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IN SILICO ANALYSIS AND OVARY FATTY ACID COMPOSITION OF COBIA FISH (RACHYCENTRON CANADUM)

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ABSTRACT
There has been not much information available related to the diet formulation for female Cobia *(Rachycentron canadum)* fish. This study characterized ovary fatty acid composition of female Cobia in their different gonad maturation stages and predicted the mechanism of HUFA (DHA/docosahexaenoic acid, EPA/eicosapentaenoic acid, and AA/arachidonic acid) in supporting gonad maturation through an *in silico* analysis. Fish samples consisted of three (3) categories: (1) prospective broodstocks – immature (initial development); (2) prospective broodstocks – maturing (middle development); and (3) broodstocks – mature (advanced development). The *in silico* analysis was done through a molecular docking between ligand (DHA, EPA, and AA) and receptors Lpl, Fatp, Fabp, and Mttp. Our findings showed that fatty acids tended to increase along with the development of the ovary. The highest fatty acids for group 1 was C16:0>C18:1n9c>DHA, for group 2 was C18:1n9c>C16:0>DHA, and for group 3 was DHA>C16:0>C18:1n9c. DHA increased significantly during gonad maturation and the vice versa happened to EPA, while AA increased significantly at the end of gonad maturation. This affected, among others, the ratio of DHA/EPA and DHA/AA—DHA/EPA ration increased along with gonad maturation, while the opposite happened for DHA/AA ratio. The results of the *in silico* analysis showed that DHA had the smallest binding affinity for docking with Lpl, Fatp, and Fapb compared to EPA and AA. This means that DHA was predicted to have a strong binding with receptors during the oocyte lipidation. DHA also had the smallest binding affinity for docking with Mttp compared to EPA and AA. This means that DHA was predicted to have a strong binding with receptors in Vtg lipidation compared to EPA and AA.

KEY WORDS
Cobia (Rachycentron canadum), fatty acid, ovary, In Silico.

Indonesians have long known Cobia *(Rachycentron canadum)*. Cobia culture aims at providing enough animal protein and supporting national food security. Broodstocks of the high quality breed are important in the culture of any species since good broodstocks will most likely to produce good offsprings; one of the factors affecting this is the quality of feed given.

To date, fresh fish and squid have been the main feed in the culture of Cobia for gonad maturation (Nhu *et al*., 2011; Nguyen *et al*., 2012; Lee *et al*., 2015). The use of fresh fish and Threadfin Bream as feed has been due to some good reasons, such as (1) they offer lower price as compared to artificial feed, (2) they are easily digested, (3) they are more nutritious than artificial feed is, and (4) they are less polluted as compared to artificial feed. However, fresh fish as feed also results in some shortcomings, such as (1) it cannot offer stable quality

over time, (2) its price tends to fluctuate, and (3) it cannot guarantee continuous availability throughout the year (Nguyen *et al*., 2012). The nutritional quality of fresh fish is varied throughout the spawning season, so it affects the biochemical composition of Cobia eggs (Faulk and Holt, 2008; Nguyen *et al*., 2010, 2012). In relation, the use of artificial feed offers some advantages, such as (1) reduced possibility of disease transmission and (2) artificial feed offers better quality, quantity, and continuity in terms of availability compared to fresh fish as feed.

There has been not much information available related to the diet formulation for female Cobia—more studies are needed. What we have known to date is that parental nutritional status influences reproductive performance and offspring quality in several species. Biesiot *et al.* (1994) write that fat concentration in the ovaries changes during ovary development (gonad maturation) of Cobia fish. The composition of lipid fatty acids from fish gonads reflects the lipid fatty acid content in feed given to the broodstock (Fernandez-Palacios *et al*., 1995). Anido *et al.* (2015) confirm that information related to ovary fatty acid composition in different gonad maturation stages is of much importance for the diet formulation of the Broodstocks since it will guide the process of feed formulation. This study characterized ovary fatty acid composition of female Cobia in their different gonad maturation stages and predicted the mechanism of gonad maturation through an *in silico* analysis for female broodstocks of Cobia fish.

