

Growth performances and gonadal development of golden rabbitfish, *Siganus guttatus* fed with two types of floating diets in sea cages

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Abstract. A feeding trial was designed to evaluate the effects of utilizing floating pellets on the growth performances, feed utilization and gonadal maturation of the golden rabbitfish, *Siganus guttatus*. The treatments were two kinds of commercial floating pellet containing different crude protein (CP) levels which were 26% (FD1) and 33% (FD2). The tested diets were fed to golden rabbitfish. Golden rabbitfish with initial body weight of 239.7±4.8 g were stocked into six sea cages with size of 2x2x2 m³ with density of 50 fishes/cage. The feeding trial lasted for 120 days with results showed that the growth performances of weight gain and specific growth rate were significantly higher ($P < 0.05$) in fish fed FD2 diet (55.0±3.7% and 0.37±0.02 %/d) compared to those fed with FD1 diet (41.7±1.6 and 0.29±0.01%/d), respectively. Both female and male fishes had developed gonad at the end of feeding trial. The gonadosomatic index (GSI) for spermatid significantly higher (< 0.05) in fish fed FD2 (4.8±0.6%) than FD1 group (3.4±0.3%). Similarly, the GSI for oocyte of fish fed FD2 diet (8.9±1.0%) was significantly higher than the group fed FD1 diet (4.2±0.5%). The FD2 diet produced higher protein content in the whole body and fillet. In contrast, lipid content of whole body and fillet of fish fed FD1 diet was higher than FD2 diet. As conclusion, better growth performances and gonadal development was found in fish fed FD2 diet, a floating diet containing approximately 33% CP.

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1 Introduction

Rabbitfish are popular food fish in the Indo–Pacific region with a high demand in Southeast Asia and other markets like Hawaii [1, 2]. In those countries, production of rabbitfish mainly from capture fisheries which may cause overfishing and decline their population in the wild [3]. In several countries including Indonesia and Philippine, one of Siganid fish, golden rabbitfish, *Siganus guttatus* has been an emerging species for aquaculture [1, 4] despite very limited seed production from hatchery. Besides its high market value, rabbitfish has various attributes to support its aquaculture development including their tolerances to a wide range of salinities and high density [5, 6]. As herbivorous fish, rabbitfish can utilize seaweed for freshfeed [4] and artificial diet formulated with alternative plant feed ingredients [7-9].

In several areas in Indonesia including in South Sulawesi, farmers have cultured golden rabbitfish both in floating net cages and in earthen ponds. This development of golden rabbitfish culture needs to be supported by feed production. In fact, commercial feed specifically for golden rabbitfish has not yet produced up to date. In order to find alternative feeds to apply for the rabbitfish culture, feeding trial was accommodate to evaluate different kinds of commercial diet suitable for rabbitfish. Evaluation of utilizing commercial sinking pellet feed for grow-out of golden rabbitfish has been conducted [10]. The main concern of using sinking feed is the nutrients loss [11, 12] different from floating feed which is more stable in water and more digestible so that nutrients loading into the water can be minimized [13]. The benefit of applying floating diets for pelagic fish implies reduction of economic losses and environmental effect [14-16]. Various studies have reported better growth performance of floating feed than sinking feed on tilapia [17, 18, 13]. Since golden rabbitfish is pelagic fish like tilapia and milkfish, application of floating feed is also necessary to be evaluated. Therefore, this present study aimed at determining the effect of commercial floating diets on the growth performances, feed utilization and biochemical composition of whole body and fillet of golden rabbitfish. In addition, the feeding trial was terminated at prematuration stage to observe the effect of the diets on the gonadal maturation of the golden rabbitfish, *Siganus guttatus*.

2 Materials and Methods

2.1 Tested commercial diets and experimental design

Completely Randomized Design was applied to accommodate the feeding consisting of two treatments with triplicates. The treatments were two kinds of commercial floating diet containing different levels of protein which were FD1 contained crude protein (CP) minimum 25% and FD2 contained higher CP of minimum 32% based on product specification. Detail composition of the two diets according to label of the product is presented in Tabel 1.

