ISSN 2962-9632

Performa Reproduksi Induk Bandeng (*Chanos chanos*) yang Diberi Pakan Kaya Bubuk Spirulina

Reproductive performance of broodstock milkfish (*Chanos chanos*) fed with *Spirulina* powder enriched diet

Lisa Ruliaty[1], A Nur[1], I R Amalia[1] and A L Nurhayati[1]

Fishery Research Centre, National Research and Inovation Agency, Indonesia
✉Corresponding author: lsruliaty781@gmail.com

ABSTRACT

The enrichment of broodstock milkfish feed with *Spirulina* flour has been carried out at the Milkfish Hatchery Unit, MCBA Jepara. This research aims to improve the reproductive performance and quality of the milkfish's eggs. For every 10 kg of pellet feed is enriched with a mixture of 10 pieces of duck eggs, 100 mL of honey, 6 g of vitamin C, 3 g of vitamin E and *Spirulina* flour dosage of 0.6 g / kg and 1.8 g / kg. These materials are mixed and blended into an emulsion. The emulsion is sprayed evenly onto the pellet's surface and left to dry.. The amount of pellet feed for broodstock milkfish is 2-3% biomass / day both in the morning and evening. The result shows that the enrichment of broodstock milkfish feed with *Spirulina* flour affected on : (a) enhancement in the number of eggs (from normally 6.9 million eggs becomes 20 million eggs), (b) frequency of lay eggs (from normally 10 times/month becomes 18 times/month), (c). fertilization degree (from normally 48.5 % becomes 70.4 %), and (d) hatching degree (from normally 66.2 % becomes 79.2 %). However, the enhancement dosage of *Spirulina* up to three times (from 0.6 g/kg become 1.8 g/kg) does not give significant results.

Keywords: reproductive performance, *Chanos chanos*, *Spirulina*, feed enrichment

Introduction

Marine fish including milkfish do not have an enzyme system like those in freshwater fish, so marine fish urgently needs *hufa* long chain n-3 and n-6 from feed for optimum growth [1] [2]. These much-needed essential fatty acids are *eicosapentanoic acid* (EPA, 20:4n–6) and *docososahexaenoic acid* (DHA, 22:6n–3) as well as AA arachidonic acid, 20:4n–6 [3] [4] [5]. Usually this unsaturated fatty acid is synthesized from the fatty acid C-18. EPA and DHA are needed for cell membrane function, while DHA is essential for the cell membranes of nerve tissue and as precursors to the formation of *eicosanoate* i.e. several kinds of hormones. [5].

Deficiency of essential fatty acid will cause disturbance to fish health including fecundity deficiency and the inability to form an embryo, larvae death and abnormal growth, mal-pigmentation, deformed eyes, inability to eat at low light intensity, abnormal behavior and decreased membrane function at low temperature [5]. Essential fatty acid requirements for sea water species ranged between 0,5-2% from dry feed weight [6]. These requirements also very dependent on natural fish ability to break down the essential fatty acids both anabolic and katabolic [7]. Main source of essential fatty acids n-3 is fish flour [8] [9] and sea water's oil and other sources can be used as substitution of previous materials [10] [11]. Fat content in fish feed ration usually 15 %, which t is highly correlated with essential fatty acids content [12].

Essential fatty acids are very important lipid components of their nutritional value that cannot be formed in sufficient quantities by animals and must be obtained from food [6]. Its main components consist of phospholipids on membranes as well as on nerve tissues. Larvae at first meals have a very high neurosmatic index that requires (n-3 HUFA) high as well, so there will be no abnormalities in nerve formation. In addition to the two types of fatty acids above, arachidonic acid (20:4n-6 ; AA) is also urgently needed by

larvae. Both types of fatty acids namely AA and EPA are substrates needed for formation of *eicosanoate* which plays a role in various physiological functions including ion regulation and egg maturity in female broodstocks [13].

