Supplementation of Dietary Fungal Oil Containing Arachidonic Acid Improves Fatty Acid Composition and Promotes Ovarian Development in Giant Freshwater Prawn, *Macrobrachium rosenbergii* (De Man, 1879)

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Abstract

Arachidonic acid (ARA) has been used as a feed additive to improve ovarian development in crustaceans. Therefore, in this study, ARA was supplemented at various levels to observe the induced development of ovaries in the broodstock of *Macrobrachium rosenbergii*. Four experimental maturation diets were formulated with different inclusion levels of ARA (0, 1, 2, and 2.9 %), sourced from *Mortierella* sp., a fungal oil which contains 45% ARA. The graded inclusion level of fungal oil resulted in 0.03, 0.35, 0.70, and 1.17% ARA in the diets. The results showed that broodstock fed with 0.70 % ARA diet, exhibited the highest hepatopancreas somatic index (HSI) value compared to broodstock fed with other diets. The histological evaluation of the ovaries revealed that broodstock fed a 0.70 % ARA diet resulted in significantly bigger oocytes ($P < 0.05$) among all diets. Thus, higher dietary ARA resulted in higher ARA content in the tail muscle, hepatopancreas, and ovary of prawn broodstock. Therefore, a dietary level of 0.70 % ARA promoted the reproductive performance and development of ovary in prawns broodstock.

Keywords: Arachidonic acid, gonadonosomatic index, *Macrobrachium rosenbergii*, maturation diet, reproductive performance

Introduction

The Malaysian giant freshwater prawn, also known as the giant freshwater prawn (GFP), *Macrobrachium rosenbergii* (de Man, 1879), is a widely cultured species in Asia and other regions. GFP is a native species in Malaysia; exhibits a notable growth rate and a high–profit margin, contributing to its popularity in the aquaculture industry. However, a critical bottleneck in its development lies in the insufficient supply of high-quality post larvae (PL). Moreover, the availability of wild broodstock is declining over time. The inadequacy of high-quality broodstock supply remains a significant challenge for of GFP production in Asia, particularly in Malaysia. The majority of GFP PL producers in rely mainly on wild broodstock for PL production in hatchery, which are then grown in pond culture.

Domesticated broodstock is an alternative to utilizing wild broodstock. However, according to discussion with local hatchery managers in Malaysia, domesticated broodstock is often regarded as being inferior quality. Thus, the quality of domesticated broodstock needs to be improved genetically and with other important contributing factors, such as using high-quality maturation feed. Maturation feed should consist of all the essential nutrients for improving the quality of broodstock, especially for better gonad development and larval quality.

In crustaceans, the reproductive performance, such as ovarian maturation is usually under the control of prostaglandins (PGs) (Sarojini et al., 1989) or eicosanoids with hormone-like functions (Mustafa and Srivastava, 1989; Sargent, 1995; Sargent et al., 1999; Stacey & Goetz, 1982; Tocher, 2003). PGs have been reported to play a role in stimulating ovarian maturation, including vitellogenesis and spawning, in many decapod crustaceans (Tahara & Yano, 2004; Rowley et al., 2005; Sumpownon et al., 2015). Arachidonic acid (ARA, C20:4n-6) is the major prostanoid precursor. Prostanoids are a class of hormonally active fatty acids composed primarily of oxygenated C18, C20, and C22 acids, known as eicosanoids, which are derived from n-3 and n-6 polyunsaturated fatty acids ($C \geq 20$; LC-PUFA) such as arachidonic acid (ARA), docosahexaenoic acid (DHA; C22:6n-3), eicosapentaenoic acid (EPA; C20:5n-3), dihomo-gamma-linolenic acids (DGLA, 20:3n-6), and gamma-linolenic acid (GLA, 18:3n-6) through the cyclooxygenase pathways (Smith et al., 2000).

ARA is known to act as a catalyst for the development of reproductive gonads (Wouters et al., 2001; Glencross, 2009; Coman et al., 2011; Xu, Cao, et al., 2017; Xu, Zhang, et al., 2017). Coman et al. (2011) reported that ARA supplementation of a semi-moist pelleted diet increases the cumulative number of females spawning and the number of eggs produced by *Penaeus monodon* broodstock. In addition, supplementation of ARA in the diets affected the fatty acid compositions in the hepatopancreas, ovaries, and fertilised eggs of female Pacific white shrimp (*Litopenaeus vannamei*) broodstock, which enhanced the final reproductive performances (Xu, Zhang, et al., 2017).

The objectives of the present study were to determine the effect of different inclusion levels of ARA in the formulated diet on the gonad development of female GFP broodstock, *M. rosenbergii*, and the impact of dietary ARA on the fatty acid composition of broodstock tissues.

Materials and Methods

Experimental diets and chemical analysis

Four isonitrogenous and isoenergetic experimental diets were formulated with 45 % crude protein and 9 % crude lipid following the nutrient level by Cavalli et al. (1999, 2000), with an energy level of 400 kcal per 100 g diet (Nik Sin et al., 2016). Fish, shrimp, and soybean meals were used as protein sources. The inclusion of fungal emulsion ARA oil from *Mortierella* sp. was added at 0, 1, 2, or 2.9 % across the four diets. The diets were thoroughly mixed using an industrial food mixer and distilled water was added until a stiff dough was formed. This dough was screw-pressed through a 3 mm die using a locally assembled Pellet Mill and the feed pellets formed were fan-dried and stored frozen at 0 °C until use.

Four experimental diets with different ARA percentage levels of 0.03, 0.35, 0.70, and 1.17 were prepared and named as T1, T2, T3, and T4, respectively. The formulation and proximate composition of the experimental diets are presented in Table 1. The moisture, crude protein, crude lipid, and ash contents of the ingredients and experimental diets were determined using the standard method (AOAC, 1990). Meanwhile, the lipid content of the experimental diet and prawn samples were extracted using a modified Folch et al. (1957) protocol and used for fatty acid (FA) analysis. The fatty acid analysis methodology followed the same method as explained by Chee et al. (2020). Briefly, the lipid of the weighed samples was extracted with chloroform: methanol at a ratio of 2: 1 (v / v) containing potassium chloride in a separatory funnel. For separation, gravitation was applied to collect the bottom layer containing chloroform and extracted lipids with a round bottom flask. The chloroform was evaporated by using a rotary evaporator at 75 ºC. The extracted lipids were then esterified into methyl esters with methanolic boron trifluoride (BF_3), followed by the saponification-esterification method (AOAC, 1990).

The fatty acid composition of the experimental diets is presented in Table 2. Specifically, fatty acid methyl esters (FAME) were resolved and analysed by a gas-liquid chromatograph (Hewlett-Packard, Model 6890) equipped with a flame ionisation detector (GC-FID), GC Autosampler, and SP™-2560 fused silica capillary column (100 m × 0.25 mm ID, 0.2 µm film thickness, Supelco, Bellefonte, PA, USA). Initially, the column temperature was programmed to hold at 140 ºC for the first 2 min, then increased to 225 ºC at a rate of 2 ºC min^{-1} and held for 5 min, then raised again to 240 ºC at a rate of 2 ºC min^{-1} and held for 3 minutes. The carrier gas was nitrogen and the pressure was 80 kPa. Fatty acid peaks were identified by comparing the retention time with known standards (Supelco 37 Component FAME Mix, PUFA No.2, and PUFA No. 3: Supelco, Bellafonte, PA, USA). The resulting peak areas were corrected by theoretical relative flame ionisation detector response factors. The response factors were calculated by the ratios between the peak area of the individual FAME. The FAMEs were expressed as weight percentages of the total FA content (Ackman, 2002; Trbović et al., 2017).

Table 1. Formulation and proximate composition of the experimental diets (g/kg dry weight)

	Dietary arachidonic acid level (% of diet)			
	0.03	0.35	0.70	1.17
Ingredients (g / kg)	ARA1	ARA2	ARA3	ARA4
Shrimp meal[1]	111.4	111.4	111.4	111.4
Fish meal[2]	238.7	238.7	238.7	238.7
Soybean meal[3]	335.7	335.7	335.7	335.7
Soybean oil	29.1	19.1	9.1	0.0
Fungal oil (45% ARA)[4]	0.0	10.0	20.0	29.1
Fish oil	19.7	19.7	19.7	19.7
Corn starch	34.8	34.8	34.8	34.8
Soybean lecithin	10.0	10.0	10.0	10.0
Cholesterol[5]	6.0	6.0	6.0	6.0
Vitamin mix[6]	30.0	30.0	30.0	30.0
Mineral mix[7]	40.0	40.0	40.0	40.0
L-ascorbic acid[8]	7.0	7.0	7.0	7.0
Choline chloride[9]	10.0	10.0	10.0	10.0
Inositol[10]	0.1	0.1	0.1	0.1
Binder[11]	8.0	8.0	8.0	8.0
BHT[12]	5.0	5.0	5.0	5.0
Astaxanthin[13]	20.0	20.0	20.0	20.0
α-cellulose	94.6	94.6	94.5	94.6
Proximate composition (mg / g dry basis)				
Moisture	107	121	111	126
Crude protein	449	445	449	443
Crude lipid	101	101	101	100
Ash	131	129	130	127
Crude fibre	26	26	25	25
NFE[14]	293	299	295	305

1 Shrimp meal (g / kg dry weight): crude protein 808.0, crude lipid 35.2, ash 133.4.
2 Fish meal (g / kg dry weight): crude protein 754.0, crude lipid 89.5, ash 147.0.
3 Soybean meal (g / kg dry weight): crude protein 536.2, crude lipid 17.7, ash 69.4, crude fibre 25.6.
4 Fungal oil (% of total fatty acids): oil from fungi (*Mortierella alpina*) with ARA content ≥40%; Cargill, USA
5 Cholesterol BP (Feed grade), NK Ingredients Pte Ltd, Singapore.
6 Vitamin premix (g/kg of dry weight) contains ascorbic acid, 45; choline chloride, 75; inositol,5; niacin, 4.5; riboflavin, 1; pyridoxine HCL, 1; thiamine mononitrate, 0.92; d-calcium panthothenate,3; retinyl acetate,0.6; cholecalciferol, 0.083; menadione, 1.67; DL-α-tocopheryl acetate (500 IU/g), 8; d-biotin, 0.02; folic acid, 0.09; Vitamin B12, 0.00135; cellulose, 854.11
7 Mineral premix (g/kg of dry weight) contains calcium phosphate monobasic, 397.65; calcium lactate, 327; ferrous sulphate, 25; magnesium sulphate $7H_2O$, 137; potassium chloride, 50; sodium chloride, 60; potassium iodide ,0.15; copper sulphate 5H2O,0.785; manganese oxide ,0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite 5H2O, 0.2
8 Rovimix C, DSM
9, 10 Sigma, USA
11 Binder: Pegabind® dry from Bentoli AgriNutrition Co., Ltd., Thailand
12 BHT: Butylated hydroxytoluene is used as an antioxidant
13 Astaxanthin (*Haematococcus sp*)-Algatech Malaysia
14 Nitrogen-free extract =100 – (protein + lipid + ash + fiber)

Table 2. Fatty acid composition (% of total fatty acid) of the experimental diets

Fatty acid	Dietary ARA level (% of diet)			
	0.03 ARA1	0.35 ARA2	0.70 ARA3	1.17 ARA4
C14:0	3.53	4.04	3.56	4.25
C15:0	0.37	0.40	0.41	ND
C16:0	25.28	22.24	19.39	19.32
C17:0	0.32	0.34	0.35	0.38
C18:0	4.09	4.75	5.52	7.25
C20:0	0.29	0.36	0.44	0.61
C22:0	ND	0.48	0.78	1.2
16:1n-7	3.44	3.53	3.56	4.31
17:1n-7	ND	ND	ND	ND
18:1n-7	2.01	2.00	1.99	2.37
18:1n-9	23.90	20.29	16.98	0.54
20:1n-9	3.10	3.20	3.32	3.89
22:1n-9	0.30	0.31	0.32	4.93
24:1n-9	0.39	0.43	0.47	0.58
18:3n-3	1.68	1.70	1.93	3.89
18:4n-3	ND	ND	ND	ND
20:3n-3	ND	2.77	0.96	0.38
20:4n-3	ND	ND	ND	ND
20:5n-3 (EPA)	4.55	4.68	4.78	5.74
22:5n-3	ND	ND	ND	ND
C22:6n-3 (DHA)	6.96	7.26	7.53	9.05
18:2n-6	13.54	13.21	12.91	14.54
C18:3n-6	0.52	0.53	0.55	1.15
C20:2n-6	ND	ND	ND	ND
C20:3n-6	4.09	2.23	4.29	1.58
C20:4n-6 (ARA)	<u>0.31</u>	<u>3.51</u>	<u>6.96</u>	<u>11.72</u>
22:4n-6	ND	ND	ND	ND
Σ SFA[a]	33.87	32.60	30.45	33.01
Σ MUFA[b]	33.12	29.75	26.64	16.62
Σ n-3 PUFA [c]	13.18	16.40	15.20	19.07
Σ n-6 PUFA [d]	18.44	19.48	24.71	28.98
(n-3)/(n-6)	0.71	0.84	0.62	0.66
ΣPUFA (total)	31.62	35.88	39.91	48.05
Σ n-3 LC-PUFA [f]	11.51	14.70	13.27	15.18
Σ n-6 LC-PUFA[g]	4.39	5.74	11.25	13.29
ARA/EPA	0.07	0.75	1.46	2.04
ARA/DHA	0.04	0.48	0.92	1.29
EPA+DHA	11.51	11.93	12.31	14.80
EPA/DHA	0.65	0.64	0.63	0.63
DHA/EPA	1.53	1.55	1.58	1.58

[a] Σ SFA : sum of all saturated fatty acids
[b] Σ MUFA : sum of all monounsaturated fatty acids
[c] Σ PUFA : sum of all polyunsaturated fatty acids
[d] Σ n-3 PUFA : sum of all omega-3 polyunsaturated fatty acids
[e] Σ n-6 PUFA : sum of all omega-6 polyunsaturated fatty acids
[f] Σ n-3 LC-PUFA : sum of all omega-3 long chain polyunsaturated fatty acids
[g] Σ n-6 LC-PUFA : sum of all omega-6 long chain polyunsaturated fatty acids
ND: Not detected

Experimental diets and chemical analysis

Rectangular fiberglass tanks with a holding capacity of 1700 L and a working capacity of 1200 L (2.4 m length × 1.2 m width × 0.6 m height) were used in this experiment with four replicate tanks for each diet treatment. The individual samples were treated as replicate samples for chemical analysis based on their ovarian stages since the development of the broodstock ovarian stages varied within the same treatment due to many factors (Chang & Shih, 1995). Each tank was supplied with ten air stones (50 mm in height and 17 mm in diameter) for aeration.