Table 1. Proximate composition (%) of two tested commercial floating diets absed on product specification

| Nutrients (%) | Tested floating diets | |
|---------------|-----------------------|-------|
| | FD1* | FD2** |
| Moisture | <11.0 | <11 |
| Protein | >25.0 | >32 |

| | | |
|-------|-------|-----|
| Lipid | >5.5 | >6 |
| Ash | <11.0 | <11 |
| Fiber | <7.0 | <5 |

* Diet contained crude protein (CP) minimum 25%

** Diet contained higher CP of minimum 32%

Based on analysis, proximate composition of the two diest is shown in Table 2. Protein content of the FD1 and FD2 diet were 26.7 and 33.9%, respectively closely to the product specification. The other nutrient contents were relatively similar contained in the two diets.

Table 2 .Analyzed proximate composition (%) of two tested commercial floating diets

| Nutrients (%) | Tested floating diets | |
|---------------|-----------------------|-------|
| | FD1* | FD2** |
| Moisture | 7.9 | 8.4 |
| Protein | 26.7 | 33.9 |
| Lipid | 8.2 | 7.2 |
| Ash | 8.4 | 8.2 |
| NFE | 48.5 | 42.4 |

* Diet contained crude protein (CP) minimum 25%

** Diet contained higher CP of minimum 32%

2.2 Fish and feeding protocols

Two hundreds of juvenile golden rabbitfish *S. guttatus* hatched and reared in Rabbitfish Installation Hatchery of Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE) located in Barru District, South Sulawesi were selected and randomly stocked at density of 50 fish/cage. Initial body weight of the juveniles was 239 g (Tabel 3). Six floating net cages with size of 2x2x2.5 m3 used for the feeding trial were allocated in two rafts. The top of all cages was equipped with a green net to prevent floating feed from escaping from the cage before being eaten by fish. During the feeding trial, the two floating diets were handy given carefully to all cages to satiation [19]. Fish were fed twice a day in the morning (08.00) and afternoon (16.00).

Monitoring of growth and condition of fish in each cage was done by weighed individually of all fish at monthly intervals. During the sampling time, all cages were replaced with the clean ones to avoid the build-up bio-fouling and pathogen infection. Duration of the feeding trial was 120 days.

2.3 Data collection and statistical analysis

Observed parameter evaluated during the feeding trial included weight gain (WG), specific growth rate (SGR) and survival rate (SR). Utilization of feed was calculated for feed intake and feed conversion ratio (FCR). Hepatosomatic index (HSI) and gonadosomatic index (GSI) for both spermatid (male) and oocyte (female) were also measured according to [20]. Biochemical analysis of whole body and fillet of fish at the end of the feeding trial were performed for proximates analysis). Calculation of parameters evaluated follows below equation:

$$WG (g \text{ fish}^{-1}) = 100 \times (\text{final weight} - \text{initial weight}) / (\text{initial weight}) \quad (1)$$

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times (\ln W_f - \ln W_i) / T \quad (2)$$

where W_i is the mean initial weight of the fish and W_f the mean final weight of the trial, and T is the culture of days [21].

$$\text{SR (\%)} = 100 \times (\text{final number of fish} / \text{initial number of fish}) \quad (3)$$

$$\text{FCR} = \text{feed intake (g dry weight)} / \text{total body weight gain (g wet weight)} \quad (4)$$

$$\text{HSI (\%)} = 100 \times (\text{weight of liver (g wet)} / \text{weight of fish (g wet)}) \quad (5)$$

$$\text{GSI (\%)} = 100 \times (\text{weight of gonad (g wet)} / \text{weight of fish (g wet)}) \quad (6)$$

3 Results and discussion

3.1 Growth performance and feed utilization

Weight increment of golden rabbitfish after 120-d feeding trial is illustrated by Figure 1. Fish fed FD2 diet containing 33% CP grew significantly faster with final body weight of 371.8 ± 8.4 g compared to FD1 containing lower CP of 26%.

