Physical properties and protein content evaluation of fermented floating feed containing black soldier fly maggots as a potential alternative for fish feed

M F Maulana¹, S Mauladani¹,², A I Rahmawati¹,², R Ma’hadah¹, M Kamila¹, A Syarif³, Komarudin¹,², H K H Saputra¹,², H Junaedi²,³, D Cahyadi²,³, U Ahidin⁴, C Sriherwanto⁵, I Suja’i⁵, A Nadaviana⁶, D W Nugroho⁷, R Ikono⁸ and N T Rochman⁹

¹Nano Center Indonesia, Jl. Raya Serpong, South Tangerang, Banten, 15314, Indonesia
²Nanobubble Karya Indonesia, Ltd., Jl. Raya Serpong, South Tangerang, Banten, 15314, Indonesia
³Department of Agricultural Industry, IPB University, Jl. Raya Dramaga, Bogor, West Java, 16680, Indonesia
⁴Institute for Research and Community Services, Pamulang University, Jl. Surya Kencana No. 1, South Tangerang, Banten, 15417, Indonesia
⁵Agency for the Assessment and Application of Technology, 630 Building, PUSPIPTEK, South Tangerang, Banten, 15314, Indonesia
⁶Research Center for Metallurgy and Materials, Indonesian Institute of Sciences, PUSPIPTEK, South Tangerang, Banten, 15314, Indonesia

E-mail: farhan@nano.or.id or nurul@nano.or.id

Abstract. The objective of this study is to examine physical properties of experimentally formulated diets containing black soldier fly (Hermetia illucens) maggots as a protein source replacement. It is fermented by Rhizopus sp. to increase buoyancy in water. Maggots at 60% of total ingredients are incorporated into six prepared diets which received two types of agriculture wastes at three different ratios (3:7, 1:4, 3:17) and tapioca at two different levels (10% and 12%). The protein content is analyzed using proximate method and the physical properties (measured in stability and buoyancy) are evaluated by testing floatation ability under aeration and non-aeration conditions. The six formulated diets have protein content up to 28% (w/w), while the buoyancy and stability characteristics range from 70-93% and 80-95%, respectively. The diet with 3:7 ratio of agriculture wastes and 10% tapioca gives the best results in stability and protein content at 95% and 28.04% (w/w), respectively. Due to its high value properties, the maggot-based floating feed could be used as an alternative nutrition source for fish feed.

1. Introduction
Fisheries is a strategic sector in Indonesia’s development, in particular, freshwater fishery commodities. This is proven by the total national catfish production in 2017 which reached 1.8 million tons, an increase of 131.7% from the previous achievement, an upward trend over the past 6 years with
an average growth of 38% per year. Therefore, an increase in aquaculture production results is followed by an increase in community fish protein requirements.

The success of aquaculture production can be determined by several factors, one of them is the quality of feed preparation. Feed is the main factor in intensive fish farming because it comprises the biggest cost [1]. Studies about the utility of alternative feed ingredients have been carried out, such as alternative feed ingredients from plant, microbial and other animal sources [2]. There are two types of feed: floating and sinking feed. The first has several advantages compared to the latter. One of them is the Feeding Conversion Rate (FCR) value. FCR is the ratio measuring the efficiency of animal feed conversion into the desired output. Floating feed has a better FCR value of 1.05 compared to sinking feed FCR value, which is 1.15. The better buoyancy of floating feed makes it easier for fish to locate and forage the feed. Excessive feed -- which is the result of sinking feed -- will be decomposed by heterotrophic bacteria that require oxygen consumption. This decomposition of the feed leftovers increases the nitrite and ammonia content which pollutes the pond and poison the fish immediately [3].

A substantial research about the bioconversion of organic waste using Black Soldier Fly (Hermetia illucens) has been performed [4]. Due to the amount of protein (476 g kg⁻¹, dry matter (DM)) and fat (118 g kg⁻¹, DM) and the well-balanced essential amino acid (EAA) profile, maggot meal might be a suitable alternative for fish meal [5]. However, a maggot-based feed that have been produced is still classified as a sinking feed, so it still has a problem in terms of a lack of control over the amount of feed given.

On the other hand, Institute of Biotechnology (Agency for the Assessment and Application of Technology) has developed an alternative floating feed with the application of fermentation technology using yeast Rhizopus sp. Fermentation by Rhizopus sp. as a key process in fish feed production causes chemical and physical changes to the substrate, making an increase in terms of buoyancy, absorption, and stability in water. This floatation technique is a better alternative than using extruder machines that require large investment costs, operation, and maintenance of machines that are not simple and require special knowledge about the use of binders and water stabilizers [6].

