

ISSN 0215-6334 (printed)
ISSN 1907-770X (electronic)

BIOTROPIA

The Southeast Asian Journal of Tropical Biology

Vol. 27 No. 1 April 2020



Published by
SEAMEO BIOTROP
Southeast Asian Regional Centre for Tropical Biology
Bogor, Indonesia
www.biotrop.org

A Publication of the Southeast Asian Ministers of Education Organization
Southeast Asian Regional Centre for Tropical Biology
SEAMEO BIOTROP
Bogor, Indonesia

Vol. 27 No. 1

April 2020

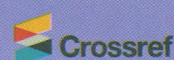
CONTENTS

Research Papers

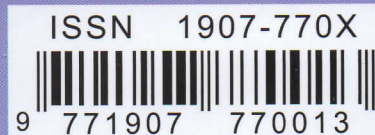
- Stemflow, Throughfall and Rainwater Interception of Eight Indonesian Tree Species
Siti Sofiah and Soejono 1 - 13
- Regulation of Adipogenesis and Key Adipogenic Gene Expression by Mangosteen Pericarp Extract and Xanthenes in 3T3-L1 Cells
Wahyu Widowati, Lusiana Darsono, Jo Suherman, Ervi Afifah, Rizal, Yukko Arinta, Tjandrawati Mozef and Tri Suciati 14 - 21
- Invasive Plant Species in the Disturbed Forest of Batukahu Nature Reserve, Bali, Indonesia
Laily Mukaromah and Muhammad Ali Imron 22 - 32
- Population Structure, Dispersion Pattern and Seedling Growth of Gwang Palm (*Corypha utan* Lam.) in Timor Island, Indonesia
Yayan Wahyu Candra Kusuma and Joko Ridho Witono 33 - 40
- The Physicochemical Properties of Several Indonesian Rice Varieties
Susiyanti, Rusmana, Yeyen Maryani, Sjaifuddin, Nanang Krisdianto and Mohamad Ana Syabana 41 - 50
- Effects of Dietary Probiotic *Bacillus* NP5 on the Growth Performances of Catfish (*Clarias* sp.)
Achmad Noerkhaerin Putra, Mustahal and Mas Bayu Syamsunarno 51 - 59
- Genetics Identification of Sea Holly (*Acanthus illicifolius*) through DNA Barcoding from Coastal Cilacap, Central Java, Indonesia
Taufan Harisam, Florencius Eko Dwi Haryono, Bintang Marhaeni, Amron, Norman Arie Prayogo and Purnama Sukardi 60 - 68
- Association of Tree Communities with Soil Properties in a Semi Deciduous Forest of Perlis, Peninsular Malaysia
Radhiah Zakaria, Mohd Nizam Mohd Said and Faridah Hanum Ibrahim 69 - 79
- Influence of Water Stress and Plant Age on the Yield and Chemical Composition of Essential Oil from *Cymbopogon winterianus* Jowitt
Muhammad Yusuf Abduh, Izni Nur Ibarahim, Ulya Atika, Robert Manurung and Dedi Suheryadi 80 - 87
- Short Communication**
- Use of Landsat Imagery to Map Spread of the Invasive Alien Species *Acacia nilotica* in Baluran National Park, Indonesia
Sutomo, Eddie van Etten and Rajif Iryadi 88 - 96

Indexed by

Scopus[®]



DOAJ
DIRECTORY OF
OPEN ACCESS
JOURNALS



BIOTROPIA

Volume 27, Number 1, April 2020

ISSN 0215-6334 (printed)

ISSN 1907-770X (electronic)

EDITORIAL BOARD

Editor-in-Chief	: Prof Dr Bambang Purwantara	Dr Jesus Corpuz Fernandez (Philippines)
Members	: Prof Dr Abu Bakar Salleh (Malaysia) Prof Dr Khatijah Mohd Yusoff (Malaysia) Prof Dr Okky Setyawati Dharmaputra Assoc Prof Dr Chew Fook Tim (Singapore) Assist Prof Dr Kobsak Wanthonchai (Thailand) Dr San Win (Myanmar) Dr Zohrah Haji Sulaiman (Brunei Darussalam)	Dr Lilian A De Las Llagas (Philippines) Dr Irdika Mansur Dr Zulhamsyah Imran Dr Sri Sudarmiyati Tjitrosoedirdjo Dr Aslan
Managing Editor	: Ms Rima Febriana, SE	
English Editor	: Dr Evelyn Varquez Bigcas	
Technical Editors	: Ms Zanne Sandriati Putri, SSi Ms Sri Ismawati Soerianegara, MSc	
Layout Designer	: Mr Asep Saepudin	

REVIEWERS (for Vol. 27, No. 1, April 2020)