MATERIALS AND METHODS OF RESEARCH

Samples of female prospective broodstocks and broodstocks of Cobia were taken from Karambia Jaring Apung (KJA) of Balai Besar Perikanan Budidaya Laut (BBPBL)[1] Lampung, Indonesia. The daily feed given was fresh Threadfin Bream. Feeding was done until the Cobia fish was full (± 3% body weight) two (2) times a day (in the morning at 08.00 and afternoon at 14.30). Fish samples consisted of three (3) categories: 1) prospective broodstocks – immature (initial development);2) prospective broodstocks – maturing (middle development); and 3) broodstocks – mature (advanced development). For each sample, three (3) samples of gonads were taken (Figure 1). The samples were kept in the freezer at -80 °C before fatty acid analyses. The analyses were done in the Laboratorium Terpadu[2] of Bogor Agricultural University, Indonesia. The analysis of total fat referred to AOAC (2012):991.36, while the analysis of fatty acids referred to AOAC (2012):969.33. Data for fatty acid content were analyzed using one-way ANOVA and followed by the Tukey test if the ANOVA test resulted in the F count > F table of 0.05 (H0 was rejected and H1 was accepted).

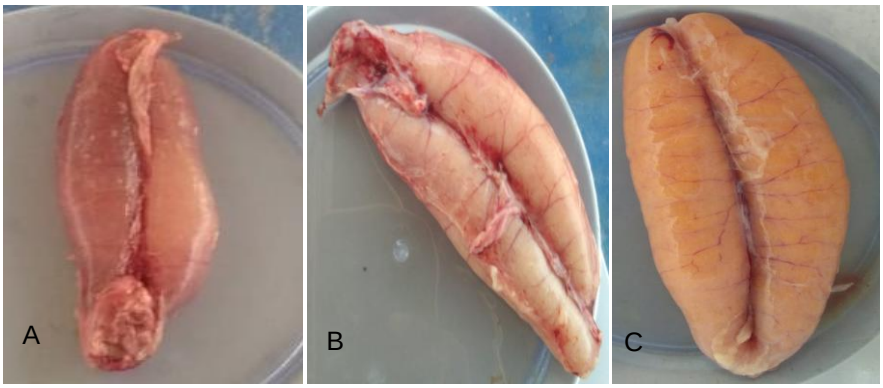

Figure 1 – The ovary morphology of Cobia
Note: (A). Group 1; (B). Group 2; (C). Group 3

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[2] *Integrated Laboratory.*

Data from fatty acid analysis results did not provide a detailed explanation of the mechanism of these fatty acids on the expression of ovary development in Cobia fish because they simply represented the results of a treatment stage. To obtain a more realistic picture and explanation, an *in silico* analysis was needed—this is an approach including molecular docking—to predict the function and pathway of a compound.

The steps included bioinformatics analysis and molecular docking. The bioinformatics analysis involved searching for ligand structures (DHA, EPA, and AA). Modeled ligands had to have a three-dimensional structure in PDB format (obtained from the webserver https://pubchem.ncbi.nlm.nih.gov). The CID number for DHA was 445580, CID for EPA was 446284, and CID for AA was 444899. The next step was to search for fatty acid binding protein (Fabp) receptors and microsomal triglyceride transfer protein (Mttp) (Lubzens *et al.*, 2017), fatty acid transfer protein (Fatp) receptors (Hiramatsu *et al*., 2015), and lipoprotein lipase receptors (Lpl) (Reading *et al*., 2018) in the PDB database and receptors were obtained in a PDB format (from the webserver http://www.rcsb.org). Lpl receptor code was 2XNL7 (Q2XNL7-TAESO), Fabp receptor code was GCID:GC06P122779, Fatp receptor code was 3ALR, and Mttp receptor code was 1LSH LIPID-PROTEIN INTERACTIONS IN LIPOVITELLIN. Furthermore, the three-dimensional structure of each fatty acid was docked onto each receptor protein using PyRx software that the binding affinity value (RMSD had to be zero) was obtained. Molecular docking simulation was done using the Autodock Vina program (De Vries *et al*., 2010) and the interaction simulation process was done using Discovery Studio 2016 software (Jayaram *et al*., 2012).

