

RESEARCH ARTICLE

Nutrient digestibility of green seaweed *Ulva* meal and the influence on growth performance of Nile tilapia (*Oreochromis niloticus*)

Lusi Herawati Suryaningrum*, Reza Samsudin

Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Sempur 1, Bogor, West Java, Indonesia

ABSTRACT

Green seaweed *Ulva* had a high growth rate and spread in all of Indonesian waters. Species *Ulva* had a complete nutrient content that made it potential to be used as an alternative fish feed ingredient. This experiment was conducted in two series of trial, digestibility measurement of *Ulva* meal and its effects on the growth performance of the Nile tilapia (*O. niloticus*). In measuring the nutrient digestibility, a protein rich diet was formulated with a test diet in which 30% of the reference diet was replaced with *Ulva* meal, used Cr_2O_3 as an inert marker. Fish with initial weight about 16 ± 0.35 g were stocked with a density of 15 fish per aquarium and collecting faeces is carried out until sufficient sample was available for analysis. The growth trial was conducted using a completely randomized design with four treatments and three replications. The experimental diets were U_0 (*Ulva* 0%), U_{10} (*Ulva* 10%), U_{20} (*Ulva* 20%) and U_{30} (*Ulva* 30%). The Nile tilapia with an average body weight of 3.42 ± 0.01 g, were stocked into the 60x50x50 cm aquaria with 25 fish in each tank and cultured for 60 days. Fish were fed three times daily on those diets at satiation level. The parameters which evaluated were specific growth rate, protein retention, lipid retention, feed efficiency, survival rate and blood hematology of the Nile tilapia. The results of digestibility measurement showed that *Ulva* meal had acceptable nutrients as an ingredient in Nile tilapia diet. Protein digestibility of *Ulva* meal was 87.36%, while lipid was 93.05%. The results of growth trial revealed that fish fed on 10% *Ulva* meal tended to have highest growth performance than those fed to the control, while the survival rate and fish health seemed not to be affected directly ($P > 0.05$).

Keywords: Growth performance; Nile tilapia; Nutrient digestibility; Survival rate; *Ulva*

INTRODUCTION

The need for alternative sources for aquafeeds has recently renewed interest in ingredients that are generally derived from plant origin, mainly terrestrial and also aquatic (Wassef et al., 2013). The use of local ingredients is an effort to reduce dependency on imported ones. The research for alternative materials has been going on for two decades (Viscaino et al., 2015). Local ingredients which have been widely used in fish feed were cassava leaves (Madalla et al., 2016), cassava peel (Tachnia et al., 2016), rubber seeds (Femi et al., 2016) and cake palm oil (Adjanke et al., 2016). However, the use of local ingredients of terrestrial plants is constrained by the high content of crude fiber and the presence of anti nutrient factors such as phytic acid and cyanide, so other materials need to be sought as fish feed ingredients.

Seaweed has potentially to be used as an alternative of fish feed ingredient because it has complete nutrient content. Seaweed has a nutrient composition that varies greatly depending on season, geography, species, harvest age and environmental conditions (Denis et al., 2010). The main content of seaweed in general is a complex of polysaccharide, protein, lipid, ash, vitamins and minerals. Seaweed is easier to digest than lignocellulosic materials from terrestrial plant (Smith et al., 2010). Research that has been carried out in order to use seaweed as fish feed ingredient, has interesting results. Pratiwy et al. (2018) stated that the addition of 8% *Sargassum* meal gave the best results on the growth of Nile tilapia. Then Shapawi and Zamry (2016) reported that 50% inclusion of seaweed meal from *Kappaphycus alvarezii*, *Eucaema denticulatum* and *Sargassum polycystum* in *Lates calcarifer* diet, did not affect the growth and feed conversion ratio. Younis et al. (2018) stated that the use of red algae (*Gracilaria arcuate*) less than 20%

*Corresponding author:

Lusi Herawati Suryaningrum. Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE). Sempur 1, Bogor, West Java, Indonesia. Tel: +62 251 8313200, E-mail: lusihera@yahoo.co.id

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in diet, gives the best results on growth performance, feed utilization and body composition of Nile tilapia.

