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EFFECTS OF DIETARY *MORTIERELLA ALPINE* MEAL LEVELS ON GROWTH, BODY FATTY ACID COMPOSITION AND ARACHIDONIC ACID RETENTION FOR ORANGE-SPOTTED GROUPER, *EPINEPHELUS COIOIDES*

Yu-Hung Lin and Man-Ting Chiu

Key words: grouper, arachidonic acid, *Mortierella alpina* meal, utilization.

ABSTRACT

This study investigated the effect of using *Mortierella alpina* meal (MAM) as a dietary arachidonic acid (ARA) source on the growth, whole-body fatty acid composition, and ARA retention of the orange-spotted grouper (*Epinephelus coioides*). Four diets supplemented with 0% (control), 1%, 2%, and 5% MAM actually contained 0.93%, 1.14%, 1.34%, and 1.93% ARA. Four experimental diets were fed to triplicate groups of fish (initial weight: 7.21 ± 0.21 g) in a recirculating system for 8 weeks. The final body weight, weight gain percentage, specific growth rate, daily feed intake, feed efficiency, and survival of fish did not significantly differ ($p > 0.05$) among treatment groups. The fatty acid level of the whole body of fish generally reflected the diet composition. The whole-body ARA concentration of grouper significantly increased with an increase in dietary MAM supplementation levels. The whole bodies of fish fed the control diet and diet containing 1% MAM had higher ARA retention levels than those fed diets containing 2% and 5% MAM. The results demonstrate that grouper can utilize ARA from MAM. However, the utilization of MAM by fish decreased with an increase in dietary supplementation levels.

I. INTRODUCTION

Marine fish are considered to require n-3 highly unsaturated fatty acids (HUFAs), mainly eicosapentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) acids, for normal development and survival (Takashi, 1997). Recently, the National Research Council (NRC, 2011) reported that marine fish also require n-6 polyunsaturated fatty acids (PUFAs) to meet physiological demands. Among n-6 PUFAs, several studies indicated the importance of arachidonic acid (ARA, C20:4 n-6) in fish metabolism because ARA is the main precursor of eicosanoids in fish (Sargent et al., 2002; Rezek et al., 2010) and one of the main components of phosphatidylinositol (Bell et al., 1983; Bell and Dick, 1990). ARA is incorporated more specifically into phosphatidylinositol than into any other lipid class (Linares and Henderson, 1991). In recent years, increasing attention has been paid to the role of ARA in fish, including larval metamorphosis (Lund et al., 2007, 2008), stress resistance (Koven et al., 2001), and growth (Rodriguez et al., 1994; Bessonart et al., 1999).

Pure ARA (chemical grade) is quite expensive. Various oleaginous zygomycetes have potential nutritional benefits because of their specific fatty acid compositions including long-chain (LC)-PUFAs, such as ARA, produced by the fungus *Mortierella alpina* (NRC, 2011). Oil extracted from *M. alpina* has been used to feed marine fish. For instance, *M. alpina* oil was used for dietary ARA enrichment in larvae of yellowtail flounder (Copeman et al., 2002), Senegal sole (Villalta et al., 2005), and common sole (Lund et al., 2007; 2008). However, whether fish can directly utilize dry *M. alpina* meal (MAM) remains unclear.

Groupers are a high-quality seafood in Asia and around the world. They are also good candidates for intensive aquaculture because of their desirable taste, hardiness in a crowded environment, and rapid growth (Chen and Tsai, 1994; Shiau and Lan, 1996). The quantitative requirement for n-6 LC-PUFAs, such as linoleic acid and ARA, in groupers has not been thoroughly investigated. Only one study reported that 0.6%~0.8% ARA is required for groupers to maintain their immunity (Wu, 2011). The current preliminary study investigated the effect of using MAM as a dietary ARA source on the growth, tissue fatty acid composition, and ARA retention of the orange-spotted grouper (*Epinephelus coioides*).

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Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung, Taiwan, R.O.C.