RESULTS AND DISCUSSION

Table 1 presents the data from the analysis of ovary fatty acids of Cobia.

Table 1 – The Results of Ovary Fatty Acids of Cobia

No	Parameter	Fish Category		
		1	2	3
Fat content (%w/w)		2.59 ± 0.08	5.52 ± 0.04	9.58 ± 0.05
Fatty Acid (%w/w)				
1	Lauric Acid. C12:0	0.07 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
2	Myristic Acid. C14:0	3.55 ± 0.04	2.08 ± 0.03	1.16 ± 0.01
3	Myristoleic Acid. C14:1	0.04 ± 0.02	0.02 ± 0.01	0.00 ± 0.00
4	Pentadecanoic Acid. C15:0	0.44 ± 0.03	0.25 ± 0.02	0.45 ± 0.01
5	Palmitic Acid. C16:0	23.49 ± 0.01^{c}	15.10 ± 0.02^{b}	12.81 ± 0.02^{a}
6	Palmitoleic Acid. C16:1	4.74 ± 0.02	3.85 ± 0.03	3.89 ± 0.04
7	Heptadecanoic Acid. C17:0	0.56 ± 0.01	0.31 ± 0.03	0.71 ± 0.01
8	Cis-10-Heptadecanoic Acid. C17:1	0.26 ± 0.03	0.27 ± 0.02	0.51 ± 0.01
9	Stearic Acid. C18:0	6.29 ± 0.01	3.19 ± 0.01	3.80 ± 0.02
10	Elaidic Acid. C18:1n9t	0.17 ± 0.03	0.17 ± 0.02	0.19 ± 0.01
11	Oleic Acid. C18:1n9c	19.51 ± 0.02	21.56 ± 0.01	10.91 ± 0.04
12	Linoleic Acid. C18:2n6c	6.11 ± 0.03	10.38 ± 0.04	1.03 ± 0.01
13	Arachidic Acid. C20:0	0.25 ± 0.02	0.11 ± 0.01	0.18 ± 0.01
14	Ɣ-Linolenic Acid. C18:3n6	0.15 ± 0.02	0.14 ± 0.01	0.18 ± 0.01
15	Cis-11-Eicosenoic Acid. C20:1	1.43 ± 0.01	0.90 ± 0.01	0.23 ± 0.01
16	Linolenic. C18:3n3	1.28 ± 0.01	1.81 ± 0.02	0.24 ± 0.02
17	Heneicosanoic Acid. C21:0	0.05 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
18	Cis-11.14-Eicosedienoic Acid. C20:2	0.38 ± 0.01	0.32 ± 0.01	0.29 ± 0.02
19	Behenic Acid. C22:0	0.12 ± 0.02	0.04 ± 0.01	0.11 ± 0.01
20	Cis-8.11.14-Eicosetrienoic Acid. C20:3n6	0.14 ± 0.05	0.14 ± 0.01	0.34 ± 0.01
21	Arachidonic Acid. C20:4n6	1.15 ± 0.02^{a}	1.12 ± 0.01^{a}	6.83 ± 0.02^{b}
22	Tricosanoic Acid. C23:0	0.03 ± 0.01	0.00 ± 0.00	0.03 ± 0.01
23	Cis-13.16-Docosadienoic Acid. C22:2	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
24	Lignoceric Acid. C24:0	0.09 ± 0.01	0.06 ± 0.01	0.13 ± 0.01
25	Cis-5.8.11.14.17-Eicosapentaenoic Acid	6.19 ± 0.02^{c}	5.46 ± 0.02^{b}	5.31 ± 0.02^{a}
24	Nervonic Acid. C24:1	0.36 ± 0.01	0.18 ± 0.01	0.15 ± 0.01
27	Cis-4.7.10.13.16.19-Docosahexaenoic Acid	10.50 ± 0.03^{a}	12.21 ± 0.02^{b}	24.47 ± 0.03^{c}
28	Fatty Acid Total	87.36 ± 0.02	79.75 ± 0.04	74.04 ± 0.01
29	Σ n-3 HUFA	17.84 ± 0.05	18.79 ± 0.07	36.61 ± 0.25
30	DHA/EPA	1.69 ± 0.07	2.23 ± 0.04	4.61 ± 0.09
31	DHA/AA	9.13 ± 0.05	10.90 ± 0.06	3.58 ± 0.02