1. Dr Titut Yulistiyarini, Technical Implementation Unit for Plant Conservation, Purwodadi Botanic Garden, Pasuruan 67163, Indonesia
2. Dr John Toland Van Stan, Department of Geology and Geography, Georgia Southern University, Statesboro, GA 30458, United States
3. Prof Madya Dr Zainul Amiruddin Zakaria, Department of Biomedical Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
4. Dr Deny Susanti, Department of Chemistry, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia
5. Dr Katja Rembold, Biodiversity, Macroecology & Biogeography, Georg-August-University of Göttingen, 37073 Göttingen, Germany
6. Asyraf Mansor, PhD, School of Biological Sciences, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia
7. Dr Andrew Powling, School of Biological Sciences, University of Portsmouth, Portsmouth PO1 2UP, United Kingdom
8. Prof Yoshio Inoue, National Institute for Agro-Environmental Sciences, Tsukuba, Japan
9. Dr Endah Yulia, Department of Agrotechnology, Universitas Padjadjaran, Sumedang 45363, Indonesia
10. Dr Sri Hidayati, Department of Agricultural Product Technology, Universitas Lampung, Bandar Lampung 35141, Indonesia
11. Dr Refflinur, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor 16124, Indonesia
12. Dr Ince Ayu Khairana Kadirah, Research Institute for Coastal Aquaculture, Maros 90512, Indonesia
13. Dr Eng Agung Sudrajad, Department of Mechanical Engineering, Universitas Sultan Ageng Tirtayasa, Serang 42124, Banten
14. Dr Alison K.S. Wee, College of Forestry, Guangxi University, Nanning 530004, Guangxi, China
15. Assoc Prof Renchao Zhou, School of Life Sciences, Sun Yat-sen University, Guangzhou, China
16. Dr Endang Hilmi, Faculty of Fisheries and Marine Science, Universitas Jenderal Soedirman, Purwokerto 53122, Indonesia
17. Dr rer.nat. Hamdan Syakuri, Faculty of Fisheries and Marine Science, Universitas Jenderal Soedirman, Purwokerto 53122, Indonesia
18. Dr Karyati, Department of Forest Management, Universitas Mulawarman, Samarinda 75119, Indonesia
19. Dr Roslan Ismail, Department of Land Management, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
20. Prof Suminar Setiati Achmadi, Department of Chemistry, IPB University, Bogor 16680, Indonesia
21. Dr Sarifah Nurjanah, Department of Agricultural Engineering, Universitas Padjadjaran, Sumedang 45363, Indonesia
22. Dr Meika Syahbana Rusli, Department of Agricultural Industrial Technology, IPB University, Bogor 16680, Indonesia
23. Prof Ing Miroslava Kačániová, PhD, Department of Microbiology, Slovak University of Agriculture in Nitra, 949 76 Nitra, Slovakia
24. Dr Meike Meilan Lisangan, Faculty of Agricultural Technology, Universitas Papua, Manokwari 98314, Indonesia
25. Prof Mohammad Afzal, PhD, 2200-Traemore Village Way, Nashville, TN 37209, United States
26. Dr N.V. Anil Kumar, Department of Chemistry, Manipal University, Manipal 576104, India
27. Dr Djong Hon Tjong, Department of Biology, Universitas Andalas, Padang 25175, Indonesia
28. Dr Eko Hanudin, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
29. Didik Suprayogo, PhD, Faculty of Agriculture, Universitas Brawijaya, Malang 65145, Indonesia
30. Venecio U. Ultra Jr., PhD, Department Earth and Environmental Science, Botswana International University of Science and Technology, Palapye, Botswana
31. Dr Shahid Adeel, Department of Chemistry, Government College University, Lahore, Punjab 54000, Pakistan
32. Prof Dr Wibowo Mangunwardoyo, Department of Biology, Universitas Indonesia, Depok 16424, Indonesia
33. Assoc Prof Dr Seri Robinson, College of Forestry, Oregon State University, Corvallis, OR 97331, United States
34. Dr Mohamad Ali Fulazzaky, Centre for Environmental Sustainability and Water Security, Universiti Teknologi Malaysia, 81310 Skudai, Johor Bahru, Malaysia
35. Prof I Nengah Surati Jaya, Faculty of Forestry, IPB University, Bogor 16680, Indonesia
36. Prof Chao-Hai Wei, PhD, College of Environment and Energy, South China University of Technology, Guangzhou, China
37. Dr Iskandar, Department of Soil Science and Land Resources, IPB University, Bogor 16680, Indonesia
38. Prof Kukuh Murtillaksono, Department of Soil Science and Land Resources, IPB University, Bogor 16680, Indonesia
39. Prof Tun Tedja Irawati, Department of Chemistry, IPB University, Bogor 16680, Indonesia
40. Prof Cahyono Agus DK, Faculty of Forestry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
41. Prof Chairil Anwar Siregar, Forestry Research, Development and Innovation Agency, Ministry of Environment and Forestry, Bogor 16118, Indonesia
42. Prof Ahmed H. El-Heneidy, Plant Protection Research Institute, Agriculture Research Center, Giza 12311, Egypt
43. Prof Edhi Martono, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
44. Dr Agung Budihardjo, Department of Biology, Universitas Sebelas Maret, Surakarta 57126, Indonesia
45. Prof Dato' Roshada Hashim, Faculty of Science and Technology, Universiti Sains Islam Malaysia, 71800 Nilai, Negeri Sembilan, Malaysia
46. Dr Siti Nor Fatimah bt Zakaria, Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, 88400 Sabah, Malaysia
47. Prof Dr.sc.agr. Suyadi, Faculty of Animal Husbandry, Universitas Brawijaya, Malang 65145, Indonesia
48. Prof Dr Mehmet Aköz, Department of Gynecology and Obstetrics, Faculty of Veterinary Medicine, University of Selçuk, Konya 42130, Turkey
49. Dr Wahyu Irawati, Department of Biological Education, Universitas Pelita Harapan, Tangerang 15811, Indonesia
50. Prof Dr Didik Widayatmoko, Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences, Bogor 16122, Indonesia
51. Prof Dr Hazzizul Rivai, Faculty of Pharmacy, Universitas Andalas, Padang 25175, Indonesia
52. Asep Hidayat, PhD, Forestry Research, Development and Innovation Agency, Ministry of Environment and Forestry, Bogor 16118, Indonesia
53. Prof I Made Sudiana, MSc, PhD, Research Centre for Biology, Indonesian Institute of Sciences, Bogor 16911, Indonesia
54. Boonhiang Promdonkoy, PhD, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Khlong Luang 12120, Pathum Thani, Thailand
55. Dr.rer.nat Adam Hermawan, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
56. Dr Riris Istighfari Jenie, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

BIOTROPIA, The Southeast Asian Journal of Tropical Biology, is a scientific publication of SEAMEO BIOTROP published three times a year. It contains scientific research articles in any subjects of the areas of tropical biology. Contribution in the form of a review article and short communication is also considered for publication. A guide for author is found on the inside back cover.

BIOTROPIA Editorial Office

SEAMEO BIOTROP, Jalan Raya Tajur Km. 6, Bogor 16134, Indonesia
E-mail: biotropia@biotrop.org; home page: <http://journal.biotrop.org/index.php/biotropia>

Cover Photo: Flower of *Melastoma malabathricum* - BIOTROP's collection.

