The effect of ethanolic extracts *Ulva lactuca* on growth performance and survival rate of milk fish (*Chanos chanos*)

N Nurfadillah 1,2,3*, H A Ningsih1, S A E Rahimi1, I Dewiyanti1,2,3, S Mellisa1,2 and A Syahril1

1Aquaculture Department, Faculty of Marine and Fisheries, Universitas Syiah Kuala, 23111 Indonesia.
2Marine and Fishery Research Center, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
3Laboratory of Marine Biology, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Indonesia
*Corresponding Author: nurfadillah@unsyiah.ac.id

Abstract. Ulva lactuca is an algae that is a source of protein for fish and can increase resistance to disease. The purpose of this study was to determine the effect of ethanolic extracts Ulva lactuca on growth and survival rate of milkfish (*Chanos chanos*). This research was conducted at Brackish Water Aquaculture Development Center, Ujung Batee, Aceh, Indonesia on April-March 2019. Data were analyzed using a completely randomized design with 6 treatments and 4 replication, namely A (0 ppm), B (100 ppm), C (200 ppm), D (300 ppm), E (400 ppm), F (500 ppm). The parameters measured were absolute weight growth, absolute length growth, specific growth rate, survival rate and water quality. The ANOVA test results showed that the Ulva lactuca extract had a significant effect (P<0.05) on absolute weight growth, absolute length growth and specific growth rate but had no effect (P>0.05) on the survival rate of milkfish (*Chanos chanos*). The concentration of 500 ppm extract Ulva lactuca (treatment F) produced the highest on absolute weight growth (1.52 g), absolute length growth (1.96 mm), specific growth rate (0.30 %/day), and survival rate (96%).

1. Introduction
The demand for milkfish in Indonesia has increased every year. Milkfish (*Chanos chanos*) cultivation shows good prospects, where at in 2015, milkfish production reached 631,125 tons, then production increased in 2016, namely 709,312 tons [1]. Milkfish production has increased significantly, which affects the availability of feed.

Fish nutritional requirements as a determining factor for growth are highly dependent on feed. One of the nutritional aspects needed in fish feed is vitamins. Vitamins are organic compounds that act as metabolite reactions. Vitamins are needed in relatively small amounts but are essential for growth. Vitamin C is a type of vitamin that has an important role and belongs to the antioxidant vitamin. Vitamin C is known to be able to normalize fish immune function, reduce stress, accelerate fish healing, increase growth rate, digestive efficiency and resistance to disease. Some algae have the same function as Vitamin C which contains antioxidants that fish need for body metabolism. Brotowidjaya et al. [2] stated that vitamin C increases fat metabolism which can lead to changes in body composition and accumulation of nutrients in fish and thus may reduce fat carcases and increase protein levels.
Various types of algae are known to be a source of protein and increase resistance to disease [3]. *Ulva lactuca* is a type of seaweed from the green algae class in Indonesia. *Ulva lactuca* has a fairly high nutritional content, which contains 17.43% protein, 62.92% carbohydrates, 2.94% ash, 5.17% fat, 0.0191% vitamin C and antioxidants of 60.975 ppm [4].

*Ulva lactuca* contains melatonin compounds. Melatonin is a type of hormone which is a powerful antioxidant. Antioxidants are compounds that can significantly prevent or inhibit damage caused by free radicals to cells. *Ulva lactuca* also contains all essential amino acids except tryptophan. Essential amino acids are known to be essential for fish growth, increase immune response and resistance to a number of pathogens [5, 2]. Hence, the purpose of this study was to determine the effect of ethanolic extracts *Ulva lactuca* on growth and survival rate of milkfish (*Chanos chanos*).

2. Research Methods

2.1. Sampling
This research was carried out in April - May 2019 at the Laboratory of the Center for Brackish Water Aquaculture (BPBAP) in Ujung Batee, Aceh Besar, *Ulva lactuca* obtained from Ulee Lheue Beach in Banda Aceh City. The main tools and materials used in research comprises: milkfish seeds, thermometer, hand refractometer, pH meter, Dissolve Oxygen meter, and aerator.

2.2. Experimental Design and Diet
The design used in this study was a completely randomized design (CRD), which consisted of 6 treatments and each treatment was given 3 replications. Addition of extract *Ulva lactuca* to the feed composition namely A (0 ppm), B (100 ppm), C (200 ppm), D (300 ppm), E (400 ppm), F (500 ppm). *Ulva lactuca* extract was made by maceration method in 70% ethanol. The ethanol extract was filtered and evaporated so that 100% pure extract was produced then *Ulva lactuca* extract was added to the feed.