Spirulina is blue green algae (cyanobacteria) which widely used as raw material in food industry because its contain of 60 – 71% protein, 8% fat, 16% carbohydrate, 1,6% *Chlorophyll-a*, 18% *Phycocyanin*, 17% ß *Carotene* and 20 – 30 % γ-linoleaic acid from total of fatty acids and vitamin [14]. High protein content in spirulina have great potency to be used as feed composition source. *Spirulina* have some advantages compared with others microalgae i.e fast reproduction, huge biomass and easy to harvest. It because of the measurement of *Spirulina*'s biomass is bigger so it can be separated from its media through filtration using filter sized 20 μm. *Spirulina* is easily digested because it has thin membrane not like cellulose which hard to digest. Those membranes are sugar chain which easily digested and absorbed [15]. *Spirulina* is widely use in aquaculture as additional supplement in to feed. Fish which is feed by additional 0,5-1% *Spirulina* show high growth rate as much 17-25% and lower death rate as much 30-50%. *Spirulina* is also used as immunostimulant for fish [6]. The research done by Hironobu [6] shows that there is reduction number of *Aeromonas hydrophila* bacteria in the liver and heart of *Cyprinus carpio* that is fed by additional *Spirulina*. This result shows that *Spirulina* has the ability to stimulate natural immunity system in fish.

Feed enrichment for broodstock milkfish with the addition of vitamin C and vitamin E which apparently does not provide fatty acid content. Thus, the addition of *Spirulina* as feed enrichment is necessary so it can improve the broodstock milkfish reproductive performance. The purpose of this test is to improve the reproductive performance of the milkfish broodstock through *Spirulina* feed enrichment.

Materials and Method

Materials and tools

Total of 80 broodstock of milkfish with body weight 5–8 kg / pc, commercial pellet feed (diameter 1 cm), *Spirulina* flour , and other feed additional materials such as duck eggs , honey, vitamins C and vitamin E. Broodstock tub with diameter of 10 m and capacity of 250 m^3, broodstock tub cover net, water pump , blower, egg collector, basin, set of blender, set of beaker glass.

Methods

Preliminary test of Spirulina dosis enrichment.

Spirulina flour that is used in this test comes from Laboratory of Live Feed,MCBA Jepara. The broodstock milkfish pellets are enriched with *Spirulina* flour according to the treatment dose. Before the test, fatty acid analysis was carried out on *Spirulina*-enriched pellets at a dose of 0.6 g / kg of feed, 1.2g / kg of feed and 1.8 g / kg Feed. While waiting for the results of fatty acid analysis the test was carried out with the addition of *Spirulina* onto pellet/feed.

Application of Spirulina.

The testing treatment is as follows: *Treatment A*: enrichment of spirulina flour as much as 0.6 g / kg of feed, and Treatment B: enrichment of *Spirulina* flour as much as 1.8 g / kg of feed. Pellet enrichment consist of mixture of 10 duck eggs, 100 mL of honey, 6 g of vitamin C, 3 g of vitamin E and *Spirulina* flour according to the treatment dose (0.6 g / kg and1.8 g / kg) are blended until it becomes an emulsion. The emulsion is sprayed to 10 kg of pellets evenly and left until it dry. All pellets are then being analyzed for its DHA / EPA fatty acids contents in the laboratory.

The test is carried out to 80 pcs broodstock milkfish with average weight 5-8 kg/pc. The broodstock milkfish is maintained on a concrete tub with a depth of ± 2.5 m, equipped with strong aeration at the bottom of the tub and covered with a net. water changes at least 200% per day while the leftovers dirts are cleaned on every week. The pellet feed is given 2 times per day (in the morning and evening) with the dose 23% of the weight of biomass per day. Milkfish eggs are harvested after being collected in *an egg collector* which is in form of net size 500 µm. Egg harvesting is done in the morning before 7 am. Collected eggs are being selected. The floating eggs are separated from settle eggs - then being spread into the larval tub.

Collection of data testing

Observations are conducted on reproductive performance i.e degree of fertilization, hatchability of eggs, egg diameter, composition of DHA/EPA fatty acids from *Spirulina* enriched pellets and water quality of broodstock milkfish culture media.

Time and testing place

The test is carried out at the Milkfish Hatchery Unit of MCBA Jepara from February to November 2021.

Results and Discussions

Spirulina dose trial

The results of *Spirulina* fatty acid analysis – at 3 different doses for broodstock milkfish feed enrichment f is shown in Figure 1 and Figure 2).