EFFECTS OF DIETARY PROBIOTIC *Bacillus* NP5 ON THE GROWTH PERFORMANCES OF CATFISH (*Clarias* sp.)**

ACHMAD NOERKHAERIN PUTRA^{1,2*}, MUSTAHAL¹ AND MAS BAYU SYAMSUNARNO¹

¹Department of Fisheries, Faculty of Agriculture, Universitas Sultan Ageng Tirtayasa, Serang 42121, Indonesia

²Indonesia-Center of Excellence for Food Security, Institute for Research and Community Services, Universitas Sultan Ageng Tirtayasa, Serang 42121, Indonesia

Received 18 July 2018 / Accepted 27 May 2019

ABSTRACT

Probiotic have long been applied to aquaculture and produce positive effects on fish and shrimp. This research aimed to evaluate the effect of probiotic *Bacillus* NP5 to promote the growth of catfish (*Clarias* sp.). Five doses *Bacillus* NP5 with 3 replicates, namely 0% probiotic (control), 0.5% probiotic, 1% probiotic, 1.5% probiotic and 2% probiotic (g/100 g feed) were used. The results showed that application of probiotic in catfish feed can promote better growth performance compared to control. Total digestibility and protease enzyme activities were significantly highest in 1% probiotic. The value of specific growth rate showed in 1% probiotic ($2.67 \pm 0.18\%$ day⁻¹), followed by 2% probiotic ($2.63 \pm 0.02\%$ day⁻¹), 1.5% probiotic ($2.42 \pm 0.07\%$ day⁻¹), 0.5% probiotic ($2.29 \pm 0.14\%$ day⁻¹) and control ($1.60 \pm 0.01\%$ day⁻¹). The addition of 1% *Bacillus* NP5 as probiotic in catfish feed showed the best result on protease enzyme activities, protein digestibility, total digestibility, final weight, specific growth rate, weight gain, feed efficiency and the protein efficiency ratio than other probiotic doses.

Keywords: *Bacillus* NP5, catfish, growth performance, probiotic

INTRODUCTION

Aquaculture is an important economic activity because it can be carried out by all levels of society in many countries. Rapid aquaculture has an important role to increase fish production for the last decades. Aquaculture is estimated to have supplied 47.6% of total world fish production in 2011 (Mathiesen 2012). Catfish (*Clarias* sp.) is an economical freshwater aquaculture commodity in several Asian countries including Indonesia. However, the intensive cultivation of catfish are faced many problems, which one is the high feed price, pathogen outbreaks (Ulkhay *et al.* 2014), low environmental quality (Yusuf *et al.* 2015), and low feed digestibility (Afrilasari *et al.* 2016). Feed costs about 50-60% of the total production costs in aquaculture, so the efficiency of feeding will reduce the cost of production (Nates 2016).

Several methods in intensive aquaculture system have been applied to improve feed digestibility, fish growth, water quality and response immune such as bio-Floch technology (Widanarni *et al.* 2012; Dauda *et al.* 2017) and dietary supplements with immuno-stimulant, anti-microbial and probiotic (Cheng *et al.* 2017).

Probiotic can be defined as additional microbe which have a positive influence on the host (Nayak 2010). In aquaculture, its application has been considered more environmentally friendly and effective to improve feed digestibility, growth, water quality and immune response (Mohapatra *et al.* 2012; Dawood *et al.* 2015; Kumar *et al.* 2016). In addition, the positive effect of probiotics is to produce inhibitory compounds to decrease the growth of pathogenic bacteria (Perez-Sanchez *et al.* 2013), water quality improvement of fish rearing media (Chumpol *et al.* 2017), enhancement fish resistance against disease (Newaj-Fyzul *et al.* 2014) and produce digestive enzymes (Zheng & Wang 2016). Amylase, protease and lipase are exogenous enzyme that

*Corresponding author, e-mail: putra.achmadnp@untirta.ac.id

**This paper was presented at the International Conference on Food Security Innovation (ICFSI) 2017, 18-20 October 2017, Serang, Banten, Indonesia

can produce by probiotics (Dawood & Koshio 2016). The exogenous enzymes can lead to an endogenous enzyme to hydrolyzes nutrients from host feeding.

In this study, *Bacillus* NP5 was used as probiotic which were added in feed. The bacteria were isolated by Putra and Widanarni (2015) from digestive tract of tilapia. The previous studies have reported that the administration of *Bacillus* NP5, could increase growth, feed efficiency and enzyme activity of tilapia (Putra *et al.* 2015). Utami *et al.* (2015) proved that dietary dried *Bacillus* NP5 Rf^R could improve growth performance of tilapia. This research aimed to evaluate the effect of probiotic *Bacillus* NP5 to promote the growth of catfish (*Clarias* sp.).

MATERIALS AND METHODS

Probiotic Preparation

Bacillus NP5 was cultured in 500 mL of TSB (Tryptic Soy Broth) in aseptic condition and incubated at room temperature (29 °C) for 18 hours. Furthermore, culture of probiotic was centrifuged at 1,000 rpm for 10 min to obtain the probiotic biomass. After that, probiotic was diluted in 1,000 mL PBS (Phosphate Buffered Saline) before it was added to the feed.

Experimental Feed Preparation

Commercial feed (protein of 29.75-30%, lipid of 8.02-8.57% and free nitrogen extract of 32.75-30%) was used in this study. These values are in accordance with the nutrient requirement of catfish (Robinson *et al.* 2001). Feed were mashed into a meal and 0.5% chromium oxide (Cr₂O₃) as indicator of digestibility and 3% tapioca as a feed binder were added to the meal before the pelleting process. The feed was

molded with a pellet machine at diameters of 1-2 mm and was dried using an oven at 40 °C for 24 h. The experimental feeds were manufactured by adding different doses of 0%, 0.5%, 1%, 1.5% and 2% (g/100 g feed) of *Bacillus* NP5 as probiotic into the feed. *Bacillus* NP5 was mixed with 2% egg yolk as a binder and was added to the feed by spraying using a syringe according to Putra *et al.* (2015). The formulation and proximate feed in this study are presented in Table 1.

