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Investigations on the use of exopolysaccharide derived from mycelial extract of *Ganoderma lucidum* as functional feed ingredient for aquaculture-farmed red hybrid Tilapia (*Oreochromis* sp.)

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ABSTRACT

There is growing interest in medicinal fungi for promoting immunomodulation benefits in humans and animals. In this study, exopolysaccharide (EPS) from *Ganoderma lucidum* was used in feed (EFF) on production of farmed-red hybrid Tilapia performance for sustainable aquaculture. The feeding trial was conducted for a period of 42 days. Tilapia weight gain (WG) was significantly the highest (18.75 ± 0.03 g/fish) using 3 g/kg of EFF compared to control (0 g/kg EFF; 12.4 g/fish). Fish fed with 3 g/kg of EFF were shown to be healthiest as attested by measured organosomatic indices that achieved an hepatosomatic index (HSI) of 2.22% and condition factor (CF) of 1.46% compared to control (1.97% HSI; 1.60 CF). Antioxidant activity of Glutathione S-transferase (GST) exhibited significantly lower activities in 1 and 2 g/kg of EFF while increasing catalase (CAT) activity was detected in higher EFF (2-3 g/kg of EPS). Muscle compositions were not affected by the EFF feeding regimes. In contrast, EFF feeding produced significantly higher haemoglobin (7.43 g/dl), haematocrit (37.5%), red blood cell (2.69 10^6/mm^3), and white blood cell (176.7 10^6/mm^3) compared to control group. In summary, EFF was beneficial in promoting growth, stimulating antioxidant enzymes, and health status of red hybrid Tilapia, which highlights the potential for future use in intensive sustainable aquaculture practices.

1. Introduction

Aquaculture is a rapidly growing food sector that has been characterised by considerable investment in many parts of the world including Malaysia (O'Neill et al., 2019; Tahar et al., 2018a; Tahar et al., 2018b; Ruiz-Salmón et al., 2020). It has been identified as a sustainable solution for addressing problems of depleting natural wild fish stock globally (O'Neill et al., 2020). Rowan and Galanaksi (2020) have also described the important role of developing intensive aquaculture practices for transitioning beyond the global economic recession, which has been caused by ongoing COVID-19 pandemic. Over the past four decades, a significant increase in fish supply has been achieved through the intensity of aquaculture production that has led to a stressful environment for the fish cultured in captivity (FAO, 2018; Naughton et al., 2020). Operational flux, such as the imposition of stressful environmental growth conditions in aquaculture, may lead to elevated susceptibility to diseases where administration of immunomodulatory therapeutics in feed has been reported previously to alleviate onset or severity including positive impact on fish gut microbiome (Carballo et al., 2019).

The utilisation of antibiotics in managing diseases is a concern in the aquaculture industry due to the increased risk of developing antimicrobial resistance in animal production (Naughton et al., 2020). A growing interest in organic farming has warranted the use of natural alternatives, such as medicinal plants and fungi as a feed supplement, as a way to reduce the adverse side effect of chemical residue from antibiotics (Baba et al., 2015; Wan-Mohtar, 2021). These prebiotic and probiotic ingredients have been reported to improve immunity as well as a growth promoter in fish and shrimp without compromising their health and also sustainable for the environment (Mohan et al., 2019a; Rufchaei et al., 2019). Probiotics are important feed supplements in aquaculture due to their capacity to enhance health and to prevent disease (Carballo et al., 2019). Carballo and co-workers (2019) recently reported that such ingredients are typically non-digestable molecules such as yeast-derived beta-glucans, which selectively modulate the intestinal microbiome by

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promoting indigenous microbial populations and commensurately prevention of unwanted pathogen proliferation. In spite of demonstrating the potential benefits of using prebiotics to improve animal health, their use in aquaculture remains limited and their mode of mechanistic action is far from understood (Dimitrogou et al., 2011; Akhter et al., 2015; Morrison et al., 2016; Ruiz-Salmón et al., 2020; Ruiz-Salmón et al., 2021)

