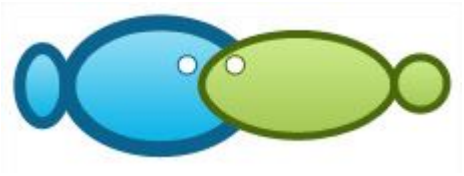

The nutritional value of *Artemia* sp. enriched with the probiotic *Pseudoalteromonas piscicida* and the prebiotic mannan-oligosaccharide

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Abstract. The application of probiotic and prebiotic to shrimp larvae can be done through bio-encapsulation or enrichment of the natural feed (*Artemia* sp.). This study aimed to evaluate the total bacterial count, the total probiotic count in the body of *Artemia* sp., and the nutritional value after being enriched with the probiotic *Pseudoalteromonas piscicida* 1Ub, the prebiotic mannan-oligosaccharide (MOS) and a synbiotic (the combination between the probiotic *P. piscicida* 1Ub and the prebiotic MOS). The enrichment was done by adding the probiotic *P. piscicida* 1Ub at a concentration of 10^6 CFU mL^{-1}, 12 mg L^{-1} prebiotic MOS, and the synbiotic (the combination between *P. piscicida* 1Ub at a concentration of 10^6 CFU mL^{-1} and 12 mg L^{-1} MOS) into the rearing medium of *Artemia* sp. for four hours. The bacterial count and the probiotic count in the enriched *Artemia* sp. were counted using SWC-agar medium for the total bacterial count and SWC-agar medium added with the antibiotic rifampicin 50 ug mL^{-1} for the total probiotic count. The nutritional content of *Artemia* sp. was determined based on the results of the proximate analysis, essential amino acid and fatty acid profiles after treatment. The results of this study revealed that the probiotic *P. piscicida* 1Ub was able to produce protease, lipase, amylase, and mannanase. *Artemia* sp. enriched with the probiotic, prebiotic, and synbiotic had the higher values of total bacterial and probiotic count than those in the control, with the highest value obtained by the synbiotic treatment: 8.6 log CFU per 0.1 gr *Artemia* for the total bacterial count and 8.6 log CFU per 0.1 gr *Artemia* for the total probiotic count. The protein content of *Artemia* sp. in the probiotic, prebiotic, and synbiotic treatment ranged from 52.68 to 53.06%, which was higher than that in the control (51.24±0.72%), the fat content range in the treatment groups (15.33 to 16.98%) was also higher than that in the control (14.05±0.12%). The highest butyric acid level was obtained by *Artemia* sp. enriched with prebiotic (0.0029±0.00014%). The enrichment of *Artemia* sp. with the synbiotic resulted in the highest value in linoleic acid level (0.142±0.0009%) and linolenic acid level (0.72±0.0023%) compared to the other treatments and the control. Similarly, the essential amino acid content of *Artemia* sp. enriched with the probiotic, prebiotic, and synbiotic showed higher values ($p < 0.05$) than the control. The highest essential amino acid portion was obtained in the leucine amino acid which ranged from 4.985 to 5.193 ppt, while the lowest portion was obtained in the histidine amino acid which ranged from 1.770 to 1.820 ppt.

Key Words: probiotic, prebiotic, synbiotic, *Artemia* sp., bio-encapsulation.

Introduction. *Artemia* is a natural feed that is commonly used in shrimp hatcheries, because its size is suitable for larvae, it has high nutritional value, and it is easy to be digested. According to John et al (2004), the newly hatched *Artemia* nauplii contains 50.6% protein, 25.7% carbohydrate, 14.2% fat, 9.4% ash and the energy value of 18.97 KJ g^{-1}. *Artemia* has a characteristic as a non-selective filter feeder, which consumes anything that enters its mouth (Mohebbi et al 2015). This fact allows it to be enriched with a variety of enrichment materials, e.g. fish oil, squid oil, vitamins or other commercial products, including probiotics and prebiotics.