The berried female GFPs were purchased from local prawn farmers at Jeneri, Sik, Kedah, Malaysia (5°53'29.2"N 100°40'26.2"E). Before bringing them into the hatchery, the prawns were screened for *Macrobrachium rosenbergii* nodavirus (MrNV). The berried female GFP of six months old was kept in a circular fiberglass tank (1.2 m in diameter × 0.5 m depth) until they hatched all the larvae. During this incubation period, a commercial shrimp pellet (2.5 mm in size, 38 % crude protein) was used to feed the broodstock and the management of broodstock was according to Daniels et al. (2010) and New (2002).

Initial samples of spent female broodstock were taken for histology analysis. Prior to the commencement of the feeding trial, the individual weight and standard length of spent female broodstock were measured, recorded, and assigned to the experimental tanks using a completely randomised design. Twenty-one pieces of GFP broodstock in the range of 18 – 35 g were randomly distributed in each experimental tank. For each experimental tank, one adult male prawn was placed in a small cage (40 cm in diameter) to trigger the maturation of the females (Nagabhushanam et al., 1989). The experimental broodstock was fed up to satiation (approximately 3 % of body weight) and two times daily (0900 and 1630 h). Every morning, feces and uneaten feed were siphoned out of the tank. The salinity of the water was in the range of 0 – 6 ppt as suggested by Yen & Bart (2008).

The water quality parameters of the experiment tanks were recorded daily for temperature, dissolved oxygen, and salinity. Ammonia, nitrate, nitrite, pH, and alkalinity were measured weekly. The water quality was monitored throughout the experimental period and remedial actions were taken to ensure the water quality parameters were in the acceptable range. Sodium bicarbonate and commercial mineral premixes were used and added weekly to maintain water alkalinity higher than 60 mgL^{-1} as suggested by Coyle et al. (2010) and Shofiquzzoha et al. (2016). The dissolved oxygen was measured with a YSI Model 550 polarographic oxygen meter (Yellow Springs Instruments Company, Yellow Springs, OH, USA). Meanwhile, ammonia, nitrite, nitrate, and alkalinity were determined according to the methods of Solorzano (1969), using a Hach Model DR-2000 spectrophotometer (Hatch Company, Ames, IA, USA).

After 50 days of feeding trials using the experimental diets, the experiment was terminated and all prawn broodstock from each tank were sampled for physical, histological, and chemical examination. The physical evaluation was carried out by measuring the individual weight and total length. Then, the treated broodstock were dissected, the hepatopancreas and the gonads were weighed to estimate the hepatopancreas somatic index (HSI) and gonadosomatic index (GSI).

The cephalothorax or head section of prawn broodstock was taken for histological samples as shown in Figure 1. Freshly dissected sample was put into Davidson's solution for further processing. Additionally, other parts of prawn broodstock such as the tail muscle, hepatopancreas, and gonad from each prawn broodstock were individually packed, labeled, and directly frozen at 0 °C for fatty acid analysis. The ovarian stages (OS) were determined based on GSI values (Chang & Shih, 1995). The individual samples of tail muscle, hepatopancreas, and gonad were then grouped according to their OS for each treatment diet.

The histological slide for the treated prawn broodstock samples was classified according to OS and analysed accordingly. The number of hepatopancreas and gonad samples from each diet treatment was limited for a particular OS, since the development of OS varies individually within the same diet treatment. The histological slide samples of hepatopancreas and ovary of OS III were available for all treated broodstock groups (n=3 of treated prawn broodstock samples for each diet). Hence, these samples were analysed and compared accordingly.

Sample collection and reproductive indices

Samples of both spent broodstock (at the beginning of the experiment) and the ARA treated broodstock (at the end of the experiment) were sacrificed and subjected to histological analysis. The standard formulae used to estimate HSI, GSI, and gonad stage index are listed below (Fatima et al., 2013; Kangpanich et al., 2016; Xu, Zhang, et al., 2017).

Hepatopancreas-somatic index (%), HSI=	(Hepatopancreas weight / body weight) × 100
Gonadosomatic index (%), GSI =	(Gonad weight / body weight) × 100
Gonad stage index =	$(\sum \boldsymbol{Si}\ \boldsymbol{x}\ \boldsymbol{ni})\ \boldsymbol{x}\ \boldsymbol{N}^{-1}$,

Where,
Si: stage of gonad (i=1-5),
ni: number of samples in stage *Si* and
N is the total number of samples counted

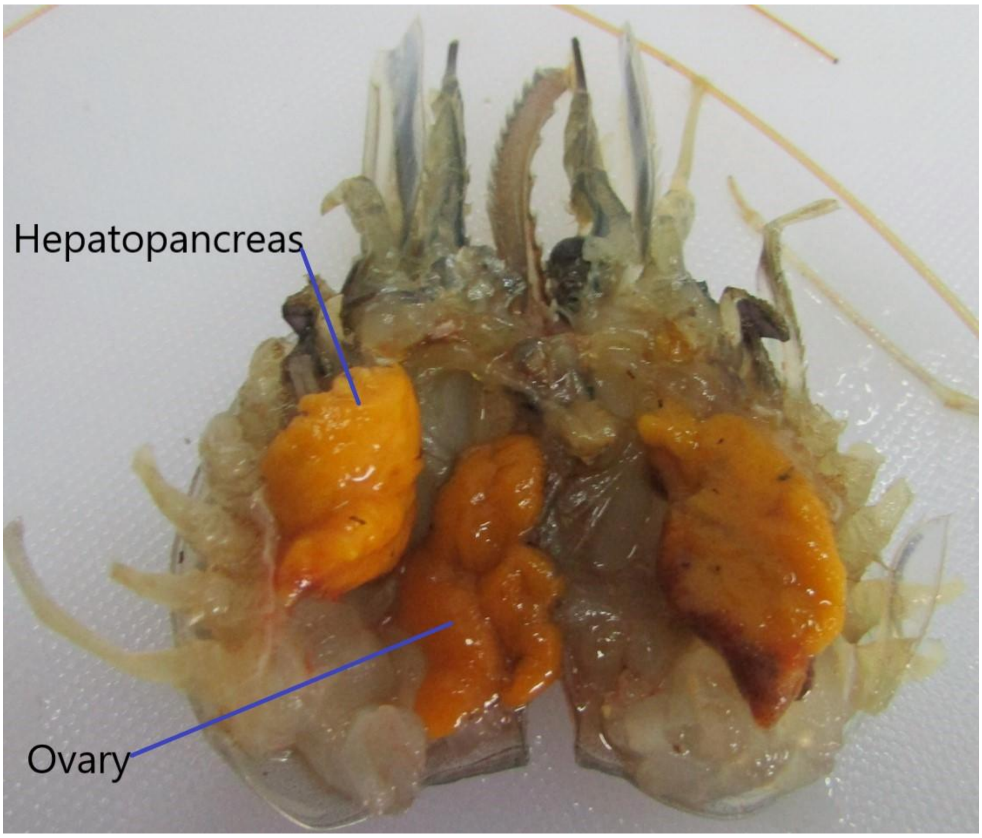

Figure 1. The head part with organs of the broodstock sample were used for histology analysis.

For histology evaluation, half of the prawn broodstock's cephalothorax section (Fig. 1) is used which consists of the hepatopancreas and ovary. The fresh sample was fixed in Davidson's solution for 24 hours before replacing with 75 % ethanol until further processing. The samples were processed (Leica ASP 300) and embedded in paraffin wax. The embedded specimens were sectioned at 5 micrometers (Leica RM2245), stained with haematoxylin and eosin (H&E), and finally mounted with DPX before examined under a microscope (Leica DM5000B) connected to a digital camera (Leica DFC 320) associated with computer software (Leica QWin). The histological techniques were performed according to Humason (1979). Histological analysis of both the ovary and hepatopancreas was performed using standard histological procedures. The cells of the hepatopancreas tubules and oocytes were chosen randomly from the histology slides for diameter measurement of both the ovary and hepatopancreas, respectively.

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SE). The data was analysed using IBM SPSS Statistics 25 program (SPSS Inc., Chicago, IL, USA). Data were subjected to one-way analysis of variance (ANOVA) and Duncan multiple comparison tests were used to determine significant differences among dietary treatments at $P < 0.05$. Before subjecting the data to ANOVA analysis, the data were checked for normal distribution and variance homogeneity under descriptive statistics and explored for normality plots to a test using the SPSS program. Whenever these assumptions were violated, non-normally distributed continuous variables were transformed using a two-step approach described by Templeton (2011). Step one involves transforming the variable into a percentile rank, which results in uniformly distributed probabilities. Step two applies the inverse normal transformation to the first step results to form a variable consisting of normally distributed z-scores.

Results

The water quality parameters recorded were in the acceptable range for freshwater prawn culture in tanks (Roustaian et al., 1999; Habashy, 2013). The parameters recorded were in the range of 26.5 – 32.5 °C for temperature, 34.0 – 136.0 mg L^{-1} for alkalinity, 0.0 – 0.58 mg L^{-1} of nitrite, 0.50 – 20.30 mg L^{-1} of nitrate, 0.0 – 0.50 mg L^{-1} of ammonia, 7.14 – 8.24 pH, and 5.10 – 8.70 mg L^{-1} for dissolved oxygen.

Experimental diets

The crude protein content of the experimental diets ranged from 443 to 449 mg g^{-1} dry weight, while the crude lipid ranged from 100 to 101 mg g^{-1} dry weight (Table 1). The fatty acid profile showed that the primary source of ARA was derived from the fungal oil *Mortierella sp.* with a concentration of 45.78 % of the total fatty acid (TFA). Other ingredients contributed to a small percentage of ARA, such as fishmeal (0.48 %), fish oil (0.21 %), and shrimp meal (1.08 %).

The fatty acid composition of the experimental diets is shown in Table 2. The diets showed gradually increased ARA by 0.31, 3.51, 6.96, and 11.72 % of TFA which were incorporated with 0.03, 0.35, 0.70, and 1.17 levels of ARA, respectively. The fatty acid composition of ARA in the experimental diets reflected the inclusion level of fungal oil used. However, a higher level of ARA was detected for the T4 diet with a 2.9 % inclusion level of fungal oil, which was higher than the calculated value of 1.05 % ARA. A similar pattern of increasing value was observed for stearic acid (C18:0), eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) with increasing dietary ARA levels (Table 2). Fish meal (18.0 % of TFA), shrimp meal (8.5 % of TFA), and fish oil (11.3 % of TFA) were found to contain a large amount of total n−3 PUFA compared to vegetable oils such as soybean meal residual oil (7.3 % of TFA) and soybean oil (0.01 % of TFA). As for vegetable oil, 50.45 % of TFA was contributed by linoleic acid (LA) in the soybean meal.

Evaluation of the treated broodstock

Physical evaluation

The physical evaluations are summarised in Table 3. The final weight of the broodstock showed no significant difference (P > 0.05) among the groups, and the value increased compared to the initial weight. No significant differences (P > 0.05) were observed for the survival of all treatment, with the value ranging from 60 – 73 % across the treatment. The HSI value of treated broodstock increased as the ARA content in the diets increased from 4.42, 4.69, and 5.25 for treated broodstock fed with T1, T2, and T3 diets, respectively. However, the HSI value decreased to 5.07 for treated broodstock fed with T4 diet. The highest HSI was recorded for the treated broodstock fed with T3 diet, which showed no significant difference compared to treated broodstock fed with other diets (P > 0.05). Further analysis of HSI was carried out by plotting the graph as shown in Figure 2. As Figure 2 illustrates, a second-order polynomial regression relationship between HSI value and dietary ARA for the treated GFP female broodstock. By applying the dose-response concept or broken line analysis, the optimal level of ARA in the diet can be extrapolated and estimated as shown in the red circle in Figure 2.

The GSI value showed an increasing pattern from treated broodstock fed with T2 and T3 diets, with a value of 3.20 and 3.52, respectively. However, the GSI value for treated broodstock fed with T4 diet showed a similar value as T3 diet of 3.51. This indicated that the ARA level in treated broodstock fed with T3 and T4 diets resulted in similar GSI value. The total HSI and GSI values of treated broodstock showed an increasing trend for treated broodstock fed with T2 and T3 diets at 7.90 and 8.77, respectively (Table 3). However, an opposite trend was observed with treated broodstock fed with T4 diet (8.58 ± 1.41). Although no significant differences were observed among treated broodstock (P > 0.05) as seen in Table 3, the value increased as higher dietary ARA was used. Further analysis of the HSI and GSI combination values, including GSI and HSI values, was carried out as illustrated in Figure 3.

Matured OS production, which is the cumulative value of OS III, IV, and V, were calculated. The values fluctuated between treatments. The maximum value of 68.89 % was recorded in treated broodstock fed with T3 diet, followed by T1, T2, and T4 diets, as shown in Figure 4.

are expressed as the mean ± standard error of the mean (SE). The data was analysed using IBM SPSS Statistics 25 program (SPSS Inc., Chicago, IL, USA). Data were subjected to one-way analysis of variance (ANOVA) and Duncan multiple comparison tests were used to determine significant differences among dietary treatments at P < 0.05. Before subjecting the data to ANOVA analysis, the data were checked for normal distribution and variance homogeneity under descriptive statistics and explored for normality plots to a test using the SPSS program. Whenever these assumptions were violated, non-normally distributed continuous variables were transformed using a two-step approach described by Templeton (2011). Step one involves transforming the variable into a percentile rank, which results in uniformly distributed probabilities. Step two applies the inverse normal transformation to the first step results to form a variable consisting of normally distributed z-scores.