Experimental Design and Procedure

The fish rearing was carried out at the Laboratory of Aquaculture, Department of Fisheries, Universitas Sultan Ageng Tirtayasa, Indonesia. Juveniles of catfish were obtained from Tri Farm, Anyer Banten Province, Indonesia. A total of 225 juvenile of catfish (initial individual weight of 5.03 ± 0.05 g) were used. They were reared in five treatments (0% probiotic as a control, 0.5% probiotic, 1% probiotic, 1.5% probiotic and 2% probiotic) with each consisting of three replicates (15 fishes/replicate). Fifteen aquariums (50 x 40 x 30 cm) were used in this study and equipped with an aeration point. Fish were reared for 45 days and before weighing the weight of fish, fish were fasted for 24 h. The weight of catfish was evaluated once every 2 weeks. Feeding was done three times daily at 08:00, 12:00 and 17:00 hours with satiation. Siphon was carried out on the remaining feed and feces in the morning before feeding time. Fifty percents of water volume in the rearing aquariums was removed every 3 days to maintain water quality. Daily water quality monitoring was conducted and in the present study, we obtained the range value of temperature from 27 °C to 30.1 °C, dissolved oxygen of 5.48-6.95 ppm and pH of 6.77-7.29.

Table 1 Formulation and proximate composition of the experimental diets

Ingredients (%)	Diet (%)				
Commercial feed	100				
Tapioca	3				
Cr ₂ O ₃	0.5				
Probiotic <i>Bacillus</i> NP5	a				
Proximate analyses (% dry matter)	Probiotic diet (g/100 g)				
	0 (control)	0.5	1	1.5	2
Crude protein	29.95	29.95	29.75	30.00	30.00
Crude lipid	8.04	8.57	8.55	8.17	8.02
NFE	32.75	33.00	33.00	33.00	33.00
Energy (Kcal/kg feed) ^b	251.78	256.74	255.88	253.64	252.42
Moisture	6.41	6.57	6.60	6.45	6.41

Notes: ^aProbiotics: 0%, 0.5%, 1%, 1.5%, 2% (g/100 g feed).

^bEnergy in digestible energy value according to NRC (1993) where protein of 3.5 Kcal, lipid of 8.1 Kcal, carbohydrate of 2.5 Kcal.

Growth Parameters

Growth parameters of the juvenile catfish were SR: survival rate (1), WG: weight gain (2), SGR: specific growth rate (3) and FE: feed efficiency (4) and were determined according to Huisman (1987). While PER: protein efficiency ratio (5) was determined according to Takeuchi (1988), by the following equations:

$$SR (\%) = NT/N0 \times 100 \quad (1)$$

$$WG (\%) = 100 \times (Ft-Fo)/Fo \quad (2)$$

$$SGR (\% \text{ day}^{-1}) = 100 \times (\ln Wt - \ln Wo) / \text{time (days)} \quad (3)$$

$$FE (\%) = 100 \times [W/F] \quad (4)$$

$$PER = W/\text{Protein feed} \quad (5)$$

Where:

NT is total of individuals at the end of experiment, N0 is total of individuals at the beginning, Ft (g) is final weight, Fo is initial weight, Wt is total biomass of final (g), Wo is total biomass on initial fish (g), W is weight gain (g) and F is the dry feed intake (g).

Digestibility Parameters

The value of nutrient digestibility is calculated by the indirect method using chromium oxidase of 0.5% (NRC 2011). Feces collection was conducted on day-7 after stocking by siphoning out the fecal material from the bottom of the aquarium. Fish feces from each treatment were collected and were stored at -4 °C until analysis. The nutrient digestibility (ND) (6) including protein digestibility (PD), fat digestibility (FD) and total digestibility (7) were calculated refer to Takeuchi (1988):

$$ND = 100 - [1 - c/c' \times d^2/d] \quad (6)$$

$$TD = 100 - [1 - c/c'] \quad (7)$$

Where, c is the value of chromium in the feed, c' is chromium in the faeces, d is the value of nutrient in the feed, and d' is the value of nutrient in the faeces.

Digestive Enzymes Activity

Enzyme activities (protease, lipase and amylase) of digestive tract of catfish were analyzed based on the method presented by Bergmeyer and Grassi (1983). At the end of the fish rearing, catfish intestine from 5 individuals per treatment were taken under low temperature condition (4 °C) and rinsed with cold water. Then, at a dose of 1 g/10 mL, it was mixed and homogenized within phosphate buffer at pH 7.5 and temperature of 4 °C. Furthermore, it was centrifuged for 15 min at temperature of 4 °C and speed of 15,000 rpm. The supernatant formed was stored at temperature of 4 °C before being analyzed.

Proximate Composition Analysis

The standard method from AOAC (1995) was used to determine the proximate composition of catfish and feces. The value of protein was determined by measuring the nitrogen content according to the Kjeldahl method. Lipid content in feed and feces was analyzed based on folch method using Soxhlet. Feed and feces were dried at 105 °C for 6 h to obtain the value of moisture. Nitrogen extract was obtained by subtracting 100% of the ingredient with percentage of other nutrients.

Statistical Analysis

All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) program for Windows (v. 16.0). Duncan's multiple range with 95% significant level was used as post hoc test in the present study.

RESULTS AND DISCUSSION

The effects of probiotics have been evaluated in aquaculture commodities and many researches recorded that the growth of cobia *Rachycentron canadum* (Geng *et al.* 2012), black tiger shrimp *Penaeus monodon* Fabricius 1798 (Hasan *et al.* 2012), carp *Cyprinus carpio* (Xu *et al.* 2014), Pangasius catfish (Van Doan *et al.* 2016), Florida pompano, common snook and red drum larvae (Hauville *et al.* 2016), grass carp (Li *et al.* 2017), giant freshwater prawn *Macrobrachium rosenbergii* (DeMan, 1879) (Ghosh *et al.* 2016), parrot fish *Oplegnathus fasciatus* (Liu *et al.* 2018) and the whiteleg shrimp *Litopenaeus vannamei* (Madani *et al.* 2018) were significantly increased. In this study, all probiotic treatment can promote better growth performance of catfish compared to control. Growth performance of catfish with different probiotic doses were presented in Table 2. In the beginning, no difference ($p > 0.05$) was obtained in feed intake between control (143.67 ± 2.08) and 0.5-2% probiotic treatments (148.00 ± 7.27 , 146.67 ± 2.08 , 144.67 ± 3.06 and 140.33 ± 2.08 ,

respectively). This is showing that dietary *Bacillus* NP5 does not affect palatability. Similar result has been found by Putra *et al.* (2015) and Elsabagh *et al.* (2018), the addition of probiotic in feed could not significantly ($p > 0.05$) increase the consumption of tilapia feed. The same results were also found d in grouper (*Epinephelus coioides*), where supplementation of *Lactococcus lactis* and *Enterococcus faecium* could not increase feed consumption (Sun *et al.* 2012).