Table 1. Formulation of the experimental diets.

Ingredient	*Mortierella alpina* meal level (%)			
	0	1	2	5
Fish meal (FM, protein 69.30%, lipids 9.34%)	52.96	52.96	52.96	52.96
Soybean meal (protein 48.53%)	17.62	16.79	15.96	13.46
Mortierella alpina meal[1]	0	1	2	5
Fish oil	0.41	0.41	0.41	0.41
Soybean oil	2.53	2.40	2.28	1.90
Squid liver meal (protein 40.90%, lipids 16.34%)	5	5	5	5
Scallop meal (protein 53.83%, lipids 13.57%)	5	5	5	5
α-Starch	9.52	9.78	10.04	10.82
Gluten (protein 74.07%)	3	3	3	3
Vitamin premix[2]	1	1	1	1
Mineral premix[3]	2	2	2	2
Choline chloride	0.1	0.1	0.1	0.1
Cellulose	0.86	0.56	0.26	0

[1] *Mortierella alpina* meal was purchased from Far-East Biochtech (Taipei, Taiwan).

[2] The vitamin mixture supplied the following (mg/g mixture): thiamin hydrochloride, 0.5; riboflavin, 1; calcium pantothenate, 1.5; niacin 2; pyridoxine hydrochloride, 0.4; folic acid, 0.2; *myo*-inositol, 40; L-ascorbyl-2-monophosphate Mg, 30; menadione, 10; alpha-tocopheryl acetate, 20; retinyl acetate, 0.5; cholecalciferol, 0.0025; biotin, 1; vitamin B_{12}, 1. All ingredients were diluted with alpha-cellulose to 1 g.

[3] The mineral mixture supplied the following (mg/g mixture): calcium biphosphate, 135.8; calcium lactate, 327; ferric citrate, 29.7; magnesium sulfate, 137; potassium phosphate (dibasic), 239.8; sodium biphosphate, 87.2; sodium chloride, 43.5; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $CuCl_2 \cdot 2H_2O$, 0.1; $MnSO_4 \cdot H_2O$, 0.80; $CoCl_2 \cdot 6H_2O$, 1; $ZnSO_4 \cdot 7H_2O$, 3.

II. MATERIALS AND METHODS

1. Experimental Diets

The formula of experimental diets is shown in Table 1. Fish meal (Pesquera Diamante, Peru) and soybean meal (defatted and dehulled,TTET Union, Taiwan), gelatinized starch (Trust River Trading Co., Ltd., Taipei, Taiwan), fish oil (semi-refined fish oil, Oleaginosa Victoria, Peru), and soybean oil (Tai-Tang Industrial, Taipei, Taiwan) were used as the main protein, carbohydrate, and lipid sources, respectively. Four isonitrogenous (46%) and isolipidic (10%) diets were supplemented with 0%, 1%, 2% and 5% MAM (FarEast Bio-Tec. Co., Ltd., Taipei, Taiwan). Because MAM is rich in n-6 fatty acids, the soybean meal and soybean oil levels were adjusted with the MAM supplementation levels to provide a similar amount of n-6 fatty acids in the experimental diets. Squid liver meal (Power Omega, Korea) and scallop meal (Japan Biofarm, Japan) were used as attractant in all diets to increase dietary palatability and acceptance. The ingredients of the experimental diets were mechanically mixed. Subsequently, cold water was added to the mixture. The mixture was further mixed until a stiff dough was obtained. The dough was then passed through a mincer with a dicer, and the resulting strands were dried using an electrical fan at 20°C. After drying, the strands were broken down, sieved into pellets (2.0 mm in diameter), and stored at -20°C until use. The proximate compositions and fatty acid profiles of the experimental diets and MAM are listed in Table 2. The methods of analysis for proximate compositions and fatty acid profiles were carried out in accordance with AOAC (1995) and Lin and Mui (2017), respectively.