2.3. Experimental fish and feeding
The milkfish seeds was purchased from the hatchery of the Ujung Batee Brackish Water Cultivation Fishery Center (BPBAP), Aceh Besar District, Aceh Province. The size milkfish seeds used are 1-2 cm. The stocking density of fish in each maintenance is 10 fish (one fish per 2 L of sea water). The frequency of feeding was 2 times a day on 8 am and 4 pm. Feeding is given 10% of the body weight of the fish. Adjustments to the amount of feed needed are made every week.

2.4. Research Parameters
The absolute weight growth was calculated based on Muchlisin et al. [6] as follows:

\[ W = W_t - W_0 \]

Where: \( W \) = absolute weight growth (g); \( W_t \) = biomass weight at the end of the research (g); \( W_0 \) = biomass weight at the start of the research (g).

The absolute length was calculated based on Muchlisin et al. [6] as follows:

\[ L = L_t - L_0 \]

Where: \( L \) = absolute length (mm); \( L_t \) = biomass length at the end of the research (mm); \( L_0 \) = biomass length at the start of the research (mm).

The specific growth rate (SGR) was calculated based on De Silva and Anderson [7] as follows:

\[ SGR = \frac{(Ln W_t - Ln W_0)}{t} \times 100 \]

Where SGR is specific growth rate (\% day\(^{-1}\)); \( W_t \) = biomass weight at the end of the research (g); \( W_0 \) = biomass weight at the start of the research (g); \( t \) is research duration (day).

The survival rate (SR) was calculated based on Goddard [8] as follows

\[ SR = \frac{N_0 - N_t}{N_0} \times 100 \]
Where SR is survival rate (%); No is total fish at the start of research; Nt is the total of fish at the end of the research.

2.5. Data analysis
The data was analyzed for the one-way analysis of variant (one-way ANOVA) test and followed by Least Significant Difference tests (LSD) and Honestly Significant Difference test (HSD).

3. Results and Discussion
Based on the Anova test showed that ethanolic extract Ulva lactuca significantly affected the growth of absolute weight, absolute length growth, specific growth rate of milkfish larvae (P<0.05) and had no significant effect on the survival rate (P>0.05). The LSD and HSD test showed the best treatment for absolute weight growth, absolute length growth and specific growth rate parameters found at treatment F (500 ppm), this values has significant different with other treatment (Table 1).

Table 1. Effect of ethanolic extracts Ulva lactuca on growth performance and survival rate of milk fish (Chanos chanos)

| Concentration of Ulva lactuca (ppm) | Absolute weight growth (g) | Absolute length growth (mm) | Specific growth rate (% day⁻¹) | Survival rate (%) |
|-------------------------------------|----------------------------|-----------------------------|--------------------------------|-----------------|
| 0                                   | 0.196±0.005ᵃ                | 1.306±0.0702ᵃ               | 0.039±0.0008ᵃ                  | 96.6 ±5.73ᵃ     |
| 100                                 | 0.416±0.005ᵇ                | 1.280±0.1501ᵃ               | 0.083±0.0008ᵇ                  | 96.6 ±5.773ᵇ    |
| 200                                 | 0.470±0.000ᶜ                | 1.320±0.0000ᵃ               | 0.093±0.0004ᶜ                  | 93.3 ±5.773ᶜ    |
| 300                                 | 0.590±0.000ᵈ                | 1.466±0.1026ᵃ               | 0.118±0.0000ᵈ                  | 96.6 ±5.773ᵈ    |
| 400                                 | 0.910±0.000ᵉ                | 1.493±0.1285ᵃ               | 0.182±0.0008ᵉ                  | 90.0 ± 10.00ᵉ   |
| 500                                 | 1.526±0.000ᶠ                | 1.966±0.1724ᵇ               | 0.305±0.0006ᶠ                  | 96.6 ±5.773ᶠ    |

Note: The mean±SD value at the same coloum with different superscript are significantly different at 95% confident level (P<0.05).

Figure 1. The absolute weight growth trend of milkfish (Chanos chanos)

The high growth performance values because Ulva lactuca contains essential amino acids. Essential amino acids are essential for fish growth, increase immune response and resistance to pathogens. Wong and Cheung [9] stated that Ulva lactuca contains essential amino acids except tryptophan. Ulva lactuca also contains high antioxidant compounds. This is confirmed by Nufus et al. [10] Ulva lactuca has antioxidant compounds, namely polyphenols, alkaloids, flavonoids, triterpenoids, steroids and has strong antioxidant activity with an IC50 value of less than 50 mg/l. Ulva lactuca also can increase
immunostimulants. Immunostimulants are substances that can increase the non-specific immune system of fish. According to Herpani et al. [11] *Ulva lactuca* can improve growth performance and non-specific immune responses in fish.