Several study results have revealed that the enrichment of *Artemia* using various materials could significantly improve the composition and nutritional value of *Artemia* biomass, so that when it is fed as a natural feed to shrimp and fish larvae, they can

support the growth and survival rate of these larvae (Sulistyowati et al 2006; Widiastuti et al 2012; Herawati et al 2014).

The application of a probiotic and a prebiotic in shrimp larvae through the enrichment of *Artemia* is an alternative that could be done to produce high quality shrimp larvae with a high survival, growth rate and resistance against several diseases (specific pathogen resistance), so that when the shrimps are stocked into the ponds, they already have a good growth response and immune system to combat various pathogens, which are found in field conditions occurred in the ponds. Several study results have proven the success of probiotics in improving the shrimp growth, survival rate, immune response and resistance (Nimrat et al 2012; Zokaeifar et al 2012; Widanarni et al 2015), while prebiotics has the ability to improve growth, survival rate, feed digestibility, feed efficiency, the composition of microflora in the intestines, and improve the immune system in shrimp (Li et al 2009; Zhang et al 2012; Aktas et al 2014).

The enrichment of *Artemia* with probiotic and prebiotic is expected to increase the accumulation of the probiotic, the bacterial population in the bodies of *Artemia* and the nutritional content of *Artemia*, so that when it is fed to shrimp larvae, they could give a positive effect on the shrimp larvae's growth, survival rate, and immune response. This study was aimed to evaluate the nutritional value of *Artemia* sp. after the enrichment with the probiotic *Pseudoalteromonas piscicida* 1Ub, the prebiotic mannan-oligosaccharide (MOS) and the synbiotic (the combination between the probiotic *P. piscicida* 1Ub and the prebiotic MOS).

Material and Method. This study was conducted on August-October 2015 located at Fish Health Laboratory, Department of Aquaculture, Bogor Agricultural University; PT. Saraswanti Indogenetic; and Microbiology Laboratory, Inter-University Center, Bogor Agricultural University, Bogor, West Java, Indonesia.

Preparation of probiotic and prebiotic. The probiotic used was *P. piscicida* 1Ub isolated from Pacific white shrimp (*Litopenaeus vannamei*) nauplii (Widanarni et al 2009). The probiotic isolate was marked with 50 ug mL^{-1} antibiotic rifampicin (*P. piscicida* 1Ub Rf^R) as a molecular marker. *P. piscicida* 1Ub Rf^R cells were grown in seawater complete (SWC)-slant agar (0.5 g bacto peptone, 0.1 g yeast extract, 0.3 mL glycerol, 1.5 g bacto agar, 75 mL seawater, and 25 mL distilled water) and were incubated at 29°C for 24 hours. Furthermore, the bacterial cells were inoculated into the SWC broth medium and were incubated in a water bath shaker at 29°C (140 rpm; 18 hours).

The prebiotic used was Bio-MOS (Alltech Inc., KY USA) which contained mannan-oligosaccharide (MOS) extracted from the cell wall of *Saccharomyces cerevisiae* with a composition of 30% crude protein, 1.4% crude fat and 13% crude fiber.

Analysis of the activity of the enzymes produced by the probiotic P. piscicida 1Ub. *P. piscicida* 1Ub cells were inoculated to 10 mL SWC broth medium, incubated in a water bath shaker at 29°C (140 rpm; 24 hours). The inoculum was then centrifuged at a speed of 11,000 rpm for 20 minutes at 4°C (Irawadi 1991). The filtrate of the crude enzyme extract was then collected to be analyzed for the amylase, lipase and protease activity. The activity of amylase was measured using 1% starch as the substrate in sodium phosphate buffer 20 mM pH 6.9, which contained NaCl 6.0 mM according to the method in Worthington (1993). Lipase activity was measured using an olive oil emulsion as the substrate and Tris-HCL as a buffer according to the method in Borlongan (1990). Protease activity was measured using casein as the substrate and phosphate buffer 0.05 M pH 7 and tyrosine 5 mmol L^{-1} as the standard according to the method in Bergmeyer (1983).