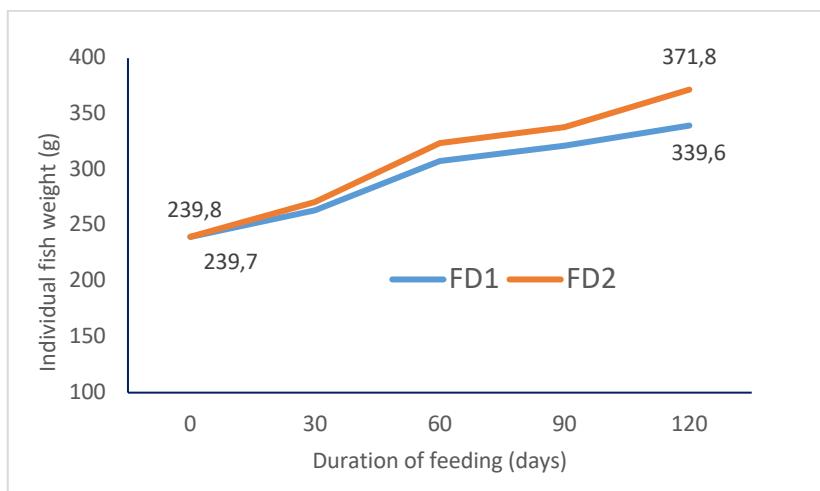


Fig. 1. Pattern of weight increment of golden rabbitfish *S. guttatus* fed two floating diets during 120 days culture. FD1 = Diet contained crude protein (CP) minimum 25%; FD2 = Diet contained higher CP of minimum 32%

Table 3 presents growth performances and feed utilization of golden rabbitfish after feeding with two floating feeds. The final weight, WGs and SGRs were significantly affected ($P < 0.05$) by the two floating diets. The WG of fish fed FD2 was $55.0 \pm 3.7\%$ significantly higher than fish fed the FD1 group ($41.7 \pm 1.6\%$). Similarly, the SGR was significantly improved in fish fed FD2 (0.37 ± 0.02 %/d) compared when fish fed the FD1 group (0.29 ± 0.01 %/d). The SR of fish fed the FD2 diet was higher than FD1 groups, the SRs however were not significantly ($P > 0.05$) influenced by both diets.

Table 3. Growth, feed intake and FCR (mean±SD) of two SW groups of golden rabbitfish *S guttatus* fed two CD diets during 120 days culture

| Parameter | FD1 | FD2 | <i>P</i> value |
|-----------------------------------|-------------------------|-------------------------|----------------|
| Initial weight (g) | 239.7±6.6 ^a | 239.8±3.8 ^a | 0.974 |
| Final weight (g) | 339.6±5.4 ^a | 371.77±8.4 ^b | 0.005 |
| Weight gain (WG, %) | 41.7±1.6 ^a | 55.0±3.7 ^b | 0.005 |
| Specific growth rate (SGR, %/day) | 0.29±0.01 ^a | 0.37±0.02 ^b | 0.006 |
| Survival rate (SR, %) | 94.0±6.0 ^a | 98.0±2.0 ^a | 0.355 |
| Feed intake (g/fish) | 281.3±19.6 ^a | 271.5±64.0 ^a | 0.812 |
| Feed conversion ratio (FCR, g/g) | 2.9±0.2 ^a | 2.1±0.5 ^a | 0.066 |

FD1 = Diet contained crude protein (CP) minimum 25%; FD2 = Diet contained higher CP of minimum 32%

Protein is a macro-nutrient in fish feed and play a crucial role in many biological processes, including growth, maintenance of body tissue and reproduction [22, 23]. With initial body weight of around 240 g, the golden rabbitfish still linearly grew in both treatment groups where the FD2 diet produced significantly higher SGR. The growth rate of golden rabbitfish at this weight seemed relatively similar with those found in the previous study that fish of 280 g had 0.37%/d of SGR [10]. The lower growth rate of fish fed floating feed containing 26% CP (FD1) obviously demonstrated that the protein level cannot support the proper growth rate of golden rabbitfish even at the bigger size. The mean final weight of 371.77±8.4 g in fish fed the FD2 diet may approach the size of maturation stage of golden rabbitfish [24]. According to [25], when fish at the reproductive stage, they reach the maximum weight achieved for adult fish or the fish are in the genetic predisposition.

