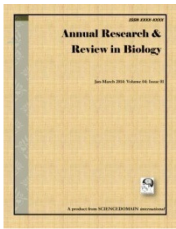

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Terrestrial Animal- and Plant-Based Ingredients as Alternative Protein and Lipid Sources in the Diets for Juvenile Groupers: Current Status and Future Perspectives

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Authors' contributions

This review work was carried out in collaboration between all authors. Author LSL managed the literature searches, designed the study, and wrote the manuscript. Author ASKY and Author RS managed the proof-reading tasks and provided suggestions to improve the manuscript. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

The Epinephelinae groupers are strict carnivorous species. They are widely cultured in the Asia Pacific region as a result of high demand and market price especially in the live reed food fish trade. Groupers are commonly raised in net cages and fed with the unsustainable low value fish. Although this feeding practice has been gradually improved by using formulated diets, the production cost of these diets is high due to the increasing prices of fish meal and fish oil. In order to find sustainable replacements for these expensive commodities, research has been conducted to evaluate alternative ingredients effects on fish growth performances and immune function. This review covers different types of alternative ingredients tested on several species of juvenile grouper. In general, animal protein sources were able to replace fish meal at higher inclusion levels than plant protein sources. Lowered digestibility, deficiency in essential nutrients, and reduced palatability are major challenges in using alternative ingredients in grouper feeds.

Keywords: Epinephelinae groupers; juvenile; protein; lipid; replacement; aquaculture feeds.

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ABBREVIATIONS

FM: fishmeal; FO: fish oil; PER: protein efficiency ratio; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; TAP: terrestrial animal proteins; PMS: processed meat soluble; IW: initial weight; WG: weight gain; FCR: feed conversion ratio; POM: poultry offal meal; FE: feed efficiency; PR: protein retention; PBM: poultry by-product meal; MBM: meat bone meal; FTM: feather meal; BM: blood meal; NRE: nitrogen retention efficiency; ERE: energy retention efficiency; TNW: total nitrogen waste outputs; FRP: fiberglass reinforced plastics; MMBM: processed meat meal and blood meal mixture; FSBM: fermented soybean meal; PE: protein efficiency; SBM: soybean meal; VO: vegetable oil; PUFA: poly-unsaturated fatty acid; SBO: soybean oil; CO: corn oil; SFO: sunflower oil; PO: peanut oil; HUFA: highly unsaturated fatty acid; MUFA: mono-unsaturated fatty acid; CPO: crude palm oil; RBDPO: refined and bleached and deodorized palm olein; CNO: canola oil; HIS: hepatosomatic index; EAA: essential amino acids; CF: condition factor; VSI: viscerosomatic index; IFR: intraperitoneal fat ratio.

1. INTRODUCTION

Groupers are classified under the sub-family Epinephelinae of the family Serranidae. They can be found in tropical and sub-tropical waters of all oceans [1]. Groupers have high a market price and demand. Therefore it is a very popular species for mariculture, especially in China, Vietnam, Malaysia, Thailand, Indonesia, Taiwan [2,3]. Unfortunately, most grouper farmers are challenged by high feed production costs. Groupers are strict carnivorous species which require high dietary protein levels (about 50%). The industry is currently relying on low value fish for feeding. However, this is not a long-term sustainable approach [4]. For example, the supply of low value fish is inconsistent, given the immense demand for this product [5]. Commercial dry formulated diets for groupers, mostly based on fish meal (FM) and fish oil (FO) as the protein and lipid sources, are available. Nonetheless, the continuous increase of FM and FO prices [6] has resulted in increased prices for formulated diets [7,8]. In order to maintain consistent feed supplies and low production costs, alternative protein and lipid sources are critically needed. Studies have been conducted to exploit the usage of protein and oil sources from terrestrial animals and plants as replacements of FM and FO in the diets for juvenile groupers with promising findings. If the diet were formulated correctly, farm-made pelleted feeds with alternative ingredients can provide better growth performance, more efficient feed utilization, and higher survival [9]. This review evaluates the acceptability and effects of these alternative protein and oil sources on the growth performance and health condition of the juvenile groupers. In addition, future grouper feeding challenges are discussed.