In the past decade, species from the genus *Ulva* have been widely used because of the high bioactive component with diverse biological activities (Taboada et al., 2010). Suryaningrum et al. (2017) reported that seaweed *Ulva* which was taken from Pulang Sawal Beach, Yogyakarta Special Region, Republic of Indonesia has a protein content of 13.65%, 0.53% lipid, 33.19% ash, 9.2% crude fiber and carbohydrate (NFE) 43.51%. Anti nutrient factors which are usually found in plant such as oxalate, phytate, tannin and carbonate are not found in *Ulva* (Abirami and Kowsalya, 2011). Amino acids contained in *Ulva* most are essential amino acids (Benjama and Masniyom, 2011), whereas fatty acid types that have double bonds reach up to 50% of all detected fatty acids (Tabarsa et al., 2012).

Seaweed *Ulva* thrives and have a very fast growth rate in locations that provide the abundance nutrients (Wassef et al., 2013). The results of the study which conducted by Chen et al. (2015) reported that the growth rate of *Ulva* reached 5.22% day⁻¹. Even some areas report the uncontrolled of *Ulva* growth (Li et al., 2016). Based on nutrient content and growth rate, *Ulva* potential to be used as fish feed ingredient. This study aims to evaluate the digestibility of *Ulva* meal and its influence on the growth and survival rate of Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

The present experiment was carried out in Fish Nutrition and Feed Technology Laboratory, Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Bogor, West Java, Indonesia. This experiment was conducted in two series of trial, digestibility measurement of *Ulva* and growth performance of Nile tilapia fed diets with different levels of *Ulva* meal.

Preparation of *Ulva* meal

Green seaweed *Ulva* was collected from Binuangeun Beach, West Java, Indonesia. Seaweed was thoroughly washed with fresh water and then sun dried for 4 h in an oven at 60°C for 48 h until complete dryness. Then the dried seaweed milled in a mechanical grinder to obtain a homogenous powder (Fig. 1). The chemical composition of *Ulva* meal was determined through laboratory analysis to AOAC (2005) as shown in Table 1.

Trial 1- Digestibility measurements in the Nile tilapia (*O. niloticus*)

Diets preparation

The digestibility measurement was carried out based on the Takeuchi (1988) method, where the experimental diets composed of 70% reference diet and 30% of *Ulva* meal as an ingredient that will be tested (Table 2). The diet was formulated to satisfy nutrients requirement of Nile tiapia. Chromic oxide (Cr₂O₃) was used as an inert marker at 0.6% in reference and test diets. All ingredients thoroughly mixed until homogenous. The 2 mm diameter pellets were wet-extruded, dried in a convection oven at 60°C for 12 h and sealed in plastic bags until used.

Faeces collection

Nile tilapia (*O. niloticus*) were obtained from the Research Station for Freshwater Fisheries Germplasm, Cijeruk, Bogor. Before starting trial, fish were acclimated and fed with the reference diet for 7 days. At the beginning of the experiment, fish with initial weight about 16±0.35 g were stocked with a density of 15 fish per aquarium. The system was provided with continuous aeration to maintain the dissolved oxygen level above saturation. Fish fed 7 days before the beginning of faecal collection. Faecal collected dried in an oven with a temperature of 60°C for 48 h, sealed in plastic container and stored in 5°C until use (Fig. 2). Collecting faeces is carried out until sufficient sample was available for chemical analysis. The diet digestibility was determined by equation proposed by Goddard and McLean (2001). The ingredient digestibility was determined by equation proposed by Bureau and Hua (2006).



Fig 1. Preparation of *Ulva* meal.

Table 1: Nutrient composition of ulva meal

Parameters	(% dry matter)
Moisturize	7.85±0.03
Crude protein	15.21±0.05
Lipid	1.07±0.07
Ash	27.84±0.11
Crude fiber	10.34±0.27
Nitrogen Free Extract (NFE) ^a	45.56±0.39
Gross Energy (GE) ^b	282.38±4.26

Values as mean±SD. ^a Calculated by difference. ^b Expressed in kcal.g⁻¹, calculated according to energy values of 1 g protein = 5.6 kcal, 1 g lipid = 9.4 kcal, 1 g carbohydrate = 4.1 kcal (Watanabe, 1988).