In term of feed utilization, both feed intake and FCR were not significantly affected by the tested diets. However, the value of FCR for fish fed FD2 diet was lower (2.1±0.5) than FD1 which was 2.9±0.2. Comparing with 2.9 of FCR recorded when golden rabbitfish fed sinking pellet containing 26% CP, the value of FCR observed in the present study was also 2.9 for floating feed containing the same protein of 26.7%. This indicated that with similar protein content, sinking and floating feed did not produce different FCR probably because the protein content of 26% did not meet the requirement of golden rabbitfish to grow and have caused similar trend of high FCRs. When the protein level higher as contained in FD2 diet (33.9%), the value of FCR was improved only 2.1 even though both were not significantly different. This may imply that 33.9% of protein was in the range of requirement level of golden rabbitfish [26, 27]. Growth and feed utilization increased exponentially at increased dietary protein level [28, 29] and will turn into a plateau when it reached the optimal range of the dietary protein requirement [28, 30, 34].

3.2 Hepatosomatic and Gonadosomatic index

Illustration of effects of the tested diets on hepatosomatic index (HSI) and gonadosomatic index of both spermatid (GSI-Sp) and oocyte (GSI-Oc) can be seen in Figure 2. The HSI of the two treatment groups were not significantly ($P>0.05$) different. However, the GSI-Sp and GSI-Oc were significantly different between the FD2 and FD1 groups. The SGI-Sp of fish fed the FD2 group (4.8%) was significantly higher ($P<0.05$) than fish fed the FD1 diet (3.4%). Furthermore, the GSI-Oc of fish fed FD2 diet was significantly higher with the value was double than fish fed FD1 group (9.0% vs 4.2%).

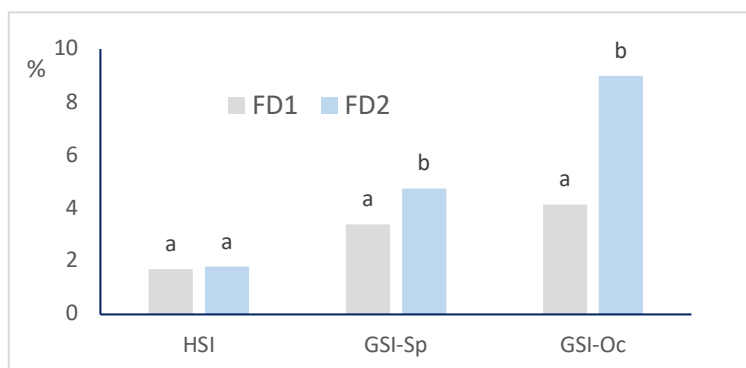


Fig. 2. HSI, GSI spermatid (GSI-Sp) and GSI oocyte (GSI-Oc) of golden rabbitfish after 120 days fed with two floating diets in sea cages. FD1 = Diet contained crude protein (CP) minimum 25%; FD2 = Diet contained higher CP of minimum 32%. Value in each bars with different alphabetic indicated significant differences ($p < 0.05$)