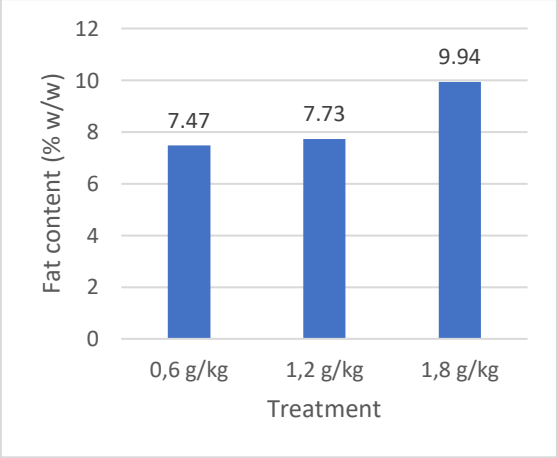

Figure 1. Fat content (% w/w) at different *Spirulina* doses

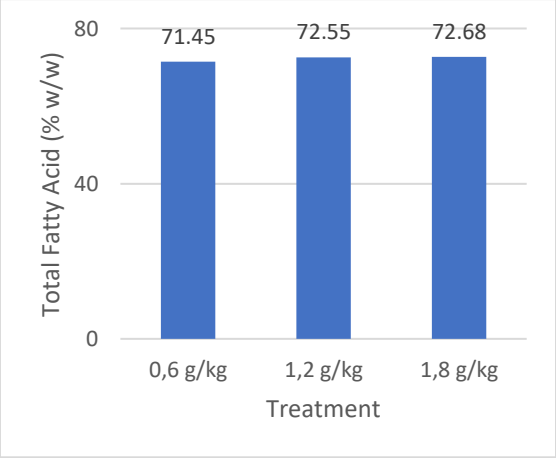

Figure 2. Total Fatty Acids (% w/w) at different *Spirulina* doses

The fat content in *Spirulina*-fortified feed increases with the number of *Spirulina* doses (Figure 2 and Figure 3). These results show that *Spirulina* is potential as feed-growing agent that is capable to enhance feed. Fat is the fatty acid ester of glycerol - stored as energy in the animal's body [6]. Fat is used for long-term energy needs, as well as for movement or energy reserved during periods of food shortage. In the body, fat provides twice energy more than protein [6].

The measurement result of fatty acids in *Spirulina*-enriched broodstock milkfish feed are shown in Table 1.

Table 1. Results of fatty acids measurement in *Spirulina*-enriched broodstock milkfish feed.

Parameters	*Spirulina* dosage treatment *(Sp)*		
	0.6 g/kg *Sp* % (w/w)	1.2 g/kg *Sp* % (w/w)	1.8 g/kg *Sp* % (w/w)
Fat Content	7.47	7.73	9.94
Fatty Acids :			
Caprilic Acid, C8:0	0.44	0.17	0.37
Capric Acid, C10:0	0.04		
Lauric Acid, C12:0	0.11	0.12	0.11
Tridecanoic Acid, C13:0	0.03	0.03	0.03
Myristic Acid, C14:0	3.4	3.33	3.54
Myristoleic Acid, C14:1	0.03	0.03	0.02
Pentadecanoic Acid, C15:0	0.3	0.3	0.28
Palmitic Acid, C16:0	16.41	16.24	16.5
Palmitoleic Acid, C16:1	3.35	3.33	3.45
Heptadecanoic Acid, C17:0	0.39	0.39	0.36
Cis-10-Heptaecanoic Acid, C17:1	0.12	0.11	0.11
Stearic Acid, C18:0	3.68	3.69	3.64
Elaidic Acid, C19:n9t	0.12	0.13	0.12
Oleic Acid, C18:1n9c	14.87	14.86	15.13
Linolelaidic Acid, C18:2n9t	0.12	0.05	0.03
Linoleic Acid, C18:2n6c	8.84	9.65	8.68
Arachidic Acid, C20:0	0.26	0.25	0.27
v-Linolenic Acid, C18:3n3	0.14	0.15	0.14
Linolenic Acid, C18:3n3	1.64	1.65	1.62
Heneicosanoic Acid, C21:0	0.08	0.1	0.05
Cis-11,14-Eicosedienoic Acid, C20:2	0.22	0.2	0.21
Behenic Acid, C22:0	0.14	0.15	0.15
Cis-8,11,14-Eicosetrienoic Acid, C20:2	0.22	0.14	0.15
Cis-11,14,17-Eicosatrienoic Acid Methyl Ester	0.14	0.14	0.16
Arachidonic Acid, C20:4n6	1.19	1.17	1.25
Tricosanoic Acid, C23:0	0.04	0.04	0.03
Cis-13,16-Docosadienoic Acid, C22:2	0.04	0.04	0.04
Lignoceric Acid, C24:0	10.15	10.54	10.86
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3	0.06	0.04	0.04
Nervonic Acid, C24:1	0.18	0.22	0.18
Cis-4,7,10,13,16,19-Docosahexaenoic Acid	4.79	5.01	4.86
Total Fatty Acid	71.45	72.55	72.68

Spirulina dose of 0.6 g/kg has 0.06 % (w/w) EPA content that higher than doses of 1.2 g/kg and 1.8 g/kg which are 0.04% (w/w) respectively. *Spirulina* dose of 1.2 g / kg obtain high DHA content while the highest ARA content is obtained at dose of 1.8 g / kg (Figure 3).