After the 45 days fish rearing, probiotic treatments showed the higher of digestive enzyme compared to control. The value of protease enzyme activity in 1% probiotic (0.70 ± 0.04 U/min/mL) was higher than the other treatments. However, there were no significant differences among the 0.5%, 1.5% and 2% probiotic treatments (0.58 ± 0.05 , 0.60 ± 0.02 and 0.62 ± 0.01 U/min/mL, respectively). The best value of lipase activity was found in 1% probiotic (0.58 ± 0.03 U/min/mL) compared to control (0.33 ± 0.03 U/min/mL), 0.5% probiotic (0.44 ± 0.03 U/min/mL) and 2% probiotic (0.52 ± 0.01 U/min/mL). However, the value was not significantly different ($p > 0.05$) between 1% probiotic and 1.5% probiotic. Furthermore, the value of amylase activity in probiotic treatment was greater ($p < 0.05$) than control. There was no difference in value of amylase activity in probiotic treatment with doses of 1-2%.

Tabel 2 Effect of dietary probiotic *Bacillus* NP5 on growth performance of catfish^a

Parameters ^b	Probiotic treatments (%)				
	0 (control)	0.5	1	1.5	2
IW (g)	5.03 ± 0.00	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01
FI (%)	143.67 ± 2.08	148.00 ± 7.27	146.67 ± 2.08	144.67 ± 3.06	140.33 ± 2.08
PA (U/min/mL)	0.48 ± 0.07 ^a	0.58 ± 0.05 ^b	0.70 ± 0.04 ^c	0.60 ± 0.02 ^b	0.62 ± 0.01 ^b
LA (U/min/mL)	0.33 ± 0.03 ^a	0.44 ± 0.03 ^b	0.58 ± 0.03 ^d	0.57 ± 0.01 ^d	0.52 ± 0.01 ^c
AA (U/min/mL)	0.22 ± 0.01 ^a	0.36 ± 0.02 ^b	0.45 ± 0.05 ^c	0.44 ± 0.03 ^c	0.43 ± 0.02 ^c
PD (%)	52.95 ± 1.71 ^a	69.78 ± 0.74 ^b	76.97 ± 0.98 ^d	75.17 ± 1.57 ^{cd}	74.54 ± 0.67 ^c
FD (%)	63.10 ± 1.13 ^a	72.82 ± 4.13 ^b	77.63 ± 2.94 ^b	75.94 ± 4.49 ^b	71.95 ± 1.41 ^b
TD (%)	44.10 ± 1.35 ^a	51.04 ± 2.39 ^b	56.03 ± 1.13 ^c	52.36 ± 2.14 ^b	52.16 ± 1.40 ^b
FW (g)	10.33 ± 0.07 ^a	11.76 ± 0.10 ^b	12.78 ± 0.56 ^d	12.02 ± 0.34 ^{bc}	12.38 ± 0.1 ^{cd}
WG (g)	79.55 ± 1.00 ^a	102.28 ± 1.59 ^b	116.62 ± 8.34 ^d	105.23 ± 5.11 ^{bc}	110.62 ± 1.61 ^{cd}
SGR (% day ⁻¹)	1.60 ± 0.01 ^a	2.29 ± 0.14 ^b	2.67 ± 0.18 ^d	2.42 ± 0.07 ^{bc}	2.63 ± 0.02 ^{cd}
FE (%)	55.37 ± 0.23 ^a	68.57 ± 4.30 ^b	79.50 ± 5.54 ^c	72.71 ± 1.98 ^b	78.83 ± 0.63 ^c
PER (%)	1.85 ± 0.01 ^a	2.29 ± 0.14 ^b	2.67 ± 0.18 ^c	2.42 ± 0.07 ^b	2.63 ± 0.02 ^c
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Notes: ^a The value in the same row followed by the same superscript are not significantly different ($p > 0.05$).

^bInitial weight (IW), final weight (FW), feed intake (FI), protease activity (PA), lipase activity (LA), amylase activity (AA), protein digestibility (PD), fat digestibility (FD), total digestibility (TD), final weight (FW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), survival rate (SR) of catfish.

Probiotic produces some extracellular/exogenous enzymes (protease, lipase and cellulase) which can improve feed utilization (Zheng & Wang 2016). The exogenous enzyme could lead endogenous enzyme to hydrolyze nutrients from host feeding. *Bacillus* NP5 are proven to produce digestive enzyme that support the nutrients absorption (Putra & Widanarni 2015). The endogenous enzyme activity will increase because probiotic can increase the colonization of beneficial bacteria in the digestive tract (Dawood & Khosio 2016; Sankar *et al.* 2016). The results indicated that *Bacillus* NP5 could improve enzyme activities in catfish intestine. Similar effects had been reported by Nimrat *et al.* (2013) where supplementation of *B. subtilis* and *Enterococcus* sp. in black tiger shrimp *Penaeus monodon* postlarvae and Adel *et al.* (2017) with the addition of *Pediococcus pentosaceus* spp. in white shrimp *Litopenaeus vannamei*.

The value of protease enzyme was different in 1% probiotic than the other treatments. This suggested that the addition of 1% probiotic in feed stimulated the growth of intestinal microbiota in catfish intestine. Digestive enzyme were secreted by beneficial bacteria in the colon to digest the nutrient contained in the feed (Buentello *et al.* 2010). This result also confirms similar hypothesis that all probiotic treatments can produce better protease enzyme than control. The results were similar to the findings in freshwater prawn (*Macrobrachium rosenbergii*) that supplementation of probiotic could increase protease activities in the colon (Gupta *et al.* 2016; Sumon *et al.* 2018; Valipour *et al.* 2019). In this study, probiotic treatment has the higher value of lipase activity. This result is in line with the findings from Allameh *et al.* (2015) in Javanese carp that the best value of enzymes lipase is shown in probiotic treatment. The results showed that the increase in probiotic dose was not followed by an increase in the value of enzyme activity. The value of digestive enzyme was the highest in the 1% probiotic than 1.5-2% probiotic. This result might be due to the limitations of bacteria conducting colonization in the intestine. Therefore, higher probiotic doses did not cause positive effect for the host (Talpur *et al.* 2013; Zhang *et al.* 2018).