Table 3. The final weight, gonadosomatic index and hepatosomatic index of the treated broodstock fed with increasing ARA levels diet[5]

	Dietary arachidonic acid level (% of diet)			
	0.03	0.35	0.7	1.17
Reproductive performance	ARA1	ARA2	ARA3	ARA4
Initial weight (g)	24.05 ± 1.16	26.07 ± 1.62	24.31 ± 0.36	24.80 ± 1.09
Final weight (g)	25.44 ± 0.55[a]	31.92 ± 2.68[b]	30.53 ± 0.80[ab]	25.88 ± 0.26[a]
Survival rate	60.79 ± 4.32	73.49 ± 6.54	69.84 ± 11.11	72.86 ± 8.55
GSI[1]	3.72 ± 1.15	3.20 ± 1.00	3.52 ± 0.82	3.51 ± 1.30
HSI[2]	4.42 ± 0.22	4.69 ± 0.23	5.25 ± 0.58	5.07 ± 0.23
Sum of (HSI +GSI)	8.14 ± 1.35	7.90 ± 0.85	8.77 ± 1.30	8.58 ± 1.41
Cumulative matured OS (%)	49.16 ± 14.13	42.01 ± 12.38	68.89 ± 17.35	34.47 ± 6.13
Gonad stages index[3]	2.28 ± 0.24	2.63 ± 0.69	2.76 ± 0.46	1.86 ± 0.07
Mature OS production (%)[4]	17.22 ± 1.47	15.56 ± 2.94	25.00 ± 5.36	13.33 ± 5.09

Note:
[1]: gonadosomatic index (%): GSI = (gonad weight/body weight) × 100
[2]: Hepatopancreatic index (%): HSI= (hepatopancreas weight/body weight) × 100
[3]: Gonad stages index = $(\sum Si\ x\ ni)\ .\ N^{-1}$, where *Si*: stage of gonad (i=1-5), *ni*: number of samples in stage Si and N is the total number of samples counted.
[4]. Mature OS production = Cumulative number of broodstock with OS III, IV & V/Initial number of broodstock used (initial stock) × 100; The number of samples, n= 31 – 40 / diet
[5]Values are the mean ± SE of the samples.
Different letters in rows indicate significant differences at P < 0.05

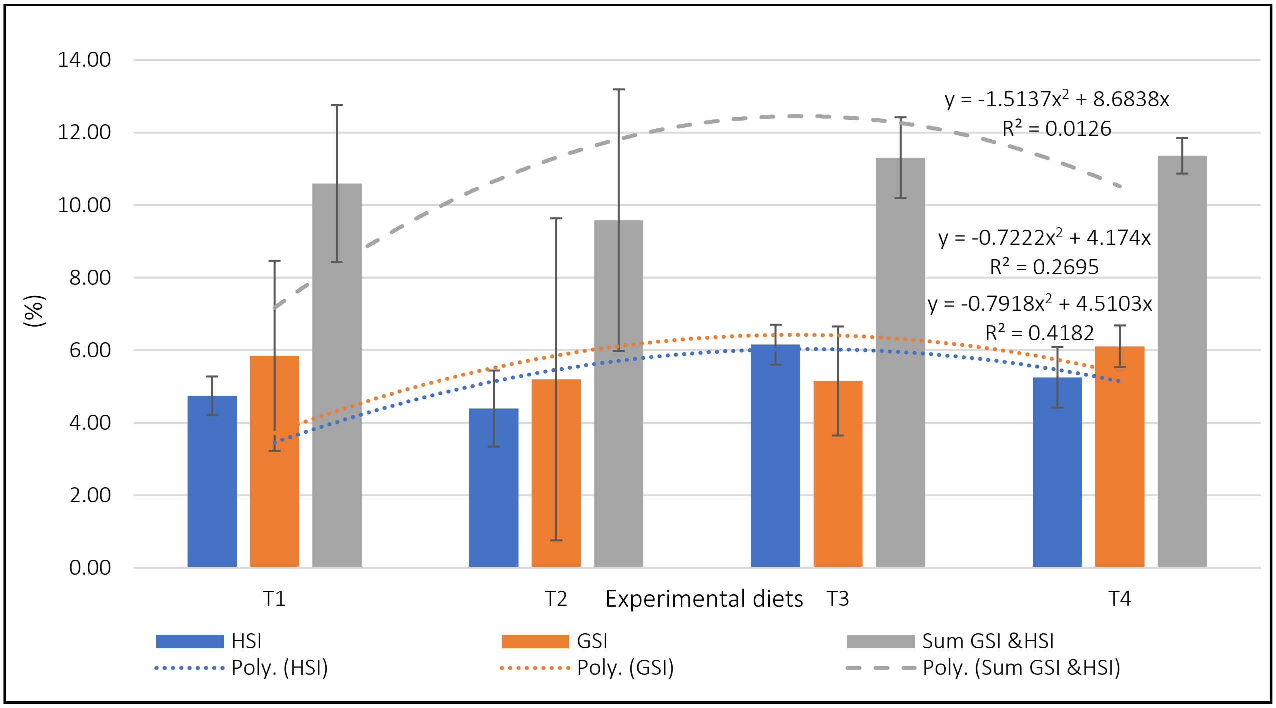

Figure 1. The relationship of GSI, HSI and sum GSI & HSI value with dietary ARA for treated GFP female broodstock as described by second-order polynomial regression

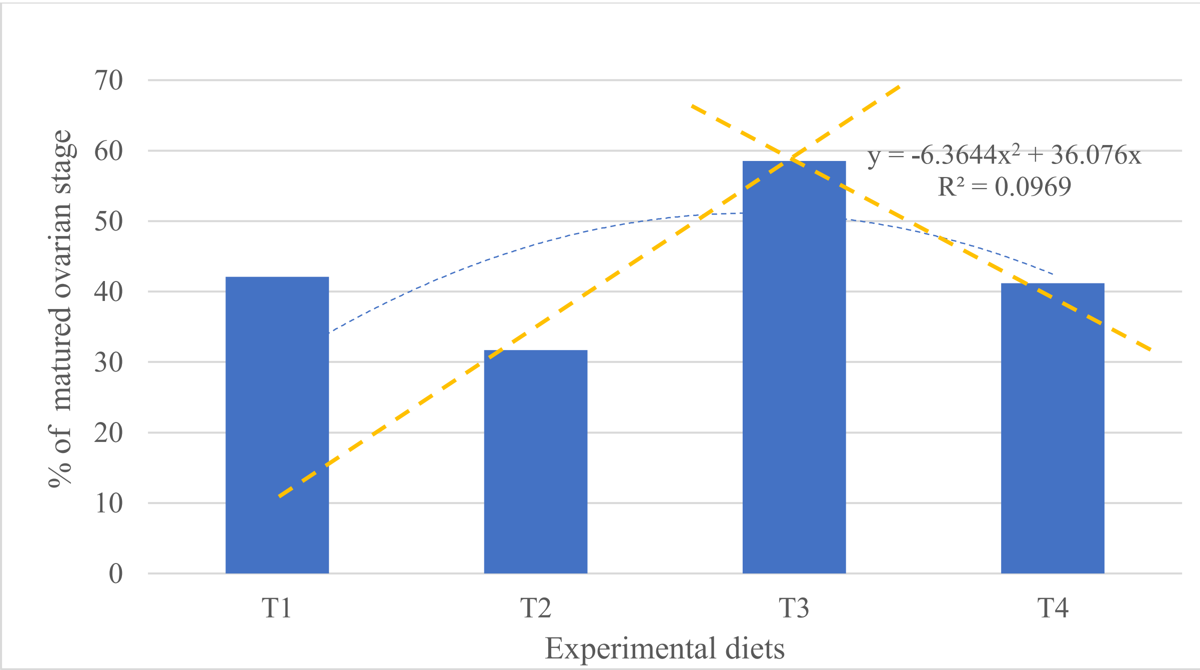

Figure 3. Relationship between production of matured ovarian stage (matured OS are OS III, IV & V) of treated GFP female broodstock and ARA content in diets as described by broken-line analysis as indicated by the dotted yellow line. The red circle is the range of optimal level of ARA that resulted the highest percentage of matured OS

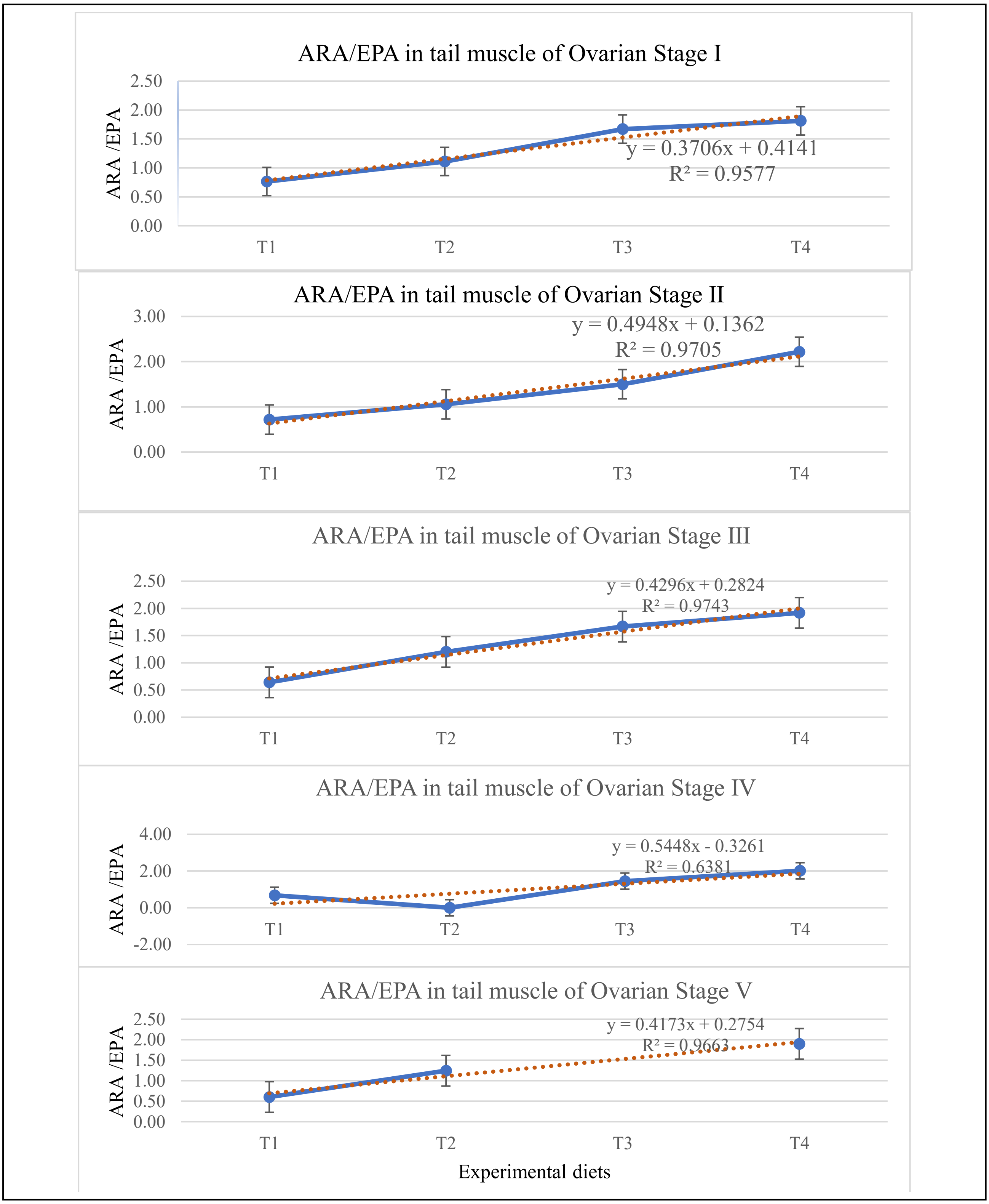

Figure 1. The relation of ARA/EPA for tail muscle of treated broodstock with increasing dietary ARA level at each ovarian stage

Tail Muscle Fatty Acid Composition

The fatty acid composition of treated broodstock tail muscle reflected the fatty acid profile of the diets used, as shown in Table 4. The significant level of fatty acid composition was tested within the same OS group with different diet treatments ($P < 0.05$). The concentrations of saturated fatty acids (SFA) in the treated broodstock of the ovarian stage (OS) I group showed no significant differences ($P > 0.05$) except for C18:0, which increased significantly ($P < 0.05$) by 10.22 %, 11.48 %, 12.02 %, and 12.63 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. Likewise, the C18:0 of the treated broodstock from other OS groups showed a similar trend of increasing across the experimental diets.

Meanwhile, the monounsaturated fatty acids (MUFA) in the tail muscle of treated broodstock for the OS I group were inversely proportioned with the dietary ARA content, whereby C18:1n9 content gradually declined with the value of 26.85, 24.04, 19.05, and 17.30 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. The same pattern was also observed for the C18:1n9 content for different OS groups when moving from T1 to T4 diet (Table 4).

The ARA content in the treated broodstock tail muscle at OS I significantly increased ($P < 0.05$) with the increasing dietary ARA content by 7.90, 10.30, 14.10, and 16.96 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. Moreover, at each ovarian stage, the highest ARA content was recorded from the treated broodstock fed with T4 diet, which was significantly higher ($P < 0.05$) compared to other treated broodstock groups. Furthermore, the ratio of ARA to EPA in the tail muscle showed an increasing trend for all OS across the experimental diets, with a range of 0.77 – 1.81 %, 0.72 – 2.22 %, 0.64 – 1.92 %, 0.68 – 2.01 %, and 0.60 – 1.90 % when moving from OS I to OS V, respectively (Figure 5).