2. Experimental Procedures

This study was conducted in accordance with the Guiding Principles for the Use and Care of Laboratory Animals and guidelines of the Institutional Animal Care and Use Committee (IACUC) of National Pingtung University of Science and Technology (NPUST; approval no. NPUST-IACUC-102-050).

Juvenile orange-spotted grouper (*E. coioides*) were obtained from a local hatchery (Pingtung, Taiwan). Upon arrival, the fish were acclimated to laboratory conditions for 4 weeks in a 3500-L fiberglass-reinforced plastic (FRP) tank with a recirculation system and fed a commercial grouper feed (Uni-President Enterprise, Tainan, Taiwan). The proximate composition (in %) of the commercial feed was as follows: moisture, 9.3; crude protein (N × 6.25), 46.7; lipid, 11.1; and ash, 12.1. At the beginning of the experiment, 12 fish (mean weight: 7.21 ± 0.21 g) were stocked in each experimental 80-L FRP tank. Each experimental diet was conducted in triplicate (four experimental diets × three tanks). Each tank was a part of a closed recirculating system containing seawater with a salinity of 30‰~32‰. The system consisted of a common filter, biofilter, protein skimmer, and ultraviolet light for maintaining water quality. The dissolved oxygen concentration was monitored weekly and maintained at about 7 mg O_2/L throughout the experimental period. Water temperature ranged 28~30°C, pH ranged 7.6~8.0, and ammonia nitrogen was < 0.5 mg/L. These water quality parameters were measured with a Multiparameter photometer (HI83203, Hanna Instruments, TX, USA). A photoperiod of 12-h light (08:00~20:00)

Table 2. Proximate composition and major fatty acid profile of the experimental diets and *Mortierella alpina* meal.

	Mortierella alpina meal level (%)				*Mortierella alpina* meal
	0	1	2	5	
Proximate composition (%)					
Moisture	7.28	7.57	7.71	8.44	6.20
Ash	12.08	11.94	11.97	12.03	9.22
Crude protein	45.47	45.69	45.46	45.77	36.35
Ether extract	9.06	8.97	8.56	8.83	13.08
Crude fiber	1.18	1.13	1.00	0.88	5.74
Nitrogen free extract[1]	26.94	24.69	25.31	24.05	28.69
Fatty acid profile (% of total lipids)					
C16:0	15.44	15.37	16.12	16.39	13.44
C18:0	4.27	4.25	4.49	4.55	7.53
C18:1 n-9	13.01	12.94	13.56	13.55	28.74
C18:2 n-6	19.35	18.56	17.14	16.85	7.65
C18:3 n-3	2.48	2.42	2.48	2.25	4.67
C20:4 n-6	0.93	1.14	1.34	1.93	13.96
C20:5 n-3	8.81	8.92	9.11	9.55	0.03
C22:6 n-3	10.01	10.15	10.26	10.73	ND
ΣSFAs[2]	24.44	24.37	25.62	26.08	23.06
ΣMUFAs	18.71	18.59	19.35	19.44	29.34
ΣPUFAs	41.75	41.37	41.67	41.87	28.32
Σn3	21.30	21.50	21.84	22.54	0.32
Σn6	20.46	19.87	19.83	19.33	28.00

[1] Nitrogen free extract = 100-moisture-ash-crude protein-ether extract-crude fiber.

[2] SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

and 12-h dark was used.

The daily rations (5% of fish body wet-weight) were divided into two equal portions and fed twice per day at 09:00 and 17:00. To record the feed intake during the feeding period, the uneaten feed was collected 30 min after feeding from each tank using a siphon, dried in an oven [drying time and temperature?], and weighed. Fish were weighed once every 2 weeks. The feeding rate was adjusted accordingly. All fish in the tank were weighed in bulk. After weighing, 50% of the rearing water was exchanged. Fish were fed the test diets for an 8-week period.