Note: Different alphabets (superscript) denoted significant differences.

Table 1 depicts that fat content increased along with gonad development. The highest fatty acid content in group 1 gonads was C16:0>C18:1n9c>DHA, group 2 gonads was C18:1n9c>C16:0>DHA, and group 3 gonads was DHA>C16:0>C18:1n9c. The DHA content increased significantly during the gonad maturation process in female broodstock, while the vice versa happened to EPA. AA content increased significantly at the end of the maturation process. This would affect, among others, the ratio of DHA/EPA and DHA/AA. The DHA/EPA ratio seemed to increase along with the gonad maturation process, while the opposite happened to DHA/AA ratio.

Comparing what Table 1 depicts with the results of the study by Nguyen *et al.* (2010) on the composition of fatty acids of Cobia from the best spawning (using fresh fish as feed), it can be concluded that the DHA, EPA, total n-3 HUFA, and DHA/EPA ratio was almost comparable. However, the AA content looked different, which was 2.19 ± 0.04% of total fatty acids, while our study resulted in a value of 6.83 ± 0.02% of total fatty acids; and DHA/AA content was ± 11.72% of total fatty acids, while our study resulted in a value of 3.58 ± 0.02% of total fatty acids. Nguyen *et al.* (2012) suggest that an increase in the value of the DHA/EPA ratio in eggs increases the proportion of normal blastomeres; also, the increase in linoleic and linolenic acid content in eggs reduces the proportion of normal blastomeres. Eggs with a degree of fertilization > 50% have a DHA/EPA ratio of 3.9 ± 0.1 µg egg^{-1}. The high AA content in eggs results in decreased fecundity and larval production. Nguyen *et al.* (2010) furthermore assert that AA content of 0.42 - 0.60% of the dry weight results in a decrease in fertilization rate and the higher need of the n-3 HUFA in the feed for Cobia broodstock, which is higher than 1.86% of the dry weight. Faulk and Holt (2008) confirm that increasing the total amount of unsaturated n-3 fatty acids will result in a decrease in the proportion of floating eggs. The results of the above studies can be taken into consideration in preparing artificial feed for female Cobia broodstock.