Table 1. Tail muscle fatty acid composition (% of total fatty acid) of female freshwater broodstock fed the diets with increasing ARA levels for ovarian stage I, II, III, IV and V[1]

	Ovarian stage I				Ovarian stage II				Ovarian stage III				One way ANOVA
Fatty acid	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	P-value OS I, II & III
C16:0	18.38	18.64	18.57	17.26	16.98 ^{a}	18.68 ^{c}	17.80 ^{ab}	18.41 ^{bc}	17.48	18.87	17.78	17.59	0.632, 0.023, 0.485
C17:0	7.50	7.48	6.02	8.53	8.35 ^{b}	7.00 ^{ab}	6.40 ^{a}	6.73 ^{ab}	7.52	7.24	7.50	7.34	0.573, 0.076, 0.492
C18:0	10.22 ^{a}	11.48 ^{b}	12.02 ^{c}	12.63 ^{d}	10.17 ^{a}	11.04 ^{ab}	11.50 ^{bc}	12.22 ^{c}	10.41	11.01	12.32	13.33	0.055, 0.031, 0.062
C18:1n9	26.85 ^{a}	24.04 ^{b}	19.05 ^{c}	17.30 ^{c}	25.01 ^{ab}	23.42 ^{b}	21.86 ^{ab}	19.75 ^{a}	24.02	24.07	19.72	18.59	0.002, 0.00, 0.121
C18:3n3 ALA	0.49	ND	0.78	ND	ND	ND	0.69	ND	0.57	ND	0.67	ND	NA
C20:5n3 EPA	10.33	9.27	8.44	9.35	10.57 ^{b}	9.52 ^{b}	8.30 ^{a}	7.86 ^{a}	10.90	8.13	8.38	9.03	0.446, 0.006, 0.072
C22:6n3 DHA	3.69	3.96	4.05	4.11	4.44 ^{a}	4.41 ^{a}	3.97 ^{b}	4.01 ^{b}	3.99 ^{a}	4.30	4.12	4.31	0.621, 0.023, 0.479
C18:2n6 LA	9.82	8.64	9.35	7.29	8.39 ^{b}	9.51 ^{b}	12.03 ^{c}	8.16 ^{a}	7.67 ^{a}	9.31 ^{b}	7.54 ^{b}	7.36 ^{b}	0.343, 0.001, 0.053
C20:4n6 ARA	<u>7.90 ^{a}</u>	<u>10.30 ^{b}</u>	<u>14.10 ^{c}</u>	<u>16.96 ^{d}</u>	7.59 ^{a}	10.05 ^{b}	12.45 ^{c}	17.43 ^{d}	<u>6.98 ^{a}</u>	<u>9.76 ^{ab}</u>	<u>13.96 ^{b}</u>	<u>17.32 ^{c}</u>	0.003, 0.00, 0.004
Σ saturated	38.33	38.91	38.61	40.71	37.70	38.09	37.68	38.55	37.34	38.43	39.86	38.25	
ΣMUFA	28.64	28.91	23.85	21.58	27.79	27.75	24.13	24.60	29.66	29.40	25.42	23.74	
ΣPUFA(n-3)	14.59	13.23	13.26	13.46	15.41	13.94	12.95	11.86	15.64	12.43	13.17	13.34	
ΣPUFA(n-6)	18.36	18.94	23.45	24.26	17.07	19.56	24.48	25.59	15.06	19.07	21.94	24.67	
ΣPUFA (total)	32.95	32.18	36.72	37.72	32.48	33.50	37.44	37.45	30.71	31.49	35.12	38.01	
ΣLC-HUFA(n-3)	14.02	13.23	12.49	13.46	15.21	13.94	12.27	11.86	15.07	12.43	12.50	13.34	
ARA/EPA	0.77	1.11	1.67	1.81	0.72	1.06	1.50	2.22	0.64	1.20	1.67	1.92	
ARA/DHA	2.14	2.60	3.48	4.13	1.71	2.28	3.14	4.35	1.75	2.27	3.39	4.02	
EPA+DHA	14.02	13.23	12.49	13.46	15.01	13.94	12.27	11.86	14.89	12.43	12.50	13.34	
EPA/DHA	2.80	2.34	2.08	2.28	2.38	2.16	2.09	1.96	2.73	1.89	2.04	2.10	
DHA/EPA	0.36	0.43	0.48	0.44	0.42	0.46	0.48	0.51	0.37	0.53	0.49	0.48	

Note:
[1]Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05., ND: not detected, ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Table 4 (continued)

Fatty acid	Ovarian stage IV ARA1	ARA2	ARA3	ARA4	One way ANOVA P-value OS IV	Ovarian stage V ARA1	ARA2	ARA3	ARA4	One way ANOVA P-value OS V
C16:0	17.51 [a]	NA	17.94 [ab]	18.87 [ab]	0.003	19.50 [c]	18.72 [bc]	NA	17.39 [ab]	0.024
C17:0	7.00	NA	6.63	6.67	0.301	7.03	7.11	NA	6.96	0.242
C18:0	9.85 [a]	NA	11.22 [a]	12.47 [b]	0.003	10.62 [ab]	12.85 [c]	NA	12.42 [bc]	0.026
C18:1n9	26.41 [a]	NA	20.52 [b]	21.31 [c]	0.003	30.41 [a]	21.97 [b]	NA	19.17 [c]	0.003
C18:3n3 ALA	0.84	NA	0.86	ND	NA	ND	ND	NA	ND	NA
C20:5n3 EPA	9.30 [ab]	NA	9.18 [ab]	7.84 [a]	0.041	10.08 [a]	8.92 [ab]	NA	8.55 [ab]	0.032
C22:6n3 DHA	3.96 [b]	NA	4.23 [b]	3.27 [a]	0.009	3.45	3.61	NA	3.58	0.359
C18:2n6 LA	9.31 [a]	NA	ND	9.36 [b]	0.003	8.89 [a]	7.55 [b]	NA	8.06 [c]	0.003
C20:4n6 ARA	6.33 [a]	NA	13.31 [b]	15.78 [c]	0.003	6.07 [a]	11.11 [b]	NA	16.24 [c]	0.003
Σ saturated [a]	36.91	NA	37.76	37.55		38.60	40.94	NA	38.89	
ΣMUFA [b]	29.84	NA	25.90	26.20		32.90	27.16	NA	24.01	
ΣPUFA(n-3) [c]	14.19	NA	14.26	11.11		13.53	12.53	NA	12.13	
ΣPUFA(n-6) [d]	16.06	NA	22.08	25.14		14.97	18.66	NA	24.30	
ΣPUFA (total)	30.25	NA	36.34	36.25		28.50	31.19	NA	36.43	
ΣLC-HUFA(n-3) [e]	13.35	NA	13.40	11.11		13.53	12.53	NA	12.13	
ARA/EPA	0.68	NA	1.45	2.01		0.60	1.24	NA	1.90	
ARA/DHA	1.60	NA	3.15	4.82		1.76	3.08	NA	4.53	
EPA+DHA	13.25	NA	13.40	11.11		13.53	12.53	NA	12.13	
EPA/DHA	2.35	NA	2.17	2.39		2.92	2.47	NA	2.39	
DHA/EPA	0.43	NA	0.46	0.42		0.34	0.40	NA	0.42	

Note:

- [a] Σ SFA : sum of all saturated fatty acids
- [b] Σ MUFA : sum of all monounsaturated fatty acids
- [c] ΣPUFA(n-3) : sum of all omega-3 polyunsaturated fatty acids
- [d] ΣPUFA(n-6) : sum of all omega-6 polyunsaturated fatty acids
- [e] Σ n-3 LC-PUFA : sum of all omega-3 long chain polyunsaturated fatty acids

[1]Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05.
ND: not detected.
ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Hepatopancreas fatty acid composition

The fatty acid composition of treated broodstock hepatopancreas samples of five OS reflected the fatty acid profile of the experimental diets (Table 5). For all OS of treated broodstock samples analysed, the SFA and MUFA contents in the hepatopancreas decreased with increasing dietary ARA. The highest value of C16:0 was recorded in the treated broodstock group fed with T1 diet compared to other diets, with a value of 26.46 %, 25.05 %, 24.18%, 23.36 %, and 22.82 % for OS I, II, III, IV, and V, respectively. Moreover, the C16:0 content of the same OS decreased as the dietary ARA content increased (Table 5).

The n-3 PUFA, particularly C20:5n3 and C22:6n3, showed a significantly higher ($P < 0.05$) value for treated broodstock fed with T1 diet compared to treated broodstock fed with other diets for OS I. However, no significant differences ($P > 0.05$) were observed for n-3 PUFA for treated broodstock groups fed with T2, T3, and T4 diets within the same OS, moving from OS I until OS V (Table 5).

The n-6 PUFA content of treated broodstock, particularly for arachidonic acid (ARA, C20:4n6), increased with increasing dietary ARA levels and was significantly higher ($P < 0.05$) in the treated broodstock groups fed with T4 diet for all OS (Table 5). Moreover, the ARA to EPA ratio recorded for each OS increased with increasing dietary ARA levels from T1 to T4 as demonstrated in Figure 6. As shown in Figure 5, the ARA to EPA ratio increased for OS 1 from 0.45, 1.79, 2.88, and 4.92 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. The OS II, OS III, OS IV, and OS V groups recorded a similar trend of increasing ARA to EPA ratio with increasing dietary ARA levels for all treated broodstock (Figure 5).

Table 2. Hepatopancreas fatty acid composition (% of total fatty acid) of female freshwater prawn broodstock fed the diets with increasing ARA levels for ovarian stage I, II, III, IV and V[1]

	Ovarian stage I				Ovarian stage II				Ovarian stage III				One way ANOVA
Fatty acid compounds	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	P-value of OS I, II & III
C16:0	26.62^{c}	22.83^{b}	23.18^{b}	19.85^{a}	25.05^{a}	23.58^{b}	23.09^{b}	21.86^{c}	24.18^{b}	23.15^{b}	22.40^{b}	20.02^{a}	0.007, 0.002, 0.012
C17:0	1.90	0.55	0.55	0.58	0.52	0.73	0.50	1.20	2.55	0.72	0.91	0.69	0.180, 0.236, 0.071
C18:0	7.29	5.77	6.81	6.80	5.91^{b}	5.74^{b}	6.71^{b}	8.15^{a}	8.02^{bc}	6.81^{a}	7.71^{b}	8.67^{c}	0.690, 0.011, 0.016
C18:1n9	28.89^{b}	25.57^{b}	28.15^{b}	19.55^{a}	30.52^{a}	27.08^{ab}	26.45^{ab}	19.13^{b}	34.37^{c}	27.34^{b}	25.86^{ab}	20.80^{a}	0.015, 0.093, 0.014
C20:1n9	2.57	3.22	2.01	3.22	2.74	2.63	2.60	3.54	1.46^{a}	3.18^{b}	2.91^{b}	3.93^{c}	0.296, 0.283, 0.002
C18:3n3 ALA	1.23	1.23	1.00	1.34	1.08	1.25	1.08	1.19	ND	1.21^{b}	1.04^{a}	1.04^{a}	0.203, 0.508, 0.032
C20:5n3 EPA	4.22^{a}	1.92^{b}	1.48^{b}	1.85^{b}	2.01^{b}	1.87^{b}	1.23^{a}	1.76^{ab}	4.87^{a}	1.71^{c}	1.32^{ab}	1.44^{b}	0.033, 0.50, 0.008
C22:6n3 DHA	3.31^{a}	2.18^{b}	1.94^{b}	2.34^{b}	2.17	2.70	1.61	2.08	3.28^{a}	1.98^{ab}	1.61^{b}	1.71^{b}	0.026, 0.060, 0.040
C18:2n6 LA	10.90	11.25	10.52	11.62	10.22^{c}	11.36^{b}	10.90^{a}	9.04^{a}	8.51	10.68	9.78	8.99	0.698, 0.004, 0.210
C20:4n6 ARA	1.90^{a}	3.44^{ab}	4.26^{b}	9.10^{c}	0.76^{a}	3.48^{b}	4.19^{b}	7.66^{c}	2.03^{a}	3.19^{b}	4.69^{c}	7.74^{d}	0.001, 0.00, 0.001
Σ saturated	39.20	35.21	35.67	33.91	35.96	34.46	35.80	38.92	37.15	35.76	36.68	36.22	
ΣMUFA	39.85	38.81	40.48	30.77	44.94	41.24	40.09	34.76	44.16	43.41	40.53	37.53	
ΣPUFA(n-3)	8.76	6.13	4.93	6.69	5.50	5.82	4.11	5.31	8.16	4.90	4.19	4.47	
ΣPUFA (n-6)	12.80	17.30	16.05	25.54	11.51	18.06	16.57	18.91	10.53	14.95	16.04	18.94	
ΣPUFA (total)	21.56	23.43	20.99	32.23	17.01	23.88	20.68	24.22	18.69	19.86	20.23	23.41	
ΣLC-PUFA(n-3)	7.53	4.26	3.55	4.64	4.18	4.57	2.84	3.84	8.16	3.70	2.93	3.15	
ARA/EPA	0.45	1.79	2.88	4.92	0.38	1.86	3.42	4.36	0.42	1.86	3.54	5.36	
ARA/DHA	0.57	1.58	2.20	3.89	I	1.29	2.60	3.67	0.62	1.61	2.92	4.53	
EPA+DHA	7.53	4.10	3.42	4.19	4.18	4.57	2.84	3.84	8.16	3.70	2.93	3.15	
EPA/DHA	1.27	0.88	0.76	0.79	0.93	0.69	0.76	0.84	1.48	0.86	0.82	0.85	
DHA/EPA	0.79	1.13	1.31	1.26	1.08	1.44	1.32	1.19	0.67	1.16	1.21	1.18	

Note:
[1]Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. ND: not detected, ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Table 5 (continued)