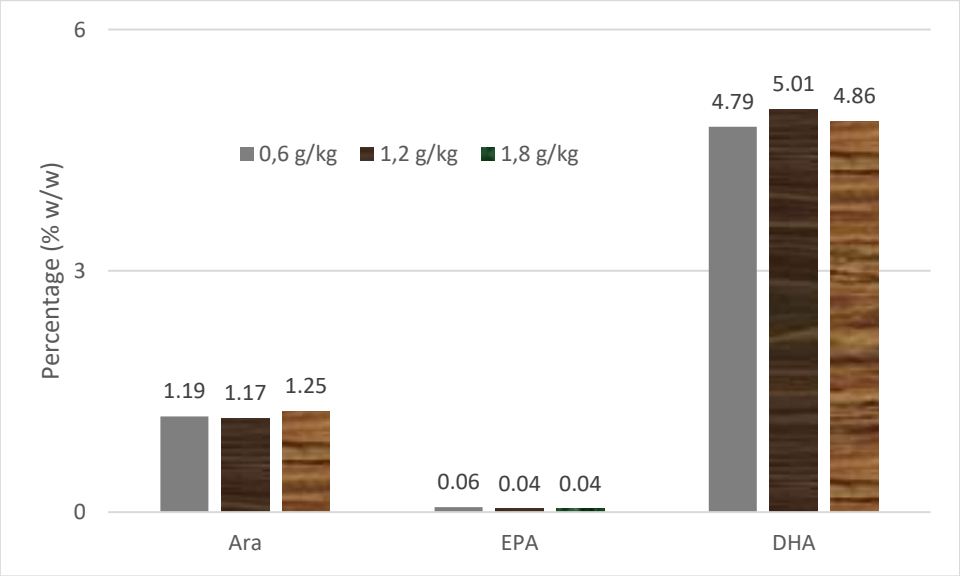

Figure 3. Percentage of *Spirulina* DHA, EPA and ARA contents

Based on the analysis result of the fatty acids at doses of 1.2 g / kg and 1.8 g / kg, then dose of Spirulina 1.8 g / kg of feed is selected for broodstock milkfish feed enrichment.

Egg laying frequency

The treatment of broodstock milkfish - feed enrichment with *Spirulina* dose of 0.6 g / kg and 1.8 g / kg is able to increase the productivity of milkfish eggs twice every month compared to no treatment feed without *Spirulina* (Figure 4).

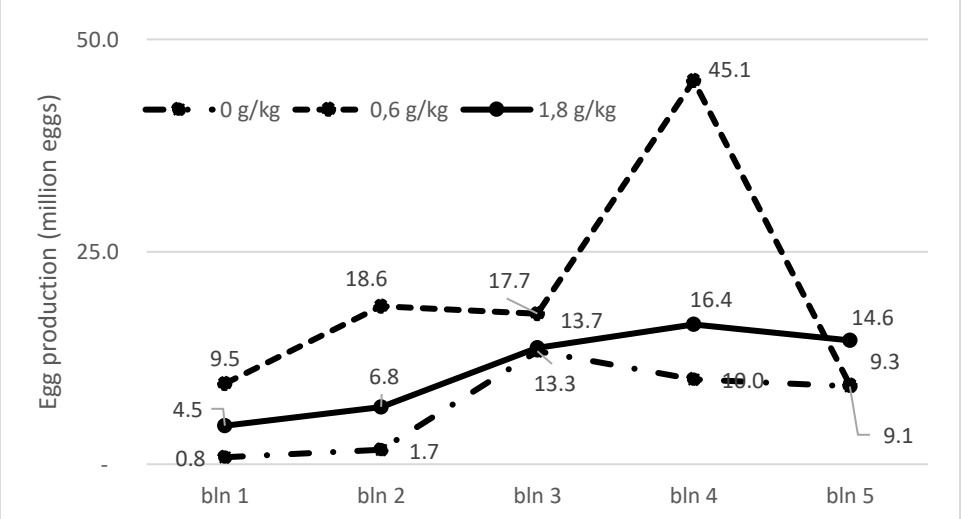

Figure 4. Egg production (million eggs) during testing

The average number of eggs that produced at dose of 0.6 g / kg or 1.8 g / kg does not make a different results (Figure 5).