Izquierdo *et al.* (2000) assert that an increase in DHA at the position of 2n of Phosphoacylglycerides (PG) and phosphatidylethanolamine (PE) in larvae in the diet for Gilthead Seabream from 0.7 to 2.6% at a constant EPA level (0.7%) resulted in significant improvement of larval growth—it was only inhibited by the incorporation of EPA into the 2n PE position and not in the other lipid class. Meanwhile, an increase in EPA levels from 0.3 to 1.1%, at constant DHA and AA levels (0.7 and 0.06% respectively), cannot replace DHA from the PG class. On the other hand, an increase in DHA at the position of 2n of phosphatidylcholine (PC) and PE, with an increase of EPA for PC and a decrease of EPA for PE results in slight improvement of larval growth. Furthermore, an increase in EPA to 1.7% at constant DHA and AA levels, 1.1 and 0.7% (1 - 0.5 DHA/EPA) respectively, replaces DHA from the 2n position of the PC and increase EPA in the 2n position from PE. This shows the importance of a balanced proportion between 20 and 22 carbon fatty acids. Takeuchi *et al.* (1992) disclose an increase in EPA (0.8 to 1.7%, without DHA) significantly reduces growth despite survival—the results were very similar between the two groups of larvae. Increased AA from 0.1 to 1.5% (DHA at 1.2%, EPA at 0.7%) not only does not displace DHA and EPA from a phospholipid (PL) of larvae but also increases the incorporation of three fatty acids into the PC and PE in the position of 2n. Interestingly, EPA levels are always higher than AA in this class of lipids, apart from low EPA levels in the diet, suggesting preferential incorporation of EPA to PC and PE (Izquierdo *et al*., 2000). Increased incorporation of EPA into the body of lipids has also been found in Japanese Flounder larvae fed with increased levels of AA (Estévez *et al*., 1997). Competition for inclusion in different lipid classes might indicate differences in each tissue because the fatty acid composition of each lipid class is very different between cellular types (Lie *et al*., 1992). For example, in juvenile Turbot fed with 0.78% AA, the incorporation of EPA into the liver and phosphatidylinositol (PI) of the brain reduces (Bell *et a*l., 1995).

Another well-known level of competition between AA and EPA is the synthesis of eicosanoids. AA and EPA are adequate substrates for lipoxygenases to produce leukotrienes series 4 and 5, and for cyclooxygenase to produce prostanoids series 2 and 3. Lipoxygenases appear to have a higher affinity for EPA, whereas AA is the preferred substrate for cyclooxygenase; there are relative levels of the two fatty acids in

phosphatidylinositol (PI) of different tissues that regulate the proportional synthesis of each series (Hwang, 1989). The products of each fatty acid have physiological differences and they compete for becoming cell receptors. Evidence for such competitive interactions has to be considered as dietary requirements.

The need for fatty acids in the gonad maturation process is different for each species. Li *et al.* (2018) confirm that in Devil Stinger (*Inimicus japonicas*), the polar fat content of the ovaries decreases during the process of sexual maturation, whereas neutral fat increases. The highest content of fatty acids in polar fat is DHA>C16:0> EPA, whereas the highest fatty acid content is C16:0>18:1n-9>DHA in neutral fat. The female parent of Gue Sole (*Cynoglossus semilaevis*) requires more EPA and less DHA for gonadal steroidogenesis than the male parent does (Xu *et al.*, 2017). Feed with high DHA content in Siberian Sturgeon (*Acipenser baeri*) will improve fecundity, hatchability, and overall larval quality (Luo *et al*., 2015). Barreto *et al.* (2012) state that the female broodstocks of Greater Amberjack (*Seriola dumerili*) have a lower DHA/EPA ratio and a higher EPA/ARA ratio compared to wild fish. These results indicate that C18:1n-9, C18:2n-6, and EFA (essential fatty acids), especially EPA and ARA, are not provided in the right proportion in cultured fish feed and will negatively affect their reproductive performance. Furthermore, Barreto *et al.* (2014) state that female broodstocks fed with an artificial feed of high C18:1n-9 content and a low EPA /AA ratio show some positive results (could support spontaneous egg release). However, the degree of fertilization is still low and the high content of C18:2n-6 in the ovaries and eggs requires improved formulations to improve reproductive performance. Cejas *et al.* (2003) state differences about the relative percentage of specific fatty acids between wild and aquaculture White Sea Bream (*Diplodus sargus*). The percentage of AA in ovaries is higher in the wild fish than in the cultured fish, whereas the opposite applies to EPA. As a result, the EPA/AA ratio is lower in ovaries of wild fish when compared to cultured fish. This difference can be seen in all lipid classes, namely TG (triacylglycerol), PC, and PE. These results indicate that the content of essential fatty acids, specifically EPA and AA in artificial feed, is not suitable for this species. Anido *et al*. (2015) reveal that in the ovary of *Rhamdia quelen*, the content of C16:0>DHA> AA is the biggest for four seasons. The AA content is higher in immature ovaries, and the content decreases throughout the maturation process. DHA and EPA increase during maturation. This variation increases the ratio of EPA/ARA and DHA/ARA in adult gonads, which can be important for breeding success. Felix *et al*. (2017) affirm that in the early stages of gonad maturation of Shortfin Corvina (*Cynoscion parvipinnis*), the largest fatty acid in a row is C16:0>DHA>AA>EPA. The n-3 fatty acid content is higher than n-6, the n-3/n-6 ratio ranges from 2.08-2.81. The biochemical composition of the gonads at the initial maturation stage between wild fish and aquaculture fish is very similar, so the data can be used as dietary requirements for the maturation of the gonads of broodstocks. Dhurmeea *et al.* (2018) write that DHA, C16:0, C18:0, and C18:1n-9 are fatty acids that dominate the gonadal, liver, and muscle tissue of Albacore Tuna (*Thunnus alalunga*). The proportion of fatty acids varies with the level of maturity and ovarian lobes, with smaller lobes having a much higher proportion of essential fatty acids, as well as C16:0 and C18:1n-9, compared to larger ones.

