Supplementation of *Kappaphycus alvarezii* Solid Waste (Bioethanol Production) in Fish Feed for *Barbonymus schwanenfeldii* Growth

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Abstract

Kappaphycus alvarezii is a renewable resource rich in dietary sources such as vitamins, proteins, carbohydrates and trace minerals. The solid waste of this marine macroalgae produced from bioethanol production was used to produce fish feed. This study aims to evaluate the efficacy of *K. alvarezii* fish feed in *Barbonymus schwanenfeldii* fish growth. The proximate analysis, micro and macronutrient and mycotoxin in the *K. alvarezii* fish feed were determined using a standard protocol. For the fish culture design, fish were randomly divided into two groups: group I – feed with commercial fish feed (control); group II – feed with *K. alvarezii* fish feed (experimental group). The initial body weight of the fish was recorded, and thereafter weekly for 12 weeks. Proximate analysis indicated that the dried *K. alvarezii* fish feed is high in nitrogen (46.30±0.1%) and low in moisture (6.40±0.1%), ash (4.50±0.1%) and fiber (4.75±0.1%) contents, while rich in macro and micronutrients. There was no mycotoxin found in the *K. alvarezii* fish feed. For the 12 weeks of the feeding of commercial and *K. alvarezii* fish feed, our results did not show any significant difference ($P > 0.05$) in the *B. schwanenfeldii* specific growth rate between the groups, 0.36±0.03% in the control group and 0.34±0.02% in the experimental group, respectively. Proximate analysis of the *B. schwanenfeldii* fish, indicated a moisture content of 19.20±0.1%, crude extract protein of 74.70±0.1% and crude lipid of 3.50±0.1% in the control group, which are significantly higher ($P < 0.05$) than in the experimental group [moisture content (15.60±0.1%), crude extract protein (70.00±0.1%) and crude lipid (2.60±0.1%)]. Although the proximate analysis in the control group is significantly higher than the experimental group, the use of *K. alvarezii* as fish feed supplement is still a good option as it utilizes the waste of *K. alvarezii* and can support towards the UN Sustainable Development Goal 12, Responsible Consumption and Production.

Keywords: *Kappaphycus alvarezii*, fish feed production, fish feed supplement, *Barbonymus schwanenfeldii*, UN SDG12

Introduction

By the year 2050, it is predicted that food production will increase by 60% to fulfil the growing human population (United Nations, 2007). Global issues such as increasing environmental conflict, limited freshwater supply, decreasing arable lands and changing eating patterns were causing food production to drop (Tacon, 1987; Duarte et al., 2009). Increasing market demand for meat and dairy products has also increased the demand for animal feed. Therefore, animal feed is leading the global food industry, enabling economic growth (FAO, 2010). Recently, the world has increased its interest in exploring the possibility of using seaweed in animal feed due to its nutrients (Lum et al., 2013).

In 2013, the total feed production in the world was estimated at 963 million tonnes which was 9 million tonnes higher than the previous year. Asia contributed the most, 348 million tonnes, followed by Europe with 227 million tonnes and North America with 189 million tonnes. The increasing production shows the increased interest in the market towards the available feeds that have health benefits to the livestock.

Seaweed is a sustainable and renewable resource as nutrient-rich food with a high growth rate. Cultivating seaweed does not require fertilizers, pesticides and freshwater and only requires one-sixth of the surface needed by plants. About 6000 species of seaweed can be found on earth, but only 5% of them are used as food for humans or animals (Chojnacka et al., 2012). Traditionally, seaweed is used as food, feed, fuel, medicine and cosmetics due to its nutraceutical value and biochemical composition. In recent, there are scientific research shows the positive effects of seaweed on human and animal health.

Seaweeds are good dietary sources high in vitamins, proteins, carbohydrates, and trace minerals (Kumar et al., 2008). Besides, seaweed is also found to be low in lipid and high in polysaccharides and bioactive molecules. As a nutrient-rich agriculture product, seaweed can improve gastrointestinal flora, improve immunity, enhance growth performance, and increase milk and meat quality and yield in livestock (Carrillo et al., 2008; Bendary et al., 2013). Furthermore, a seaweed-based diet for aquaculture improved weight gain, increased triglyceride and protein in muscle, improved resistance to disease and less mortality, better growth index, higher gutted weight, increased fish digestibility, enhanced natural pigmentation, carcass quality and starvation tolerance and decreased nitrogen output into the environment (Becker et al., 2007; Fleurence et al., 2012).