Fatty acid compound	Ovarian stage IV				One way ANOVA P-value OS IV	Ovarian stage V				One way ANOVA P-value OS V
	ARA1	ARA2	ARA3	ARA4		ARA1	ARA2	ARA3	ARA4	
C16:0	23.36[b]	NA	18.66[ab]	24.00[b]	0.035	22.82	20.20	23.43	20.88	0.390
C17:0	3.40	NA	3.26	0.82	0.193	2.17	3.23	4.24	1.34	0.386
C18:0	9.62	NA	9.66	9.34	0.317	7.72 [a]	9.44 [a]	14.39[b]	10.28 [a]	0.029
C18:1n9	31.10[a]	NA	22.32[c]	28.27[b]	0.003	31.06 [b]	28.42 [ab]	29.02 [b]	23.14 [a]	0.039
C20:1n9	1.91	NA	2.39	ND	0.860	3.00	2.25	ND	ND	0.269
C18:3n3 ALA	ND	NA	ND	ND	-	0.91	0.83	ND	0.96[a]	0.995
C20:5n3 EPA	4.78	NA	3.11	ND	0.071	4.16	3.50	ND	1.43	0.170
C22:6n3 DHA	1.45	NA	2.79	ND	0.136	1.99	3.26	ND	1.79	0.170
C18:2n6 LA	9.05	NA	8.59	8.05	0.407	8.02	9.10	8.67	8.27	0.582
C20:4n6 ARA	1.04[a]	NA	7.90[b]	3.23[c]	0.009	2.71 [a]	5.77 [ab]	10.01 [b]	7.20 [b]	0.030
Σ saturated	39.74	NA	36.31	42.07		35.74	35.74	42.06	39.29	
ΣMUFA	43.55	NA	37.78	41.89		44.20	41.26	39.72	35.63	
ΣPUFA(n-3)	6.67	NA	7.35	0.00		6.67	7.47	0.00	4.17	
ΣPUFA(n-6)	10.04	NA	17.99	9.57		10.57	15.54	18.22	17.68	
ΣPUFA (total)	16.71	NA	25.34	9.57		17.24	23.00	18.22	21.85	
ΣLC-PUFA(n-3)	6.00	NA	5.92	0.00		5.81	6.65	0.00	3.21	
ARA/EPA	<u>0.22</u>	NA	<u>2.54</u>	ND		<u>0.65</u>	<u>1.65</u>	ND	<u>5.05</u>	
ARA/DHA	0.69	NA	2.71	ND		1.36	1.77	ND	4.03	
EPA+DHA	6.00	NA	5.76	ND		5.81	6.65	ND	3.21	
EPA/DHA	3.14	NA	1.07	ND		2.10	1.07	ND	0.80	
DHA/EPA	0.32	NA	0.94	ND		0.48	0.93	ND	1.25	

Note:
[1]Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. ND: not detected, ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

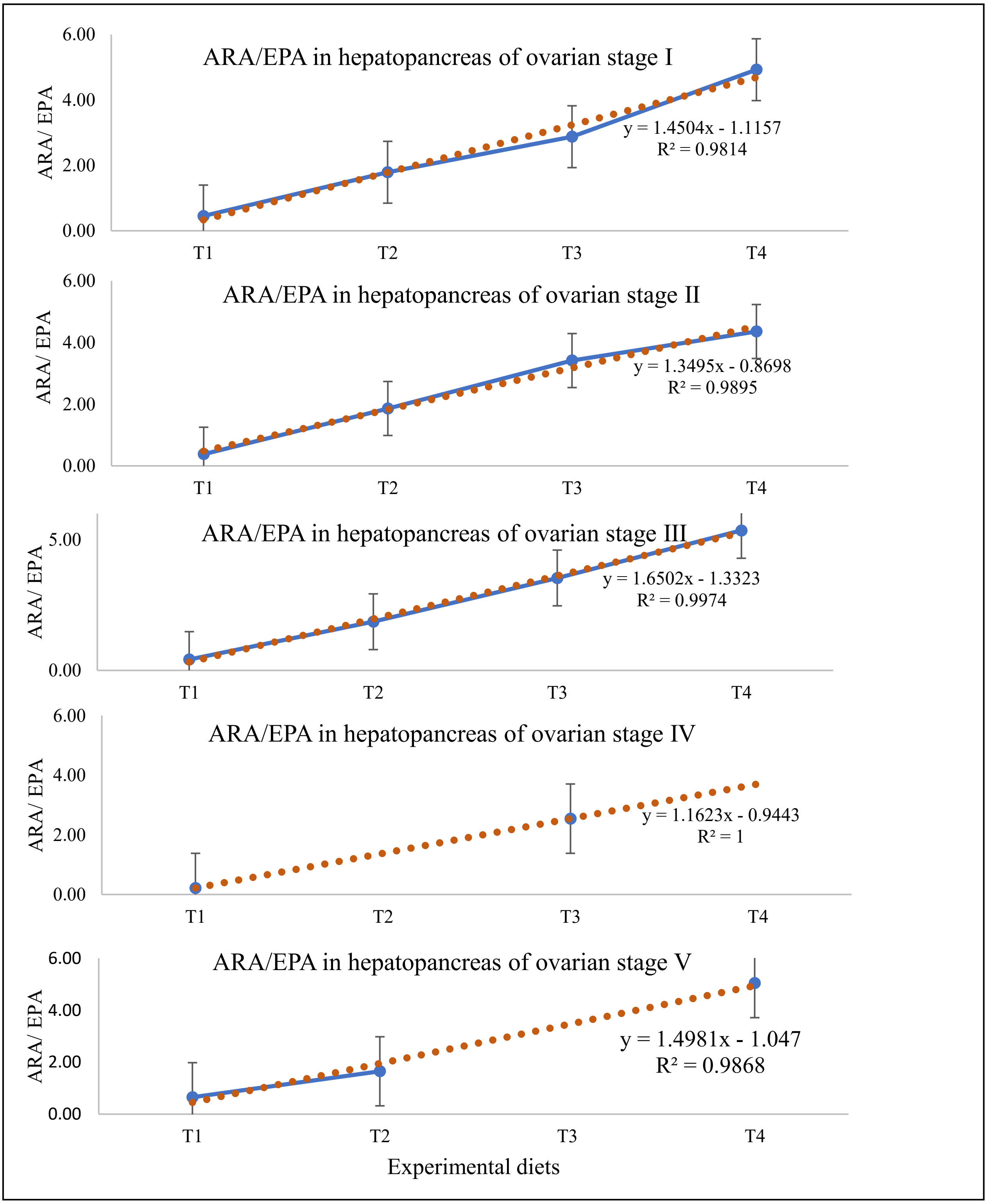

Figure 5. The relation of ARA/EPA for hepatopancreas of treated broodstock with increasing dietary ARA level at each ovarian stage

Gonad fatty acid composition

The gonad fatty acid composition of treated broodstock for all experimental groups are shown in Table 6. For each OS, the SFA content of the treated broodstock fluctuated across the experimental diets. For OS I, C16:0 content of 24.50 %, 21.25 %, 22.26 %, and 23.05 % were recorded for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. Meanwhile, the MUFA, C18:1n9 for OS I, decreased as treated broodstock dietary ARA increased, from 38.31 % to 31.89 %, 32.73 %, and 30.12 % for broodstock fed with T1, T2, T3, and T4 diets, respectively. The EPA value for OS I also decreased for treated broodstock with increasing ARA levels. Similar patterns were recorded for EPA content for all OS of the gonad samples within the same OS samples.

Like the ARA content in the tail muscle and hepatopancreas of treated broodstock, the ARA content in the gonad increased as the dietary ARA increased. The ARA values of OS I recorded were 5.92 %, 5.85 %, 8.06 %, and 9.93 % for treated broodstock fed with T1, T2, T3, and T4 diets, respectively. In all other OS, the ARA contents increased in treated broodstock fed with T1 to T4 diets, as shown in Table 6. The highest ARA content was recorded in the gonad of the treated broodstock fed with T4 diet, with a value of 9.93 %, 10.75 %, 10.17 %, 9.25 %, and 8.91 % for OS I, II, III, IV, and V, respectively, as compared to other diets.

The ARA to EPA ratio recorded in the gonad for each OS was increased with increasing dietary ARA levels from T1 to T4 as demonstrated in Figure 7. The ARA to EPA ratios of tail muscle, hepatopancreas, and gonads of the treated broodstock fed with experimental diets for each OS are presented in Figures 4, 5, and 6, in which the ARA to EPA ratio shows an increasing trend as the dietary ARA level increased in the hepatopancreas, gonads, and tail muscles of treated broodstock for all OS. Although no samples were available for a certain OS, the prediction can still be made with the available data, as shown in Figure 5 for OS V. However, the ARA to EPA ratio in the gonads of the treated broodstock showed a different pattern for OS III, whereby the ARA to EPA ratio in the gonads increased for treated broodstock fed with T1, T2, and T3 diets, and then decreased sharply in the treated broodstock fed with T4 diets (Figure 7).

Table 3. Gonad fatty acid composition (% of total fatty acid) of female freshwater prawn broodstock fed the diets with increasing dietary ARA levels for ovarian stage I, II, III, IV and V[1]

Fatty acid compound	Ovarian Stage I				Ovarian Stage II				Ovarian Stage III				One way ANOVA
	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	ARA1	ARA2	ARA3	ARA4	P-value OS I, II & III
C16:0	24.50^{b}	21.25^{a}	22.26^{a}	23.05^{ab}	22.80	20.37	21.89	22.08	22.89	20.97	21.12	20.47	0.037, 0.126, 0.333
C17:0	ND	1.48	0.69	1.36	0.89	1.53	1.26	2.40	1.17^{a}	1.18^{a}	0.68^{a}	1.34^{b}	0.043, 0.537, 0.036
C18:0	8.22^{bc}	6.03^{a}	7.80^{b}	8.69^{c}	5.84^{a}	7.12^{b}	7.35^{b}	8.98^{c}	5.98	6.38	8.02	8.23	0.00, 0.005, 0.393
C18:1n9	38.31	31.89	32.73	30.12	39.92^{c}	31.76^{b}	32.30^{b}	27.87^{a}	40.41^{a}	32.36^{b}	31.53^{b}	27.41^{b}	0.061, 0.003, 0.026
C20:1n9	ND	0.83	ND	ND	ND	1.03	ND	ND	ND	1.12^{b}	1.18^{a}	1.35^{c}	NA, NA, 0.001
C18:3n3 ALA	ND	1.22	1.34	1.31	1.30	1.31	1.28	1.44	0.95	1.29	1.14	1.28	0.010, 0.311, 0.19
C20:5n3 EPA	6.58	4.14	3.41	3.15	4.02	3.75	3.68	3.22	4.72	3.41	1.32	2.90	0.112, 0.339, 0.09
C22:6n3 DHA	ND	3.62	3.86	3.56	3.37	4.06	4.03	4.28	3.26^{a}	3.76^{b}	3.31^{a}	3.68^{b}	0.266, 0.521, 0.00
C18:2n6 LA	14.21	12.39	13.26	12.12	13.62	12.86	13.66	12.93	11.34	12.42	11.95	12.09	0.068, 0.171, 0.19
C20:4n6 ARA	5.92^{a}	5.85^{a}	8.06^{ab}	9.93^{b}	1.28^{a}	5.35^{b}	9.03^{c}	10.75^{c}	2.02^{a}	4.79^{b}	6.91^{c}	10.17^{d}	0.025, 0.001, 0.00
Σ saturated	32.57	31.14	32.26	34.90	30.96	30.97	32.05	35.12	31.65	30.47	31.95	32.58	
ΣMUFA	41.20	37.07	37.07	33.71	44.41	36.57	35.48	29.15	45.29	37.78	38.94	33.61	
ΣPUFA(n-3)	6.46	9.47	8.58	8.02	8.69	9.67	8.99	8.94	8.93	8.72	7.19	8.11	
ΣPUFA(n-6)	19.77	19.27	21.25	22.05	14.90	19.28	22.69	23.68	13.36	18.68	20.17	24.22	
ΣPUFA (total)	26.23	28.73	29.83	30.07	23.59	28.95	31.67	32.62	22.29	27.40	27.36	32.34	
ΣLC-HUFA(n-3)	6.46	8.11	7.24	6.71	7.39	8.22	7.71	7.50	7.98	7.27	6.05	6.83	
ARA/EPA	0.90	1.42	2.37	3.15	0.32	1.43	2.45	3.34	0.43	1.40	5.22	3.50	
ARA/DHA	NA	1.62	2.09	2.79	0.38	1.32	2.24	2.51	0.62	1.27	2.09	2.77	
EPA+DHA	6.46	7.75	7.24	6.71	7.39	7.81	7.71	7.50	7.98	7.17	4.63	6.58	
EPA/DHA	NA	1.14	0.88	0.89	1.19	0.92	0.91	0.75	1.45	0.91	0.40	0.79	
DHA/EPA	0.00	0.87	1.13	1.13	0.84	1.08	1.09	1.33	0.69	1.10	2.50	1.27	

Note:
[1]Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stage indicate significant differences at P<0.05. P-value between group each OS. ND: not detected, ARA1 =0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

Table 6 (continued)

Fatty acid compound	Ovarian stage IV				One way ANOVA P-value OS IV	Ovarian stage V				One way ANOVA P-value OS V
	ARA1	ARA2	ARA3	ARA4		ARA1	ARA2	ARA3	ARA4	
C16:0	22.64^{a}	NA	21.53^{b}	20.40^{c}	0.003	21.93^{b}	19.87^{a}	21.09^{b}	19.75^{a}	0.009
C17:0	1.26	NA	1.38	1.31	0.375	0.34^{a}	1.30^{b}	0.58^{a}	1.42^{b}	0.008
C18:0	6.37^{ab}	NA	7.86^{b}	8.05^{b}	0.043	5.65^{a}	6.96^{b}	7.37^{bc}	7.93^{c}	0.010
C18:1n9	37.16^{c}	NA	31.79^{bc}	30.92^{ab}	0.008	38.16^{c}	11.65^{a}	31.18^{b}	30.63^{a}	0.004
C20:1n9	ND	NA	1.24	0.00	-	0.00^{b}	1.09^{a}	1.30^{c}	1.20^{a}	0.009
C18:3n3 ALA	1.13	NA	1.06	1.14	0.227	1.11^{a}	1.17^{a}	1.28^{b}	1.30^{b}	0.007
C20:5n3 EPA	3.54^{a}	NA	2.77^{b}	2.85^{b}	0.029	3.83^{b}	3.42^{ab}	3.00^{a}	2.81^{a}	0.034
C22:6n3 DHA	3.15	NA	2.99	3.46	0.279	3.15^{ab}	3.40^{b}	3.47^{b}	2.94^{a}	0.048
C18:2n6 LA	11.49	NA	10.88	11.93	0.184	11.46^{a}	11.50^{a}	12.23^{b}	10.84^{c}	0.006
C20:4n6 ARA	1.37^{a}	NA	6.27^{b}	9.25^{c}	0.000	1.09^{a}	4.07^{b}	7.06^{c}	8.91^{d}	0.000
Σsaturated	32.65	NA	33.80	32.07		30.21	30.48	31.90	31.27	
ΣMUFA	44.56	NA	38.30	35.43		45.55	40.03	38.30	36.90	
ΣPUFA(n-3)	7.82	NA	6.94	7.45		8.61	8.31	7.74	7.41	
ΣPUFA(n-6)	13.46	NA	18.65	22.81		13.29	16.70	21.05	21.58	
ΣPUFA (total)	21.28	NA	25.59	30.26		21.91	25.01	28.80	28.99	
ΣLC-HUFA(n-3)	6.69	NA	5.88	6.31		7.32	6.97	6.47	6.11	
ARA/EPA	0.39	NA	2.27	3.25		0.29	1.19	2.35	3.17	
ARA/DHA	0.43	NA	2.10	2.67		0.35	1.19	2.04	3.03	
EPA+DHA	6.69	NA	5.75	6.31		6.97	6.83	6.47	5.75	
EPA/DHA	1.12	NA	0.93	0.82		1.22	1.00	0.87	0.96	
DHA/EPA	0.89	NA	1.08	1.22		0.82	1.00	1.15	1.05	

Note:
[1]Values are the mean ± SE of the duplicate samples. Different superscripts in the same row for each group of ovarian stages indicate significant differences at P<0.05. ND: not detected, NA = Sample is not available, ARA1 = 0.03% ARA diet; ARA2 = 0.35% ARA diet, ARA3 = 0.70% ARA diet, ARA4 = 1.17% ARA diet.