3. Growth Performance and Assay Methods

At the end of the feeding trial, fish in each tank were weighed individually to calculate the percentage of body weight gain [100 × (final body weight – initial body weight)/initial body weight], specific growth rate [(ln final body weight – ln initial body weight)/day × 100], feed efficiency [FE; (final body weight – initial body weight)/feed intake], daily feed intake [total feed intake/(initial body weight + final body weight)/2/day × 100], and survival [100 × (final fish number/initial fish number].

4. Analysis of the Proximate Composition and Fatty Acid Analysis

After the final weighing, three fish were randomly selected from each tank, and their whole-body fatty acid profiles were determined. The fish were homogenized before the determination. The proximate composition, including moisture, ash, crude protein, and ether extract of the whole body were analyzed according to the AOAC (1995). The whole-body fatty acid profiles were determined according to the method of Lin and Mui (2017) using gas chromatography (Thermo Fisher Scientific, Italy). Briefly, total lipids were extracted from samples using chloroform: methanol (2:1) (Folch et al., 1957). Extracted lipid (0.1 g) was saponified with 2 mL 0.5 N KOH-methanol (Sigma Chemical, St. Louis, MO, USA) for 10 min. The saponified lipid was transmethylated with 2 mL 0.7 N HCl-methanol and 3 mL BF_3 in methanol (Sigma Chemical) to yield methyl ester. The fatty acid analysis was conducted with a gas chromatograph (Thermo Trace 2000, Thermo Fisher Scientific S.P.A., Italy) equipped with a flame ionization detector and a glass capillary column (100 m × 0.25 mm × 0.2 × m film thickness, Supelco, Bellefonte, ST, USA). The column temperature was programmed from 140 to 240°C at a rate of 4°C min^{-1} and was held at the final temperature for 15 min. The injection and detector temperatures were both maintained at 260°C. Fatty acids were identified by comparison with known standards (Supelco 37-Component FAME Mix, 47885-U, Sigma Chemical). Moreover, ARA re-

Table 3. Growth performance of grouper fed the different diets for 8 weeks[1].

	Mortierella alpina meal level (%)			
	0	1	2	5
Initial body weight (g)	7.11 ± 0.21	7.36 ± 0.21	7.20 ± 0.28	7.12 ± 0.08
Final body weight (g)	46.02 ± 1.53	50.88 ± 1.73	48.35 ± 3.40	42.45 ± 6.41
Weight gain (%)	548 ± 28	591 ± 31.39	572 ± 51	545 ± 34
Specific growth rate (%)	2.78 ± 0.05	2.88 ± 0.07	2.84 ± 0.11	2.67 ± 0.20
Feed intake (g/fish)	37.93 ± 1.13	41.70 ± 0.86	40.52 ± 2.70	39.61 ± 1.83
Daily feed intake (%)	2.14 ± 0.04	2.14 ± 0.02	2.18 ± 0.02	2.19 ± 0.07
Feed efficiency	1.03 ± 0.04	1.04 ± 0.02	1.01 ± 0.02	0.98 ± 0.03
Survival (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0

[1] Values are the mean ± SD of three groups of fish (n = 3), with 12 fish per group.

Table 4. Whole-body proximate composition and fatty acid (FA) profile of grouper fed the different diets for 8 weeks[1].