Therefore, seaweed could be a good source of nutrient supply. Seaweed is abundantly used as dietary supplementation in the aquaculture Fields (Hashim & Saat, 1992; Cruz-Suárez et al., 2000; Valente et al., 2006). Generally, seaweed is divided into three types; red seaweed, green seaweed and brown seaweed (Guiry & Guiry, 2016). *Kappaphycus alvarezii* is a type of red seaweed which is abundantly found in the Indo-Pacific region spreading from the area of eastern Africa to

Guam, and it is most concentrated in the Southeast Asian Region. In Malaysia, *K. alvarezii* is abundantly cultivated in Sabah state (Trono, 1992).

Fish is a good protein source for human usage because it provides high-quality protein for people (Tidwell & Allan, 2001). Besides, the demand for fish is also increasing from 151 thousand tonnes in 2000 to 202 thousand tonnes in 2016 in Malaysia. Fish feed is the main component of growing aquaculture production, where the feed influences the growth and quality of the fish (Pereira et al., 2012). Fish feed generally consists of proteins, amino acids, carbohydrates, lipids, energy, minerals and vitamins. Fish requires the balance of nutrients in the feed for growth, energy and reproduction.

Barbonymus schwanenfeldii is a freshwater fish found in Southeast Asia, such as the Mekong river, Sumatera, Borneo and Peninsular Malaysia (Christensen, 1992). The species is classified as an omnivore, where it consumes both meaty and plant-based foods. It consumes smaller fish, small invertebrates and aquatic plants in the wild. An adult *B. schwanenfeldii* could grow up to approximately 35.5 cm total length. The optimal water conditions for *B. schwanenfeldii* are pH 6.0 to 7.5 and temperature 20.4 to 33.7^{0}C. This study is aimed at evaluating the efficacy of fish feed produced from *K. alvarezii* solid waste in the freshwater fish species *B. schwanenfeldii*.

Materials and Methods

Sample preparation

Solid wastes of *Kappaphycus alvarezii* from bioethanol production (acid hydrolysis) was collected and washed with distilled water. The *K. alvarezii* solid waste was dried and weighed until a constant weight was obtained. The dried *K. alvarezii* solid waste was stored at -20□C until further use.

Fish feed preparation

The fish feed formulation was made with proteins of vegetable origin, mainly soybean meal. The list of the ingredients is expressed in weight (kg), as shown in Table 1. Only 3% *K. alvarezii* solid waste was included in the fish feed production. of the dry ingredients were weighed and mixed homogeneously. The soybean oil was added to the mixture and mixed thoroughly for 5 minutes. Water was added to the mixture, and a soft but non-sticky dough was obtained. The dough was steamed cooked for 15 minutes until gelatinization and then shaped into the desired pellet size (0.4+0.1cm) using the meat mincer. The pellets were dried at 75□C for 45 minutes before storing in an airtight plastic container.

Table 1. Recipe for a 10 kg-fish feed using plant-based protein

Feed ingredient	Weight (kg)	Percentage of total feed (%)
Corn meal	1.0	10
Wheat flour	1.0	10
Soybean meal	6.7	67
soybean oil	0.2	2
Wheat bran	0.7	7
K. alvarezii solid waste	0.3	3
Total amount	**10.0**	**100**

Proximate Analysis of Fish Feed and Fish Samples

Moisture content determination

The sample's moisture content was determined by the weight loss of the sample when it was dried to a constant weight in an oven (Bhuiyan et al., 2016). The sample's initial weight was recorded and placed in an oven at 90^0C for 24 hours. The sample was cold and weighted. The drying and weighing continue until a constant weight is obtained. The moisture content can be calculated by the formula below:

$$\text{Moisture content (\%)} = \frac{initial\ sample\ weight - final\ sample\ weight}{initial\ sample\ weight} \times 100\%$$

Ash content determination

The initial weight of the sample was obtained. The sample was placed into the ashing furnace at 600□C for 4 hours until the whitish-grey ash was obtained (Bhuiyan et al. 2016). The remaining ash was cool and weight. The following formula can calculate the ash content:

$$\text{Ash content (\%)} = \frac{weight\ of\ ash}{initial\ weight\ of\ sample} \times 100\%$$