Probiotic addition to feed is aimed to increase the population of probiotic in the catfish tract so that the action mechanism of the probiotic in producing exogenous enzymes for digestion will increase (Merrifield *et al.* 2010). Subsequent digestibility of fish diets is important because it influences the energy and nutrient availability, absorption and utilization. The protein digestibility in control ($52.95 \pm 1.71\%$) was lower than that of the 0.5-2% probiotic treatments ($69.78 \pm 0.74\%$, $76.97 \pm 0.98\%$, $75.17 \pm 1.57\%$, $74.54 \pm 0.67\%$, respectively). Furthermore, fat digestibility also differed among the treatments. Total digestibility was significantly higher in the 1% probiotic ($56.03 \pm 1.13\%$) as compared with the other treatments but no significant difference existed among the 0.5% probiotic, 1.5% probiotic and 2% probiotic.

Digestibility of fish diets is important because it can influence energy and nutrient availability, absorption and utilization. The value of protein digestibility in control ($52.95 \pm 1.71\%$) was lower ($p < 0.05$) than that of the 0.5-2% probiotic treatments ($69.78 \pm 0.74\%$, $76.97 \pm 0.98\%$, $75.17 \pm 1.57\%$, $74.54 \pm 0.67\%$, respectively). Furthermore, difference in fat digestibility between 0.5% probiotic, 1% probiotic, 1.5% probiotic and 2% probiotic. Total digestibility was significantly higher in 1% probiotic ($56.03 \pm 1.13\%$) compared to the other treatment but there was no difference between 0.5% probiotic, 1.5% probiotic and 2% probiotic. The results showed that the digestibility of protein, fat and dry matter in the 0.5-2% probiotic were greater than control ($p < 0.05$). This result caused the value of digestive enzyme in probiotic treatments greater than control. Similar results were found in white shrimp (*Litopenaeus vannamei*) that probiotic *Bacillus subtilis* in their feed can increase the digestibility of protein, lipid dan dry matter/total digestibility (Tsai *et al.* 2019).

The value of final weight and weight gain in control were lower ($p < 0.05$) than that of the 0.5-2% probiotic. The result showed that feed efficiency of the control was $55.37 \pm 0.23\%$, and the value was smaller than that obtained by 0.5-2% probiotic (68.57 ± 4.30 , 79.50 ± 5.54 , 72.71 ± 1.98 , 78.83 ± 0.63 , respectively) and there was no difference between the 1% probiotic and 2% probiotic. The increased

nutrient digestibility (protein, fat and total digestibility) in catfish has a positive correlation to final weight, weight gain, PER and SGR. The high value of digestive enzyme and nutrient digestibility has affected growth and feed efficiency (Afrilasari *et al.* 2016). The highest ($p < 0.05$) of SGR was found in 1% probiotic ($2.67 \pm 0.18\% \text{ day}^{-1}$), followed by 2% probiotic ($2.63 \pm 0.02\% \text{ day}^{-1}$), 1.5% probiotic ($2.42 \pm 0.07\% \text{ day}^{-1}$), 0.5% probiotic ($2.29 \pm 0.14\% \text{ day}^{-1}$) and control ($1.60 \pm 0.01\% \text{ day}^{-1}$). In the previous study, probiotic could stimulate the growth of intestinal microbial and secretion of digestive enzyme to help feed digestion (Wang *et al.* 2012; Zokaefar *et al.* 2012). Therefore, growth increased of catfish was strongly related to the increased digestive enzyme activities in the colon. It is shown in the higher value of digestive enzyme and nutrient digestibility in probiotic treatments than that in control. In the present study, the high concentrations of probiotic-supplemented (1.5-2% probiotic treatments) in diets may not further promote the SGR of catfish. This might be related to the lack of enzyme activity (protease, lipase, and amylase) in catfish intestine of 1.5-2% probiotic compared to 1% probiotic treatment. Similar results had been reported by Sun *et al.* (2010) in the addition of *Bacillus* spp. in *Epinephelus coioides*. Probiotic in the very high dose will cause negative effect of the microflora in the digestive tract (Ramos *et al.* 2013). In this study, supplementation of probiotic did not influence survival rate, which indicated that administration of *Bacillus* NP5 did not have impact on fish physiology of catfish. Similarly, Kumar *et al.* (2013) found that the survival rate of giant freshwater prawn with the addition of *B. licheniformis* in the diet and Garcia-Bernal *et al.* (2018) also reported that supplementation of probiotic could not decrease the survival rate of *Litopenaeus vannamei*.

CONCLUSION

The addition of probiotics to feed can promote better growth performance of catfish compared to control. The specific growth rate, protease activity and protein digestibility were significantly highest ($p < 0.05$) in 1% probiotic

($2.67 \pm 0.18\% \text{ day}^{-1}$, $0.70 \pm 0.04 \text{ U/min/mL}$, $76.97 \pm 0.98\%$, respectively) than other treatments. The addition of 1% probiotic in feed showed the best result on enzyme protease activities, protein digestibility, total digestibility, final weight, specific growth rate, weight gain, feed efficiency and protein efficiency ratio among other treatments.

ACKNOWLEDGEMENTS

The authors want to thank Mr Heri Endra from Tri Farm in Anyer, Banten, Indonesia, for his guidance in catfish culture.

REFERENCES

- Adel M, Yeganeh S, Dawood MAO, Safari R, Radhakrishnan S. 2017. Effects of *Pediococcus pentosaceus* supplementation on growth performance, intestinal microflora and disease resistance of white shrimp, *Litopenaeus vannamei*. *Aquac Nutr* 23(6):1401-9.
- Afrilasari W, Widanarni, Meryandini A. 2016. Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.). *Hayati J Biosci* 23:168-72.
- Allameh SK, Ringo E, Yusoff FM, Daud HM, Ideris A. 2015. Dietary supplement of *Enterococcus faecalis* on digestive enzyme activities, short-chain fatty acid production, immune system response and disease resistance of Javanese carp (*Puntius gonionotus*, Bleeker 1850). *Aquac Nutr* 23(2):331-8.
- AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis of AOAC International, 16th edition, volume II. Arlington (VA, US): AOAC International.
- Bergmeyer HU, Grassi. 1983. Methods of enzymatic analysis, 2nd volume. Weinheim (DE): Verlag Chemie.
- Buentello JA, Neil WH, Gatlin DM III. 2010. Effects of dietary probiotics on the growth, feed efficiency and non-specific immunity of juvenile red drum *Sciaenops ocellatus* fed soybean-based diets. *Aquac Res* 41:411-8.
- Cheng AC, Lin HL, Shiu YL, Tyan YC, Liu CH. 2017. Isolation and characterization of antimicrobial peptides derived from *Bacillus subtilis* E20⁻fermented soybean meal and its use for preventing *Vibrio* infection in shrimp aquaculture. *Fish Shellfish Immunol* 67:270-9.