The results of the analysis indicated that DHA was the highest highly unsaturated fatty acid (HUFA) compared to EPA and AA in the ovary maturation process of Cobia fish. To predict the mechanism of action of the three fatty acids in the gonad maturation process, the *in silico* analysis was done. Affinity binding is the ability of a compound to bind to a target protein; the smaller the value of binding affinity, the higher activity the compound does. The results of the *in silico* analysis between DHA, EPA, and AA as ligands with several receptors namely Lpl, Fatp, Fabp, and Mttp are as follows.

Table 2 – Molecular Docking of Lpl with DHA, EPA, and AA

No	Ligand	Binding Affinity				Average	SD	Rank
		1	2	3	4			
1.	DHA	-7.7	-8.1	-7.7	-8.0	-7.88	0.22	1
2.	EPA	-7.9	-7.8	-7.7	-7.7	-7.77	0.01	2
3.	AA	-7.1	-7.6	-7.6	-7.7	-7.50	0.27	3

Table 2 confirms that DHA has the highest binding affinity, followed by EPA and AA.

Table 3 – Molecular Docking of Fatp with DHA, EPA, and AA

No	Ligand	Binding Affinity						Average	SD	Rank
		1	2	3	4	5	6			
1.	DHA	-6.4	-5.9	-6.1	-6.4	-5.6	-5.9	-6.05	-0.32	1
2.	EPA	-6.0	-6.1	-6.2	-6.0	-5.8	-6.1	-6.03	-0.14	2
3.	AA	-5.1	-5.7	-5.5	-5.1	-5.8	-5.7	-5.48	-0.31	3

Table 3 confirms that DHA has the highest binding affinity, followed by EPA and AA.

Table 4 – Molecular Docking of Fabp with DHA, EPA, and AA

No	Ligand	Binding Affinity				Average	SD	Rank
		1	2	3	4			
1.	DHA	-5.5	-5.4	-5.0	-5.4	-5.33	0.22	1
2.	EPA	-3.3	-5.9	-5.5	-5.8	-5.13	1.22	3
3.	AA	-4.9	-5.5	-5.4	-5.0	-5.20	0.29	2

Table 4 confirms that DHA has the highest binding affinity, followed by AA and EPA.

Table 5 – Molecular Docking of Mttp with DHA, EPA, and AA

No	Ligand	Binding Affinity				Average	SD	Rank
		1	2	3	4			
1.	DHA	-6.9	-7.2	-7.2	-7.2	-7.13	0.15	1
2.	EPA	-7.2	-6.6	-7.1	-6.5	-6.85	0.35	2
3.	AA	-6.7	-6.5	-6.6	-6.9	-6.68	0.17	3

Table 5 confirms that DHA has the highest binding affinity, followed by EPA and AA.