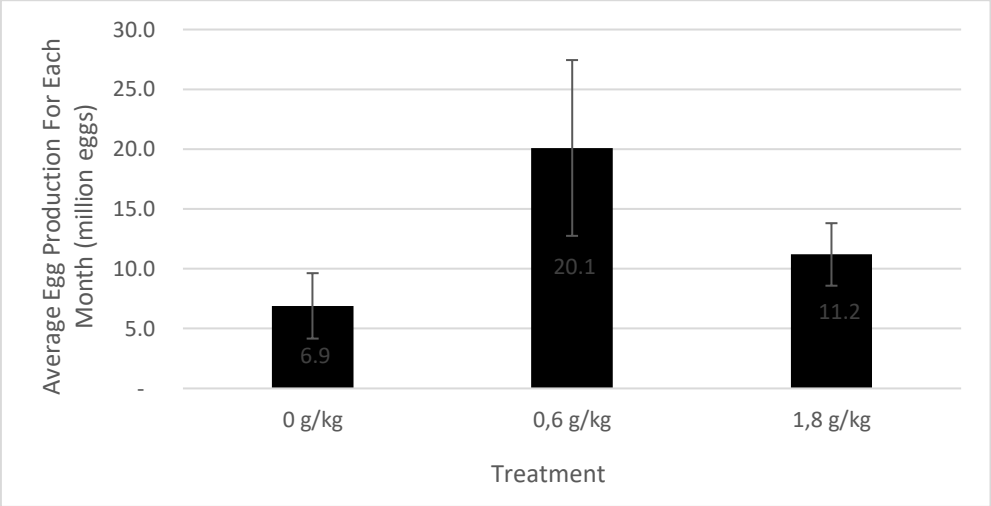

Figure 5. Average of egg production (million eggs) among treatments.

Fatty acids are very important in broodstock milkfish reproduction's process until - embryo development. Nutrition has real impact to ovary's development, number of egg and larvae development [16], however it still needs more testing in nutrition for broodstock fish [17]. Formula arrangement for broodstock feed needs lipid for reproduction function of female broodstock, as well as embryo development, and the survival at the time of yolk absorption [16]. Other information which can be used to arrange feed formula for broodstock is life cycle, eating habit and the fish's habitat [18], as well as composition of fatty acids in fish egg can be guide to arrange broodstock fish feed ration [19]. The lipids especially from group of PUFA *i.e eicosapentaenoic acid* (20:5 n-3; EPA) and *docosahexaenoic acid* (22:6 n-3; DHA) have proven to be correlated with fish reproduction success [20] [21]. Some study shows that the highest lipid requirement is when *vitellogenesis* (egg yolk formation). The requirement of EPA is started on *previtellogenesis* period until ovulation process. Thus, EPA and DHA are very essentials to be added as additives in broodstock feed in order to gain normal larvae growth and development. Additional essential fatty acids in broodstock feed can be done through enriched life feed or commercial feed which formula has been decided [22]. Right combination between DHA and AA can speed up the spawning rate and hatching rate; and also increase larvae survival rate [23] [24] [25]. Spirulina enrichment into broodstock milkfish feed can increase egg laid frequency at double in a month compare to feed without *Spirulina* enrichment. *Spirulina* enrichment gives different effect compare to feed without enrichment. However, enrichment dose of 0,6 g/kg and 1,8 g/kg does not give different effect to egg laying frequency of broodstock milkfish (Figure 6).