For the analysis of mannanase enzyme activity, *P. piscicida* 1Ub cells were cultured in 100 mL BSM medium which contained 0.5% mannan solution (locust bean gum), incubated in a water bath shaker at 29°C (140 rpm; 24 hours). The inoculum was then centrifuged at a speed of 5000 rpm for 10 minutes. The crude enzyme filtrate extract was then collected for the mannanase enzyme activity analysis (Hossain et al 1996).

Test of the ability of MOS in stimulating the growth of the probiotic P. piscicida 1Ub. The test was conducted *in vitro* using 50 mL SWC broth medium that had been reduced its nutrient at an amount of 50% and had been added 0.2 g MOS as a prebiotic. The probiotic *P. piscicida* 1Ub was cultured in this medium and was incubated in a water bath shaker at 29°C (140 rpm) for 16, 18, and 20 hours. The probiotic inoculum was then grown on the SWC agar medium using the total plate count method. The MOS's ability to stimulate the growth of the probiotic *P. piscicida* 1Ub could be measured by comparing the number of probiotic colonies that grew on the medium containing MOS to the number growing on medium without MOS (the control).

Hatching and the enrichment of Artemia sp. *Artemia* cysts were hatched in 2 g L^{-1} seawater (30 ppt), strongly aerated, and the *Artemia* were harvested after 24 hours. The enrichment of *Artemia* sp. was conducted on the instar 2 stadium (approximately four hours after the harvesting) in a plastic container that had been filled with 1 L seawater (30 ppt). The density of *Artemia* sp. in each container was 100 individuals per mL. The enrichment was conducted by adding *P. Piscicida* 1Ub Rf^R at a concentration of 10^6 CFU mL^{-1}, 12 mg L^{-1} MOS, and synbiotic (the combination between 10^6 CFU mL^{-1} *P. piscicida* 1Ub Rf^R and 12 mg L^{-1} MOS) into each *Artemia* sp. enrichment container for four hours.

Bacterial population in Artemia sp. The bacterial population in the enriched *Artemia* sp. was observed using SWC-agar medium for the total bacterial count and SWC-agar that had been added with 50 ug mL^{-1} antibiotic rifampicin for the total probiotic *P. piscicida* 1Ub Rf^R count. The bacterial population was enumerated using the total plate count method (Madigan et al 2003).

The nutritional value of Artemia sp. The nutritional value of *Artemia* was analyzed based on proximate analysis, the fatty acid profile, and the essential amino acid profile in *Artemia* that had been enriched with the probiotic, prebiotic, synbiotic, and *Artemia* without any enrichment (the control). The proximate analysis was conducted on protein, fat, crude fiber, ash, and nitrogen free extract content (Takeuchi 1988). The analysis of amino acid profile was conducted using the UPLC method, while the analysis of fatty acid profile was done using gas chromatography (GC).

Statistical analysis. The nutritional content of *Artemia* in each treatment was analyzed through ANOVA. If there was a difference among treatments, the statistical analysis will be continued with the Duncan's test at a 95% confidence level using the SPSS 12 program. The data for the activity of enzymes produced by the probiotic *P. piscicida* 1Ub Rf^R, the ability of MOS in stimulating the growth of the probiotic *P. piscicida* 1Ub Rf^R, and the bacterial population in *Artemia* were analyzed descriptively.

Results and Discussion

Activity of the enzymes produced by the Probiotic P. piscicida 1Ub. The probiotic *P. piscicida* 1Ub Rf^R is able to produce a number of exogenous enzymes such as protease, lipase, amylase, and mannanase. The analysis results for the activity of protease, lipase, amylase, and mannanase in *P. piscicida* 1Ub Rf^R are presented in Table 1.

