high inclusion limited growth. However, there were no significant differences between RM-FO6 and RP-FO6 and RM-FO3 and RP-FO3 treatments ($p > 0.05$). These results suggested that replacing FO with SO may be done up to 50%, without affecting individual growth. On the contrary, total biomass (2780-4003 g $tank^{-1}$), total weight gain (2105-3328 g $tank^{-1}$), and protein efficiency ratio (PER: 1.79-1.92) were significantly affected by both FO replacement and polyculture ($p = 0.01$ and 0.001, respectively), with FO replacement having a negative effect and polyculture a positive effect. Even though in the polyculture tanks considerable more feed was supplied (see Material and Method) over the experimental period, the FCR was lower than in the respective monocultures.

Table 2

Effect of FO replacement with SO and polyculture on growth, biomass, and nutritive utilization of rabbitfish and white shrimp cultured for ten weeks

Indicator	*Treatment*						*Significance (p-value)*[a]		
	RM-FO6	*RM-FO3*	*RM-FO1*	*RP-FO6*	*RP-FO3*	*RP-FO1*	*FO*	*P*	*FO x P*
Stocking									
Fish									
- Density (fish m^{-3})	30	30	30	15	15	15	-	-	-
- Initial weight (g $fish^{-1}$)	4.50±0.09	4.50±0.14	4.50±0.16	4.51±0.11	4.50±0.18	4.51±0.12	-	-	-
- Biomass (g $tank^{-1}$)	675.35±4.05	675.45±2.65	675.40±5.45	338.35±5.63	337.48±4.37	338.27±5.84	-	-	-
Shrimp									
- Density (shrimp m^{-3})	-	-	-	54	54	54	-	-	-
- Initial weight (g $shrimp^{-1}$)	-	-	-	1.25±0.14	1.25±0.10	1.25±0.11	-	-	-
- Biomass (g $tank^{-1}$)	-	-	-	337.05±6.14	337.86±5.10	337.14±5.92	-	-	-
Combined biomass (g $tank^{-1}$)	675.35±7.05	675.45±5.65	675.40±8.15	675.40±6.95	675.34±8.55	675.41±7.14	-	-	-
Harvesting									
Fish									
- Biomass (g $tank^{-1}$)[a]	2985±52.22[b]	2926±41.77[b]	2780±45.24[b]	1521±37.58[a]	1505±50.39[a]	1451±74.81[a]	ns	***	ns
- Final weight (g $fish^{-1}$)[a]	22.96±0.15[b]	22.80±0.26[b]	21.83±0.52[a]	23.05±0.75[b]	22.92±0.61[b]	21.86±0.39[a]	**	ns	ns
- Daily growth rate (g day^{-1})[a]	0.31±0.00[b]	0.31±0.01[b]	0.29±0.01[a]	0.31±0.01[b]	0.30±0.01[b]	0.29±0.01[a]	**	ns	ns
- Survival (%)[a]	87±3[a]	86±4[a]	85±3[a]	88±1[a]	88±2[a]	88±3[a]	ns	ns	ns
Shrimp									
- Biomass (g $tank^{-1}$)[b]	-	-	-	2482±13.85[b]	2448±26.96[b]	2199±12.26[a]	-	-	-
- Final weight (g $shrimp^{-1}$)[b]	-	-	-	10.33±1.11[b]	10.17±1.47[b]	9.23±1.12[a]	-	-	-
- Daily growth rate (g day^{-1})[b]	-	-	-	0.15±0.01[b]	0.15±0.00[b]	0.13±0.01[a]	-	-	-
- Survival rate (%)[b]	-	-	-	89±1[a]	89±2[a]	88±2[a]	-	-	-
Combined									
- Total biomass (g $tank^{-1}$)[a]	2985±52.22[b]	2926±41.77[b]	2780±45.24[a]	4003±43.51[d]	3953±17.69[d]	3650±67.66[c]	**	***	ns
- Total weight gain (g $tank^{-1}$)[a]	2310±54.87[b]	2250±41.85[b]	2105±71.64[a]	3328±58.04[d]	3278±13.21[d]	2975±61.88[c]	**	***	ns
- Overall FCR[a]	1.75±0.03[c]	1.76±0.04[c]	1.80±0.02[d]	1.67±0.02[a]	1.69±0.03[a]	1.73±0.02[b]	**	***	ns
- PER (%)[a]	1.83±0.03[b]	1.83±0.04[b]	1.79±0.02[a]	1.92±0.02[d]	1.91±0.03[d]	1.86±0.02[c]	**	***	ns