2. PROTEIN AND LIPID REQUIREMENT OF JUVENILE GROUPERS

Following the continuous expansion of grouper aquaculture, many studies have been conducted to determine grouper nutritional requirements to optimize the performances of formulated diets. Those studies have been collectively reviewed by Boonyaratpalin [10], Chen [11], Luo et al. [12] and the latest by Williams [4]. These reviews highlighted the requirements for protein (including essential amino acids) and lipid. In general, for optimum growth, juvenile groupers require relatively high dietary protein levels of 46 – 56% (per 100 g diet) and moderately low lipid levels at 10 – 12%. Apart from the literature cited in the review by Williams [4], Yoshii et al. [13] found that approximately 15% dietary lipids were necessary for optimum growth and protein efficiency ratio (PER) for the juvenile kelp grouper

Epinephelus bruneus. Recently, Shapawi et al. [14] demonstrated that 50% and 16% were the optimum dietary protein and lipid levels, respectively, required for juvenile brown-marbled grouper *Epinephelus fuscoguttatus.* However, the lipid levels reported by Yoshii et al. [13] and Shapawi et al. [14] are slightly higher than the levels generally reported for juvenile groupers. Higher lipid levels could provide a protein sparing effect [14].

The role of dietary lipid in enhancing the immune function of juvenile malabar grouper *Epinephelus malabaricus* [15] and orange-spotted grouper *Epinephelus coioides* [16] have also been studied. Both studies [15,16] reported that dietary lipid supplementation can enhance grouper immune responses. Wu et al. [17] stated that docosahexaenoic acid (DHA) was more essential than eicosapentaenoic acid (EPA) for the growth of grouper *E. malabaricus*, based on observations of enhanced growth when the DHA/ EPA ratio was greater than 1. In more recent work, Wu and Chen [18] demonstrated that dietary levels of 2% linolenic and linoleic acids at a ratio of 3:1 led to improved weight gain and non-specific immune responses in juvenile *E. malabaricus*.

3. REPLACEMENT OF FISH MEAL AND FISH OIL IN THE DIETS FOR JUVENILE GROUPERS

Early reviews on the replacement of FM and FO in the diets for juvenile groupers with alternative ingredients from terrestrial animals and plants have been presented by Luo et al. [12] and Williams [4]. Nevertheless, some of the important information such as the growth performance of fish fed on diets with plant proteins, the effects of plant oils on the fish's immune function, and the constraints of replacing FM and FO in the diets with these alternative ingredients were not covered in those reviews. All this information is crucial to the development of practical aquafeeds for groupers culture. Therefore, those earlier and the more recent works are critically re-examined in the following sections.

3.1 Terrestrial Animal Proteins (TAP)

Table 1 shows types of evaluated TAP with their proximate composition, and optimum portions that can be used to replace FM protein in the diets of various sizes and species of juvenile groupers.

In general, all TAP used in these studies contained high levels of crude protein (~ 60 - 90 % dry matter); however, the possible replacement levels which caused no detrimental effect to the cultured grouper were only about 50% or below. Millamena and Golez [19] replaced the Chilean FM in the practical diets for juvenile *E. coioides* with commercial poultry by product meal (PBM)-based processed meat solubles (PMS), by formulating eight isonitrogenous diets with increasing levels of PMS protein (0%, 10%, 20%, 30%, 40%, 60%, 80% and 100%). This was fed to *E. coioides* with initial weight (IW) 2.0±0.15 g in a flow through seawater system for 60 days. The results showed that 40% FM protein could be replaced by the PMS with no adverse effects on the fish weight gain (WG), survival and or feed conversion ratio (FCR).

Usman et al. [20] determined the possibility of poultry offal meal (POM) to replace FM in the diets formulated for the humpback grouper *Cromileptes altivelis* (IW 285±20 g). The fish were maintained in floating net cages that were suspended from a floating platform, and fed with five isonitrogenous and isoenergetic diets for 20 weeks. The five diets contained POM proteins at increasing levels (0%, 16%, 32%, 48% and 64%) replacing the FM protein. This study concluded that up to 48% of the FM protein in the diet can be replaced by POM without compromising the WG, feed efficiency (FE), protein retention (PR), total feed intake and survival rate.