Different protein content of diets has obvious effects on development of gonad in golden rabbitfish found in this present study where FD2 diet contained 33% CP enhanced development of both spermatid and oocyte. The 33% CP diet produced higher GSI compared with the FD1 diet containing lower 26% CP. This finding supported many previous studies revealing the role of protein on growth and reproduction in fish [35,22, 23]. The findings also demonstrated that application of floating diets containing CP content closely to requirement level for grow-out (33%) can also stimulate the gonadal development of rabbitfish up to the earlier stage of maturation. The GSI of oocyte obtained in this present study was lower (9%) compared when golden rabbitfish fed with maturation diet contained 40% CP [24]. This indicated that protein requirement for maturation stage of golden rabbitfish is higher than for grow-out. High amount of protein in both oocyte and spermatid which were 68.5 and 76.7%, respectively [24], also implied that reproduction stage required higher protein requirement. Moreover, type of protein sources and other ingredient used for formulating maturation diet significantly affected the reproductive performances of fish [36] including golden rabbitfish [24].

3.3 Whole body and fillet proximate composition

Proximates content of whole body and fillet of golden rabbitfish fed the two floating diets are presented in Table 4. The CP of whole body significantly ($P < 0.05$) increased when fish fed higher CP diet of FD2 (62.1%). Ash content was also significantly higher in fish fed the FD2 diet. In contrast, the lipid content of fish fed lower CP diet (FD1) was significantly higher ($P < 0.05$) compared to fish fed FD2 diet.

Table 4. Whole body and fillet proximate analysis (% dry matter) of golden rabbitfish *S guttatus* fed two floating diets

| Nutrient | Whole body | | P value | Fillet | | P value |
|----------|------------|-----|---------|--------|-----|---------|
| | FD1 | FD2 | | FD1 | FD2 | |

| | | | | | | |
|---------------|-----------------------|-----------------------|-------|-----------------------|-----------------------|-------|
| Crude protein | 56.9±0.7 ^a | 62.1±2.6 _b | 0.029 | 80.5±1.9 ^a | 87.1±0.7 _b | 0.006 |
| Lipid | 22.3±1.4 ^b | 15.5±3.2 _a | 0.029 | 12.3±2.7 ^b | 6.0±0.4 ^a | 0.018 |
| Ash | 11.9±0.6 ^a | 15.1±0.8 _b | 0.005 | 4.8±0.9 ^a | 5.3±0.2 ^a | 0.372 |
| NFE | 8.8±1.5 ^a | 7.3±0.3 ^a | 0.138 | 2.4±0.3 ^a | 1.6±0.7 ^a | 0.380 |

FD1 = Diet contained crude protein (CP) minimum 25%; FD2 = Diet contained higher CP of minimum 32%.

Similar to whole body CP content, fillet CP in fish fed the FD2 diet was significantly higher ($P<0.05$) compared to FD1 diet, while lipid content was significantly ($P<0.05$) lower in fish fed the FD2 diet. In addition, ash and NFE of the fillet were not significantly different ($P>0.05$).

Trend of CP content in both whole body and fillet of golden rabbitfish found in the present study clearly showed the influence of higher CP of 33.9% in FD2 diet compared to 26% in FD1 diet. In contrast, different responses were found when the golden rabbitfish fed with sinking diet containing 23.8 and 26.4% that CP content in fillet were not significantly different and produced lower fillet CP approximately 76.9% [10]. Furthermore, the lipid content in whole body and fillet in fish fed FD1 diet was approximately 6-7% higher than in whole body and fillet in fish fed FD2 indicating that when fish fed higher CP content, the lipid content in the fish declined. The trend of high lipid content in cultured fish than in wild fish was also observed in the present study supporting the previous study in golden rabbitfish [37].

4 Conclusion

Commercial floating diet containing 33.9% CP (FD2) produced better growth performances with WG and SGR of $55.0\pm 3.7\%$ and $0.37\pm 0.02\%/d$, respectively. The FD2 diet also increase the GSI of oocyte double than the FD1 containing lower CP of 26%.