Crude protein determination

Crude protein was determined by the Kjeldahl method. The sample was first digested using concentrated sulphuric acid, copper sulphate, sodium sulphate and a speck of selenium tablet until the digest became pale green. The mixture was left completely cold, and distilled water was added. The digest was undergoing distillation; sodium hydroxide was added to the marshal distillation apparatus and allowed to boil. The mixture was distilled into 2% boric acid containing screened methyl red indicator. In the end, the alkaline ammonium borate formed was titrated directly with 0.1N HCl. The titre value of the acid used was recorded, and the crude protein was calculated by the formula below:

$$\text{N (\%)} = \frac{14 \times VA \times 0.1 \times w}{1000 \times 100} \times 100$$

VA = volume of acid used; w = weight of the sample

Crude protein (%) = N (%) x 6.25

Crude lipid determination

Extraction of ether was carried out by mixing the sample with anhydrous diethyl ether and the mixture was boiled for 4 hours (adopted from Bhuiyan et al., 2016 with modification). After the extraction, the sample was dried at 65□C for 4 hours. The sample was allowed to cold and weighted.

$$\text{Crude lipid (\%)} = \frac{weight\ of\ extract}{weight\ of\ initial\ sample} \times 100\%$$

Crude fiber determination

The fat-free sample from ether extraction was used to determine the crude fiber in the sample (modified from Oladipo & Bankole, 2013). The fat-free sample was added to pre-heated 1.25% H_2SO_4 and boiled for 30 minutes. The sample was filtered and washed with hot water and ethanol. The residue was dried at 65□C for 24 hours. The cooled sample was weighed and recorded. The residue was ashed in a muffle furnace at 600□C for 4 hours. The ash was cooled and weighed.

$$\text{Crude fiber (\%)} = \frac{dry\ weight\ of\ residue\ before\ ashing - weight\ of\ residue\ after\ ashing}{weight\ of\ sample} \times 100$$

Nitrogen-free extract (NPE) determination

Nitrogen content in the sample was determined by subtracting the percentage of all the nutrients already determined from 100 (Bhaskar et al. 2015).

$$\text{NPE (\%)} = 100 - [Moisture\ content\ (\%) + Crude\ fiber\ (\%) + Crude\ protein\ (\%)\ \ Crude\ lipid\ (\%) + Ash\ (\%)]$$

Mycotoxin determination in a fish feed sample

The fish feed sample was ground into powder. The mycotoxins in fish feed were extracted with 10mL methyl cyanide (MeCN) containing 2% formic acid. The mixture was then centrifuged, and the supernatant was obtained. The sample was then purified, and the solvent was exchanged with MeOH: H_2O (50:50, v/v). For mycotoxins quantification, 10□L of the sample was injected into the

HPLC column, which was heated to 45□C. 10mM ammonium formate and MeOH were used as mobile phases with a flow rate of 300□L/min. The sample was run for 21 minutes (including 5 minutes of equilibration). The mycotoxin determination method was adapted from the Association of Official Analytical Chemists (AOAC) official method 991.31. The tested parameters are aflatoxin (B1, B2, G1, G2), deoxynivalenol (DON), zearalenone, t-2 Toxin, fumonisins (B1, B2) and ochratoxin A. All the test parameters were expressed in ppb.

Macro and micronutrients in K. alvarezii fish feed

The macro and micronutrients in *K. alvarezii* fish feed were carried out using the Association of Official Analytical Chemists (AOAC) official method 999.11. AOAC standard analytical methods are used to analyze different sources, including fertilizers, food and waste related to agriculture and the environment. The macro and micronutrients are phosphorus, potassium, calcium, sulfur, magnesium, chloride, manganese, iron, zinc and copper. All the test parameters were expressed in ppm.

Culture tank design and set up

The aquaculture system consists of a fish tank and a settling tank. Water from the fish tank was directed to a settling tank to remove all the solid waste produced from the fish and food. The settling tank consisted of a pump, a biofilter and a compartment for filtered water. The recirculating water system was operated for 12 weeks with 9 hours light/15 hours dark.

Thirty *B. schwanenfeldii* fish (≈20g) was randomly divided into two groups: group I – feed with commercial fish feed (control); group II – feed with *K. alvarezii* fish feed (experimental group). All the fishes were fed twice per day (0900 and 1600), and the following equation determined the feeding amount:

Amount of feed = average fish weight (g) x 5% of fish weight x total number of fish

The body weight of *B. schwanenfeldii* fish was measured weekly. At 12th week, the fish starved for 24 hours, and sacrificed by chilling on ice. The *B. schwanenfeldii* carcasses were stored at -80□C for further usage.