	Mortierella alpina meal level (%)			
	0	1	2	5
Proximate composition (% wet basis)				
Moisture	70.05 ± 0.68	69.95 ± 1.17	70.19 ± 0.69	70.16 ± 0.63
Ash	4.23 ± 1.53	4.40 ± 0.52	4.50 ± 1.24	4.98 ± 0.54
Crude protein	16.96 ± 0.44	17.51 ± 1.08	17.63 ± 0.96	16.89 ± 0.34
Crude lipids	5.15 ± 1.04	5.33 ± 0.98	4.60 ± 1.29	4.25 ± 1.28
Fatty acid profile (% total lipids)				
C14:0	4.01 ± 0.14	4.26 ± 0.21	4.14 ± 0.35	4.21 ± 0.21
C16:0	18.86 ± 0.22^{ab}	19.25 ± 0.82^{ab}	19.90 ± 0.88^{b}	18.60 ± 0.32^{a}
C18:0	5.12 ± 0.15	5.14 ± 0.15	5.47 ± 0.36	5.05 ± 0.20
C20:0	0.26 ± 0.00	0.26 ± 0.00	0.25 ± 0.02	0.27 ± 0.01
C22:0	0.15 ± 0.00^{ab}	0.13 ± 0.01^{a}	0.16 ± 0.01^{b}	0.17 ± 0.02^{b}
C16:1	5.01 ± 0.22	4.95 ± 0.08	5.12 ± 0.46	4.86 ± 0.05
C18:1 n-9	16.35 ± 0.27^{a}	16.54 ± 0.75^{a}	17.72 ± 0.51^{b}	16.19 ± 0.30^{a}
C20:1 n-9	0.86 ± 0.12	0.89 ± 0.12	0.81 ± 0.56	0.95 ± 0.02
C22:1 n-9	0.30 ± 0.02	0.29 ± 0.01	0.28 ± 0.05	0.33 ± 0.02
C24:1 n-9	0.19 ± 0.03	0.20 ± 0.05	0.20 ± 0.04	0.19 ± 0.00
C18:2 n-6	17.81 ± 0.47^{b}	17.48 ± 0.89^{ab}	16.45 ± 1.23^{a}	16.24 ± 0.19^{a}
C20:4 n-6	0.76 ± 0.03^{a}	0.85 ± 0.03^{b}	0.95 ± 0.05^{c}	1.47 ± 0.00^{d}
C18:3 n-3	2.19 ± 0.14	2.22 ± 0.19	2.05 ± 0.12	2.03 ± 0.06
C20:5 n-3	6.24 ± 0.12	6.18 ± 0.33	6.60 ± 0.39	6.51 ± 0.06
C22:6 n-3	9.63 ± 0.13	9.41 ± 0.25	9.06 ± 0.15	9.49 ± 0.19
Other FAs	10.74 ± 0.42	10.50 ± 0.33	10.22 ± 3.51	11.34 ± 0.69
ΣSFAs[2]	29.56 ± 0.14	30.25 ± 0.78	31.16 ± 1.61	29.72 ± 0.64
ΣMUFAs[2]	23.05 ± 0.41	23.11 ± 0.73	24.50 ± 1.05	22.80 ± 0.18
ΣPUFAs[2]	36.91 ± 0.81	36.47 ± 1.52	35.53 ± 1.07	36.33 ± 0.10
Σn-3	18.13 ± 0.37	17.88 ± 0.72	17.78 ± 0.38	18.09 ± 0.19
Σn-6	18.78 ± 0.47	18.59 ± 0.83	17.75 ± 1.25	18.25 ± 0.18

[1] Values are presented as the mean ± SD of three groups of fish (n = 3), with three fish per group. Different superscripts (^{a,b,c,d}) in the same row indicate a significant ($p < 0.05$) difference between different dietary treatments.

[2] SFAs saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

tention was calculated by changes in the initial and final whole-body ARA contents and ARA intake levels of the grouper. Because some ARA can be biosynthesized from 18:2 n-6, the calculation represented apparent retention. The equation of apparent ARA retention was [100 × (final whole-body ARA content – initial whole-body ARA content)/total ARA intake]. The initial whole-body ARA concentration was 1.58% of total lipids.

5. Statistical Analysis

Each experimental diet was fed to three groups of fish according to a completely randomized design. The results were analyzed through a one-way analysis of variance using SAS (SAS Institute, Cary, NC, USA). Significance was set at $p < 0.05$. Duncan's new multiple-range test was used for multiple comparisons among means.