Wang et al. [21] determined the potential of using a blend of poultry by-product meal (PBM), meat bone meal (MBM), feather meal (FTM) and blood meal (BM) to replace herring meal in the practical diets formulated for *E. malabaricus* (IW 51.0±0.2 g). The blended meal comprised of 50% PBM, 20% MBM, 20% FTM and 10% BM. Five diets with 0%, 25%, 50%, 75% and 100% of blended meal replacing the FM protein were prepared and fed to fish that were reared in net pens for 10 weeks. The fish fed with the 25% and 50% blended meal protein diets showed no significant differences compared to those fed with the control diet in WG, FCR, nitrogen retention efficiency (NRE), energy retention efficiency (ERE) and total nitrogen waste outputs (TNW). The fish fed with diets containing 75% and 100% blended meal protein had poorer FCR and TNW, and lower WG, NRE and ERE. This led to a conclusion that the maximum level of herring fish meal protein replacement with the blended meal protein was 50%. An expansion of this study has been conducted by Li et al. [22], examining the results of using PBM, MBM and FTM proteins individually to replace the herring meal protein. A control and seven replacement diets (25%, 50% and 75% PBMprotein; 25% and 50% MBM protein; 25% and 50% FTM protein replacing FM protein) were prepared and fed to the fish (IW 50.2 g) in net pens for 10 weeks. The results showed nosignificant differences in WG, NRE, ERE and TNW between those fish which consumed diets containing 25, 50 and 75% PBM, 25 and 50% MBM, 25% FTM and those that consumed the control diet. The fish fed diet with 50% FTM protein showed lower WG, NRE and ERE but higher TNW. On the other hand, the fish fed diets with 50% MBM, 25% and 50% FTM diets yielded poorer FCR than those fed control diet. In conclusion, the authors recommended that only 24% out of the total FM protein could be replaced by the PBM, MBM, or FTM in the diets formulated for *E. malabaricus* although there are no significant differences in growth and feed utilization among the fish fed with the control, 75% PBM or 50% MBM diets.

Recently, Gunben et al. [23] evaluated the potential of pet food-graded PBM to replace FM in the diet for juvenile *E. fuscoguttatus* (IW 26.2±2 g). A fully FM based diet and another three diets with increasing level of PBM protein as the substitution of the FM protein (50%, 75% and 100%) were prepared and fed to the fish. The fish were maintained in cylindrical cages placed in a 150 ton fiberglass reinforced plastic (FRP) tank. The feeding trial was conducted for 16 weeks. The WG of fish fed diets with 50% PBM inclusion level showed the best result in terms of WG, PER and FCR among all treatments. The PER value for 50% PBM treatment (1.7) was significantly higher than the control treatment (1.0), indicating that the PBM protein was well utilized by the fish.

Nevertheless, there are exceptional cases where the TAP could replace more than half of the total amount of FM used in the grouper diets. Millamena [24] replaced the Chilean FM in the practical diets formulated for juvenile *E. coioides* with processed meat meal and blood meal mixture (MMBM) at a ratio of 4: 1. The experimental designs and protocols were similar to those applied by Millamena and Golez [19]; however in this case, bigger sized fish (IW 6.1±0.55 g) were used. The results showed that the MMBM can be used to replace up to 80% of the FM protein in the diets with no adverse effects on fish survival, WG and FCR.

Table 1. Studies on fish meal replacement in the diets for juvenile groupers with alternative ingredients from terrestrial animals

Terrestrial animal proteins	Proximate composition (% dry matter)	Total protein levels (/100 g diet)	Fish species	Initial weight (g)	Feeding trial duration	Weight gain (%)	Portion of FM protein replaced	Reference no.
Processed meat solubles (PMS) from abbatoir by product	Protein= 72-76; Lipid= 10-12; Ash= 10-12 Moisture= 3-5	44	*E. coioides*	2.0 ± 0.15	60 days	535.0	40%	19
Poultry offal meal (viscera)	Protein= 66.40; Lipid= 5.52 Ash= 20.86; Moisture= 7.27	47	*C. altivelis*	285 ± 20	20 weeks	31.3	48%	20
Poultry by product meal (50%) + meat bone meal (20%) + feather meal (20%) + blood meal (10%)	N/A	52 - 53	*E. malabaricus*	51.0 ± 0.2	10 weeks	155.4	50%	21
Poultry by product meal	Protein= 71.9; Lipid= 12.2 Ash= 13.6; Dry matter= 96.3	52	*E. malabaricus*	50.2	10 weeks	198.4	24%	22
Meat bone meal	Protein= 59.6; Lipid= 14.0 Ash= 23.8; Dry matter= 95.3					184.7		
Feather meal	Protein= 92.8; Lipid= 5.3 Ash= 1.2; Dry matter= 92.5					194.9		
Pet food graded poultry by-product meal (National Renderers Association, Inc., USA)	Protein= 67.58; Lipid= 10.11; Ash= 15.52; Moisture= 3.12	50	*E. fuscoguttatus*	26.2 ± 2	16 weeks	337.6	50%	23
Feed-grade poultry by-product meal (Dindings Ltd., Malaysia)	Protein= 69.25; Lipid= 10.91; Ash= 12.67; Moisture= 4.44	50	*C. altivelis*	12.4 ± 0.2	8 weeks	154.3	75%	25
Pet food graded poultry by product meal (National Renderers Association, Inc., USA)	Protein= 67.58; Lipid= 10.11; Ash= 15.52; Moisture= 3.12					156.7		