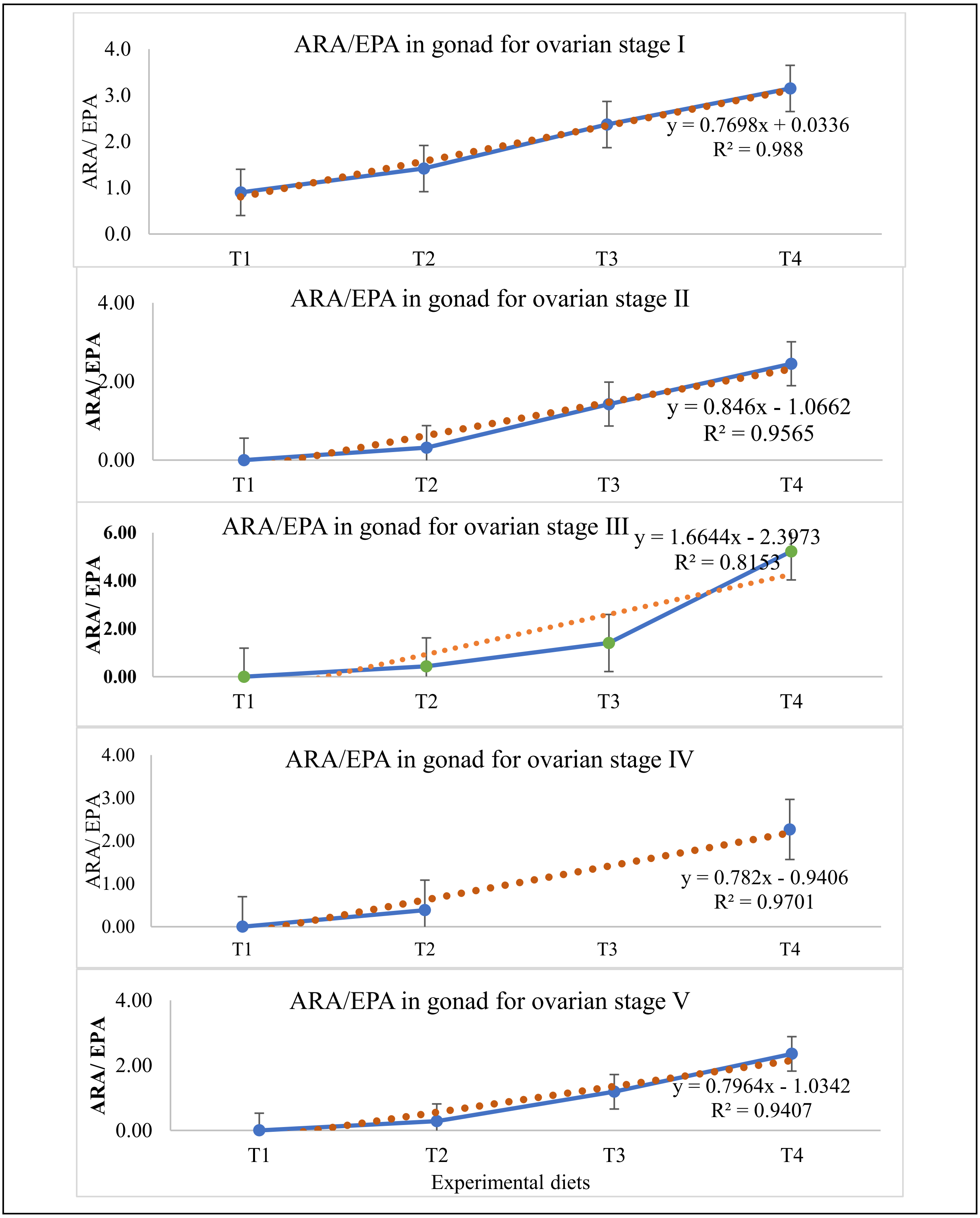

Figure 1. The relation of ARA/EPA for gonad of treated broodstock with increasing dietary ARA level at each ovarian stage

Histological evaluation

Figure 7 shows the representative histological cross-sections of the hepatopancreas and gonad of the initial sample. The cross-section of the initial sample indicated that the ovary was at OS I and II, as described by Soonklang et al. (2012), Sumpownon et al. (2015), and Shen et al. (2020). The embryonic cell (E-cell) and fibrillenzellen cell (F-cell) for the initial sample of hepatopancreas and gonad are shown in Figure 7, and the identification of cells was referred to the work done by Silva et al. (2018). The histological slide images for the final samples are shown in Figure 8 for hepatopancreas and ovary of treated broodstock fed with T2 and T3 diets at OS III. Basically, the final cells of the ovary fed with T3 and T4 diets showed healthy and well-arranged cells. Figure 8 shows the representative cells in the hepatopancreas and ovary that were measured, and the results were as tabulated in Table 7.

Table 7 summarises the analysed parameters from the histological slides of the hepatopancreas and ovary. The hepatopancreas tubule diameter of treated broodstock fed with T2 diet was recorded at 96.54 μm, which was significantly lower than the treated broodstock fed with T4 diets ($P < 0.05$); but showed no significant different compared to treated broodstock fed with T1 and T3 diets ($P > 0.05$). The hepatopancreas tubule area of 6643.89 μm^2 was recorded for the treated broodstock fed with T1 diet, which showed no significant difference ($P < 0.05$) compared to other groups. Meanwhile, the hepatopancreas tubule diameter of 108.28 μm was recorded for treated broodstock fed with T4 diet and was significantly higher ($P < 0.05$) than the treated broodstock fed with T3 diet, but did not significantly differ ($P > 0.05$) with treated broodstock fed with T1 and T2 diets.

The oocyte diameter recorded for treated broodstock fed with T3 diets showed a significantly higher ($P < 0.05$) value of 207.64 μm compared to other treatment groups. Moreover, oocyte diameters of 126.57 μm, 169.54 μm, and 139.84 μm were recorded for treated broodstock fed with T1, T2, and T4 diets, respectively, yet no significant difference were found between these groups ($P > 0.05$) (Table 7). Based on the oocyte diameter, it showed a pattern of increasing in size as dietary ARA increased up to T3, and then decreased in T4 (Figure 9). In Figure 9, it illustrates a second-order polynomial regression relationship between oocyte diameter and dietary ARA for the treated GFP female broodstock. The optimal level of ARA in the diet, as represented by the red circle in Figure 9, can be estimated by applying the dose-response concept or broken line analysis to the diameter size of the oocyte.

Table 4. The diameter and area for tubule in hepatopancreas (tubule) and ovary (oocyte) of the experimental broodstock after feeding trial. (n=57, Mean ± S.E.)[1]

	Hepatopancreas		Ovary
Diet	Tubule diameter (µm)	Tubule area (µm^2)	Oocyte diameter (µm)
Initial	113.35 ± 2.44	8605.51 ± 255.68	41.50 ± 1.12
ARA1	102.57 ± 2.85^{ab}	6643.89 ± 329.39^{ab}	126.57 ± 12.67^{a}
ARA2	96.54 ± 1.85^{ab}	6507.60 ± 322.12^{ab}	169.54 ± 3.28^{a}
ARA3	102.25 ± 2.54^{a}	6016.02 ± 222.26^{a}	207.64± 4.59^{b}
ARA4	108.27 ± 3.18^{b}	7307.58 ± 366.43^{b}	139.84 ± 6.07^{a}

[1]Values are the mean ± SE of a group of representative histological slide samples (n=57). Different superscripts in the same column indicate significant differences at P<0.05. Initial samples were excluded from statistical analysis.

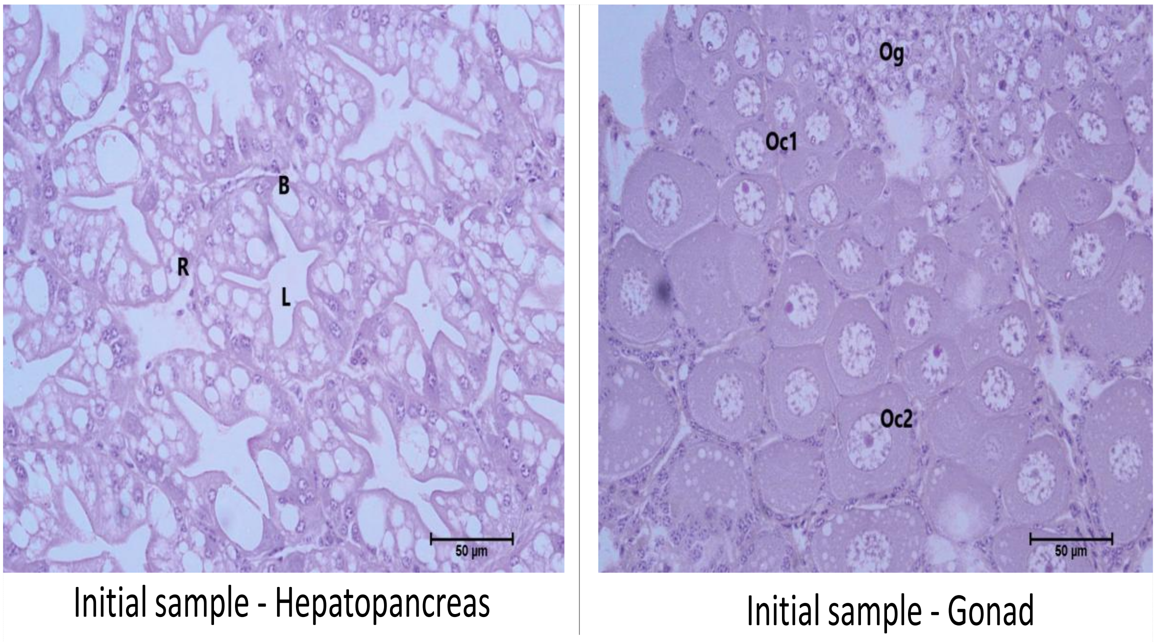

Figure 7. Representative histological cross-sections of the hepatopancreas of initial prawn showing the arrangement of hepatopancreatic tubules and cells (on the left) and gonad (on the right). (20 × Magnification) Og = oogonia, Oc1 & Oc2 = previtellogenic oocytes, L = Lumen of tubule, B = B- cell and R = R- cell

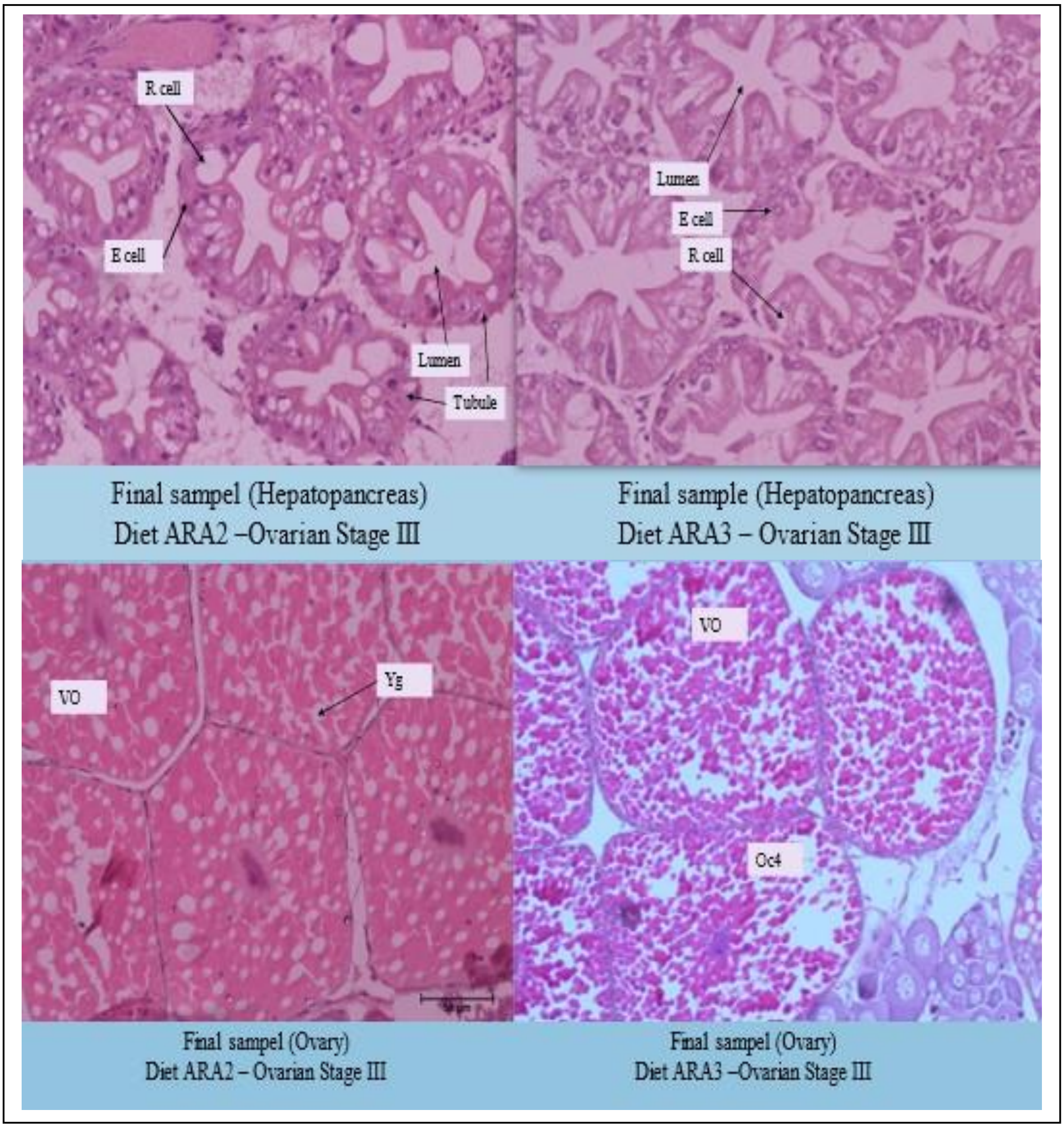

Figure 8. Representative final histological sample of ovary and hepatopancreas for treated female broodstock fed the diets T2 and T3 diets at ovarian stage III (20 x magnification) VO = vitellogenic oocyte, Yg = yolk granules, Oc4 = mature oocytes