The data correspond to the mean of three replicates ± standard deviation. Mean values in the same row with different superscript letters differ significantly ($p < 0.05$); FO = fishoil; P = polyculture; FO x P = fishoil x polyculture interaction; Ns = not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; [a] Results from split-plot two-way ANOVA and Tukey's test; [b]one-way ANOVA and Tukey's test ($p < 0.05$); FCR = feed conversion ratio; PER = protein efficiency ratio.

Table 3

Effect of FO replacement with SO and polyculture on proximate composition content of rabbitfish

Composition (%)	*Stocking fish*	*Harvesting fish*						*Significance (p-value)[a]*		
		RM-FO6	*RM-FO3*	*RM-FO1*	*RP-FO6*	*RP-FO3*	*RP-FO1*	*FO*	*P*	*FO x P*
Moisture	74.30±1.16	73.82±1.58[a]	74.31±1.47[a]	75.19±1.21[a]	74.80±1.29[a]	74.75±1.40[a]	74.78±1.72[a]	ns	ns	ns
Crude protein	21.89±0.82	21.73±1.48[a]	21.72±1.26[a]	21.07±1.01[a]	21.98±1.02[a]	21.82±0.99[a]	21.81±0.54[a]	ns	ns	ns
Crude lipid	7.10±0.86	7.47±0.34[a]	7.42±0.52[a]	7.07±0.20[a]	7.50±0.55[a]	7.31±0.34[a]	7.11±0.30[a]	ns	ns	ns
Ash	2.70±0.89	2.78±0.62[a]	2.85±0.31[a]	2.87±0.32[a]	2.81±0.62[a]	2.84±0.29[a]	2.83±0.24[a]	ns	ns	ns

The data correspond to the mean of three replicates ± standard deviation; mean values in the same row with different superscript letters differ significantly ($p < 0.05$); FO = fish oil; P = polyculture; FO x P = fish oil x polyculture interaction; Ns = not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; [a] Results from split-plot two-way ANOVA and Tukey's test ($p < 0.05$).

Table 4

Effect of FO replacement with SO and polyculture on the fatty acid composition of rabbitfish cultured for ten weeks