III. RESULTS AND DISCUSSION

1. Growth Performance

The growth performance of grouper fed different diets for a period of 8 weeks is presented in Table 3. Grouper receiving the various diet treatments showed no significant differences ($p > 0.05$) in the final body weight, percentage weight gain, specific growth rate, daily feed intake, feed efficiency, or survival. The whole-body moisture, ash, crude protein, and ether extract did not significantly differ among the diet treatments (Table 4).

The protein (46%) and lipid (9%) levels of the treatment diets used in the study were proven to be adequate for grouper (Chen and Tsai, 1994; Shiau and Lan, 1996; Lin and Shiau, 2003). Our feed formulation and rearing conditions resulted in normal growth and feed utilization by grouper, yielding weight gain of 545%~591% and feed efficiency of 0.98~1.04. Comparable results were reported in our previous study with a similar stage (juvenile stage, initial wt: 8.48 ± 0.06 g) for *E. coicodes* (Lee et al., 2018). The results indicated that grouper fed the diets with MAM did not exhibit altered growth performance or whole-body proximate composition.

Even in diets supplemented with up to 5% MAM, the feed intake in fish was still similar ($p > 0.05$) among all dietary treatments. Chou et al. (2008) indicated that the feed intake of fish, particularly carnivorous fish, is generally reduced by inclusion of plant ingredients in the feed. To prevent low feed intake with the MAM-containing diet, an attractant (consisting of 5% squid liver meal and 5% scallop meal) was added to the experimental diets. The feed intake did not significantly differ among the dietary treatments, suggesting that the diets were well accepted by the fish.

Our study indicated that 0.93% ARA seemed to satisfy the requirement of grouper. Wu (2011) reported that 0.6%~0.8% ARA is required for grouper to maintain their immunity. These results are similar to the range of ARA requirements (0.5%~1%) characteristic of other marine fish species: turbot (Castell et al, 1994), gilthead seabream (Bessonart et al., 1999), and Japanese flounder (Xu et al., 2010). It can be inferred that grouper requires a maximum level of 1% ARA, and they do not need to have additional ARA included in the diet.

2. Fatty Acid Composition

The linoleic acid (C18:2 n-6) concentration in the whole body was higher in fish fed the MAM-free control diet than those in fish fed with diets containing 2% and 5% MAM (Table 4). The whole-body ARA concentration was highest in fish fed the diet containing 5% MAM, followed by fish fed diets containing 2% and 1% MAM. The whole-body ARA concentration was lowest in fish fed the control diet.

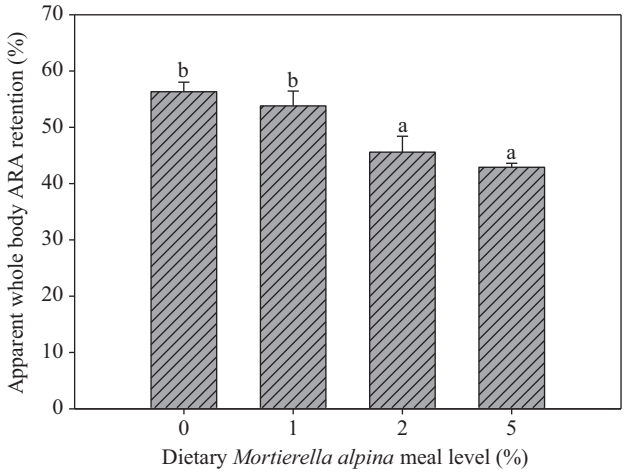

Fig. 1. Apparent whole-body arachidonic acid (ARA) retention of grouper fed the different diets for 8 weeks. Different superscripts (^{a,b}) in a bar indicate a significant ($p < 0.05$) difference between different dietary treatments.Values are the mean of three groups of fish ($n = 3$), with three fish per group.

The decreasing trend of whole-body linoleic acid concentrations reflected linoleic acid levels in the diet (Table 2). This is because deposition of fatty acids is strongly affected by dietary fatty acids (Bell et al., 1995). MAM was rich in n-6 fatty acids (28% of total lipids, Table 2). Therefore, soybean meal and soybean oil were adjusted according to the MAM supplementation level. The linoleic acid concentration in MAM was only 7.65% of total lipids. Thus, with an increase in MAM supplementation levels from 0% to 5%, the linoleic acid concentration in the diet decreased from 19.35% to 16.85% of total lipids. This could explain the decreasing trend of the whole-body linoleic acid concentration.