Table 2: Ingredient of the reference and test diets (% dry matter)

Ingredients	Reference diet	Test diet
Basal diets	96.4	66.4
Ulva meal	0.0	30.0
CMC	3.0	3.0
Cr ₂ O ₃	0.6	0.6
Total	100.0	100.0

**Fig 2.** Faecal collection.

Trial 2- Growth performance of Nile tilapia

Experimental diets

Growth performance measurement of Nile tilapia was conducted using a completely randomized design (CRD) with 4 treatments and 3 replications, i.e. U₀ (Ulva 0%), U₁₀ (Ulva 10%), U₂₀ (Ulva 20%), U₃₀ (Ulva 30%). Those diets were isonitrogenous (28±0.5%) and isoenergetic (4100±100 kcal.kg⁻¹) which fulfills the requirement for tilapia. The experimental diets for growth performance was shown in Table 3, while proximate analysis was in Table 4. All dietary ingredients were blended thoroughly, moistened, pelleted and dried at 60°C for 24 h. Diets were stored in hermetic plastic until fed (Fig. 3).

Experimental fish, rearing condition and feeding regime

The Nile tilapia (*O. niloticus*) were collected from the Research Station for Freshwater Fisheries Germplasm, Cijeruk, Bogor. Fish were acclimatized for 14 days prior to the feeding trial. Each experimental diet was fed to triplicate group of 25 Nile tilapia with an average initial body weight of 3.27±0.11 g. The growth trial lasted for 60 days and was carried out in 150 L glass aquaria which equipped with a recirculation system (Fig. 4). The diets were fed to apparent satiation level three times daily. The weight of each fish was measured at the beginning and at the end

Table 3: Ingredient composition of experimental diets (% dry matter) containing ulva meal in different levels

Ingredients	Experimental diets			
	U ₀	U ₁₀	U ₂₀	U ₃₀
Fish meal	17.12	16.49	15.91	13.80
Ulva meal	0.00	10.00	20.00	30.00
Soyabean meal	35.08	30.55	26.00	23.00
Wheat bran meal	37.84	33.00	28.00	23.00
Cassava meal	3.00	3.00	3.00	3.00
Fish oil	0.60	0.50	0.50	0.50
Corn oil	0.00	0.20	0.40	0.60
Vegetable oil	3.30	3.20	3.10	3.00
Vitamin/mineral mix	3.06	3.06	3.06	3.06
Total	100.00	100.00	100.00	100.00

Table 4: Proximate analysis of experimental diets (% dry matter) containing ulva meal in different levels

Parameters	Experimental diets			
	U ₀	U ₁₀	U ₂₀	U ₃₀
Crude protein	28.83	28.76	28.65	28.61
Lipid	5.79	5.96	5.65	5.86
Ash	10.01	11.21	12.50	14.05
Crude fiber	5.63	5.62	5.58	5.35
Nitrogen Free Extract (NFE) ^a	49.73	48.46	47.63	46.13
Gross Energy (GE) ^b	4197.73	4157.66	4088.33	4044.37

Values as mean ± SD. ^a Calculated by difference. ^b Expressed in kcal.g⁻¹, calculated according to energy values of 1 g protein = 5.6 kcal, 1 g lipid = 9.4 kcal, 1 g carbohydrate = 4.1 kcal (Watanabe, 1988).

of the experimental period. Water quality parameters were monitored and remained at acceptable levels throughout the experimental period, temperatures ranged 27–29°C, pH 6.9–7.3 and dissolved oxygen 6.1–6.9 mg.L⁻¹. Hematology parameters of experimental fish was carried out at the end of the experimental period.

Determination of growth parameters

Following parameters calculated from the data were: feed efficiency (FE) = (Wt - Wo) × F⁻¹, protein retention (PR) = 100 × (Pt - Po) × P⁻¹, lipid retention (LR) = 100 × (Lt - Lo) × L⁻¹; specific growth rate (SGR) = 100 × (ln Wt - ln Wo) × t⁻¹, survival rate (SR) = 100 × (final number of fish × initial number of fish⁻¹). The symbols in the equations are as the follows: Wo and Wt—the initial and the final average fish body mass (g); t—number of days of feeding trial; F—the total amount of feed per fish consumed during growth test (g); P—protein intake (g); Pt—final protein content in fish body (g); Po—initial protein content in fish body (g); Lt—final lipid content in fish body (g); Lo—initial lipid content in fish body (g); L—lipid intake (g).