The final body weight of *B. schwanenfeldii* fish was measured. The specific growth rate (SGR) was calculated according to the following equation:

$$\text{SGR (\% body wt. gain/day)} = \frac{Log(final\ weight) - Log(initial\ weight)}{t} \times 100\%$$

Water parameters in an aquaculture system

Water parameters in the aquaculture tank were determined using LAQUA Twin Compact Meters (pH, temperature, ammonia, nitrite and nitrate) and a dissolved oxygen meter weekly. All the test parameters were expressed in ppm.

Microbe on the B. schwanenfeldii

B. schwanenfeldii fish were harvested and undergoing microbe examination. For microbe examination, aerobic plate counts (AOAC official method 990.12), yeast (AOAC official method 2014.05), mould (AOAC official method 2014.05), coliform (AOAC official method 990.08 & 991.14), *E. coli* coliform (AOAC official method 990.08 & 991.14), *Staphylococcus aureus* (AOAC official method 2003.11, 2003.07 & 2003.08) and salmonella (AOAC official method 2014.01) were examined. The samples were examined by using the AOAC official method accordingly.

Results

Proximate analysis of K. alvarezii fish feed

Proximate analysis was carried out for the *K. alvarezii* fish feed sample (dry). The fish feed sample is found to be high in nitrogen content and low in moisture, ash and fiber content. The proximate analysis for *K. alvarezii* fish feed is shown in Table 2.

Table 2. Proximate analysis of *K. alvarezii* fish feed

Parameters	***K. alvarezii* fish feed** (mean□SD %)
Moisture content	6.40□0.1
Ash	4.50□0.1
Crude protein	27.00□0.1
Crude lipid	11.10□0.2
Crude fiber	4.75□0.1
Nitrogen free extract	46.30□0.1

Mycotoxin in fish feed

Mycotoxin in fish feed was carried out. The tested mycotoxins are Aflatoxin (B1, B2, G1, G2), deoxynivalenol (DON), zearalenone, t-2 Toxin, fumonisins (B1, B2) and ochratoxin A. None of the tested mycotoxins was found in the fish feed sample. The mycotoxin in fish feed is shown in Table 3.

Table 3. Mycotoxin in fish feed

Test parameters	Unit	Reading
Aflatoxin (B1, B2, G1, G2)	ppb	ND (< 0.1)
Dexoynivalenol (DON)	ppb	ND (< 5)
Zearalenone	ppb	ND (< 10)
t-2 Toxin	ppb	ND (< 5)
Fumonisins (B1, B2)	ppb	ND (< 50)
Ochratoxin A	ppb	ND (< 0.5)

Value are mean□SD. ND denotes not detected.

The *K. alvarezii* solid wastes are rich in phosphorus, potassium, calcium and sulfur. The macro and micronutrients in *K. alvarezii* solid waste is shown in Table 4.

Table 4. The macro and micronutrients in *K. alvarezii* solid waste

Test parameters	Reading
Phosphorus	404.3□0.14 ppm
Potassium	1230.0□0.07 ppm
Calcium	969.0□0.42 ppm
Sulfur	4692.7□66.26 ppm
Magnesium	297.0□0.07 ppm
Chloride	207.2□0.14 ppm
Manganese	2.4□0.02 ppm
Iron	50.2□0..01 ppm
Zinc	9.56□0.03 ppm
Copper	11.96□0.02 ppm

Value are mean□SD. ND denotes not detected.

The specific growth rate of B. schwanenfeldii fish

The specific growth rates for *B. schwanenfeldii* fish in the control and experimental group were not significantly different ($P > 0.05$). However, no significance was found between the groups but the specific growth rate in control group is relatively higher than the specific growth rate in the experimental group. The specific growth rate for *B. schwanenfeldii* fish in the control and experimental group is shown in Table 5.

Table 5. Specific growth rate between the control group and experimental group

Group	Specific growth rate (%)
Control group	0.36□0.03[a]
Experimental group	0.34□0.02[a]

Values are mean□SD. Different superscript letters in each row indicate a statistical significantly different at $P < 0.05$.

Proximate analysis of B. schwanenfeldii fish in the control and experimental group.