The fatty acid composition in fish generally reflects the feed composition (NRC, 2011). The whole-body ARA composition in the grouper completely reflected the dietary ARA level (Tables 2 and 4). These results were similar to those reported in juveniles of turbot (Castell et al., 1994), Japanese seabass (Xu et al., 2010), larvae of common sole (Lund et al., 2007; 2008), and Senegal sole (Villalta et al., 2005). Whole-body ARA reflecting dietary ARA levels indicates that grouper can utilize ARA from MAM. However, the availability needs more investigation.

3.Apparent ARA Retention

Although the data demonstrate that grouper can absorb ARA from MAM, the utilization of MAM by these fish remains unclear. Thus, the apparent whole-body ARA retention was calculated to evaluate the utilization of MAM by fish. The apparent ARA retention levels (ratio of dietary ARA intake to the increase in the whole-body ARA content; Fig. 1) in groups fed 0% (control),

1%, 2%, and 5% MAM were 56.32%, 53.80%, 45.59%, and 42.90%, respectively. The apparent whole-body ARA retention was higher in fish fed the control diet and diet containing 1% MAM than in those fed diets containing 2% and 5% MAM (Fig. 1). However, the whole-body ARA concentration and retention showed an opposite result. The low ARA retention in high MAM-supplemented groups suggests the poor availability of MAM to grouper. The MAM (Far-East Biochtech, Taipei, Taiwan) is a spray-dried product formulated without breaking the cell walls. The cell walls remaining intact might have caused poor absorption of ARA from MAM and resulted in low ARA retention in the grouper body. Thus, removing (or breaking) the cell walls of MAM is necessary for improving the utilization of ARA-rich MAM.

In conclusion, results of this study demonstrate that MAM can be used as an ARA source for grouper. The utilization of MAM by fish decreased with an increase in the dietary supplementation level. Additional studies aiming to improve the utilization of MAM by fish are required.

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REFERENCES

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

Bell, M.V. and J. R. Dick (1990). Molecular species composition of phosphatidylinositol from brain, retina, liver and muscle of cod (*Gadus morhua*). Lipids 25, 691-694.

Bell, M.V., C. M. F. Simpson and J. R. Sargent (1983). (*n*-3)and (*n*-6) polyunsaturated fatty acids in the phosphoglycerides of salt-secreting epithelia from two marine fish species. Lipids 18, 720-725.

Bell, J. G., J. D. Castell, D. R. Tocher, F. M. MacDonald and J. R. Sargent (1995). Effects of different dietary arachidonic acid: docosahexaenoic acid ratios on phospholipid fatty acid compositions and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). Fish Physiology and Biochemistry 14, 139-151.

Bessonart, M., M. Izquierdo, M. Salhi, C. M. Hernandez-Cruz, M. M. Gonzalezand and H. Fernandez-Palacios (1999). Effect of dietary arachidonic acid levels on growth and survival of gilthead sea bream (*Sparus aurata* L.) larvae. Aquaculture179, 265-275.

Castell, J. D., J. G. Bell, D. R. Tocher and J. R. Sargent (1994). Effect of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot. Aquaculture 128, 315-333.

Chen, H. Y. and J. C. Tsai (1994). Optimum dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. Aquaculture 119, 265-271.

Chou, H. Y., Y. H. Lin and S. Y. Shiau (2008). Nutrition, immunology and health management of groupers. In: Grouper Aquaculture (eds. by IC. Liao & E. M. Leaño). Asian Fisheries Society, Manila, Philippines, World Aquaculture Society, Louisiana, USA, The Fisheries Society of Taiwan, Keelung, Taiwan, and National Taiwan Ocean University, Keelung, Taiwan, 189-205.