According to the study by Shapawi et al. [25], good quality PBM can be used to replace more than half the protein from FM in the diets formulated for *C. altivelis*. In the study, the fish (IW 12.4±0.2 g) were maintained in cylindrical cages placed in a 150 ton seawater polyethylene tank. Six experimental isolipidic and isonitrogenous diets (0%, 50%, 75%, 100% feed grade PBM, 75% and 100% pet food-graded PBM) were formulated for the eight weeks feeding trial. The results of this study were similar to those of Millamena [24], since *C. altivelis* fed on diets with up to 75% of PBM replacement level showed no significant differences in growth performance, survival, feed utilization efficiency, and condition factors compared with that in the control group.

The quality of the terrestrial animal protein meals could be significantly influenced by the animal's origin and processing method used [26]. In addition, the response of the targeted fish might be species specific. Therefore, the full potential of these commodities should be investigated before they can be used in manufacturing fish diets.

3.2 Plant Proteins

Research on the substitution of FM with plant proteins in the practical diets for grouper is rather limited. Luo et al. [27] examined the potential of using fermented soybean meal (FSBM) to replace FM in the diets for *E. coioides* (IW 9.4±0.1 g). A control diet without FSBM inclusion and four isonitrogenous diets with 14, 28, 43 and 57% of FM protein replaced by FSBM protein, respectively, were prepared and fed to fish reared in floating net cages for 56 days. At the end of the feeding trial, the fish that fed on 14% FM protein replaced-diet gained the heaviest weight but was not significantly different from that fed on the 28% FM protein replaced-diet and the control diet. However, the fish FE and protein efficiency (PE) decreased with increased replacement levels of FM. Result from the broken-line regression analysis on WG versus replacement level of FM with FSBM showed that 19% of FM protein in the diets can be replaced by the FSBM protein with no effect on fish growth, FE, PE and body composition.

A recent study by Shapawi et al. [28] reported that soybean meal (SBM) can be used to replace up to 20% to 30% of FM protein in the diets for *E. fuscoguttatus* (IW 13.9±0.7 g). Five isonitrogenous diets were formulated by replacing FM protein at 0%, 10%, 20%, 30% and 40%, and fed to fish reared in tanks with a stocking density of 10 fish per tank. Each experimental diet was fed to fish in tanks in triplicates for 10 weeks. The WG, SGR, FCR, PER and survival of fish fed on diets containing 20% and 30% of SBM were not significantly different from those in the control treatment (0% SBM). Except for the body lipid, the body proximate composition of fish fed on diets with 20% and 30% SBM also showed no significant difference from those in the control treatment. On the other hand, Shiu et al. [29] reported that 30% of FM protein in the diet formulated for juvenile *E. coioides* can be replaced with *Bacillus subtilis* E20-fermented SBM protein without compromising its growth performance and feed efficiency. Apart from this information, little is known about the performance of plant proteins to replace FM in the diets for juvenile grouper.

Morphological changes in the liver and distal intestine were detected in juvenile *E. coioides* fed on diets containing SBM [29]. According to the previous studies on other fish species, dietary SBMs were also reported to cause inflammations of the distal intestine in common carp *Cyprinus carpio* L. [30] and in Atlantic salmon *Salmo salar* L. [31]. Nevertheless, no sign of enteritis was found in other species, such as the juvenile Egyptian sole *Solea aegyptiaca* [32] and cobia *Rachycentron canadum* [33]. SBM-induced morphological changes in liver were reported to occur in rainbow trout *Oncorhynchus mykiss* but not in pacu *Piaractus mesopotamicus* [34]. Apparently, the occurrence of dietary SBM induced-morphological changes in liver and intestine varied among different species. Such changes can be pathological to the fish [35]. Therefore, studies needed to be done to improve the SBM performance before it can be used in the diet formulations for juvenile groupers (as discussed in the following section).