Table 1

The activity of protease, lipase, amylase, and mannanase produced by *P. piscicida* 1Ub Rf^R

Isolate name	*Enzyme activity (U mL^{-1} $minute^{-1}$)*			
	Protease	*Lipase*	*Amylase*	*Mannanase*
P. piscicida 1Ub Rf^R	0.0311±0.0302	0.1978±0.0579	0.008±0.0034	0.0185±0.0036

The ability of *P. piscicida* 1Ub Rf^R to produce exogenous enzymes indicates that *P. piscicida* 1Ub Rf^R has the ability to utilize protein, fat, carbohydrate, and MOS as a source

of additional nutrition to support its growth. This was in line with the statement by Wang et al (2008) that probiotics can produce a number of exogenous enzymes for feed digestion, including amylase, protease, lipase, and cellulase. Tzuc et al (2014) reported *Pseudoalteromonas* sp. isolated from the stomach, intestines, and hepatopancreas of Pacific white shrimp could produce amylase, lipase, and chitinase. Hakamada et al (2014) reported that various microorganisms, bacteria, yeast, and fungi, can produce mannanase. Ivanova et al (2002) also reported that a mesophilic and psychrophilic bacterial species, *Pseudoalteromonas issachenkonii*, was known to utilize D-Mannose with a help from mannanase.

The protease can hydrolyze protein into peptides, and bacteria produce peptidase which decomposes peptides into amino acids that are needed for metabolism. The amylase hydrolyzes amylum and assists the digestion of organisms. Lipases are a group of enzymes that generally play a role in hydrolyzing fats, monoglycerides, diglycerides, and triglycerides to produce free fatty acids and glycerol (Falony et al 2006). The main functions of lipase are to digest fats and lipids, to maintain the healthy function of the gall bladder, to maintain the electrolyte balance in the body, to help in maintaining optimum cell permeability, allowing the nutrition required by the cell to enter and assist metabolism.

The ability of MOS in stimulating the growth of the probiotic P. piscicida 1Ub. The ability of MOS in stimulating the growth of the probiotic *P. piscicida* 1Ub was evaluated based on the comparison between the total *P. piscicida* 1Ub count using SWC plate agar medium containing MOS and SWC plate agar medium without MOS. The total *P. piscicida* 1Ub count grown on the SWC plate agar medium containing MOS during the 16-20 hour incubation period ranged between 11.2-11.8 log CFU mL^{-1}, while the total *P. piscicida* 1Ub count grown on SWC plate agar medium without MOS ranged between 9.2-9.5 log CFU mL^{-1} (Figure 1).