Proximate analysis of *B. schwanenfeldii* fish in control and experimental groups was carried out. Moisture content, ash, crude protein and fat in the *B. schwanenfeldii* fish were measured. The proximate analysis for the *B. schwanenfeldii* fish is shown in Table 6.

Table 6. Proximate analysis of *B. schwanenfeldii* fish for the control and experimental groups

Parameters	Control group	Experimental group
Moisture content	19.20□0.1[a]	15.60□0.1[b]
Ash	7.00□0.2[a]	10.50□0.2[b]
Crude protein	74.70□0.1[a]	70.00□0.1[b]
Crude lipid	3.5□0.1[a]	2.60□0.1[b]

Values are mean□SD. Different superscript letters in each row indicate a statistical significantly different at $P < 0.05$.

Water parameters in an aquaculture system

For the 12 weeks of study, the water quality of the aquaculture was maintained at pH (7.25□0.10), temperature (24.10□0.21□C), DO (6.51□0.03ppm), ammonia (0.00ppm), nitrite(0.00ppm) and nitrate (59.42□3.12ppm).

Microbiology tests on the B. schwanenfeldii fish

For the microbiology test on *B. schwanenfeldii* fish, aerobic plate counts, yeast, mould, coliform, *E. coli*, *Staphylococcus aureus*, and *salmonella* were tested. Results of the microbiology examination of *B. schwanenfeldii* fish is shown in Table 7.

Table 7. Microbiological test on *B. schwanenfeldii* fish

Microbes	Reading
Aerobic plate counts	1.3×10^4 (35□C, CFU/g)
Yeast	20 (25□C, CFU/g)
Mould	90 (35□C, CFU/g)
Coliform	40 (35□C, CFU/g)

Table 7. Continued

Microbes	Reading
E. coli	ND
Staphylococcus aureus	ND
Salmonella	ND

ND denotes not detected.

Discussion

Moisture content in the feed is a common property of food materials, and it is important to ensure feed quality and stability. The moisture content in *K. alvarezii* fish feed is 6.40□0.1%, and the suggested moisture content is usually less than 10%. Thus, the moisture content in the *K. alvarezii* fish feed fits the standard. The moisture content in the feed will interfere with the microbial stability where the tendency of microorganisms to grow in the feed relies on the water container. Besides, the fish feed quality, such as texture, taste and stability, also depends on the water contained. Furthermore, the feed's moisture content impacts feed intake and nutrient utilization of the animal (Oehme et al., 2014).

The growth performance of the fishes is dependent on the feed nutrients. Thus the correct balance of proteins, carbohydrates, fats, vitamins and minerals is required for the fish to grow and be healthy. The crude protein in the *K. alvarezii* fish feed is 27.00□0.1%. Protein is the most important component in the fish feed, which helps to build up the fish mass. Omnivorous fish such as *B. schwanenfeldii* need around 25 to 35% of their diet's protein to grow at optimal levels. Proteins are built by different amino acids to form other structures and enzymes in living organisms (Gatlin, 2010). Therefore, *K. alvarezii* fish feed would supply a good source of protein for the *B. schwanenfeldii*.

Ash content represents the total mineral content in the feed, and the mineral content such as Ca, Na, K and Cl are the specific inorganic components present. The ash content in *K. alvarezii* fish feed is 4.50□0.1%. Generally, the feed's ash content is approximately 5% (Hoffman, 1993). The ash content in feed correlated with the quality, microbiology stability and nutrition in the fish feed. High mineral content in the feed can be used to prohibit the growth of certain microorganisms, and the mineral elements such as Ca, P, K, and Na are needed for the fishes to grow in a healthy (Shearer et al., 1992; Cai-Juan et al., 2016).

Furthermore, lipid nutrition in fish feed is essential to supply essential fatty acids such as long chain polysaturated fatty acids (PUFA), -Linolenic Acid (LNA, 18:3ω3), Linoleic Acid (LA, 18:2ω6), eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). In this study, fatty acid experiments were not included. The crude lipid content in *K. alvarezii* fish feed is 11.10□0.2%, whereas the suggested lipid content in fish feed is 10 to 15%. Dietary lipid is needed for

membrane structural components and acts as precursors of steroid hormones and prostaglandins in fish (Craig & Helfrich, 2002; Hixson, 2014).