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References

1. P.A. Caballero, E.B. Coniza, R. Dayrit. *Nursery and grow-out culture of rabbitfish siganus guttatus in brackishwater ponds*. *Aquaculture Extension Manual No. 70*. Southeast Asian Fisheries Development Center, Aquaculture Department Tigbauan 5021, Iloilo, Philippines. (2022)
2. FAO. *Rabbitfish farming in Asia*. Bangkok. (2022)

3. FAO. FishStatJ – software for fishery and aquaculture statistical time series. In: FAO, Fisheries and Aquaculture Division. Rome. Cited 26 November (2021)
4. H.A. Sulaeman, Z. Zainuddin and A. Laining. *Evaluation of seaweed. Ulva lactuca as a fresh diet for nursery stage of golden rabbitfish.* In *Siganus guttatus* IOP Conf. Ser. Earth Environ. Sci. 1119 012057 (2022).
5. S.R.M. Tabugo, J.P. Sendaydiego, E.A. Requieron, M.D. Int. Rch. J. of Bio. Sci. Vol. 1(8), 65-70, December (2012).
6. R. Syah, Makmur, B.R. Tampangallo, M.C. Undu, A.I.J. Asaad, A. Laining. *Rabbitfish (Siganus guttatus) culture in floating net cage with different stocking densities.* in IOP Conf. Series: Earth and Environmental Science 564, 012022 (2020).
7. A. Laining, Usman, R. Syah. *AACL Bioflux* 9 **352** (2016).
8. C. Mendoza, J.P. de la Cruz, M. Bautista, R.S. Abalos. *J. of Natural and Allied Sciences.* Vol. IV **1**, pp. 73-81, (2020)
9. M.I.C. Cabanilla-Legaspi, R.F.M. Traifalgar, E.G.T. Jesus-Ayson, K.G.S. *Andrino-Felarca, R.E.P Mamauag. Changes in iodide and thyroid hormone levels of hatchery-reared orange-spotted rabbitfish Siganus guttatus (Bloch 1787) during early larval development.* Aquaculture Reports 20 (2021) 100674.
10. A. Nawang, Ramadhan, Rosni, S R H Mulyaningrum, Lideman, M C Undu, M Safri, S Ardyansyah, T Asriani, A Laining. *A comparative study on the use of two commercial diets for grow-out of golden rabbitfish, Siganus guttatus reared in Sea Cages.* in IOP Conf. Ser. Earth Environ. Sci. (in review) (2023).
11. B.A. Falayi, B.A.M. Adebayo, C.T. Madu. *J. of Sustainable Tropical Agriculture.* **9** 104-108 (2003).
12. A.M. Orire, S. O. E. Sadiku. *J. of Int. Sci. Publications: Agriculture & Food* 2, 521-523 (2014)
13. E.Y. Mohammady, M.R. Soaudy, M.M. Ali, M.A. El-Ashry, M.S.A. El-Karim, S. Jarmolowicz, M.S. Hassaan. *Response of nile tilapia under biosfloc system to floating or sinking feed and feeding rates: water quality, plankton community, growth, intestinal enzymes, serum biochemical and antioxidant status.* Aquaculture Reports 29 (2023)
14. T. Aas, B. Terjesen, T. Sigholt, M. Hillestad, J Holm, S. Refstie, G. Baeverfjord, Ka. Rørvik, M. Sørensen, M. Oehme. *Aquac. Nutr.* **17**, 657–670 (2011)
15. S. Ma, H. Wang, J. Yang, J. Li, M. Xue, H. Cheng, F. Zou, C. Blecker. *Aquac. Rep.* **23**, (2022)
16. S. Xing, X. Liang, H. Wang, X. Xie, P.A. Wierenga, J.W. Schrama, M. Xue. *Aquaculture* **570** 739442 (2023).
17. Md.I. Hossain, N. Haque, A.S.A.F. Alam, H. Begum, M.B. Mokhtar. *Turk. J. Fish. Aquat. Sci.* (19) **1**, 71–80 (2018).
18. A.M. Abdelhamid, M.F. Salem, M.El.Sh. *Egypt. J. Aquat. Biol. Fish.* (23) **2**, 347–361. (2019)
19. A. Laining, U. Usman, R. Syah. *J. Appl. Aquac.* **29** (2017)
20. S. Duchaud, S. Ternengo, D.H.E. Durieux, B. Bianchini, M. Garrido, A. Antoine Aiello, R. Romain Bastien, V. Pasqualini. *Regional Studies in Marine Science* **42**, 101645 (2021)
21. C. Schulz, U. Knaus, M. Wirth, and B. Rennert. *Aquaculture Nutrition* **11**: 403–413 (2005)
22. H. Rodríguez-González, M. García-Ulloa, A. Hernández-Llamas, H. Villarreal. *Aquaculture* **257** (1–4), 412–419 (2006)
23. S.A. Watts, A.L. Lawrence, J.M. Lawrence. *Nutrition. Sea Urchins: Biology and Ecology*, 191–208 (2020)
24. A. Laining, I. Trismawanti, Usman, M. H. Masruri. *Indonesian Aquaculture Journal (IAJ)* (14) **1**, 31-38 (2019)
25. S. Mejri, R. Tremnlay, G. Vandenberg, M. Moren, I.B. Khemis, C. Auder. *Canadian Journal of Zoology*, (95) **5**, 299-310. (2017).