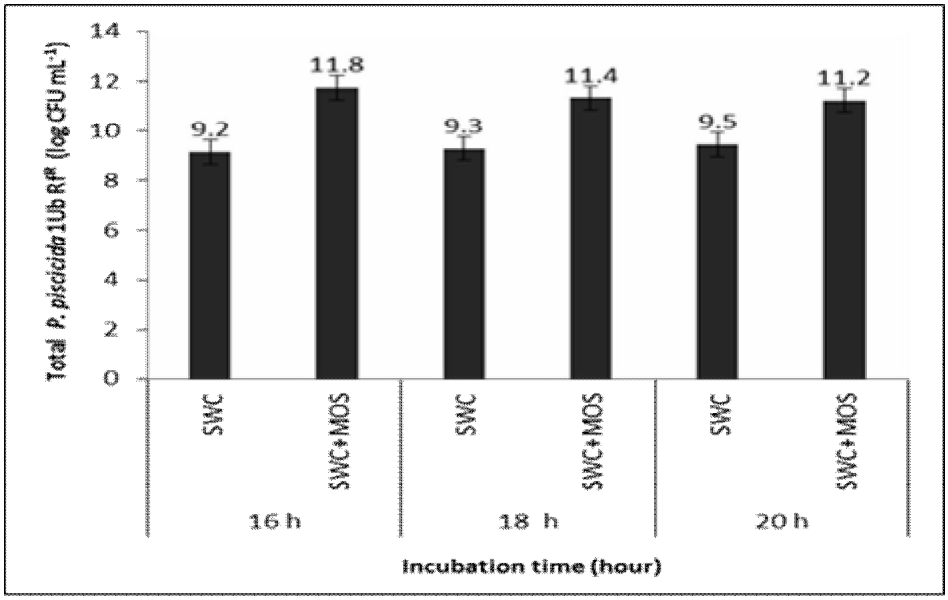

Figure 1. The total *P. piscicida* 1Ub count on the SWC plate agar medium and on the SWC+MOS plate agar medium.

The prebiotic MOS used in this study was able to stimulate the growth of the probiotic *P. piscicida* 1Ub Rf^R. This was indicated by the total *P. piscicida* 1Ub Rf^R count grown on the SWC plate agar medium containing MOS which was greater than the total *P. piscicida* 1Ub Rf^R count on the SWC plate agar medium without MOS. The higher total *P. piscicida* 1Ub Rf^R count grown on the SWC medium containing MOS was due to the availability of MOS as a source of additional nutrition for *P. piscicida* 1Ub Rf^R and the ability of *P. piscicida* 1Ub Rf^R to utilize MOS through the activity of mannanase produced by this

probiotic bacteria. Cerezuela et al (2011) stated that prebiotics are indigestible feed materials which have a beneficial effect on their host by stimulating the growth and the activity of particular bacteria in the intestines, improving the health of the host. There are many prebiotics that have been studied and applied in aquaculture, including inulin, mannan-oligosaccharides (MOS), fructooligosaccharides (FOS), short-chain fructooligosaccharides (scFOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), trans-galactooligosaccharides (TOS), and isomaltooligosaccharides (IMO) (Ringø et al 2010).

Bacterial population in Artemia sp. The administration of the probiotic *P. piscicida* 1Ub Rf^R, the prebiotic MOS, and the synbiotic (the combination between the probiotic *P. piscicida* 1Ub Rf^R and the MOS) to *Artemia* sp. could modulate the growth of microflora in the bodies of the *Artemia*, so the bacterial population in the bodies of the *Artemia* given the probiotic, prebiotic and synbiotic were higher than that in the control. The total bacterial count in the synbiotic treatment was 8.6 log CFU per 0.1 g *Artemia*, 8.0 log CFU per 0.1 g *Artemia* in the probiotic treatment, 7.7 log CFU per 0.1 g *Artemia* in the prebiotic treatment, and 6.8 log CFU per 0.1 g *Artemia* in the control. Moreover, *P. piscicida* 1Ub Rf^R administered were also able to survive and colonize in the bodies of *Artemia*. The total *P. piscicida* 1Ub Rf^R count in the bodies of the *Artemia* treated with the probiotic was 6.4 log CFU per 0.1 g *Artemia*, and in those treated with the synbiotic was 6.9 log CFU per 0.1 gr *Artemia* (Figure 2).