There are various sizes and complexity of carbohydrates that are reduced by plants reduces. Inexpensive carbohydrates are used as an energy source for the fish. The crude fiber content in *K. alvarezii* fish feed is 4.75□0.1%, and the general fish feed content is usually less than 7%. This is because fish cannot digest high content of crude fiber; thus, the crude fiber must be kept at a very low-level (Gatlin, 2010). The nitrogen-free extract in *K. alvarezii* fish feed is 46.30□0.1%, where the nitrogen-free extract is made up of carbohydrates such as sugars and starches. It usually contained 25 to 45% in fish feed to provide good integrity and stability and make the pellet less dense (Dhaneesh et al., 2012).

Mineral elements in fish feed are essential for tissue formation, osmoregulation and metabolic functions. *K. alvarezii* solid waste is rich in calcium, phosphorus, magnesium, chloride, potassium and sulfur. The minerals are macro minerals needed in large quantities in the diet and stored in the body. Among the macro minerals, phosphorus is the most critical macro mineral. This is because phosphorus only exists a little in water. Phosphorus is an essential mineral to build up the bone and scales in various biochemical. Besides, chloride and potassium are crucial electrolytes for osmoregulation and acid-base balance in the body. Furthermore, magnesium is needed for intra and extracellular homeostasis and cellular respiration (Tacon, 1987; Warne, 2014; Mohanty et al., 2016). Micro mineral or trace minerals found in *K. alvarezii* solid waste included copper, iodine, iron, manganese and zinc. Micro minerals are needed in low amounts. Although these minerals are required in low doses, they must form a complete diet for the fish to grow.

Mycotoxins are toxic chemicals produced by several species of moulds such as *Aspergillus*, *Penicillium* or *Fusarium* genera. In this study, the *K. alvarezii* fish feed is free from mycotoxin. Mycotoxin is Aflatoxin (B1, B2, G1, G2), deoxynivalenol, zearalenone, t-2 Toxin, fumonisins (B1, B2), and ochratoxin A. Contamination of mycotoxin in fish feed is usually related to the storing method. When the fish feed is exposed to the air with high moisture conditions, it may produce mycotoxin. Ingestion of mycotoxin-contaminated feed might cause the fish to lose weight, immune impairment and organ lesions (Manning, 2010; Anater et al., 2016; Marijani et al., 2017; Matejova et al., 2017). Fish feed is the nutrient source for the fish. Thus, ensuring that the fish feed is free from mycotoxin is essential.

After 60 days of feeding of *K. alvarezii* and commercial feed for experimental and control groups, the specific growth rate for *B. schwanenfeldii* fish in the control and experimental groups is not significantly different. The growth of the *B. schwanenfeldii* fish is highly correlated with the supplemented fish feed. *K. alvarezii* fish feed provides a good source of protein, fat, and macro and micronutrients. It thus can supply sufficient nutrients that are needed by the *B. schwanenfeldii* to

grow. Compared to commercial feed fed to the control group, no significant difference was found in the specific growth rate. Therefore, *K. Alvarezii* fish feed is suitable for *B. schwanenfeldii* fish.

Although no significant difference was found in the specific growth rate between the control and experimental groups, there is a significant difference in proximate analysis. According to the commercial fish feed labelling, the proximate analysis for the fish feed is ash (≤ 17%), crude protein (≥ 40%), crude lipid (≥ 9%) and crude fiber (≤ 2%) (Sutharshiny and Sivashanthini 2011; Njinkoue et al. 2016). Compared to *K. alvarezii* fish feed, the commercial fish feed has a higher crude protein and lipid content; thus, it has more protein and fat content for the *B. schwanenfeldii* fish. In Table 5, crude protein and crude lipid in the control group are significantly higher than in the experimental group. Since the commercial fish feed has higher protein and fat content, thus *B. schwanenfeldii* fish in the control group tend to have higher protein and fat content than the experimental group.