It is necessary to do a technological innovation regarding the substitution of fish meal in feed and the application of fermentation methods to produce floating feed without the extrusion process with high temperature and pressure. The objective of this study is to examine the physical properties of experimentally formulated diets containing maggots as a protein source replacement and fermented by Rhizopus sp. to make floating feeds. The physical properties tested in this study are buoyancy and stability in water.

2. Materials and methods

2.1. Maggot cultivation

The stage of the maggot cultivation process begins with the breeding of flies. In their life cycle, adult male flies mate with female adult flies within ± 3 days. Black soldier fly has a fairly short adult life phase, 6-8 days. BSF lays eggs for 3 days. The bioconversion process lasts for approximately 2-3 weeks. In this process, mini larvae will convert organic waste. During the larval phase, the maggot will continue to eat until it approaches the pupal phase. In this phase, insects stop eating and will leave their food source, followed by finding a place to shelter until entering the pupal phase. The pupal phase lasts for 6-7 days and after that, the insect will metamorphose into an adult insect.

The harvest process is done when the maggot is brownish yellow. In this process, the maggot is harvested before turning to pupae. After going through the harvest process, the output produced is the harvest of maggot and fertilizer. Maggot yields are used as material for making feed, while fertilizer is used for the agricultural industry.
2.2. Feed formulation
The feed ingredients are made from maggots, a mixture of agricultural wastes, tapioca flour, and additives such as vitamin mix. From these ingredients, the formulations of each diet can be seen in Table 1.

| Formula | Ingredients          | Maggot (%) | Agriculture wastes | Tapioca flour (%) | Additive (%) |
|---------|----------------------|------------|-------------------|-------------------|-------------|
| F1C1    | 60                   | 3:7        | 10                | 2                 |
| F1C2    | 60                   | 1:4        | 10                | 2                 |
| F1C3    | 60                   | 3:17       | 10                | 2                 |
| F2C1    | 60                   | 3:7        | 12                | 2                 |
| F2C2    | 60                   | 1:4        | 12                | 2                 |
| F2C3    | 60                   | 3:17       | 12                | 2                 |

In the making of maggot meal, harvested fresh maggots are ensured to be stored below 4°C. Then, maggots are removed from the cooler and allowed to stand for a while. In order to reduce the initial water content, maggot is steamed and then dried using an oven for at least 45 minutes at 100 °C. After that, maggots are blended until it becomes smooth. After all the ingredients were ready, then it is mixed and stirred evenly and put into a pelleting machine.

2.3. Floating feed optimization
Optimization of floating feed is carried out using the fermentation method by *Rhizopus* sp. as much as 2% of the amount of feed. *Rhizopus* sp. inoculum was supplied from BPPT Institute of Biotechnology, Serpong, South Tangerang. The material mixture was incubated at 30 °C for 24 hours. After that, fermented feed was dried using an oven at 40 °C for 24 hours. Dry fermented feed was evaluated for their physical properties such as buoyancy and water stability.

2.4. Buoyancy and water stability tests
Buoyancy tests are performed at two small plastic-covered ponds, with an aerator system connected to one of them. Twenty pellet-shaped feed pieces were dropped into the water and observed for 60 minutes at five minutes intervals. The percentage of the pieces afloat is calculated by dividing the initial number of pieces afloat from the number of pieces afloat at the specified time, times 100. Water stability of the feed pieces was observed by calculating the number of intact pieces (not leached) over total number of pieces.

3. Results and discussion
Table 2 shows protein content of each formulation used in this study. From the results of feed testing, it can be concluded that F1C1 formulation has the largest protein content, which is 28.04% (w/w). This shows that maggot and combination of two types of agriculture wastes as much as 3:7 can produce feed with the highest protein content. All formulations that use 12% of tapioca flour except F2C1 have a higher protein content than formulations with 10% of tapioca flour.

| Sample | Protein yield (%w/w) |
|--------|----------------------|
| F1C1   | 28.04                |
| F1C2   | 19.90                |
| F1C3   | 23.86                |
| F2C1   | 26.05                |
| F2C2   | 25.79                |
| F2C3   | 27.82                |
In order to compare the buoyancy performance of the feed, the test includes putting the feed pellets in both aerated and non-aerated condition. Figure 1 shows the buoyant performance of the feed in the non-aerated condition. Fermented feed samples are able to increase buoyant feed count to be almost equivalent to the extruded feed. Until the 15th minute, fermented feed has a buoyancy ranging between 70-100%. The formulation that has the highest buoyancy performance is F2C2 since it still maintains 100% buoyancy until 15th minute mark.