Several studies have stated that *Ulva lactuca* is very effective in enhancing growth performance. Mahasu et al. [12] explained that the use of *Ulva lactuca* up to 12% in tilapia feed did not decrease growth performance, whereas the study of Soler-Vila et al. [13] stated that the use of *Ulva lactuca* 30% affected growth, muscle gain and feed conversion ratio in trout. According to Menghe et al. [14] giving 2% *Ulva lactuca* flour affects body composition and feed utilization in catfish. *Ulva lactuca* has good protein digestibility for fish. Mahasu et al. [12] stated that tilapia was able to digest *Ulva lactuca* flour by 66.28% and protein digestibility of 83%. The digestibility value of *Ulva lactuca* protein is almost the same as the digestibility of pollard protein by 82.87% [15]. Burtin [16] adds *Ulva lactuca* flour contains polysaccharide fibers of xylan and ulvan types so that it is easily digested by intestinal bacteria.

Based on the results of the ANOVA (analysis of variance) test, the ethanol extract of *Ulva lactuca* did not significantly affect the survival rate in each treatment (P>0.05). In general, each treatment has a value of 90% - 96.67%, this shows that the addition of *Ulva lactuca* has not significantly affect the survival rate of tilapia. Natify et al. [18] stated that the use of *Ulva lactuca* up to 10% in tilapia feed was proven not to interfere with the growth and survival performance of tilapia.

4. Conclusion
Ethanolic extract *Ulva lactuca* can increase the growth performance of milkfish (*Chanos chanos*). The best treatment shown at the 500 ppm ethanol extract *Ulva lactuca*.

Reference
[1] Kementerian Kelautan dan Perikanan (KKP) 2016 *Statistika Perikanan Tangkap, budidaya dan eksport-impor seluruh Indonesia* (Jakarta:Kementerian Kelautan dan Perikanan Republik Indonesia) p 178
[2] Brotowidjoyo M D, Djoko T dan Eko M 1995 Pengantar Lingkungan dan Budidaya Air (Yogyakarta: Liberty)
[3] Liao W R, Lin J Y, Shieh W Y and Jeng W L 2003 *Journal of Industrial Microbiology & Biotechnology* 30 433-439
[4] Costa J F, Merdekaawi W and Otu R F 2015 *Bioteknologi* 12 34-45
[5] Burrells C, Williams P D and Forno P F 2001 *Aquaculture* 199 159-169
[6] Muchlisin Z A, Afrido F, Murda T, Fadli M, Muchlisin Z A, Jalil Z and Yulvizar C 2016 *Biosains Shell* 8 172-177
[7] De Silva S S and Anderson T A 2005 *Fish nutrition in aquaculture* (London (GB): Chapman and Hall) p 319
[8] Goddard S 1996 *Feed management in intensive aquaculture* (New York (US): Chapman and Hall)
[9] Wong K H and Cheung P C K 2000 *Food Chemistry* 71 475-482
[10] Nufus C, Nurfannah and Abdullah A 2017 *Jurnal Pengolahan Hasil Perikanan Indonesia* 20 620-623
[11] Herpani E, Santoso I, Sari W R and Purba O D 2015 *Marine Science Research* 7 65-64
[12] Mahasu N H, Jusadi D, Setiaiwati M and Giri I N A A 2016 *Jurnal Ilmu dan Teknologi Kelautan Tropis* 8 259-267
[13] Soler-Vila A, Coughlan S, Guiry M D 2009 *Journal of Applied Phycology* 21 617-624
[14] Menghe H L, Robinson E H, Tucker C S, Manning B B and Khoo L 2009 *Aquaculture* 292 232-236
[15] Ribeiro F B, Lanna EAT, Delmondes MA, Bompim, Donzele J L, Quadros M, Cunha P S L, Takishita S S and Vianna R A 2013 *Revista Brasileira de Zoologia* 41 1075-1081
[16] Burtin P 2003 *Journal of Agricultural and Food Chemistry* 2 1–6
[17] Azaza, Mensi F, Ksouri J, Dhraief M N, Brini B, Abdelmouleh A and Kraiem M M 2008 *Journal of Applied Ichthyology* **24** 202-207

[18] Natify W, Droussi M, Berday N, Araba N and Benabid M 2015 *International Journal of Agronomy and Agricultural Research* **7** 85-92