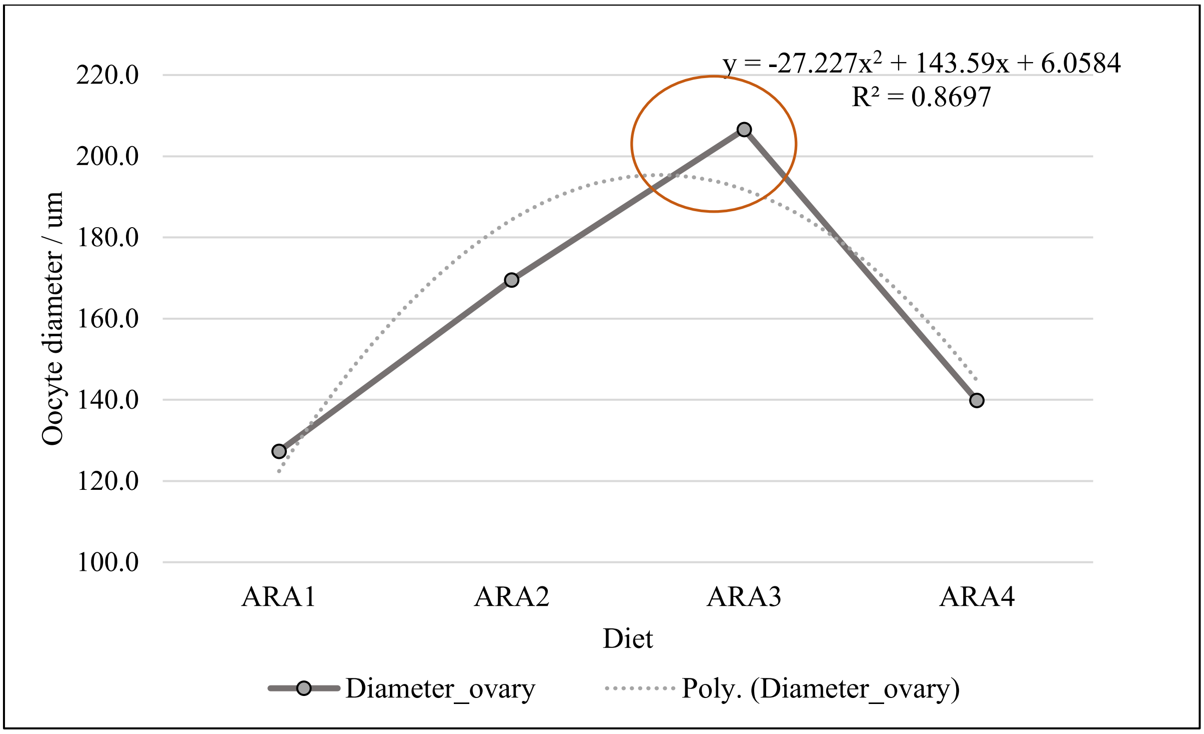

Figure 9. The relationship between oocytes diameter and ARA content in diets for GFP female broodstock was described as second-order polynomial regression

Discussion

The supplementation of fungal oil containing ARA at different levels (0.03, 0.35, 0.70, and 1.17 %) resulted in a significantly higher GSI, particularly at 0.70 % ARA. Nutrient storage in the hepatopancreas is transported to the muscle, gonads, and other tissues during the growth and reproductive stages of prawns (Jiang et al., 2009; Wang et al., 2014). Thus, the nutrients (particularly, fatty acids) stored in the hepatopancreas were found to be reduced at the last stage of ovarian development. The recorded total HSI and GSI reflected the ARA levels in the diets. This can be correlated with the gonad index stage and the oocyte size of the treated broodstock.

Similar results were reported by Kangpanich et al. (2016), where the increasing level of ARA in the diet resulted in increasing GSI and HSI values for *M. rosenbergii* broodstock. Other studies also reported the increasing value of GSI with increasing ARA in the diets of tiger shrimp (*P. monodon*) (Coman et al., 2011), Pacific white shrimp (*Litopenaeus vannamei*) (Xu, Zhang, et al., 2017), red claw crayfish (*Cherax quadricarinatus*) (Shehata et al., 2020), European sea bass juveniles (*Dicentrarchus labrax*) (Torrecillas et al., 2017), and Pacific oyster (*Crassostrea gigas*) (Seguineau et al., 2011). Several researchers also reported on fish and shrimp reproductive performance with different dietary ARA levels (Meunpol et al., 2005; Hoa et al., 2009;

Norambuena et al., 2012; Chimsung, 2014; Norambuena, Estévez, et al., 2013; Norambuena, Morais, et al., 2013; Xu et al., 2017a; Zuo et al., 2018). Like in the present study, the GSI value for sea urchin fed diets of 1 % ARA and 2 % ARA showed a constant value, meaning that the higher supply of ARA in diets beyond a certain level showed no beneficial effects and can have negative effects on reproduction (Zuo et al., 2018).

The fatty acid profile of tail muscle reflected the dietary ARA content in the present study, where the ARA content increased with dietary ARA levels for all OS. A similar result was reported for *M. rosenbergii* which was fed diets of increased dietary ARA levels for all OS (Kangpanich et al., 2016). Moreover, the marine shrimp *P. monodon* showed a higher value for the percentage of spawning and the number of eggs produced when fed with a higher level of ARA diet than a lower ARA (Coman et al., 2011).

This study indicates that the fatty acid profile of the hepatopancreas and ovary of treated broodstock showed increasing value of ARA content (% of total fatty acid) with increasing dietary ARA. Similar results were reported by Kangpanich et al. (2016) on *M. rosenbergii* for hepatopancreas and ovary for all OS. Similar results were also reported for the Pacific white shrimp, *Litopenaeus vannamei*, broodstock where increased ARA content was found in the hepatopancreas and ovary as dietary ARA increased (Xu, Zhang, et al., 2017).

This present study showed that supplementation of ARA in the diets affected the fatty acid deposition in tail muscle, hepatopancreas, and ovary of treated broodstock. Consequently, studies on the requirement of LC-PUFA in freshwater prawn maturation feed could provide more insights on the improvement of gonadal development and productivity of female broodstock.

In this present study, the highest ARA level used was 1.17 % in the diet (T4), whereas Kangpanich et al. (2016) used the highest level of 0.80 % ARA. Thus, based on the results obtained from this and Kangpanich et al. (2016) studies, we can suggest that the optimal ARA level in the diet is around the range of 0.70 – 0.80 % for better gonad development, reproductive performance, and larvae production. As exhibited by the present study, groups fed with the diet of 1.17 % ARA (T4) had significantly inferior HSI, GSI, and ovary size cells compared to treated broodstock fed with other diet groups ($P < 0.05$). The results of the present study indicated that there is no additional benefit in supplementing ARA beyond 0.70 % of TFA in terms of prawn reproductive performance. Eicosanoids derived from omega 6 fatty acids tend to be pro-inflammatory, while those derived from omega 3 fatty acids tend to be anti-inflammatory, as described by Davinelli et al. (2018) and Tsoukalas et al. (2019). Thus, a higher level of ARA may impart negative results on reproductive performance since ARA synthesizes pro-inflammatory eicosanoids.

ARA is a precursor of signalling molecules necessary for crustacean reproduction and prostaglandins E and F of series II (PGE2 and PGF2α) in the cyclooxygenase pathway. PGE2 production in the ovaries of *M. rosenbergii* was reported to be reflected by the retention of ARA in ovarian tissues throughout the maturation stages (Goodall, 2016; Kangpanich et al., 2016). Although the PGE2 level was not analysed in the present study, the retention level of ARA in ovaries increased for prawn broodstock fed with increasing dietary ARA diets for all OS. The ovarian ARA levels obtained from the present study were comparable to those detected in ovaries at different OS of *M. rosenbergii* (Kangpanich et al., 2016) and *L. vannamei* (Xu, Zhang, et al., 2017). The supplementation of ARA in diets did affect the prostaglandin synthesis (Goodall, 2016). The role of ARA in improving reproductive performance with the direct utilisation of ARA as the primary precursor for the synthesis of eicosanoids, namely series II prostaglandins, bioactive lipid mediators with an essential role in the pathway of reproduction in invertebrates (Wimuttisuk et al., 2013; Kangpanich, 2016; Thongbuakaew et al., 2021). The pathway of ARA serves as a precursor for the synthesis of eicosanoids and LC-PUFA is described by Wimuttisuk et al. (2013), Trevor et al. (2003), and Goodall (2016) in their studies.

Meanwhile, based on the histological analysis, the oocyte from the initial sample was at the primary stage with a diameter size of 41.50 µm, which is similar to that described by Martins et al. (2007) and Soonklang et al. (2012). The final samples showed the oocytes diameter of 127.3 – 206.5 µm with the biggest oocytes recorded in treated broodstock fed with T3 diet, and was significantly higher than other treatment groups ($P < 0.05$). All oocytes samples final size were in the development stage of vitellogenic oocytes (Martins et al., 2007). The final sample of oocyte diameter suggested an optimal ARA content in the diet is approximately 0.7 %, resulting in a bigger size compared to other diets. This finding can improve the size of newly hatched larvae generated by domesticated broodstock of *M. rosenbergii*, which were previously claimed to be smaller than those produced by wild broodstock (per comm with local hatchery managers). The smaller newly hatched larvae require special attention, and the artemia used must be small enough for these tiny larvae. By offering *M. rosenbergii* broodstock a maturation diet containing around 0.7 % ARA, the quality of larvae from domesticated broodstock can be improved and comparable to wild broodstock larvae.

The biological evaluation data showed that a dietary ARA level of 0.70 % exhibited better reproductive performance than other diets. Meanwhile, the fatty acid analyses showed that with higher dietary ARA level, the ARA content in tail muscle, hepatopancreas, and ovary of treated broodstock was also higher. Additionally, the histological evaluation revealed that dietary 0.70

% ARA resulted in significantly bigger oocytes in the ovary among treated broodstock fed with various experimental maturation diets (P<0.05).

Conclusion

The results obtained from the present study suggest a dietary level of ARA that promotes the best reproductive performance, with the ovary development of female GFP at 0.70 %. A higher level of ARA (1.17 %) in the diet showed a counterproductive result for the reproductive performance of female GFP. Therefore, it is suggested that the optimal concentration of ARA in the diet should be maintained around 0.70 %.

Recommendation

The optimal inclusion level of ARA in the maturation diet for GFP broodstock has been determined. However, the price of *Mortierella* sp fungi, the source of ARA used in this experiment, is relatively high for the time being. Thus, it is imperative to look for cheaper sources of ARA, for example, alga (*Parietochloris incisa*), polychaeta and terrestrial animal liver, such as bovine liver meal.

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Conflict of Interest

The author(s) do not have any conflict of interest.

References

Ackman, R. G. (2002). The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century. Analytica Chimica Acta, 465(1–2), 175–192. https://doi.org/10.1016/S0003-2670(02)00098-3

AOAC. (1990). AOAC: **Official Methods of Analysis** (Volume 1). In K. Helrich (Ed.), AOAC International (15th ed., Vol. 1, Issue Volume 1).

Cavalli, R. O., Lavens, P., & Sorgeloos, P. (1999). Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. Aquaculture, 179(1–4), 387–402. https://doi.org/10.1016/S0044-8486(99)00173-8

Cavalli, R. O., Menschaert, G., Lavens, P., & Sorgeloos, P. (2000). Maturation performance, offspring quality and lipid composition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. Aquaculture International, 8(1), 41–58. https://doi.org/10.1023/A:1009220932268

Chang, C. F., & Shih, T. W. (1995). Reproductive cycle of ovarian development and vitellogenin profiles in the freshwater prawns, *Macrobrachium rosenbergii.* Invertebrate Reproduction and Development, 27(1), 11–20. https://doi.org/10.1080/07924259.1995.9672429

Chee, W. L., Turchini, G. M., Teoh, C. Y., & Ng, W. K. (2020). Dietary arachidonic acid and the impact on growth performance, health and tissues fatty acids in Malabar red snapper (*Lutjanus malabaricus*) fingerlings. Aquaculture, 519(November), 734–757. https://doi.org/10.1016/j.aquaculture.2019.734757

Chimsung, N. (2014). Maturation diets for black tiger shrimp (*Penaeus monodon*) broodstock: A review. Songklanakarin Journal of Science and Technology, 36(3), 265–273.

Coman, G. J., Arnold, S. J., Barclay, M., & Smith, D. M. (2011). Effect of arachidonic acid supplementation on reproductive performance of tank domesticated *Penaeus monodon*. Aquaculture Nutrition, 17(2), 141–151. https://doi.org/10.1111/j.1365-2095.2009.00716.x

Coyle, S.W., Alston, D.E. and Sampaio, C.M.S. (2010). **Freshwater Prawns Biology and Farming** (M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, & M. N. Kutty, Eds.; 1st edition). Wiley Blackwell, A John Wiley & Sons, Ltd., Publication.

Daniels, W. H., Cavalli, R. O., & Smullen, P. (2010). **Freshwater Prawns Biology and Farming** (M. B. New, W. C. Valenti, J. H. Tidwell, L. R. D'Abramo, & M. N. Kutty, Eds.; 1st edition). Wiley Blackwell, A John Wiley & Sons, Ltd., Publication.