Chemical analysis

Proximate analysis was carried out on feedstuffs samples, experimental diets, dried feces and whole-body fish before and after experimental period using the AOAC method (2005). Analysis of chromium (Cr₂O₃) in experimental

diet and dried faeces was carried out using the Takeuchi (1988) method. The following methodology was adopted for blood analysis: erythrocytes and leucocytes (Blaxhall and Daisley, 1973), hemoglobin (Wedemeyer and Yasutake, 1977) and hematocrit (Anderson and Siwicki, 1993).

Statistical analysis

The value of parameters was presented as means \pm standard deviation (SD). Experimental data were analyzed as one-way completely randomized designs to ANOVA ($P < 0.05$) and Duncan's multiple range test using SPSS version 23.00 statistical package.

RESULTS

The results revealed that Ulva meal had acceptable nutrient content as an ingredient in Nile tilapia diet. Lipid showed the highest, then followed by protein. These result was exhibited in Table 5.

Growth performance and feed utilization of diets during experimental period is shown in Table 6. The results showed that the feed intake was not significantly different for all

treatments ($P > 0.05$). During the 60 days of experimental period, the weight of tilapia increased 5.62 to 7.03 times from the initial weight. Fish fed with diet containing 10% Ulva (U_{10}) had the largest body weight at 7.03 times at the end of experimental period ($P < 0.05$). While fish fed with diet containing of 30% Ulva (U_{30}) had the lowest growth ($P < 0.05$). Feed efficiency, protein retention and specific growth rate have the same pattern. Thus the tilapia fed with diet containing of 10% Ulva (U_{10}) has the highest growth performance when compared with other treatments ($P < 0.05$). Lipid retention in fish bodies tends to have inversely proportional patterns compared to other growth performance parameters. The survival rate of tilapia ranges from 98.00 to 98.67% and not significantly different for all treatments ($P > 0.05$).

Blood hematology parameters can be used to diagnose fish health status. The results of hematological measurement of experimental fish are shown in Table 7. Total erythrocytes ranged from $1.34\text{--}2.79 \times 10^6 \text{ mm}^{-3}$, leucocytes 8.04--



Fig 3. Experimental diet.

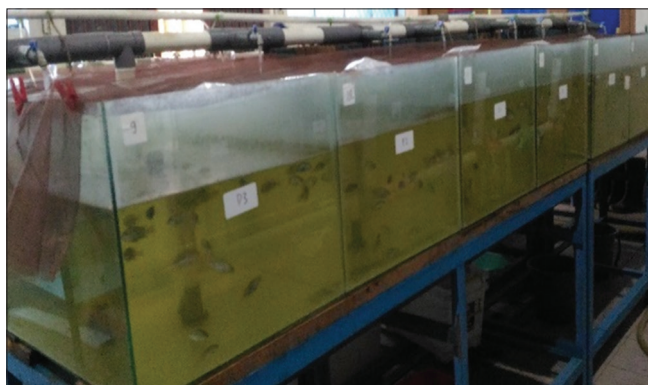


Fig 4. Growth trial.

Table 5: Nutrient digestibility of ulva meal in the nile tilapia

Parameters	(%)
Protein	87.36 \pm 3.14
Lipid	93.05 \pm 1.39

Values as mean \pm SD

Table 6: Growth parameters and survival rate of Nile tilapia fed diets with different levels of Ulva meal