Main fatty acids (% total fatty acids)	*Treatment*						*Significance (p-value)[a]*		
	RM-FO6	*RM-FO3*	*RM-FO1*	*RP-FO6*	*RP-FO3*	*RP-FO1*	*FO*	*P*	*FO x P*
14:0	2.52±0.12[b]	2.03±0.48[a]	2.44±0.09[b]	2.60±0.09[b]	2.24±0.18[a]	2.47±0.07[b]	*	ns	ns
16:0	19.47±0.25[a]	19.55±0.46[a]	20.53±0.46[b]	19.22±0.75[a]	19.26±0.30[a]	20.19±0.20[b]	**	ns	ns
18:0	4.28±0.35[a]	4.48±0.16[a]	5.81±0.73[b]	4.13±0.29[a]	4.87±0.40[a]	5.81±0.52[b]	***	ns	ns
16:1	4.05±0.07[a]	4.50±0.47[a]	5.41±0.34[b]	4.17±0.10[a]	4.20±0.23[a]	5.49±0.32[b]	***	ns	ns
18:1	22.88±0.20[a]	23.80±0.30[ab]	24.02±0.44[b]	22.48±0.15[a]	22.69±0.49[ab]	23.92±0.89[b]	*	ns	ns
18:2n-6	13.02±0.32[a]	13.03±0.11[a]	17.54±0.56[b]	13.11±0.14[a]	13.14±0.21[a]	16.76±0.36[b]	***	ns	ns
18:3n-6	1.35±0.21[a]	1.41±0.18[a]	1.79±0.19[b]	1.39±0.18[a]	1.45±0.12[a]	1.66±0.08[b]	**	ns	ns
18:3n-3	0.90±0.02[a]	1.12±0.09[b]	1.28±0.14[c]	0.89±0.05[a]	1.11±0.10[b]	1.32±0.13[c]	***	ns	ns
20:3n-6	0.24±0.01[a]	0.26±0.02[a]	0.47±0.08[b]	0.23±0.01[a]	0.27±0.03[a]	0.54±0.07[b]	***	ns	ns
20:4n-6 (ARA)	1.28±0.04[b]	1.19±0.04[b]	0.87±0.08[a]	1.24±0.11[b]	1.13±0.07[b]	0.93±0.07[a]	***	ns	ns
20:4n-3	1.13±0.08[b]	0.77±0.08[a]	0.75±0.06[a]	1.14±0.05[b]	0.76±0.07[a]	0.72±0.06[a]	***	ns	ns
20:5n-3 (EPA)	3.34±0.10[b]	3.21±0.28[b]	1.60±0.03[a]	3.37±0.23[b]	3.22±0.19[b]	1.74±0.06[a]	***	ns	ns
22:6n-3 (DHA)	16.43±0.22[c]	15.74±0.23[b]	7.70±0.32[a]	16.27±0.16[c]	15.67±0.31[b]	8.00±0.20[a]	***	ns	ns
ΣSFA	27.76±0.22[a]	28.64±0.94[b]	30.43±0.63[c]	27.53±0.69[a]	29.16±0.75[b]	30.31±0.66[c]	***	ns	ns
ΣMUFA	29.80±0.40[a]	30.96±0.28[a]	33.54±0.73[b]	29.74±0.67[a]	30.47±0.62[a]	33.97±0.97[b]	***	ns	ns
ΣPUFA	38.00±0.20[b]	37.34±0.65[b]	33.02±0.79[a]	38.00±0.42[b]	37.28±0.42[b]	32.63±0.37[a]	***	ns	ns
Σn-3 PUFA	21.82±0.11[c]	21.08±0.50[b]	11.33±0.42[a]	21.67±0.13[c]	20.94±0.52[b]	11.79±0.23[a]	***	ns	ns
Σn-6 PUFA	16.18±0.12[a]	16.26±0.33[a]	21.69±0.72[b]	16.31±0.25[a]	16.34±0.18[a]	20.84±0.60[b]	***	ns	ns
n-3/n-6 PUFA	1.35±0.01[c]	1.30±0.02[b]	0.52±0.04[a]	1.33±0.03[c]	1.28±0.05[b]	0.57±0.03[a]	***	ns	ns
EPA/DHA	0.20±0.01[a]	0.21±0.02[a]	0.21±0.01[a]	0.21±0.02[a]	0.21±0.01[a]	0.22±0.01[a]	ns	ns	ns

Data represent the mean±standard deviation of three replicates. Values on the same line and with different superscripts are significantly different ($p \leq 0.05$); SFA (saturated fatty acids): 14:0, 15:0, 16:0, 18:0, 19:0, 20:0, 22:0, 24:0; MUFA (monounsaturated fatty acids): 16:1 18:1, 20:1, 22:1, 24:1; PUFA (polyunsaturated fatty acids): 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:4, 22:5, 22:6; total n-3: 18:3n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:4n-3, 22:6n-3; total n-6: 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6; ARA: arachidonic acid (20:4n-6), EPA: eicosapentaenoic acid (20:5n-3); DHA: docosahexaenoic acid (22:6n-3).

The whole fish body composition. Proximate composition of the whole fish body, at the end of the experiment, including the contents of crude protein (21.07-21.98%), crude lipid (7.07-7.50%), ash (2.70-2.87%), and moisture (73.82-75.19%) was not affected by either polyculture/FO replacement or the interaction between polyculture and FO replacement ($p > 0.05$) (Table 3). The results indicated that replacing FO by SO up to 50% and even 85% has no negative influence on the proximate composition of the fish.