- Chumpol S, Kantachote, Nitoda T, Kanzaki H. 2017. The roles of probiotic purple nonsulfur bacteria to control water quality and prevent Acute Hepatopancreatic Necrosis Disease (AHPND) for enhancement growth with higher survival in white shrimp (*Litopenaeus vannamei*) during cultivation. *Aquac* 473:327-36.
- Dauda AB, Romano N, Karim M, Natrah I, Kamarudin MS, Ekasari J. 2017. Different carbon sources affect biofloc volume, water quality and the survival and physiology of African catfish *Clarias gariepinus* fingerlings reared in an intensive biofloc technology system. *Fish Sci* 83(6):1037-48.
- Dawood MAO, Koshio S, Ishikawa M, Yokoyama S. 2015. Effects of heat-killed *Lactobacillus plantarum* (LP20) supplemental diets on growth performance, stress resistance and immune response of red sea bream *Pagrus major*. *Aquac* 442:29-36.
- Dawood MAO, Koshio S. 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. *Aquac* 454:243-51.
- Elsabagh M, Mohamed R, Moustafa EM, Hamza A, Farrag F, Decamp O, ... Eltholth M. 2018. Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*. *Aquac Nutr* 24(6):1613-22.
- Garcia-Bernal M, Medina-Marrero R, Rodriguez-Jaramillo C, Marrero-Chang, Campa-Cordova AI, Media-Garcia R, Mazon-Suastegui J. 2018. Probiotic effect of *Streptomyces* spp. on shrimp (*Litopenaeus vannamei*) postlarvae challenged with *Vibrio parahaemolyticus*. *Aquac Nutr* 24(2):865-71.
- Geng X, Dong XH, Tan BP, Yang QH, Chi SY, Liu HY, Liu XQ. 2012. Effects of dietary probiotic on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. *Aquac Nutr* 18:46-55.
- Ghosh AK, Bir J, Azad MAK, Hasanuzzaman AFM, Islam MS, Huq KA. 2016. Impact of commercial probiotics application on growth and production of giant fresh water prawn (*Macrobrachium rosenbergii* DeMan, 1879). *Aquac Reports* 4:112-7.
- Gupta A, Verma G, Gupta P. 2016. Growth performance, feed utilization, digestive enzyme activity, innate immunity and protection against *Vibrio harveyi* of freshwater prawn, *Macrobrachium rosenbergii* fed diets supplemented with *Bacillus coagulans*. *Aquac Int* 24(5):1379-92.
- Hasan BMA, Guha B, Datta S. 2012. Efficiency of probiotic on growth and sustainable production of black tiger shrimp, *Penaeus monodon* Fabricius 1798 in brackish water ponds of West Bengal, India. *Asian Fish Sci* 25:303-16.
- Hauville MR, Zambonino-infante JLO, Bell JG, Migaud H, Main KL. 2016. Effects of mix of *Bacillus* sp. as a potential probiotic for Florida pompano, common snook and red drum larvae performances and digestive enzyme activities. *Aquac Nutr* 22(1):51-60.
- Huisman EA. 1987. Principles of fish production. Wageningen (NL): Department of Fish Culture and Fisheries, Wageningen University and Research.
- Kumar NR, Raman RP, Jadhao SB, Brahmchari RK, Kumar K, Dash G. 2013. Effect of dietary supplementation of *Bacillus licheniformis* on gut microbiota, growth and immune response in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquac Int* 21(2):387-403.
- Kumar V, Roy S, Meena DK, Sarkar UK. 2016. Application of probiotics in shrimp aquaculture: Importance, mechanisms of action, and methods of administration. *Rev Fish Sci Aquac* 24:342-68.
- Li ZJ, Chen YH, Zhang JZ, Zhu X, Zhang JS, Chen DX, ... Chu WY. 2017. Effects of dietary *Bacillus natto* supplementation on growth performance and the growth-related gene/microRNA expression in the skeletal muscle of grass carp (*Ctenopharyngodon idella*). *Aquac Nutr* 23(1):46-53.
- Liu CH, Wu K, Chu TW, Wu TM. 2018. Dietary supplementation of probiotic, *Bacillus subtilis* E20, enhances the growth performance and disease resistance against *Vibrio alginolyticus* in parrot fish (*Oplegnathus fasciatus*). *Aquac Int* 26(1):63-74.
- Madani NSH, Adorian TJ, Farsani HG, Hoseinifar SH. 2018. The effects of dietary probiotic Bacilli (*Bacillus subtilis* and *Bacillus licheniformis*) on growth performance, feed efficiency, body composition and immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) postlarvae. *Aquac Res* 49(5):1926-33.
- Mathiesen AM. 2012. The state of the world fisheries and aquaculture. Rome (IT): FAO.
- Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker RTM, Bogwald J, ... Ringgo E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquac* 302:1-18.
- Mohapatra S, Chakraborty T, Prusty AK, Das P, Paniprasad K, Mohanta KN. 2012. Use different microbial probiotic in the diet of rohu, *Labeorobita fingerlings*: Effect on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. *Aquac Nutr* 18:1-11.
- Nates FS. 2016. Introduction. In: Nates FS, editor. *Aquafeed Formulation*. Oxford (UK): Academic Press. p. xiii-xxii.