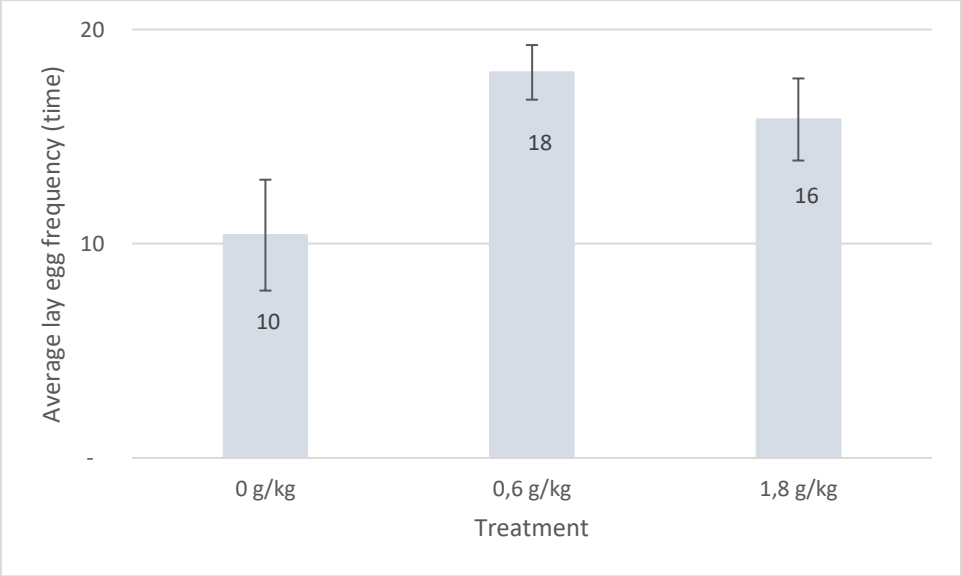

Figure 6. Frequency of egg laying from female broodstock milkfish (times /month)

Degrees of fertilization and hatching

The fertilization degree increase as high as the dose of *Spirulina* feed enrichment.. The highest egg fertilization degree (FR) value is 70.4% obtained from 1.8 g/kg dose, while dose 0.6 g/kg obtain only 57.7%. Both doses of fertilization value are higher than the dose of 0 g / kg (without *Spirulina*) which is at 48.5%. On hatching - degree of eggs (HR), enrichment with *Spirulina* dose of 0.6 g / kg and dose of 1.8 g / kg give higher degree of hatching than without *Spirulina*. Doses of 0.6 g/kg and 1.8 g/kg show no different effect on fertilization degrees (Figure 7).

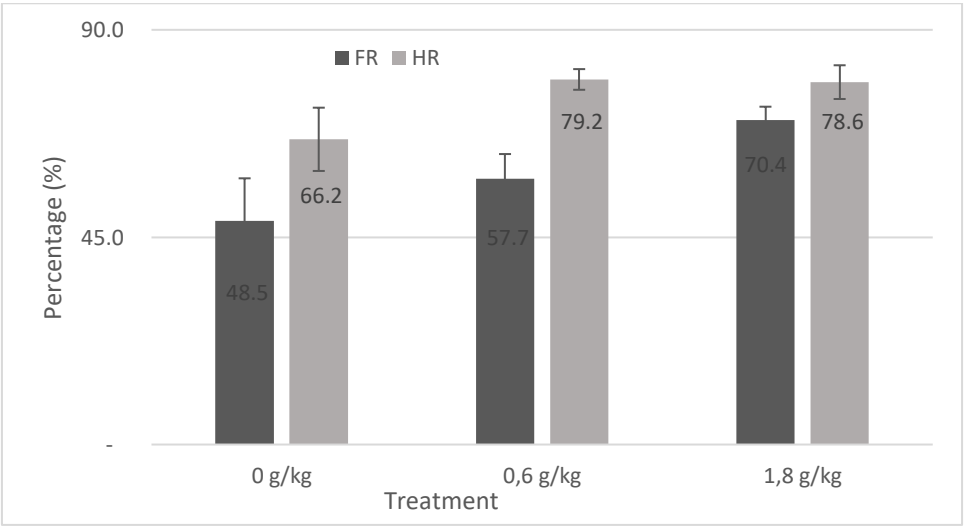

Figure 7. Degree of fertilization (FR) and degree of hatching (HR)

Fertilized eggs in milkfish have characteristics such as transparent yellow, round shaped and floating on the surface and in the water column. Unfertilized eggs are cloudy white, orwhen it has transparent yellow there are still slight patches / stains of cloudy white,eggs are not round and settle.

Egg diameter

Eggs diameter measurement results shows that during 5 months *Spirulina* feed enrichment at dose 0.6 g / kg and 1.8 g / kg did not give much difference in yield. (Figure 8).