26. M.M. Parazo. *Siganus guttatus* Aquaculture. **86**, 41-49 (1990).
27. A Laining, I. Trismawanti, M.C. Undu, S.R.H. Mulyaningrum, Usman, R. Syah, Ramadhan, Rosni. *Development of rabbitfish culture. Accelerating the development of finfish mariculture in Cambodia through south-south research cooperation with Indonesia (ACIAR FISH/2016/130) (Technical report of Research Institute for Coastal Aquaculture and Fisheries Extension*. Agency for Research and Human Resources. Ministry of Marine Affairs and Fisheries) p 34 (219b). (In Bahasa)
28. S. Zehra, M.A. Khan. *Aquacult International* **20**:383–395 (2012).
29. M. Ren, K. Ji, H. Liang, X. Ge, H. Mi. *The Israeli Journal of Aquaculture - Bamidgeh, IJA* **69**.2017.1424, 9 pages. (2017)
30. C. Ye, Y. Wu, Z Sun, A. Wang. *Aquaculture Research* 1–10 (2016).
31. Susanto, J. Hutabarat, S. Anggoro, Subandiyono. *AACL Bioflux Volume 12, Issue 1*. (2019).
32. N. Ahmad, P.J.A. Siddiqui, A. Ali, K.M. Khan, R. Masroor, N. Akbar , M. Amin, M. Attaullah. *Pakistan Journal of Zoology*, **51**(3), p.1003. (2019)
33. X. Lu, D. Peng, X. Chen, F. Wu, M. Jiang, J. Tian, W. Liu, L. Yu, H. Wen, K. Wei. *Aquaculture Reports* **18**, 100542 (2020).
34. M. Hussain, H. U.I. Hassan, M.A.M. Siddique, K. Mahmood, M.F.A. Abdel-Aziz, M. Y. Laghari, N. A. Abro, K. Gabol, Nisar, S. Rizwan, Halima. *Egyptian Journal of Aquatic Research* **47**, 329–334 (2021).
35. R.P. Wilson. *Amino acid and proteins in Halver*, J.E. (Eds) p 143-179. Fish nutrition. Third edition. The United State of America: Academic Press, 824 pp. (2002).
36. D. Gonzalez-Silvera, D. Izquierdo-Gomez, P. Sanchez-Jerez, M.T. Elbal, J.A. Lopez-Jimenez, F.J. Martínez-Lopez. *Influence of aquaculture waste on fatty acid profiles and gonad maturation of wild fish aggregations at fish farms*. *Marine Environmental Research* **156**, 104902 (2020)
37. Laining A. Usman, Syah R. *AACL Bioflux Volume 9 Issue 2*, 352-359. (2016)