Use of medicinal mushrooms has been previously reported to provide health benefits in both animals and humans through immunomodulatory function (Smith et al., 2002; Rowan et al., 2003; Sullivan et al., 2006; Masterson et al., 2020; Murphy et al. 2020a; Murphy et al., 2020b; Galanakis et al., 2020). *Ganoderma lucidum* is one such medicinal mushroom known for its valuable properties to treat many diseases (Cao et al., 2018). Regarded as a 'marvellous herb', *G. lucidum* is widely used in many countries, particularly in East Asia due to its therapeutic effects (Kozarski et al., 2019; Wan-Mohtar et al., 2020). It contains functional metabolites that possess antifungal, antibacterial and anti-inflammatory properties (Rowan et al., 2003; Cao et al., 2018; Wan-Mohtar et al., 2017). The bioactive metabolites, particularly polysaccharide extracted from *G. lucidum,* have demonstrated immunomodulatory and anticancer properties (Meng et al., 2014). However, the low yield of fungal polysaccharides extracted from fruiting bodies and mycelium of *G. lucidum* is appreciated as a technological limitation: consequently, it has prompted advance research in the area of fungal exopolysaccharide (EPS) through submerged liquid fermentation (Supramani et al., 2019b). Technically, the production of EPS is viewed to be less-harsh during the extraction process, hence reduce product degradation and resulted in cost-effectiveness (Yuan et al., 2012).

Tilapia production is projected to increase from 58% in 2016 to 62% in 2030 (FAO, 2018) In Malaysia, red hybrid tilapia (*Oreochromis* sp.) is considered as the top commercial species besides catfish (DOF, 2018). However, there are many constraints in developing sustainable, yet intensive culture of tilapia. One of the top challenges is the disease threat that could results in economic loss. Several bacteria disease such as Streptococcosis, Columnaris and Haemorrhagic septicaemia are amongst common infections of tilapia culture in Malaysia. Viral infection such as Tilapia Lake Virus (TiLV) is currently a significant threat to global tilapia production and major outbreak has also been reported in Malaysia. The interaction of pathogenic bacteria and TiLV was reported to be synergistic, which resulted in strengthening of both pathogens and increasing severity of the disease (Amal et al., 2018; Elumalai et al., 2019; Jansen et al., 2019). According to USDA (2019) there is no vaccine available for the treatment of TiLV and appropriate alternative mitigation and biosecurity measures are needed to address this challenge. Hence, to reduce the challenges in the stressful event during intensive tilapia culture, introducing feed additive containing immunostimulant properties would be an advantage to boost their immunity. Therefore, this constitutes the first study to examine the potential of EPS fish-feed (EFF) extracted from the mycelium of *G. lucidum* on growth, antioxidant responses, body composition and haematological indices of Malaysian red hybrid tilapia.

2. Materials and method

2.1. Production of fungal exopolysaccharides from the mycelium of Ganoderma lucidum

Fungal biomass was pre-grown in a 5-L stirred-tank bioreactor system (STR, Fermetec, Malaysia) under controlled submerged fermentation strictly according to Usuldin et al. (2019) using *Ganoderma lucidum* strain QRS 5120 (Supramani et al., 2019a) obtained from Functional Omics and Bioprocess Development Laboratory, Institute of Biological Sciences, Universiti Malaya. The fermented *G. lucidum* mycelial biomass was subjected to cold 95% (v/v) ethanolic extraction at 1:4 ratio and left for 48 hours at 4 °C for macromolecules precipitation. The method of extraction was conducted according to Hassan et al. (2019) whereas the centrifuged (9000 rpm, 15 min) macromolecule precipitates was freeze-dried to produce crude EPS.

Table 1
Formulation and nutritional composition of exopolysaccharide fish feed (EFF) originated from the mycelium of *Ganoderma lucidum.*

Ingredients (g/kg)	Control [5]	1 g/kg	2 g/kg	3 g/kg
Fish meal (g)	300.0	300.0	300.0	300.0
Corn meal (g)	193.9	193.5	193.1	192.7
Rice bran meal (g)	199.3	198.9	198.6	198.2
Soybean meal (g)	236.8	236.6	236.3	236.1
Exopolysaccharide (g)	0.0	1.0	2.0	3.0
Lysine (g)	10.0	10.0	10.0	10.0
Methionine (g)	5.0	5.0	5.0	5.0
Vitamin premix(g) [1]	1.0	1.0	1.0	1.0
Mineral premix(g) [2]	3.0	3.0	3.0	3.0
Dicalcium phosphate (g)	10.0	10.0	10.0	10.0
Fish Oil (ml)	40.0	40.0	40.0	40.0
Total (g)	1000	1000	1000	1000
Nutritional composition				
Protein (%)	37.86	36.30	37.02	35.19
Lipid (%)	6.29	6.29	4.84	6.67
Fiber (%)	1.63	1.70	1.92	1.76
Dry matter (%)	85.65	85.69	85.73	85.77
Ash (%)	12.00	12.86	12.40	12.45
Carbohydrate (%) [3]	42.22	42.85	43.82	43.93
Gross energy (kJ/g) [4]	18.98	18.72	18.49	18.80