Fatty acids profiles. After 70 days of the feeding trial, the fatty acid compositions of the whole body of the fish were clearly influenced by dietary fatty acids but were not affected by polyculture, and thus displayed significant differences among dietary treatments ($p < 0.05$) (Table 4). The fatty acids 16:0, 18:1, 18:2, and 22:6n-3 (DHA) were, respectively, the most abundant SFA, MUFA, and LC-PUFA in the whole body composition. Replacement of FO with SO (by either 50% or 85%) increased the fish's SFA by 5.94% and 10.10%, respectively, MUFA by 4.10% and 14.22%, respectively, and total n-6 LC-PUFA by 0.49% and 34.05%, respectively as compared to 100% dietary FO. FO replacements reduced the fish's LC-PUFA by 1.74% and 13.11%, respectively, and total n-3 LC-PUFA by 3.39% and 48.08%, respectively. The fish lipid contents of 18:2n-6, 18:3n-6, and 20:3n-6 showed no significant differences between 4 treatments RM-FO6, RP-FO6, RM-FO3, and RP-FO3 ($p > 0.05$). However, these values were significantly different from those in the RM-FO1 and RP-FO1 groups ($p < 0.01$). The same trend can be seen in 20:4n-6 (ARA) and 20:5n-3 (EPA) which were not significantly different between RM-FO6, RP-FO6, RM-FO3, and RP-FO3 treatments ($p > 0.05$) but significantly different as compared with RM-FO1 and RP-FO1 ($p < 0.01$). Values of 22:6n-3 (DHA) and n-3/n-6 PUFA were significantly different among treatments. Yet the decrease of 22:6n-3 (DHA) and n-3/n-6 PUFA was not proportional with the respective decrease in the feed content. The values in the RM-FO3 and RP-FO3 were very close to the values in RM-FO6, and RP-FO6. These fatty acid profiles suggested that replacing FO with SO may be done up to 50% without a drastic impact on the fatty acid profile of the rabbitfish. Hence, in summary, 50% replacement of FO by SO does not influence production and fatty acid profile drastically.

Discussion. In the present study, rabbitfish were grown in monoculture or together with white shrimp in polyculture. In addition, it was verified to what extent fish oil replacement with soybean oil would influence the outcome either in monoculture or polyculture. It appears that rabbitfish growth at the individual level is not affected by the culture system and only slightly affected by a drastic replacement of FO by SO (85%). Thus, rabbit fish growth is to a very large extent independent from the presence of shrimp. At the tank level, much more rabbitfish is produced in the monoculture, but that can be largely explained by the fact that the starting biomass was double relative to the polyculture (Table 2). Significant differences in production parameters at the tank level, such as total weight gain, overall FCR, and PER were observed among the monoculture and polyculture treatments, with the polyculture being slightly more efficient in converting feed into harvestable biomass, even though the feed supplied to the polyculture was approximately 1.4 times higher than to the monoculture (see Material and Method section). These results are consistent with previous studies in which finfish polyculture systems showed higher production as compared to monoculture systems. Luong et al (2014) reported that the yield in shrimp /rabbitfish polyculture (blue shrimp *L. stylirostris* with goldlined rabbitfish *Siganus lineatus*) was 1.7 times larger than in monoculture. Polyculture of *Oreochromis niloticus* and *L. vannamei* increased by 1.2 times in productivity (Junior et al 2012). The total biomass of white shrimp *L. vannamei* and gray mullet *Mugil cephalus* integrated farming was 1.4 times higher than that in white shrimp monoculture (Hoang et al 2018). Until now, there is very little information on the effects of diet FO replacement by vegetable oils on growth performance, feed utilization, and body/fillet nutritional traits of the rabbitfish. A few studies have worked with other marine herbivorous fish species such as rabbitfish *Siganus rivulatus* (Saoud et al 2010; Monzer et al 2017), milkfish *Chanos chanos*, striped mullet *Mugil cephalus* (Turchini et al 2009), and rabbitfish *Siganus canaliculatus* (Xu et al 2012). It appears