3.3 Plant (Vegetable) Oils

Table 2 presents the fatty acid composition in the experimental diets formulated for juvenile groupers, which were substituted (50% of total dietary lipid content) with several types of vegetable oil (VO). VOs contain higher level of Omega 6, the n-6 poly-unsaturated fatty acid (PUFA) but lower level of Omega 3, the n-3 PUFA than fish oils (see [36] in Table 2), and its inclusion in the diets will affect the fatty acid composition of fish muscle and liver. Lin et al. [37] conducted feeding trial on *E. coioides* (IW 13.2±0.02g) with diets that contained FO, soybean oil (SBO), corn oil (CO), sunflower oil (SFO) or peanut oil (PO) and evidenced that the muscle of fish which fed on FO diet had significantly higher ($P<0.05$) levels of 14:0, 16:0, 16:1n-7 and 20:5n-3 (EPA) and DHA+EPA but lower levels of 18:1n-9, 18:2n-6 and DHA/EPA ratios than those that fed on diets that contained either SBO, CO or SFO. Similar results were found in the muscle of fish fed on diet with PO but interestingly, no significant difference was shown in the levels of 16:0 and DHA+EPA with the muscles of fish fed on FO diet. Livers of fish fed on FO diets contained significantly higher levels of 18:0, 20:5n-3, 22:6n-3, n-3 highly unsaturated fatty acids (HUFA) and DHA+EPA but lower levels of 18:1n-9, 18:2n-6 and mono-unsaturated fatty acid (MUFA) than the livers of fish that fed on the vegetable oils. On the other hand, Shapawi et al. [36] found out that the muscle and liver of *C. altivelis* (IW 10.6±2.2 g) fed on diets with either crude palm oil (CPO), refined, bleached and deodorized palm olein (RBDPO), SBO or canola oil (CNO) contained lower n-3 HUFA and increased levels of 18:2n-6 than those fed on FO diet. Despite these differences, no significant differences were found in fish survival, WG, FCR, PER, hepatosomatic index (HSI) and lipid levels in muscle and liver between the fish fed on diets with VOs and that fed on the control (FO) diet. Such results concluded that groupers can efficiently utilize these dietary oils for growth. Therefore, VOs can be used to substitute FO in the diets formulated for juvenile groupers.

Replacement of FO with VOs was also reported to enhance the immune functions of the juvenile grouper. Lin and Shiau [38] examined the growth and non-specific immune responses of *E. malabaricus* fed on diets which contained corn oil as a substitute. Five semi-purified diets supplemented with FO (F4), corn oil (C4) and a blend of fish oil with corn oil at ratios of 3: 1 (F3C1); 1: 1 (F2C2) and 1: 3 (F1C3), respectively, were prepared and fed to the fish (IW 10.26±0.14 g) that were maintained in aquaria. The WG of fish fed with diets F3C1 and F2C2 showed no significant differences compared with those fed with the control diet (F4). However, the leukocyte superoxide anion (O_2^-) production ratio, plasma lysozyme activity and plasma alternative complement activity (ACH50) in fish fed with F3C1 and F2C2 were significantly higher than those fed with the control diet. The leukocyte superoxide anion is one of the most important anti-microbial components in the defense of phagocytes; lysozyme and alternative complement activities are the humoral immune indicators in fish. The higher the production ratio of leukocyte superoxide anions and activities of the plasma lysozyme and alternative complement represent the better the functioning of the fish's non-specific immune system. Therefore, corn oil can be used to replace up to the half portion of FO in the diet and enhance the non-specific immune responses of grouper. Other than this knowledge, there is no information about the effects of other plant oils on the immune response of juvenile groupers.

Table 2. Fatty acids composition of experimental diets with different types of VO used for the juvenile groupers