Copeman, L. A., C. C. Parrish, J. A. Brown and M. Harel (2002). Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture 210, 285-304.

Folch, J., M. Lees and G. H. Sloane-Stanley (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497-509.

Koven, W. M., S. Kolkovski, E. Hadas, K. Gamsiz and A. Tandler (2001). Advances in the development of microdiets for gilthead seabream, *Sparus aurata*: A review. Aquaculture194, 107-121.

Lee, M. C., L. C. Zhuo and Y. H. Lin (2018). Effects of dietary docosahexaenoic acid sources, microalgae meal and oil, on growth, fatty acid composition, and docosahexaenoic acid retention of orange-spotted grouper, *Epinephelus coioides*. Aquaculture Research 49, 30-35.

Lin, Y. H. and S. Y. Shiau (2003). Dietary lipid requirement of grouper, *Epinephelus malabaricus*, and effects on immune response. Aquaculture 225, 243-250.

Lin, Y. H. and J. J. Mui (2017). Evaluation of dietary inclusion of housefly maggot (*Musca domestica*) meal on growth, fillet composition and physiological responses for barramundi, *Lates calcarifer*. Aquaculture Research 48, 2478-2485.

Linares, F. and R. J. Henderson (1991). Incorporation of ^{14}C-labeled polyunsaturated fatty acids by juvenile turbot, *Scophthalmus maximus* (L.) in vivo. Journal of Fish Biology 38, 335-348.

Lund, I., S. J. Steenfeldt and B. W. Hansen (2007). Effect of dietary arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid on survival, growth and pigmentation in larvae of common sole (*Solea solea* L.). Aquaculture 272, 532-544.

Lund, I., S. J. Steenfeldt, G. Banta and B. W. Hansen (2008) The influence of dietary concentrations of arachidonic acid and eicosapentaenoic acid at various stages of larval ontogeny on eye migration, pigmentation and prostaglandin content of common sole larvae (*Solea solea* L.). Aquaculture 276, 143-153.

National Research Council (NRC) (2011). Nutrient Requirement of Fish and Shrimp. National Academic Press, Washington, DC, 376.

Rezek, T. C., W. O. Watanabe, M. Harel and P. J. Seaton (2010). Effects of dietary docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) on the growth, survival, stress resistance and fatty acid composition in black sea bass *Centropristis striata* (Linnaeus 1758) larvae. Aquaculture Research 41, 1302-1314.

Rodriguez, C., J. A. Perez, M. S. Izquierdo, J. Mora, A. Lorenzo and H. Fernandez-Palacios. (1994). Essential fatty acid requirements of larval gilthead sea bream *Sparus aurata* (L). Aquaculture and Fisheries Management 25, 295-304.

Sargent, J. R., Tocher, D. R. and J. G. Bell (2002). The lipids. In: Fish Nutrition, 3th edition (eds. by J.E. Halver & R. Hardy). Academic Press, San Diego, 181-257.

Shiau, S. Y. and C. W. Lan(1996).Optimum dietary protein level and protein to energy ratio for growth of grouper (*Epinephelus malabaricus*). Aquaculture 145, 259-266.

Villalta, M., A. Estévez and M. P. Bransden (2005). Arachidonic acid enriched live prey induces albinismin Senegal sole (*Solea senegalensis*) larvae. Aquaculture 245, 193-209.

Wu, S. T. (2011). Effects of arachidonic acid on growth performance and immune responses in juvenile grouper, *Epinephelus coioides*. Master thesis, National Sun Yat-sen University.

Xu, H., Q. Ai, K. Mai, W. Xu, J. Wang, H. Ma, W. Zhang, X. Wang and Z. Liufu (2010). Effects of dietary arachidonic acid on growth performance, survival, immune response and tissue fatty acid composition of juvenile Japanese seabass, *Lateolabrax japonicas*. Aquaculture 307, 75-82.