Furthermore, the growth of *B. schwanenfeldii* fish also depends on the water quality. *B. schwanenfeldii* is warm water fish that can be found in Southeast Asia. In general, the water quality for warm water fish is suggested to maintain at temperature (22-32□C), DO (4-6ppm), pH (6-8.5), ammonia (< 3ppm), nitrite (< 1ppm) and nitrate (< 400ppm). For temperature, it is correlated with the solubility of gases in water, the reaction rate of chemicals and the toxicity of ammonia to the fish. When the water temperature increases, the solubility of oxygen will decrease. Oxygen is one of the factors for the growth and well-being of fish. Oxygen is needed for fish respiration. At optimal temperature, the DO is around 4 to 6ppm. When the DO in water is less than 4ppm, the fish will stop feeding, growth slows down and stress-related begins. Besides that, the pH value in the water is also crucial for fish health. The fish can die from pH shock resulting from the sudden change of pH in the water. Despite that, the ammonia content in the water is excreted from the fish gills and as urine. Besides, solid waste contributes to the ammonia content in the water. The high ammonia content is toxic to fish, destroying the fish's central nervous system and gills, resulting in impaired respiration and convulsions. The toxicity of ammonia is also correlated with pH and temperature, where at higher pH value and temperature, the ammonia is more toxic to the fish. Ammonia in the water will be nitrified by bacteria and converted into nitrite and nitrate. Nitrite is toxic to the fish, and a high nitrite level may cause the fish to die rapidly. Furthermore, nitrate is less toxic and more tolerable to the fish. The tolerating level of nitrate for the fish is as high as 400ppm (Myrick, 2011; Kasnir et al., 2014; Moffat, 2017). Thus, the water quality in the study was maintained at pH (7.25□0.10), temperature (24.10□0.21□C), DO (6.51□0.03ppm), ammonia (0.00ppm), nitrite(0.00ppm) and nitrate (59.42□3.12ppm).

The microbiological testing for food samples is to ensure food safety. Aerobic plate count, yeast, mould, coliform, *E. coli*, *Staphylococcus aureus* and *Salmonella* are the most common food safety microbiological tests. Aerobic plate count is one of the most common tests used to investigate the microbial quality of the food (Broekaert et al., 2011; Sanjee & Karim, 2016). In general, the

microbial quantity should be less than 10^6 CFU/g. The aerobic plate count for the *B. schwanenfeldii* fish sample is 1.3.104 CFU/g which fits the food safety guideline. Yeast and mould are always found in food and water. Existing of this bacteria in food is always correlated with food spoilage. Thus, the microbiological quantity for yeast and mould should always be less than 1000 CFU/g. For *B. schwanenfeldii* fish sample, the yeast (20 CFU/g), Mould (90 CFU/g) are much lower than the limit (van den Broek et al. 1984). Furthermore, coliform is naturally found in the intestines of animals, and the presence of coliform indicates the possibility of being contaminated by the faecal (Varga & Anderson 1968). The contaminant limit for coliform is less than 100 CFU/g, and the coliform found in *B. schwanenfeldii* fish sample is 40 CFU/g (HPA, 2009).

Despite that, the pathogenic bacteria; *E. coli*, *Staphylococcus aureus* and *Salmonella* are absent in the *B. schwanenfeldii* fish sample. *E. coli* is an undesirable bacterial in food, indicating poor hygienic conditions. In nature, *E. coli* should not be found in fish except in grossly polluted waters (Teophilo et al., 2002). Besides, *Staphylococcus aureus* is naturally present in human skin, hair and superficial mucous but not on fish and fish products. The presence of it indicates the presence of enterotoxin and the sanitary or production practice (Simon & Sanjeev, 2007; Saito et al., 2011; Obaidat et al., 2015). Lastly, the presence of Salmonella in food also indicates poor food handling, such as cross-contamination. *Salmonella* can cause fever, diarrhoea, intestinal cramps and vomiting. Thus it is important to eliminate salmonella from the food, especially in the ready-to-eat food (Heinitz et al., 2000; Zhang et al., 2013).

Conclusion

Kappaphycus alvarezii fish feed is rich in macro and micronutrients where it can supply balanced nutrients for the *B. schwanenfeldii* fish. Besides, the *K. alvarezii* fish feed could provide a good source of protein and fat for the *B. schwanenfeldii* fish to grow. Furthermore, the *K. alvarezii* fish feed is free from mycotoxin; thus, it is safe to be consumed by the *B. schwanenfeldii* fish. The specific growth rate for both control and experimental groups was not significantly found. Although the crude protein and fat content in the control group are significantly higher than in the experimental group, K. alvarezii fish feed is still a potential fish feed that could be used to grow *B. schwanenfeldii* fish. Lastly, there are no pathogenic bacteria found in *B. schwanenfeldii* fish. Although a small amount of yeast, mould and coliform were detected, the microbial quantity is within limits.

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