Reference no.	37					36				
Fish species	*Epinephelus coioides*					*Cromileptes altivelis*				
Basal diet	White fishmeal					Danish fishmeal, Solvent extracted soybean meal				
Protein and lipid levels	Protein level = 48-49%, lipid level = 10-11%					Protein level = 50%, lipid level = 10%				
Diets with different lipid sources and fish samples	FO	SBO	CO	SFO	PO	CLO	CPO	RBDPO	SBO	CNO
Fatty acids										
C10:0	N/A	N/A	N/A	N/A	N/A	TR	0.3	0.4	TR	TR
C12:0	N/A	N/A	N/A	N/A	N/A	TR	0.9	TR	TR	TR
C14:0	2.5	0.8	0.8	0.9	0.9	8.7	2.7	3.2	3.0	4.5
C14:1	N/A	N/A	N/A	N/A	N/A	0.9	0.4	0.1	0.4	0.8
C16:0	13.7	10.9	11.5	9.3	12.4	15.9	38.2	36.5	15.7	19.7
C16:1	4.2	1.5	1.7	1.5	1.4	8.4	3.9	3.2	5.6	4.8
C18:0	3.0	3.1	2.9	2.8	3.5	4.6	4.8	4.1	4.8	3.5
C18:1n-9	23.4	28.9	31.8	34.9	42.0	19.7	28.8	35.2	21.0	31.5
C18:2n-6	12.7	30.2	33.8	30.6	22.4	4.1	9.2	9.9	31.5	19.7
C18:3n-3	0.1	0.4	0.7	0.5	0.5	1.2	0.3	0.3	3.5	4.2
C20:0	N/A	N/A	N/A	N/A	N/A	0.4	0.2	0.2	0.6	0.5
C20:1	N/A	N/A	N/A	N/A	N/A	10.8	1.1	1.0	1.3	2.7
C20:1n-11	4.6	2.8	2.6	3.1	3.1	N/A	N/A	N/A	N/A	N/A
C20:2	N/A	N/A	N/A	N/A	N/A	TR	TR	TR	TR	0.2
C20:4n-6	0.8	0.5	0.6	0.5	0.6	0.8	0.3	TR	0.6	0.7
C20:5n-3 (EPA)	7.0	2.4	2.5	2.3	2.1	8.5	1.6	1.4	1.9	2.5
C22:1n-9	N/A	N/A	N/A	N/A	N/A	6.1	1.7	TR	4.4	0.2
C22:5n-6	3.1	0.6	0.5	0.8	0.7	N/A	N/A	N/A	N/A	N/A
C22:6n-3 (DHA)	17.6	6.9	6.6	6.3	7.0	9.5	3.5	3.7	5.0	3.5
Total saturates	22.2	14.8	15.2	13.0	16.8	29.7	47.6	44.4	24.4	28.7
Total monoenes	32.2	33.2	36.1	39.5	46.5	46.0	35.8	39.6	32.7	40.2
n-3 HUFA	24.7	9.7	9.8	10.1	9.6	N/A	N/A	N/A	N/A	N/A
DHA/EPA	2.5	2.9	2.6	2.7	3.3	1.1	2.2	2.6	2.6	1.4
DHA + EPA	24.6	9.3	9.1	8.6	9.1	18.0	5.1	5.1	6.9	6.0
Total PUFA	N/A	N/A	N/A	N/A	N/A	24.3	15.0	15.4	42.5	30.8
Total n-3	N/A	N/A	N/A	N/A	N/A	19.2	5.4	5.4	10.4	10.2
Total n-6	N/A	N/A	N/A	N/A	N/A	5.0	9.5	10.0	32.1	20.4
n-3/ n-6	N/A	N/A	N/A	N/A	N/A	3.9	0.6	0.5	0.3	0.5

N/A = not available; TR = Trace

Although the replacement of FO with plant oils in diets are also reported to be of no harm to the immune responses of other fish species, some studies report on the contrary that (see review by Kiron [39]). Further investigations should be carried out on other species of juvenile grouper with more types of plant oil to understand the fish tolerance to plant oils as dietary lipid sources.

4. CONSTRAINTS OF FISH MEAL REPLACEMENT IN JUVENILE GROUPER DIETS

In general, high potential of protein sources from terrestrial animals and plants can be used as alternative protein in the diets of juvenile groupers with lack of adverse effect on fish growth performance and feed utilization. However, the acceptable substitution levels of these proteins in the diets are considerably low, particularly for the plant proteins. Factors that contribute to this limitation are reviewed in the following section.

4.1 Lacking Certain Essential Amino Acids (EAA)

In many cases, the juvenile groupers fed on diets with increased levels of TAP meals attained lower growth than those fed on FM-based diets. Such results could be partly due to the shortage of certain EAA in the TAP meals, compared to that present in the FM used. Table 3 presents the EAA profiles in different types of TAP meals and FM used in the feeding trials on juvenile groupers. Generally, the EAA content in the TAP meals is lower than that in the FM, except for the arginine. The amino acid profiles of groupers may also be used to determine the EAA requirement of the fish as carried out by Millamena and Golez [19] and Millamena [24] to determine the lacking EAA that could possibly contribute to the fish's slow growth.