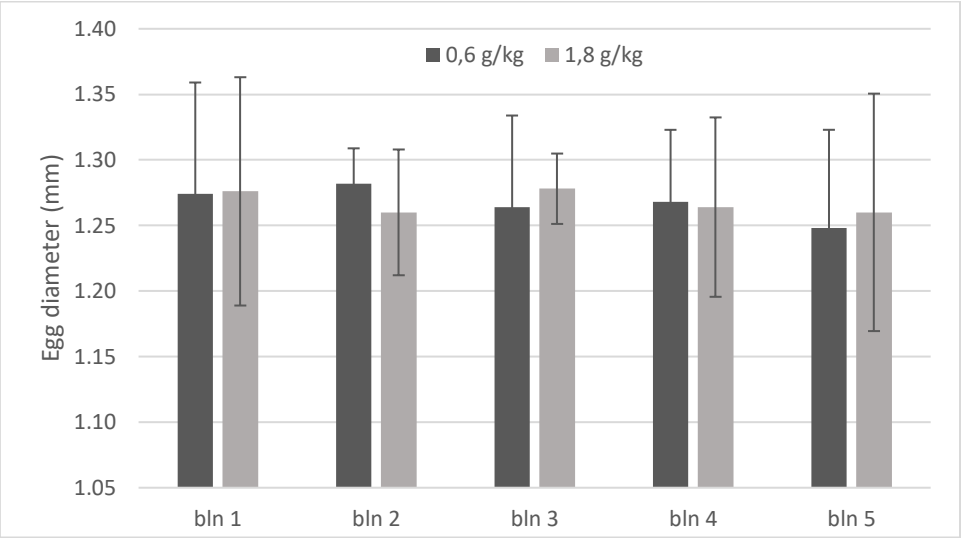

Figure 8. Egg diameter (mm)

Enrichment dose of 0,6 g/kg feed, has produced egg with diameter around 1,25 – 1,28 mm. Almost similar result also found in feed enrichment dose 1,8 g/kg feed, *i.e* 1,26 – 1,28 mm. Based on SNI [26], diameter of milkfish's egg is 0,9 – 1,2 mm. This is related with *egg yolk* contain in milkfish egg, where the bigger diameter has good quality of egg.

Conclusions

The result of feed enrichment with *Spirulina* flour are: (a) Number of eggs increase from 6,9 million to 20 million; (b) Egg laying frequency increase from 10 time/month to 18 time/month; (c) Degree of fertilization increase from 48,5 % to 70,4 %; (d) Hatching rate increase from 66,2 % to 79,2 %; (e) dose of 0,6 g/kg and 1,8 g/kg do not give different result.

Suggestion

It needs further test to know the effect of *Spirulina* feed enrichment to milkfish larvae performance especially in gonad and larvae stages development.

References

A. G. J. Tacon and M. Metian 2008 Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects Aquaculture 285 1–4 doi: 10.1016/j.aquaculture

Badan Standardisasi Nasional 2011 SNI 6148.3:2013 Ikan Bandeng (*Chanos chanos* Forskal) - Bagian 3 : Produksi benih pp 1–13, 2011, [Online]. Available: www.bsn.go.id

C. Ibeas, C. Rodríguez, P. Badía, J. R. Cejas, F. J. Santamaría, and A. LorenzoEfficacy of dietary methyl esters of n - 3 HUFA vs. triacylglycerols of n - 3 HUFA by gilthead seabream (*Sparus aurata* L.) juveniles 2000 *Aquaculture* 190 3–4 doi: 10.1016/S0044-8486(00)00399-9.

M. Yildiz 2008 Fatty acid composition of some commercial marine fish feeds available in Turkey *Turkish J. Vet. Anim. Sci.* 32 3

D. A. Higgs and F. M. Dong 2000 *Lipids and fatty acids. Encyclopedia of aquaculture* (London: Wiley-Interscience)

M. E. B. Seiffert, V. R. Cerqueira, and L. A. S. Madureira 2001 Effect of dietary (n-3) highly unsaturated fatty acids on growth and survival of fat snook (*Centropomus parallelus*, Pisces: Centropomidae) larvae during first feeding,*Brazilian J. Med. Biol. Res.* 34 5 doi: 10.1590/S0100-879X2001000500013

D. R. Tocher 2003 Metabolism and functions of lipids and fatty acids in teleost fish *Reviews in Fisheries Science*11 2 doi: 10.1080/713610925

H. Pangkey 2011 Kebutuhan asam lemak esensial pada ikan laut *J. Perikan. dan Kelaut. Trop*7 2 doi: 10.35800/jpkt.7.2.2011.185

M. I. Gladyshev, N. N. Sushchik, and O. N. Makhutova 2013 Production of EPA and DHA in aquatic ecosystems and their transfer to the land *Prostaglandins other lipid …*[Online]. Available: https://www.sciencedirect.com/science/article/pii/S1098882313000208

R. D. Miles and F. A. Chapman 2006 Benefits of fish meal in aquaculture diets *EDIS* 12 doi: 10.32473/edis-fa122-2006

G. W. Olsen, J. M. Burris, D. J. Ehresman2007Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers *Environ*.doi: 10.1289/ehp.10009.