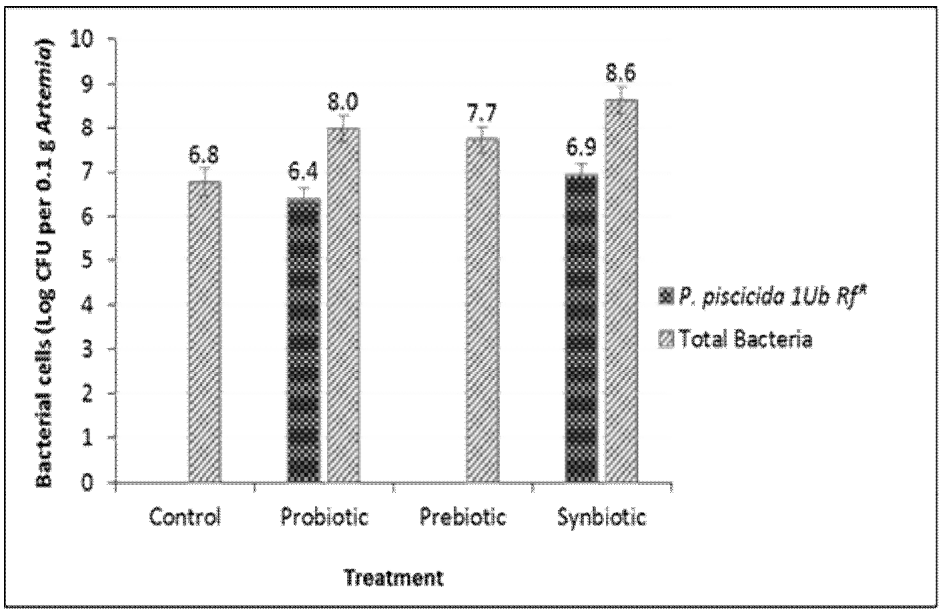

Figure 2. Total bacterial count and total *P. piscicida* 1Ub Rf^R count in the bodies of *Artemia*.

The ability of *P. piscicida* 1Ub Rf^R to survive and colonize in the bodies of *Artemia* sp. was demonstrated by the total bacterial count and the total *P. piscicida* 1Ub Rf^R count in the bodies of the *Artemia*. Based on the average values of total bacterial count and total probiotic *P. piscicida* 1Ub Rf^R count in the bodies of the *Artemia*, those revealed that the administration of the synbiotic resulted in higher total bacterial count and total *P. piscicida* 1Ub Rf^R count compared to other treatments and the control. The high values of total bacterial count and total *P. piscicida* 1Ub Rf^R count in the bodies of *Artemia* treated with the synbiotic were possibly caused by the ability of *P. piscicida* 1Ub Rf^R to survive and colonize in the bodies of *Artemia* and its ability to utilize mannan-oligosaccharide due to the presence of mannanase produced by *P. piscicida* 1Ub Rf^R. This was in line with the results of the study by Widanarni et al (2015) who reported that the application of the synbiotic (the combination between the probiotic *Vibrio alginolyticus* SKT-b and the prebiotic from sweet potato extract) resulted in the highest total bacterial count and total probiotic count in the bodies of Pacific white shrimp larvae. The application of synbiotics

is known to be able to optimize the function and gain the number of beneficial bacteria in the host's intestines (Delgado et al 2011).

The nutritional value of Artemia sp. The results of the proximate analysis and fatty acid profile of *Artemia* sp. enriched with the probiotic *P. piscicida* 1Ub Rf^R, the prebiotic MOS, and the synbiotic (the combination between *P. piscicida* 1Ub Rf^R and MOS) are presented in Tables 2 and 3. The enrichment of *Artemia* sp. with the probiotic, prebiotic, and synbiotic had a significant effect ($p < 0.05$) on the protein and fat content in the *Artemia*, but did not have a significant effect ($p > 0.05$) on the crude fiber, ash, and nitrogen free extract content. The protein content of the *Artemia* treated with the probiotic, prebiotic, and synbiotic ranged between 52.68-53.06%, while the fat content of the *Artemia* ranged between 15.33-16.98%.

Based on the fatty acid profile (Table 3), it could be concluded that the administration of the probiotic, prebiotic, and synbiotic had a significant effect ($p < 0.05$) on the butyric acid, linoleic acid, and linolenic acid content of *Artemia*. The highest butyric acid ($p < 0.05$) was observed in the *Artemia* enriched with the prebiotic (0.0029±0.00014%), followed by those enriched with the synbiotic (0.0009±0.00007), while in the probiotic treatment and control, butyric acid was not detected. The enrichment of *Artemia* with the synbiotic resulted in the higher content ($p < 0.05$) of linoleic acid and linolenic acid compared to the other treatments and the control. The linoleic acid and linolenic acid content of the *Artemia* enriched with the synbiotic were 0.142±0.0009% and 0.72±0.0023%, respectively.