The dietary amino acid profiles can influence the growth performance and body composition of juvenile groupers [40]. By feeding the E. coioides with formulated diets that contain FM and soybean protein concentrate as the basal diet, and manipulating the dietary EAA content with crystalline amino acids, Luo and colleagues [41,42] evidenced that dietary methionine and lysine levels could influence fish growth performance, feed utilization, whole body protein and lipid contents, condition factor (CF), viscerosomatic index (VSI) and intraperitoneal fat ratio (IFR). Based on the results of the broken-line analysis, the dietary methionine and lysine requirements for juvenile grouper were determined to be 1.31% of the diet (2.73% of dietary protein on a dry weight basis) and 28.3 g kg^{-1} of the diet or 55.6 g kg^{-1} of dietary protein, respectively. Luo et al. [43] also demonstrated that the dietary arginine level plays an important role in supporting weight gain, specific growth rate and body retention of dietary EAA in *E. coioides*. The dietary arginine requirement for the juvenile grouper was determined to be 2.7% of the diet (5.5% of dietary protein), based on the broken-line analysis. In a more recent study by Zhou et al. [44], similar results were obtained on the dietary arginine requirement of juvenile yellow grouper *E. awoara*, which was 2.8% of the diets (6.5% of dietary protein), based on the quadratic regression analysis.

Table 3. Comparisons of EAA profiles among different types of dietary TAP with FM or fish body sample in previous studies

Ingredients and fish samples	Origin / Source	Essential amino acids profile										EAA that lacked in animal meals		Reference no.
		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Tryp	vs. FM used	vs. Fish body	
Processed meat solubles (PMS) from abbatoir by product	daka a.m.b.a, Danish-based commercial feed company	5.82	1.78	2.69	9.74	6.55	1.46	4.39	4.36	3.65	0.35	N/A	His, Ile, Lys, Met, Phe, Thr, Tryp	19
E. coioides body sample	N/A	4.72	2.26	3.14	9.49	8.65	3.43	4.66	5.20	0.15	3.56			
Poultry offal meal (viscera)	Local slaughters, Makassar, South Sulawesi	4.71	0.83	2.26	4.44	3.24	ND	2.04	2.62	2.75	ND	All except Arg and Tryp	N/A	20
Fish meal (5-01-985)	N/A	4.26	1.75	3.09	5.25	5.10	0.13	2.53	3.06	3.34	ND			
Poultry by product meal	National Renderers Association, Inc., USA	4.78	2.21	2.73	4.78	4.32	1.44	2.68	2.67	3.07	1.96	All except Arg and Val	N/A	22
Meat bone meal		3.90	1.69	2.01	3.85	3.29	0.97	2.09	2.16	2.40	1.39	All except Arg	N/A	
Feather meal		6.63	1.10	4.22	7.02	1.93	0.56	4.38	4.26	5.87	2.38	His, Lys, Met	N/A	
Blood meal		4.72	4.88	2.65	9.33	7.21	1.18	5.47	4.09	5.31	2.75	Ile, Met	N/A	
Herring meal	Tianbang Feed Company, Shanghai, China	3.87	2.40	2.95	4.94	6.01	1.98	2.81	2.89	3.06	2.15			
Feed-grade poultry by-product	Dindings Ltd., Malaysia	6.00	1.48	3.10	5.55	3.51	1.43	3.30	3.15	4.14	0.62	All except Arg and Val	N/A	25
Pet food grade poultry by-product	National Renderers Association, Inc., USA	5.64	1.57	2.80	4.99	4.12	1.76	2.84	2.70	3.30	0.63	All except Arg	N/A	
Danish fish meal	N/A	5.05	1.62	3.66	6.11	5.67	2.07	3.48	3.38	4.05	0.69			

Arg = Arginine; His = Histidine; Ile = Isoleucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Val = Valine; Tryp = Tryptophan

Among all types of TAP that have been used to replace FM in juvenile grouper diets, PBM, MBM and FTM were reported to be deficient mainly in methionine and lysine contents [19,20,22,24,25]. In addition, FTM was also deficient in histidine [22]. According to Wang et al. [21], blood meal was rich in lysine hence can be used to balance the dietary lysine level in the diets with PBM, MBM and FTM inclusions. Other than that, supplementation of the crystalline amino acids into these diets can be done [40,41,42,43,44]. More research is necessary to determine the requirement for other EAAs in different species of groupers so that appropriate supplementation can improve the utilization of TAP-based diets in juvenile groupers.

4.2 Apparent Digestibility and Anti-Nutritional Properties of Feed Ingredients

Apparent digestibility of the dietary ingredients is essential to be known to ensure maximum utilization of dietary nutrients by fish. Several studies have been conducted to evaluate the apparent digestibility of many types of ingredients in the diets of juvenile groupers [7,45,46,47] and this information has been well reviewed [4].