M. S. Izquierdo, E. Atalah, T. Benítez-Santana, and ...2007 *Feeding marine fish larvae with lipid sources alternative to fish oil* [Online] Available: https://accedacris.ulpgc.es/handle/10553/77040

M. A. Litzow, K. M. Bailey, F. G. Prahl, and R. Heintz 2006 Climate regime shifts and reorganization of fish communities: The essential fatty acid limitation hypothesis *Mar. Ecol. Prog. Ser.* 315 doi: 10.3354/meps315001.

L. A. Copeman, C. C. Parrish, J. A. Brown, and M. Harel 2002 Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): A live food enrichment experiment," *Aquaculture* 210 1–4 doi: 10.1016/S0044-8486(01)00849-3.

S. T. and R. D. L. Jongkon P 2008 Phytoremediation of kitchen wastewater by *Spirulina platensis* (Nordstedt) Geiteler: pigment content, production variable cost and nutritonal value *Maejo Int. J. Sci. Technol.* 2 1

H. Desmorieux and N. Decaen 2006 Convective drying of Spirulina in thin layer *J. Food Eng.*77 1 doi: 10.1016/j.jfoodeng.2005.05.060

C. Mazorra *et al.*, 2003 Dietary lipid enhancement of broodstockstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*) *Aquaculture* 227 1–4. doi: 10.1016/S0044-8486(03)00493-9

M. S. Izquierdo, H. Fernández-Palacios, and A. G. J. Tacon 2001 Effect of broodstockstock nutrition on reproductive performance of fish *Aquaculture* 197 1–4 doi: 10.1016/S0044-8486(01)00581-6

M. P. Bransden, S. C. Battaglene, R. M. Goldsmid, G. A. Dunstan, and P. D. Nichols 2007 Broodstockstock condition, egg morphology and lipid content and composition during the spawning season of captive striped trumpeter, *Latris lineata Aquaculture* 268 1-4 doi: 10.1016/j.aquaculture.2007.04.026

R. Rosa, R. Calado, L. Narciso, and M. L. Nunes 2007 Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies and habitats: A fatty acid approach *Mar. Biol.* 151 3 doi: 10.1007/s00227-006-0535-6

T. Watanabe and R. Vassallo-Agius 2003 Broodstockstock nutrition research on marine finfish in Japan *Aquaculture* 227 1–4. doi: 10.1016/S0044-8486(03)00494-0

Y. Y. Li, W. Z. Chen, Z. W. Sun, J. H. Chen, and K. G. Wu 2005 Effects of n-3 HUFA content in broodstockstock diet on spawning performance and fatty acid composition of eggs and larvae in Plectorhynchus cinctus *Aquaculture* 245 1–4 doi: 10.1016/j.aquaculture.2004.12.016

R. L. Lane and C. C. Kohler 2007 Comparative fatty acid composition of eggs from white bass fed live food or commercial feed *N. Am. J. Aquac.* 69 1 doi: 10.1577/a06-014.1

H. Furuita, T. Yamamoto, T. Shima, N. Suzuki, and T. Takeuchi 2003 Effect of arachidonic acid levels in broodstockstock diet on larval and egg quality of Japanese flounder *Paralichthys olivaceus Aquaculture* 220 1–4 doi: 10.1016/S0044-8486(02)00617-8

O. Karadal, D. Güroy, and G. Türkmen 2017 Effects of feeding frequency and *Spirulina* on growth performance, skin coloration and seed production on kenyi cichlids (*Maylandia lombardoi*) *Aquac. Int.* 25 1 doi: 10.1007/s10499-016-0017-x

W. A. Shawky, H. S. El-Sayed, N. E. Saleh, A. A. Ismael, and A. F. M. El-Sayed 2021 Evaluation of microalgae-supplemented diets and enriched decapsulated artemia cyst powder as novel diets for post-weaned common sole (*Solea solea*) larvae *Aquac. Nutr.* 27 4 doi: 10.1111/anu.13245