Parameters	Experimental diets			
	U_0	U_{10}	U_{20}	U_{30}
W_0 (g fish ⁻¹)	3.42 \pm 0.01 ^a	3.42 \pm 0.00 ^a	3.41 \pm 0.01 ^a	3.42 \pm 0.01 ^a
W_t (g fish ⁻¹)	21.52 \pm 0.54 ^b	24.04 \pm 0.22 ^c	21.84 \pm 1.91 ^b	19.21 \pm 0.50 ^a
FI (g fish ⁻¹)	21.20 \pm 0.15 ^a	20.95 \pm 0.08 ^a	20.60 \pm 0.08 ^a	20.67 \pm 0.14 ^a
FE (%)	84.82 \pm 1.15 ^b	88.55 \pm 1.16 ^c	83.70 \pm 0.76 ^b	76.41 \pm 0.49 ^a
PR (%)	35.17 \pm 1.68 ^b	37.94 \pm 1.24 ^c	35.37 \pm 0.58 ^b	32.33 \pm 0.88 ^a
LR (%)	45.11 \pm 0.98 ^b	42.65 \pm 0.80 ^a	44.61 \pm 0.36 ^b	47.47 \pm 0.47 ^c
SGR (% g.day ⁻¹)	3.07 \pm 0.05 ^b	3.25 \pm 0.02 ^c	3.02 \pm 0.03 ^b	2.88 \pm 0.04 ^a
SR (%)	98.67 \pm 2.31 ^a	98.67 \pm 2.31 ^a	98.00 \pm 2.00 ^a	98.67 \pm 2.31 ^a

Keterangan: Values as mean \pm SD. Values in the same line with different superscript letters are significantly different ($P < 0.05$). W_0 = initial weight, W_t = final weight, FI = feed intake, FE = feed efficiency, PR = protein retention, LR = lipid retention, SGR = specific growth rate, SR = survival rate.

Table 7: Hematology parameters of Nile tilapia fed diets with different levels of ulva meal

Parameters	Pakan uji			
	U_0	U_{10}	U_{20}	U_{30}
Erythrocytes (10^6 mm^{-3})	1.34-2.11	1.92-2.13	2.51-2.76	2.36-2.79
Leucocyte ($\times 10^4 \text{ cell. mm}^{-3}$)	8.74-9.34	8.43-9.03	8.81-9.66	8.04-8.41
Hemoglobin (g.L ⁻¹)	6.11-6.79	5.65-5.99	6.95-7.83	6.08-6.54
Hematocrit (%)	28.14-28.82	26.17-29.89	32.11-34.28	29.47-33.19

$9.66 \times 10^4 \text{ mm}^{-3}$ cells, hemoglobin $5.65\text{--}7.83 \text{ g.L}^{-1}$ and hematocrit $26.17\text{--}34.28\%$.

DISCUSSIONS

The determination of nutrient digestibility is the first step in evaluating the potential of an ingredient for use in the diet of an aquaculture species (Zhou and Yue, 2012). Digestibility measurement shows the amount of nutrients that can be digested and used by fish for metabolism and growth (NRC 2011). Tilapia could digest protein of Ulva at $87.36 \pm 3.14\%$. This was not much different from pollard which is equal to 82.87% (Ribeiro et al., 2011). This result is in line with the research of Koprucu and Ozdemir (2005) who reported that protein digestibility of some ingredients in Tilapia ranged from $75\text{--}95\%$. Koprucu and Ozdemir (2005) and Ribeiro et al. (2011) reported that protein digestibility is an indicator of amino acid digestibility.

Tilapia could digest lipid of Ulva meal at $93.05 \pm 1.39\%$. This result is in line with Sklan et al. (2004) which reported that lipid digestibility in tilapia ranged from 7.20 to 97.50% . Although the lipid content in seaweed is relatively low, but lipid digestibility depends on the composition of fatty acids and the degree of saturation. More carbon atoms in the chain of fatty acids, will decrease the digestibility of lipid, and more double bonds will increase their digestibility. Tabarsa et al. (2012) reported that about 50% of the fatty acids found in Ulva is group of $n\text{-}3$ and $n\text{-}6$ PUFA. In addition, high lipid digestibility is also related to the high activity of lipase enzymes in Nile tilapia (Sargent et al., 1989).

The results of the digestibility measurement showed that tilapia is capable of digesting nutrients contained in Ulva meal, so it can be used as feed ingredients for Nile tilapia. Then Ulva meal used up to 30% in diet to measure the effect of nutrients inside on the growth performance and survival rate of Nile tilapia.

The results of variance analysis showed that using Ulva meal up to 30% in diet did not affect survival rate of tilapia significantly ($P > 0.05$) (Table 6). It showed that the addition of Ulva meal does not have a negative influence on the water quality parameters, so it can support the survival rate of tilapia.