In silico test results showed that DHA had the smallest binding affinity for docking with Lpl, Fatp, and Fabp compared to EPA and AA (Table 2, 3, and 4); this means that DHA was predicted to have a strong bond with the receptor in the oocyte lipidation process. DHA also had the smallest binding affinity for docking with Mttp compared to EPA and AA (Table 5); this means that DHA was predicted to have a strong bond with the receptor in the vitellogenin (Vtg) lipidation process compared to EPA and AA.

The gonad maturation of female broodstock Cobia with HUFA (DHA, EPA, and AA) stimulation through the *in silico* analysis was done employing two pathways namely oocyte lipid pathway and Vtg lipidation.

Oocyte lipid pathway:

- Very low-density lipoprotein (VLDL) circulated from the liver into the circulatory system, then VLDL was metabolized by the action of Lpl (a key enzyme of VLDL metabolism), in ovarian follicle cells (especially granulosa cells) to produce free fatty acids (FFA). FFA was represented here by n-3 HUFA consisting of DHA and EPA, as well as from the n-6 HUFA group, AA. From the results of docking in Table 2, it appeared that DHA had a high binding activity with Lpl, then followed by EPA and AA;
- FFA (DHA, EPA, and AA) entered the oocyte by crossing the oolemma by binding to Fatp. The docking results presented in Table 3 showed that DHA had the highest binding activity with Fatp, followed by EPA and AA;
- FFA (DHA, EPA, and AA) were then brought into the RE by binding to the cytosolic Fabp in the ooplasm. The docking results presented in Table 4 showed that DHA had the highest binding activity with Fabp, followed by AA and EPA;
- FFA (DHA, EPA, and AA) were synthesized into neutral fatty acids in the endoplasmic reticulum (RE). The FFA underwent esterification and were stored in lipid droplets or inclusions located throughout the ooplasm. Lipid droplet biogenesis requires coordination of fatty acid activation by acyl-CoA synthetase, *de novo* neutral fat synthesis, the lands cycle of phospholipid remodeling, Kennedy pathway from phospholipid synthesis, and accessory protein function (Pol *et al*., 2014).

The process of lipidation on Vtg was via Mttp. In lipid transfer, Mttp was required for lipoprotein assembly. This resulted in newborn competent apoB secretions and might be involved in TG imports into the RE lumen. Mttp interacted on several sides in the N-terminal β α1 apoB structural domain. Lipids modulated protein interactions between apoB and Mttp. Lipids associated with Mttp increased the binding of apoB - Mttp while lipids associated with ApoB reduced this binding. The docking results between Mttp and DHA, EPA and AA were presented in Table 5, with DHA had the highest activity, and followed by EPA and finally AA. The apoB-Mttp binding pathway in lipoprotein assembly refers to Hussain *et al.* (2002).

CONCLUSION

The results of the analysis of the ovary fatty acids of Cobia fish at several maturation stages were as follows. First, the highest fatty acids for prospective broodstocks (immature stage) was C16:0>C18:1n9c>DHA. Second, the highest fatty acids for prospective broodstocks (maturing stage) were C18:1n9c>C16:0>DHA. Third, the highest fatty acids for broodstocks (mature stage) were DHA>C16:0>C18:1n9c. DHA showed a significant increase during the gonad maturation process, yet the opposite happened for EPA; AA showed a significant increase at the end of the maturation process. This affected, among others, the ratio of DHA/EPA and DHA/AA—DHA/EPA ratio increased along with gonad maturation, while the opposite happened for DHA/AA ratio. The results of the *in silico* analysis showed that DHA was predicted to have a strong binding with receptors during the oocyte lipidation and Vtg lipidation compared to EPA and AA.

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