Danish, M., & Nizami, M. (2019). Complete fatty acid analysis data of flaxseed oil using the GC-FID method. Data in Brief, 23, 103845. https://doi.org/10.1016/j.dib.2019.103845

Davinelli, S., Corbi, G., Righetti, S., Sears, B., Olarte, H. H., Grassi, D., & Scapagnini, G. (2018). Cardioprotection by cocoa polyphenols and ω-3 fatty acids: A disease-prevention perspective on aging-associated cardiovascular risk. Journal of Medicinal Food, 21(10), 1060–1069. https://doi.org/10.1089/jmf.2018.0002

Fatima, H., Ayub, Z., Ali, S. A., & Siddiqui, G. (2013). Biochemical composition of the hemolymph, hepatopancreas, ovary, and muscle during ovarian maturation in the penaeid shrimps *Fenneropenaeus merguiensis* and *F. penicillatus* (Crustacea: Decapoda). Turkish Journal of Zoology, 37(3), 334–347. https://doi.org/10.3906/zoo-1201-12

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol Chem, 226, 497–509.

Glencross, B. D. (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture, 1, 71–124. https://doi.org/10.1111/j.1753-5131.2009.01006.x

Goodall, J. (2016). Improving the Reproductive Performance of Domesticated Giant Tiger Shrimp, *Penaeus monodon*. The University of Queensland.

Habashy, M. M. (2013). On the breeding behaviours and reproduction of the freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879) (Decapoda-Crustacea) under laboratory conditions. Aquaculture Research, 44(3), 395–403. https://doi.org/10.1111/j.1365-2109.2011.03044.x

Humason, G. L. (1979). Animal Tissue Techniques (4th edition). W.H. Freeman, San Francisco.

Kangpanich, C. (2016). Effects of Alternative Oil Sources, n-3 to n-6 Ratios and Arachidonic Acid Supplementation in Feed on Growth and Reproduction of Giant Freshwater Prawn (Issue December). BURAPHA UNIVERSITY.

Kangpanich, C., Pratoomyot, J., Siranonthana, N., & Senanan, W. (2016). Effects of arachidonic acid supplementation in maturation diet on female reproductive performance and larval quality of giant river prawn (*Macrobrachium rosenbergii*). PeerJ, 4, e2735. https://doi.org/10.7717/peerj.2735

Martins, J., Ribeiro, K., Rangel-Figueiredo, T., & Coimbra, J. (2007). Reproductive Cycle, Ovarian Development, and Vertebrate-Type Steroids Profile in the Freshwater Prawn *Macrobrachium rosenbergii*. Journal Of Crustacean Biology, 27(2), 220–228.

Mustafa, T., Srivastava, K. C. (1989). Prostaglandins (eicosanoids) and their role in ecto- thermic organisms. Advances in Comparative and Environmental Physiology, 5, 157–207.

Nagabhushanam, R., Machale, P. R., & Sarojini, R. (1989). Pheromonal and hormonal control of reproduction in the freshwater prawn, *Macrobrachium kinesis*. Proceedings: Animal Sciences, 98(2), 95–98. https://doi.org/10.1007/BF03179632

New, M. B. (2002). Farming freshwater prawns: A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). In FAO Fisheries Technical Paper (Vol. 428). FAO.

Nik Sin, N. H., Seok Kian, A. Y., & Shapawi, R. (2016). Effects of different protein sources in the broodstock diet on reproductive performance of giant freshwater prawn (*Macrobrachium rosenbergii*). International Journal of Aquatic Science, 7(2), 87–94.

Norambuena, F., Estévez, A., Mañanós, E., Bell, J. G., Carazo, I., & Duncan, N. (2013). General and Comparative Endocrinology Effects of graded levels of arachidonic acid on the reproductive physiology of Senegalese sole (*Solea senegalensis*): Fatty acid composition, prostaglandins and steroid levels in the blood of broodstock bred in captivity. General and Comparative Endocrinology, 191(June), 1–9. https://doi.org/10.1016/j.ygcen.2013.06.006

Norambuena, F., Estévez, A., Sánchez-Vázquez, F. J., Carazo, I., & Duncan, N. (2012). Self-selection of diets with different contents of arachidonic acid by Senegalese sole *(Solea senegalensis*) broodstock. Aquaculture, 364–365, 198–205. https://doi.org/10.1016/j.aquaculture.2012.08.016

Norambuena, F., Morais, S., Estévez, A., Bell, J. G., Tocher, D. R., Navarro, J. C., Cerdà, J., & Duncan, N. (2013). Dietary modulation of arachidonic acid metabolism in senegalese sole (*Solea Senegalensis*) broodstock reared in captivity. Aquaculture, 372–375, 80–88. https://doi.org/10.1016/j.aquaculture.2012.10.035

Roustaian, P., Kamarudin, M. S., Omar, H., Saad, C. R., & Ahmad, M. H. (1999). Changes in fatty acid profile during larval development of freshwater prawn *Macrobrachium rosenbergii* (de Man). Aquaculture Research, 30, 815–824.

Rowley, A. F., Vogan, C. L., Taylor, G. W., & Clare, A. S. (2005). Prostaglandins in non- insectan invertebrates: recent insights and unsolved problems. Journal of Experimental Biology, 208, 3–14.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., & Tocher, D. (1999). Lipid nutrition of marine fish during early development: Current status and future directions. Aquaculture, 179(1–4), 217–229. https://doi.org/10.1016/S0044-8486(99)00191-X

Sargent, J. R. (1995). Origins and functions of egg lipids: nutritional implications. In R. J. Bromage, N.R., Roberts (Ed.), Broodstock Management and Egg and Larval Quality (pp. 373 – 398). Blackwell Science, Oxford, UK, pp. 373 – 398.

Sarojini, R., Sambasiva Rao, S., & Jayalakshmi, K. Nagabhushanam, R. (1989). Effect ofPGF1a on ovarian maturation in a penaeid prawn *Metapenaeus affinis*. Journal of Zoological Research 2:7 – 11., 2 7–11.

Seguineau, C., Racotta, I. S., Palacios, E., Delaporte, M., Moal, J., & Soudant, P. (2011). The influence of dietary supplementation of arachidonic acid on prostaglandin production and oxidative stress in the Pacific oyster *Crassostrea gigas*. Comparative Biochemistry and Physiology, Part A., 160(1), 87–93. https://doi.org/10.1016/j.cbpa.2011.05.011

Shehata, A. I., Wang, T., Jibril Habib, Y., Wang, J., Fayed, W. M., & Zhang, Z. (2020). The combined effect of vitamin E, arachidonic acid, *Hematococcus pluvialis*, nucleotides, and yeast extract on growth and ovarian development of crayfish (*Cherax quadricarinatus*) by the orthogonal array design. Aquaculture Nutrition, 26(6), 1–16. https://doi.org/10.1111/anu.13142

Shen, S. Q., Li, J. W., Xu, H. J., Yang, J. S., Ma, W. M., & Qian, G. Y. (2020). Sexual characteristic development and sex identification of juvenile prawns, *Macrobrachium rosenbergii*. Aquaculture Research, 51(9), 3718–3728. https://doi.org/10.1111/are.14721

Shofiquzzoha, A. F. M., Haque, S. M., & Wahab, M. A. (2016). Reproductive performance of freshwater prawn *Macrobrachium rosenbergii* (De Man 1879) broodstocks grown on different diets. Journal of the Bangladesh Agricultural University, 14(2), 229–234. https://doi.org/10.3329/jbau.v14i2.32698

Silva, M. A. S., Almeida Neto, M. E., Ramiro, B. O., Santos, I. T. F., & Guerra, R. R. (2018). Histomorphologic characterisation of the hepatopancreas of freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879). Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 70(5), 1539–1546. https://doi.org/10.1590/1678-4162-10497

Smith, W. L., DeWitt, D. L., & Garavito, R. M. (2000). Cyclooxygenases: Structural, Cellular and Molecular Biology. Annual Review of Biochemistry, 69, 145–182.

Solorzano, L. (1969). Determination Of Ammonia in Natural Waters by the Phenolhypochlorite Method. Limnology and Oceanography, 14(5), 799–801. https://doi.org/10.4319/lo.1969.14.5.0799

Soonklang, N., Wanichanon, C., Stewart, M. J., Stewart, P., Meeratana, P., Hanna, P. J., & Sobhon, P. (2012). Ultrastructure of differentiating oocytes and vitellogenesis in the giant freshwater prawn, *Macrobrachium rosenbergii* (de man). Microscopy Research and Technique, 75(10), 1402–1415. https://doi.org/10.1002/jemt.22081

Stacey, N. E., & Goetz, F. W. (1982). Role of prostaglandins in fish reproduction. Canadian Journal of Fisheries and Aquatic Science, 39, 92–98. https://doi.org/10.1139/f82-011

Sumpownon, C., Engsusophon, A., Siangcham, T., Sugiyama, E., Soonklang, N., Meeratana, P., Wanichanon, C., Hanna, P. J., Setou, M., & Sobhon, P. (2015). Variation of prostaglandin E2 concentrations in ovaries and its effects on ovarian maturation and oocyte proliferation in the giant freshwater prawn, *Macrobrachium rosenbergii*. General and Comparative Endocrinology, 223, 129–138. https://doi.org/10.1016/j.ygcen.2015.04.019

Tahara, D., & Yano, I. (2004). Maturation-related variations in prostaglandin and fatty acid content of ovary in the kuruma prawn (*Marsupenaeus japonicus*). Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 137(4), 631–637. https://doi.org/10.1016/j.cbpb.2003.12.005

Templeton, G. F. (2011). A two-step approach for transforming continuous variables to normal: Implications and recommendations for IS research. Communications of the Association for Information Systems, 28(1), 41–58. https://doi.org/10.17705/1cais.02804

Thongbuakaew, T., Sumpownon, C., Engsusophon, A., Kornthong, N., Chotwiwatthanakun, C., Meeratana, P., & Sobhon, P. (2021). Characterisation of prostanoid pathway and the control of its activity by the eyestalk optic ganglion in the female giant freshwater prawn, *Macrobrachium rosenbergii*. Heliyon, 7(1), 1–10. https://doi.org/10.1016/j.heliyon.2021.e05898

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science, 11, 107–184.

Torrecillas, S., Román, L., Rivero-Ramírez, F., Caballero, M. J., Pascual, C., Robaina, L., Izquierdo, M. S., Acosta, F., & Montero, D. (2017). Supplementation of arachidonic acid rich oil in European sea bass juveniles (*Dicentrarchus labrax*) diets: Effects on leucocytes and plasma fatty acid profiles, selected immune parameters and circulating prostaglandins levels. Fish and Shellfish Immunology, 64, 437–445. https://doi.org/10.1016/j.fsi.2017.03.041

Trbović, D., Polak, T., Demšar, L., Parunović, N., Dimitrijević, M., Nikolić, D., & Đorđević, V. (2017). Determination of the fatty acids in fish tissue and feed — comparison of different methods and statistical evaluation. Acta Chromatographica, 30(3), 175–179. https://doi.org/10.1556/1326.2017.00165

Tsoukalas, D., Alegakis, A. K., Fragkiadaki, P., Papakonstantinou, E., Tsilimidos, G., Geraci, F., Sarandi, E., Nikitovic, D., Spandidos, D. A., & Tsatsakis, A. (2019). Application of metabolomics part II: Focus on fatty acids and their metabolites in healthy adults. International Journal of Molecular Medicine, 43(1), 233–242. https://doi.org/10.3892/ijmm.2018.3989

Wang, W., Wu, X., Liu, Z., Zheng, H., & Cheng, Y. (2014). Insights into Hepatopancreatic Functions for Nutrition Metabolism and Ovarian Development in the Crab *Portunus trituberculatus* : Gene Discovery in the Comparative Transcriptome of Different Hepatopancreas Stages. 9(1). https://doi.org/10.1371/journal.pone.0084921

Wimuttisuk, W., Tobwor, P., Deenarn, P., Danwisetkanjana, K., Pinkaew, D., Kirtikara, K., & Vichai, V. (2013). Insights into the Prostanoid Pathway in the Ovary Development of the Penaeid Shrimp *Penaeus monodon*. PLoS ONE, 8(10), 1–15. https://doi.org/10.1371/journal.pone.0076934

Wouters, R., Lavens, P., Nieto, J., & Sorgeloos, P. (2001). Penaeid shrimp broodstock nutrition: an updated review on research and development. Aquaculture, 202, 1–21. https://doi.org/10.1016/S0044-8486(01)00570-1

Xu, H., Cao, L., Zhang, Y., Johnson, R. B., Wei, Y., Zheng, K., & Liang, M. (2017). Dietary arachidonic acid differentially regulates the gonadal steroidogenesis in the marine teleost, tongue sole (*Cynoglossus semilaevis*), depending on fish gender and maturation stage. Aquaculture, 468, 378–385. https://doi.org/10.1016/j.aquaculture.2016.11.002

Xu, H., Zhang, Y., Luo, K., Meng, X., Luan, S., Cao, B., Chen, B., Liang, M., & Kong, J. (2017). Arachidonic acid in diets for early maturation stages enhances the final reproductive performances of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture, 479, 556–563. https://doi.org/10.1016/j.aquaculture.2017.06.037

Yen, P. T., & Bart, A. N. (2008). Salinity effects on reproduction of giant freshwater prawn *Macrobrachium rosenbergii* (de Man). Aquaculture, 280(1–4), 124–128. https://doi.org/10.1016/j.aquaculture.2008.04.035

Zhang, H., Wang, Z., & Liu, O. (2015). Development and validation of a GC-FID method for quantitative analysis of oleic acid and related fatty acids. Journal of Pharmaceutical Analysis, 5(4), 223–230. https://doi.org/10.1016/j.jpha.2015.01.005

Zuo, R., Li, M., Ding, J., & Chang, Y. (2018). Higher Dietary Arachidonic Acid Levels Improved the Growth Performance, Gonad Development, Nutritional Value, and Antioxidant Enzyme Activities of Adult Sea Urchin (*Strongylocentrotus intermedius*). Journal of Ocean University of China, 17(4), 932–940. https://doi.org/10.1007/s11802-018-3606-7