The amount of feed intake that was not significantly different ($P > 0.05$) showed that the addition of Ulva meal up to 30% in diet does not affect palatability and appetite for fish. Fish fed with diet containing 10% of Ulva meal gives the best specific growth rate compared to other treatments ($P < 0.05$). It showed that fish fed with diet containing 10%

of Ulva meal is more optimal in utilizing energy from non-protein energy sources (lipids and carbohydrates) for metabolic and maintenance activities, so the protein contained in diet can be used for growth (Hardy 2010; Sikorska et al., 2012). It meaning more efficient of feed utilization. This is supported by data on protein retention, feed efficiency and lipid retention. Protein retention in fish fed with diet containing 10% of Ulva meal have the highest value compared to other treatments, meaning that the protein stored more in the whole-body fish. The more efficient use of diet is shown by data on feed efficiency (Table 6). Lipid retention value in Nile tilapia decreases along with the increasing of Ulva meal in diet. Setiawati et al. (2003) reported that the decrease in lipid retention indicates that fish can utilize carbohydrates and lipids well, so the protein in diet can be used by cell for protein synthesis. The maximum use of energy derived from lipid and carbohydrates can support the use of protein as a component of fish growth. The growth of experimental fish can also be seen from their weight gain.

The results also showed that the utilization of Ulva meal in diet by 30% produces low growth which is significantly different ($P < 0.05$) with control. This is reinforced by the low feed efficiency value. The growth of Nile tilapia is getting lower along with the increasing Ulva meal in diet, it caused by the higher ash content in the experimental diets (Table 4). According to the statement of Sugiura et al. (1998), consumption of high ash content in diet decreased nutrient absorption and growth.

The decrease in fish growth caused by the use of seaweed in diets was found in previous research results. The use of Ulva rigida seaweed in diet by 20% cause the lower goldfish growth (Diler et al., 2007). Tilapia diet with containing of 15% Ulva rigida cause growth and feed intake as well as low energy utilization (Kut-Guroy et al., 2007). Low growth, survival rate and body composition also occur in rainbow trout with 10% of Sargassum ilicifolium in diet (Zamannejad et al., 2016). Differences in the optimal level of supplementation of seaweed in fish diet can occur due to differences in fish species, size of fish and different research conditions.

Hematological parameters are used to detect the health status of fish (Al-Attar 2005). Fish infected with the disease will experience changes in the number of erythrocytes, leukocytes, hemoglobin and hematocrit. The test results showed that the hematology of fish all treatments was in the range of the value of healthy tilapia. Healthy tilapia has a number of erythrocytes ranging from $2.0 \times 10^4\text{--}3.0 \times 10^6 \text{ mm}^{-3}$ cells; leucocytes $2.0 \times 10^4\text{--}1.5 \times 10^5 \text{ mm}^{-3}$ cells; hemoglobin $5.05\text{--}8.33 \text{ g}\%$ (Salasia et al., 2001) and hematocrit $5\text{--}60\%$ (Sasongko 2001). Overall, the results of

the blood hematology test showed that the utilization of *Ulva* up to 30% in diet proved not to affect the fish health.

The results in this study are in line with several previous research. Natify et al. (2015) reported that the use of *Ulva* up to 10% in Nile tilapia diet, proved not to interfere with growth performance, feed conversion, protein efficiency ratio and somatic index. The results of the study by Mahasu et al. (2016) reported that the use of *Ulva lactuca* up to 12% in Nile tilapia diet not decreased growth performance, while the study of Azaza et al. (2008) reported that the use of *Ulva* meal in tilapia diet up to 20% does not have a negative impact on growth performance, survival, feed conversion and protein efficiency ratio, use of *Ulva* meal will have a negative impact on growth when the inclusion level in diets reaches 30%.

CONCLUSION

The result of this study showed that green seaweed *Ulva* was found to be digested and utilized by Nile tilapia. Dietary inclusion at 10% *Ulva* meal could be potentially used and further investigations are required to evaluate the optimum dietary inclusion level between 10-20% of *Ulva* meal in Nile tilapia diets to enhance growth performance, feed utilization and survival rate.

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Author's contributions

Lusi Herawati Suryaningrum, collected the experimental data, interpreted & analyzed the results and drafted the manuscripts. Reza Samsudin, assisted in conducting the experiments, paper writing and corrected the english text.

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