that 50% diet FO replacement with SO does not affect growth performance, FCR, and PER. However, 85% diet FO replacement significantly reduced the final weight (FW), DGR, PER, and overall FCR, yet that effect was limited to a maximum of 10%. Interestingly, the inclusion of SO in the fish diets from 0 to 85% did not affect the survival rate of animals (fish and shrimp). These results are in agreement with previous studies indicating that the replacement ratio of FO by SO may be done up to 50% of total dietary lipid in the diets of rainbow trout *Oncorhynchus mykiss* (Figueiredo-Silva et al 2005), European seabass *Dicentrarchus labrax* (Özşahinoğlu et al 2013), and 60% of total dietary lipid in the diet of sharpsnout seabream *Diplodus puntazzo* (Piedecausa et al 2007), or even up to 100% of dietary lipid in the diet of rabbitfish *S. rivulatus* (Saoud et al 2010), mandarin fish *Siniperca scherzeri* (Sankian et al 2019) without any adverse effect on growth performance and FCR. The replacement of FO with ≥ 50% SO in the diet of rabbitfish *S. rivulatus* (Monzer et al 2017) or 80% SO in the diet of gilthead seabream *Sparus aurata* gave lower growth rate values (Izquierdo et al 2005). On the contrary, Xu et al (2012) reported that rabbitfish *S. canaliculatus*, a marine herbivorous species, fed the dietary FO replaced with 33%, 67% and 100% SO resulted in better growth performance compared with the fish fed 100% FO diet. This is probably because *S. canaliculatus* may harbor a physiological system capable of digesting plant materials and thus could utilize SO more effectively than marine carnivorous species (Xu et al 2012). Also, *S. canaliculatus* has been documented to have the ability to convert C18 PUFA into LC-PUFA (Xie et al 2015). It is not known to what extent *S. guttatus*, the species used here, is equally capable of converting C18 PUFA into LC-PUFA, something that remains to be verified.

Under the mono- and polyculture systems, the replacement of the dietary FO with three different levels of SO, in the present study, did not alter the whole fish body composition parameters which are often used to evaluate the nutritional values of fish (Table 3). This was in agreement with previous studies in other marine fish species, which documented that the partial or complete replacement of dietary FO with SO did not affect the body composition of seabass *Dicentrarchus labrax* (Hunt & Tekelioglu 2008), herbivorous species *Siganus canaliculatus* (Xu et al 2012), rabbitfish *Siganus rivulatus* (Saoud et al 2010), and fat snook *Centropomus parallelus* (Bendhack et al 2014). Lipid content in the carcass of sea bass *Dicentrarchus labrax* fed with dietary vegetable oils (soybean, sunflower, corn oils) was also found higher than that in FO groups (Yildiz & Sener 2004). This is probably due to the levels of n-3 PUFA in the diets. Fish oil, in general, have more n-3 PUFA while vegetable oils contain more n-6 PUFA. The diets enriched in n-3 PUFA can suppress fatty acids synthesis, stimulate fatty acid β-oxidation, activate lipoprotein lipase, and reduce triacylglycerol synthesis, resulting in an overall reduction in lipid deposition (Al-Hasani & Joost 2005).

The results indicate that there was a linear relationship between the fatty acid composition of the whole body of the fish and the dietary fatty acid composition. Basically in the treatment in which in the feed, 50% of the FO was replaced with SO, a logically strong reduction in e.g. EPA and DHA was measured (by nearly 50% relative to the FO6 treatments). Yet in the whole body of the fish (FO3 treatments), these fatty acids only slightly decreased (approximately 5% relative to FO6) (Table 1 and 4). This might indicate that rabbitfish, when offered a diet with lower EPA and/or DHA inclusion, may partly produce these fatty acids or may selectively catabolize other fatty acids so that e.g. DHA become enriched. The experimental design here does not allow to distinguish between these two possibilities. Anyhow it indicates that FO can be partially replaced by SO without a drastic effect on the nutritional value of the fish. It is interesting to note that relative to the feed, EPA is lower, ARA and DHA are higher in the fish and their ratio is higher in the feed than in the fish. In the present study, levels of ARA and DHA in the whole body of the fish were 1.4 and 1.8 times, respectively higher than those in the feeding diets. The same case was also explored by Caballero et al (2002) for rainbow trout *O. mykiss*. Several studies have reported that the concentrations of DHA in the tissue of European sea bass *D. labrax* (Mourente & Bell 2006), gilthead sea bream *S. aurata* (Menoyo et al 2004), turbot *Psetta maxima* (Regost et al 2003) and rabbitfish S. *canaliculatus* (Xu et al 2012) were 1.5, 1.6, 1.4 and 2 times, respectively higher than