Table 2

The results of the proximate analysis of *Artemia* sp. enriched with the probiotic, prebiotic, and synbiotic

Treatment	*Protein (%)*	*Fat (%)*	*Crude fiber (%)*	*Ash (%)*	*Nitrogen-free extract (%)*
Control	51.24±0.72^{a}	14.05±0.12^{a}	0.65±0.07^{a}	20.60±1.98^{a}	12.02±1.91^{a}
Probiotic	53.06±0.23^{b}	15.77±0.64^{ab}	2.00±2.12^{a}	18.80±0.57^{a}	10.38±3.09^{a}
Prebiotic	52.88±0.19^{b}	16.98±0.51^{b}	2.02±1.10^{a}	23.44±3.20^{a}	4.69±2.41^{a}
Synbiotic	52.68±0.26^{b}	15.33±0.92^{ab}	1.66±0.88^{a}	19.15±3.18^{a}	12.63±4.26^{a}

Means±standard deviations in the same column with different superscript letters indicate significantly different results ($p < 0.05$).

Table 3

The fatty acid profile of *Artemia* sp. enriched with probiotic, prebiotic, and synbiotic

Treatment	*Butyric acid (%)*	*Linoleic acid (%)*	*Linolenic acid (%)*	*EPA (%)*	*DHA (%)*
Control	Nd	0.117±0.0013^{a}	0.53±0.0023^{a}	0.018±0.0023^{a}	nd
Probiotic	Nd	0.141±0.0018^{c}	0.65±0.0127^{c}	0.020±0.0031^{a}	nd
Prebiotic	0.0029±0.00014^{a}	0.127±0.0009^{b}	0.58±0.0078^{b}	0.023±0.0009^{a}	nd
Synbiotic	0.0009±0.00007^{b}	0.142±0.0009^{c}	0.72±0.0023^{d}	0.046±0.0001^{a}	0.018±0.0006

Means±standard deviations in the same column with different superscript letters indicate significantly different results ($p < 0.05$); nd = not detected.

On the other hand, the analysis of the essential amino acid profile of *Artemia* sp. (Table 4) revealed that the administration of the probiotic, prebiotic, and synbiotic had a significant effect ($p < 0.05$) on the essential amino acid content of *Artemia* sp. The essential amino acid that took the highest portion was leucine that ranged between 4.985-5.193 ppt, while the essential amino acid that took the lowest portion was the amino histidine that ranged between 1.770-1.820 ppt.

Table 4

The essential amino acid profile in the *Artemia* sp. enriched with the probiotic, prebiotic, and synbiotic

Essential amino acid	*The concentration of essential amino acid (ppt)*			
	Control	*Probiotic*	*Prebiotic*	*Synbiotic*
Histidine	1.630±0.051[a]	1.770±0.012[b]	1.820±0.009[b]	1.809±0.003[b]
Threonine	1.630±0.051[a]	1.770±0.012[b]	1.820±0.009[b]	1.809±0.003[b]
Arginine	2.920±0.010[a]	3.297±0.034[c]	3.128±0.046[b]	4,565±0.061[d]
Valine	3.415±0.029[a]	3.695±0.043[c]	3.531±0.055[ab]	3.611±0.047[bc]
Phenylalanine	3.358±0.042[a]	3.477±0.087[a]	3.783±0.019[b]	3.893±0.131[b]
Isoleucine	3.193±0.056[a]	3.540±0.065[c]	3.398±0.027[b]	3.425±0.018[bc]
Leucine	4.839±0.161[a]	5.193±0.078[b]	5.018±0.043[ab]	4.985±0.075[ab]
Lysine	2.014±0.037[a]	4.843±0.002[d]	2.732±0.015[b]	4.102±0.025[c]

Means±standard deviations in the same column with different superscript letters indicate significantly different results ($p < 0.05$).