The methods of processing can affect the digestibility of the ingredients [4]. Suitable methods of processing [48] or supplementation of enzyme [49] can enhance the ingredients' digestibility. Such information is critically important, especially to the plant-based ingredients that usually contain variety of anti-nutritional properties. The anti-nutritional properties can affect the fish's health and production by limiting the ingredients utilization, mainly on its proteins, minerals and vitamins [50]. Therefore, it should be eliminated or minimized before the ingredients are used for fish feed production. In fact, SBM contains many anti-nutritional properties, including protease inhibitors, lectins, saponins and phytic acid. Shiu et al. [29] reported that juvenile *E. coioides* fed on the diet with *Bacillus subtilis* E20-fermented SBM attained higher activity levels of digestive enzymes in the stomach (pepsin) and in the distal intestine (trypsin, chymotrypsin, amylase, and lipase), and lesser histopathological changes in the liver and distal intestine than those fed on the diet with un-treated SBM. Luo et al. [27] demonstrated that *E. coioides* fed with diets that contained same inclusion level of FSBM attained higher growth, FE and PE than those fed with the non-processed one. Rachmansyah et al. [51] supplemented phytase into the partial SBM-based diets for *C. altivelis* (BW 61.3±0.4 g) and found that it could improve the protein and phosphorus availability and reduce the loading of nitrogen and phosphorus into the environment. Shapawi et al. [28,52] also demonstrated that phytase supplementation into the SBM-based diets can improve the growth performances and feed utilizations of *E. fuscoguttatus* (IW 13.9±0.7 g and 44.57±0.3 g, respectively). More studies should be carried out to determine the effects of plant-based ingredients with different processing methods, and supplementation of different types of enzyme on the growth performance of juvenile groupers. Such comparative studies are necessary to find out the most efficient approach in preparing practical diets with plant proteins for juvenile groupers.

4.3 Poor Palatability of the Diets

Palatability is another concern for the formulated diets with reduced amount of FM since this will affect the feed intake and growth performance of the fish. Usman et al. [20] and Shapawi et al. [25] reported the high inclusion levels (64% and 100%, respectively) of the terrestrial animal proteins did not reduce feed intake in *C. altivelis*. Nevertheless, contradicting results were found in the study by Li et al. [22], using FTM and PBM at 50% and 75% inclusion levels, respectively, on *E. malabaricus*. Such variation could be highly due to not only the quality, source, and processing method of the alternative protein, but also the fish species-specific taste preferences [53]. For the plant proteins, Luo et al. [27] demonstrated that *E. coioides* which were fed with higher inclusion levels of soybean meal attained lower feeding efficiency, and the authors proposed that one of the reasons was that the higher soybean meal content might cause the diet to be less attractive and palatable. In fact, incorporating high contents of SBM in diets also reduced the feed intake of other marine carnivorous species, such as European sea bass *Dicentrarchus labrax* [54], Asian sea bass *Lates calcarifer* [55], red snapper *Lutjanus campechanus* [56], Japanese flounder *Paralichthys olivaceus* [57] and tiger puffer *Takifugu rubripes* [58]. Nevertheless, specific feeding stimulants or attractants for some of these fish species have been identified and have proven to be effective in promoting their feed intake on diets with alternative protein sources [54,57,58,59,60]. Wang et al. [21] reported that supplementation of squid viscera meal into diets with a blend of rendered animal protein ingredients cannot stimulate feed intake of juvenile *E. coioides*. Apart from this, there is no study on determining potential feeding stimulants for groupers. Therefore, investigation on the suitable feeding stimulants to be supplemented in the terrestrial animals or plants proteins-based diets for juvenile groupers should be considered.

5. CONCLUSION

In general, FM and FO in diets formulated for juvenile groupers can be partially replaced with proteins and lipids of terrestrial animals and plants origins to reduce the feed production costs and dependency on fish-based ingredients. High quality proteins from terrestrial animals can be used to fully replace FM with supplementation of some EAAs, particularly lysine and methionine. The usage of plant proteins to replace FM in groupers diets seems to be very limited but is full of potential and yet to be exploited. The usage of SBM can be expanded through some treatments, such as through fermentation process, and by the supplementation of feed enzyme into the diets to improve its digestibility. Nevertheless, more information is needed to ensure the success of these diets with alternative ingredients. In fact, globally scientists are exploiting the usage of plant proteins in the feed of many other fish species. Plants are much easier to be mass cultivated than any terrestrial animals, therefore its supply and price is usually more consistent and cheaper than that of terrestrial animal. However, groupers are strict carnivorous species and they have low tolerance to diets with plant proteins. Studies should focus on how to improve the performance of plant proteins-based feeds for juvenile groupers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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