those in the dietary concentrations. The ratios of n-3 PUFA and n-6 PUFA in the fish, in our study, steadily decreased with an increase in dietary SO and were strongly influenced by the corresponding diet's n3/n6 ratio. This is probably due to an increase in FO replacement by SO resulting in a reduction of n-3 PUFA levels in the fish, while a reverse trend has been detected in levels of n-6 PUFA. This is in agreement with Piedecausa et al (2007) who reported that substitution of FO with vegetable oils, particularly SO, greatly reduced the n-3/n-6 fatty acid ratio of the feeding diet, leading to a reduction of the fish n-3/n-6 lipid ratio. The inclusion of vegetable oils (canola, corn, sunflower, and peanut oils) in the diet of rainbow trout *O. mykiss* led to decreased n-3/n-6 lipid ratio (Dernekbaşı et al 2021). In addition, SFA in the fish is lower than in the feed, MUFA is higher than in the feed and PUFA are more or less stable (with an exception for DHA which increases). All indications are that SFA are preferentially metabolized with a saving effect at the level of DHA. However, a drastic replacement of FO by SO in the diet up to 85%, significantly reduced levels of ARA, EPA, DHA, and n-3 PUFA in the whole body composition of the fish. A reverse trend can be seen in concentrations of total SFA, MUFA, and n-6 PUFA which increased with the increasing replacement of FO by SO. Hence it appears that rabbitfish under the current conditions has a limited ability to convert C18 into n-3 PUFA. These results were in agreement with the previous study of Xu et al (2012) who reported that the increased replacement of dietary FO by SO decreased the dietary levels of ARA, EPA, and DHA resulting in a reduction of these fatty acids in the muscle of rabbitfish *S. canaliculatus*. And, full or partial replacement of FO by vegetable oils (cottonseed and canola oils) in the feeding diets for marine fish species, that are well-known to produce no or very limited amount of DHA, such as rainbow trout *O. mykiss* significantly reduced the lipid contents of ARA, EPA, DHA, and n-3 PUFA (Yıldız et al 2018). The other study by Yıldız et al (2014) also working with rainbow trout, documented the replacement of FO with 100% vegetable oils (sunflower, sesame oils) resulted in a decrease of total n-3 PUFA levels and increased levels of n-6 PUFA in both fillet and liver. The fatty acid composition of tissue lipids in Atlantic salmon has been reported to be influenced by the dietary fatty acid composition (Torstensen et al 2000; Bell et al 2001). It is also clear from the present and previous studies conducted by Bell et al (2001) that up to 50% of dietary FO can be replaced with vegetable oils without or with only modest decreases in the concentrations of ARA, EPA, or DHA in the fish.

Conclusions. The replacement of fish oil with soybean oil as a lipid source in fish diets can be done up to 50% without adversely affecting the growth performance or the whole body composition of rabbitfish *Siganus guttatus*. Especially, the concentration of DHA in the fish lipid was not or only marginally affected upon FO replacement in the rabbitfish diet by SO (50%). However, FO replaced by SO (≥ 85%) modified the fatty acid profiles of the fish lipid, reducing the levels of ARA, EPA, and DHA. The different cultivation models (mono/polyculture) significantly affected the growth performance of rabbitfish *Siganus guttatus* but did not alter the whole fish body composition parameters.

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Conflict of interest. The authors declare that there is no conflict of interest.

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