- Nayak SK. 2010. Probiotics and immunity: A fish perspective. *Fish Shellfish Immunol* 29:2-14.
- Newaj-Fyzul A, Al-Harbi AH, Austin B. 2014. Review: Developments in the use of probiotics for disease control in aquaculture. *Aquac* 431:1-11.
- Nimrat S, Tanutpongpalin P, Sritunyaluksana K, Boonthai T, Vuthiphandchai V. 2013. Enhancement of growth performance, digestive enzyme activities and disease resistance in black tiger shrimp (*Penaeus monodon*) postlarvae by potential probiotics. *Aquac Int* 21(3):655-66.
- NRC (National Research Council). 1993. Nutrient requirement of fish. Washington DC (US): National Academic Press.
- NRC (National Research Council). 2011. Nutrient requirements of fish and shrimp. Washington DC (US): National Academies Press.
- Perez-Sanchez T, Ruiz-Zarzuola I, Blas ID, Balcazar JL. 2013. Probiotics in aquaculture: A current assessment. *Rev Aquac* 5:1-14.
- Putra AN, Widanarni. 2015. Screening of amylolytic bacteria as candidates of probiotics in tilapia (*Oreochromis* sp.). *Res J Microbiol* 10:1-13.
- Putra AN, Widanarni, Utomo NBP. 2015. Growth performance of tilapia (*Oreochromis niloticus*) fed with probiotic, prebiotic and synbiotic in diet. *Pakistan J Nutr* 14(5):263-8.
- Ramos MA, Webwe B, Gobcalves JF, Santos GA, Rema P, Ozorio ROA. 2013. Dietary probiotic supplementation modulated gut microbita and improved growth of juvenile rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol Part A, Mol Integr Physiol* 166:302-7.
- Robinson EH, Li MH, Manning BB. 2001. A practical guide to nutrition, feeds, and feeding of catfish. Mississippi State (US): Mississippi Agricultural & Forestry Experiment Station.
- Sankar H, Philip B, Philip R, Singh ISB. 2016. Effect of probiotic on digestive enzyme activities and growth of cichlids, *Ectropus suratensis* (Pearl spot) and *Oreochromis mossambicus* (Tilapia). *Aquac Nutr* 23(4):852-64.
- Sumon MS, Ahmmed F, Khushi SS, Ahmmed MK, Rouf MA, Chisty MAH, Sarower MG. 2018. Growth performance, digestive enzyme activity and immune response of *Macrobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets. *J King Saud Univ-Sci* 30:21-8.
- Sun YZ, Yang HL, Ma RL, Lin WY. 2010. Probiotic applications of two dominant gut *Bacillus* strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*. *Fish Shellfish Immunol* 29(5):803-9.
- Sun YZ, Yang HL, Ma RL, Song K, Li JS. 2012. Effect of *Lactococcus lactis* and *Enterococcus faecium* on growth performance, digestive enzymes and immune response of grouper (*Epinephelus coioides*). *Aquac Nutr* 18(3):281-9.
- Takeuchi. 1988. Laboratory work-chemical evaluation of dietary nutrients. In: Watanabe, editor. *Fish Nutrition and Mariculture*. Kanagawa (JP): Kanagawa International Fisheries Training, Japan International Cooperation Agency (JICA). p. 179-233.
- Talpur AD, Ikhwanuddin M, Abdullah MDD, Bolong A-MA. 2013. Indigenous *Lactobacillus plantarum* as probiotic for larviculture of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758): Effects on survival, digestive enzyme activities and water quality. *Aquac* 416-7:173-8.
- Tsai CY, Chi CC, Liu CH. 2019. The growth and apparent digestibility of white shrimp, *Litopenaeus vannamei*, are increased with the probiotic, *Bacillus subtilis*. *Aquac Res* 50(5):1475-81.
- Ulkhag MF, Widanarni, Lusastuti AM. 2014. Application of *Bacillus* probiotic to prevent *Aeromonas hydrophilla* infection in catfish. *Jurnal Akuakultur Indonesia* 13(2):105-14.
- Utami DAS, Widanarni, Suprayudi MA. 2015. Quality of dried *Bacillus* NP5 and its effect on growth performance of tilapia (*Oreochromis niloticus*). *Pakistan J Biol Sci* 18:88-93.
- Valipour A, Nedaei S, Noori A, Khanipour AA, Hoseinifar SH. 2019. Dietary *Lactobacillus plantarum* affected on some immune parameters, air exposure stress response, intestinal microbiota, digestive enzyme activity and performance of narrow clawed crayfish (*Astacus leptodactylus*, Eschscholtz). *Aquac* 504:121-30.
- Van Doan H, Doolgindachbaporn S, Suksri A. 2016. Effect of *Lactobacillus plantarum* and Jerusalem artichoke (*Helianthus tuberosus*) on growth performance, immunity and disease resistance of *Pangasius catfish* (*Pangasius bocourti*, Sauvage 1880). *Aquac Nutr* 22(1):444-56.
- Wang YB, Fu LL, Lin J. 2012. Probiotic (*Bacillus coagulans*) cells in the diet benefit the white shrimp *Litopenaeus vannamei*. *J Shellfish Res* 31(3):855-60.
- Widanarni, Ekasari J, Maryam S. 2012. Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp. cultured at different stocking densities. *Hayati J Biosci* 19(2):73-80.
- Xu Y, Wang Y, Lin J. 2014. Use of *Bacillus coagulans* as a dietary probiotic for the common carp, *Cyprinus carpio*. *J World Aquac Soc* 25(4):403-11.
- Yusuf MW, Utomo NBP, Yuhana M, Widanarni. 2015. Growth performance of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp. *J Fish Aquatic Sci* 10(6):523-32.
- Zhang C, Zhang J, Fan W, Huang M, Liu M. 2018. Effects of dietary *Lactobacillus delbrueckii* on

- growth performance, body composition, digestive and absorptive capacity, and gene expression of common carp (*Cyprinus carpio* Huanghe var). *Aquac Nutr* 25(1):166-75.
- Zheng CN, Wang W. 2016. Effects of *Lactobacillus pentosus* on the growth performance, digestive enzyme and disease resistance of white shrimp, *Litopenaeus vannamei* (Boone, 1931). *Aquac Res* 48(6):1-11.
- Zokaeifar H, Balcazar JL, Saad CR, Kamarudin MS, Sijam K, Arshad A, Nejat N. 2012. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol* 33:683-9.