In the aerated water conditions, the best buoyancy is demonstrated by the F1C3 formulation -- at the 5th minute, the feed still maintain its floating state. Yet, it drops to 57% at the 15th minute. Air bubbles can accelerate the sinking process of fermented feed, since the turbulent water may cause the feed to increase its density quickly. Based on a weighted-average calculation, it can be concluded that F1C3 is the best formulation that can bring out the highest buoyancy performance since it excels in both conditions.

Figure 1. Buoyancy test in aeration (left) and non-aeration condition (right).

During the fermentation process, feed substrate metabolism is carried out by Rhizopus sp. and some of these substrates undergo biochemical processes into volatile products so the feed loses mass due to this fermentation. Volatile compounds produced during the fermentation of Rhizopus sp. including esters, alcohols, aldehydes, ketones, furans, aromatic compounds of pyrazines, sulfur compounds [7] and carbon dioxide [8]. This may reduce the overall water-pellet density ratio, which explains the increased buoyancy performance of the fermented pellets.

In addition, fermentation by Rhizopus sp., which is characterized by the formation of white mycelia that bind and unite the substrate, strengthen the texture of the fermentation product, so that the empty space between the substrate particles becomes more densely filled with mycelia and is able to slow down water absorption [9]. In addition to buoyancy testing, water stability test of the feed pellets is also carried out to determine the physical endurance of the feed in the water. Water stability is an important quality trait for slow eating aquatic animals when the feed has to be soaked in water for hours with minimum leaching of nutrients. Feed that has low water stability will be easily destroyed and can pollute the pond. The following are the results of the water stability test of fermented feed.
Figure 2. Stability test of experimental feed.

From the graph (Figure 2), it can be observed that the water stability of fermented feed tends to be stable at 80-95%. This shows that the stability of floating feed with aeration and without aeration is not significantly different. Meanwhile, water stability of feed without fermentation was observed to be decreasing. This indicates that the fermentation process is very influential on feed stability.

4. Conclusion
Based on the analysis of the data above, it can be concluded that fermentation by *Rhizopus* sp. is able to alter feed physical characteristics, including buoyancy and water stability of the feed. The best formulation that contains a high value of physical properties and protein content is F1C3 formulation. This formulation contains 23.86% of protein, maintains 93% buoyancy level in non-aerated condition, and reaches a 90% water stability level. Due to its high value properties, the maggot-based floating feed could be used as an alternative nutrition source for fish feed.

5. References
[1] Sørensen M 2012 A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods *Aquaculture Nutrition* **18** 233-248
[2] Magalhães R, Sánchez-López A, Leal R, Martínez-Llorens S, Oliva-Teles A and Peres, H 2017 Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*) *Aquaculture* **476**
[3] Somerville C, Cohen M, Pantanella E, Stankus A and Lovatelli A 2014 Fish in aquaponics. In: Small-scale aquaponic food production: integrated fish and plant farming *FAO Fisheries and Aquaculture Technical Paper* **589** 103–121
[4] Liu Z, Minor M, Morel P C H and Najar-Rodriguez A J 2018 Bioconversion of Three Organic Wastes by Black Soldier Fly (Diptera: *Stratiomyidae*) Larvae *Environmental Entomology* **47** 1609–1617
[5] Kroeckel S, Harjes A G E, Roth I, Katz H, Wuertz S, Susenbeth A and Schulz C 2012 When a turbot catches a fly: evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute - growth performance and chitin degradation in juvenile turbot (*Psetta maxima*) *Aquaculture* **364/365** 345-352
[6] Zaman A B, Sriherwanto C, Yunita E and Suja’i I 2018 Physical characteristics of non-extruded floating fish feed produced through *Rhizopus oryzae* fermentation *Jurnal Bioteknologi dan Biosains Indonesia* **5** 27-35
[7] Chukeatirote E, Eungwanichayapant PD and Kanghae A 2017 Determination of volatile components in fermented soybean prepared by a co-culture of Bacillus subtilis and Rhizopus oligosporus Food Res. 1 225–233

[8] Christen P, Bramorski A, Revah S and Soccol CR 2000 Characterization of volatile compounds produced by Rhizopus strains grown on agro-industrial solid wastes Bioresour. Technol. 71 211–215

[9] Leiskayanti Y, Sriherwanto C and Suja’i I 2017 Fermentation using tempe starter as a biological method for providing buoyancy to fish feed Jurnal Bioteknologi dan Biosains Indonesia 4 54-63

Acknowledgment
This research is funded by grants from Industrial Technology Development Program (PPTI) of Ministry of Research, Technology, and Higher Education and supported by Nano Center Indonesia. Mr. Hardi Junaedi is supported by scholarship from Indonesia Endowment Fund for Education (LPDP).