The enrichment of *Artemia* sp. with the probiotic *P. piscicida* 1Ub Rf^R, prebiotic MOS, and synbiotic (the combination between *P. piscicida* 1Ub Rf^R and MOS) could also improve the nutritional value of *Artemia* sp., especially the protein content, fat content, fatty acid profile, and essential amino acid profile. The improvement of the nutritional value of *Artemia* sp. enriched with the probiotic, prebiotic, and synbiotic were most probably due to the colonization by a number of beneficial bacteria in the bodies of the *Artemia* sp. and the ability of probiotic to produce a number of exogenous enzymes, including protease, lipase, amylase, and mannanase. This was in accordance with the statement by Balcázar et al (2006) who stated that the beneficial effects of probiotics, prebiotics, and synbiotics for the host are postulated to be due to the colonization of a number of beneficial bacteria in the host's digestive tract. Probiotics have also been demonstrated to produce protease, amylase, and lipase, vitamins, fatty acids and amino acid cofactors in the digestion process which will improve feed efficiency and growth performance, shown by the improvement in the nutritional values of the host body. Abdelhamid et al (2009) reported that an increased dose of probiotics could increase the protein, fat, and energy content in the fish body. This indicates that the application of a substance that can modulate the bacterial population in the digestive tract of aquatic organisms (probiotics, prebiotics, and synbiotics) would induce the improvement in the host's nutritional value.

Fat is needed as a source of metabolic energy (ATP) and as a material to maintain the structure and integrity of the cell membrane in the form of phospholipids. Phospholipids consist of fatty acids. There are two fatty acids that build fat: non-essential fatty acids that can be synthesized by the body and essential fatty acids that must be obtained from the exogenous sources (Jobling 2001). Essential fatty acids, especially those from the HUFA (highly unsaturated fatty acids) and PUFA (polyunsaturated fatty acids) groups, have important roles in the body's metabolic activities, because they are the components of membranes (phospholipids and cholesterol), hormones (metabolism of steroids and vitamin D), activate certain enzymes, are a precursor of prostanoids and leukocytes, maintain the structure and function of the cell membrane and have the function as the precursor for eicosanoids (Sargent et al 1999; Ibeas et al 1996). Fatty acids that are essential for crustaceans are 18:2n-6 (linoleic), 18:3n-3 (linolenic), 20:5n-3 (eicosapentaenoic, EPA) and 20:6n-3 (docosahexaenoic, DHA) (D'Abramo 1997). EPA and DHA play important roles in supporting the growth and survival rate of crustaceans (D'Abramo & Sheen 1993; Suprayudi et al 2004).

Brown & Robert (2002) and Herawati (2014) stated that leucine, isoleucine, valine and lysine are essential amino acids that are important for the growth of fish and shrimp larvae. Leucine plays important roles in maintaining the nitrogen balance, the degradation and production of protein. Isoleucine is a building component of protein, while valine has a function to replace glutamic acid that plays a role to bind oxygen effectively. Lysine plays a role in the production of vitamin B1, has anti-viral properties, helps the absorption of calcium, forms antibody hormones, stimulates the appetite, and

facilitates the production of carnitine which is needed to convert fatty acids into energy (Herawati et al 2012).

Conclusions. The enrichment of *Artemia* sp. with the probiotic *P. piscicida* 1Ub Rf^R, the prebiotic mannan-oligosaccharide, and the synbiotic (the combination between the probiotic *P. piscicida* 1Ub Rf^R and the prebiotic mannan-oligosaccharide) could increase the bacterial population and the nutritional value of the *Artemia* sp., with the best results demonstrated by the application of the synbiotic.

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