[1] The vitamin premix supplied the following per 100g diet: vitamin A, 500IU; vitamin D_3, 100IU; vitamin E, 0.75 mg; vitamin K, 0.02 mg; vitamin B_1, 1.0 mg; vitamin B_2, 0.5 mg; vitamin B_3, 0.3 mg; vitamin B_6, 0.02 mg; folic acid, 0.1 mg; biotin, 0.235 mg; pantothenic acid, 1.0 mg; inositol, 2.5 mg.

[2] The mineral premix supplied the following per kg diet: selenium, 0.2 mg; iron, 8.0 mg; manganase, 1.0 mg; zinc, 8.0 mg; copper, 0.15 mg; potassium chloride, 0.4 mg; magnesium oxide, 0.6 mg; sodium bicarbonate, 1.5 mg; iodine, 1.0 mg; cobalt, 0.25 mg. [3]Carbohydrate = 100 – (% protein+ % lipid + % fiber + % ash).

[4] Gross energy was calculated as 23.9, 39.8, and 17.6 kJ/g for protein, lipid, and NFE, respectively.

[5] Feed: Control = 0.0 g/kg of EPS,

2.2. Preparation of experimental diets

Four experimental EFF were formulated to be isoenergetic composition (~19g/kJ) containing 0 (control), supplementation of 1(1E), 2(2E) and 3(3E) g EPS per kg diet (Table 1). The supplements (in dried powder form) were uniformly mixed with dry ingredients and pelleted with extruder machine (KCM, Y132M-A). The pelleted feed was then oven-dried at 60^0C and later stored in 4^0C until further use.

2.3. Experimental animal and setup

Red hybrid Tilapia fingerlings were purchased from a local fish farm in Sungai Buloh, Malaysia and acclimated for two weeks prior to the feeding trial. Fish were hand-fed with commercial diet, *ad libitum*, twice per day during the acclimatisation period. One hundred twenty fish (16.19 ± 0.24 g) were stocked into each of the eight tanks (100 L) with a density of 15 fish per treatment in duplicate cultures as justified by Wan-Mohtar et al., (2020). Feeding was performed twice daily at a 3% body weight ratio throughout the 42-days of feeding trial and feeding intake was recorded.

Each tank was equipped with filtration and constant aeration with dechlorinated water throughout the study. Water temperature were maintained at 28–29°C, pH at 6.0–6.8, DO above 4.0, ammonia <0.80 mg/L, and nitrate <1.9 mg/L. Approximately 25-30% of water was changed every two days, and uneaten feed and faeces were collected every day by siphoning at the bottom of the tanks to maintain water quality.

All procedures of fish rearing and handling were performed according to the Universiti Malaya Animal Care and Use Policy (UM ACUP)

(IACUC-002), which was established according to the Malaysian Animal Act 1953 (Act 647).

2.4. Growth performance and organosomatic indices

After termination of the feeding trial at day 42, fish were starved for 24 hours before weighing. All the fish were anaesthetised with clove oil and weigh individually to determine the growth performance including body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR) and feed intake (FI). Organosomatic indices including visceromatic index (VSI), hepatosomatic index (HSI) and condition factor (CF) were also measured post-feeding trial.

$$BWG\ (g/fish) = final\ weight\ (g) - initial\ weight\ (g)$$

$$FI\ (g/fish) = total\ feed\ for\ the\ feeding\ period\ (g)/number\ of\ fish\ alive$$

$$SGR\ (\%) = \left[(Ln\ final\ weight) - (Ln\ initial\ weight)/days\ of\ trial\right] \times 100$$

$$FCR = feed\ intake\ (g)/weight\ gain\ (g)$$

$$SR\ (\%) = final\ number\ of\ fish\ alive/initial\ number\ of\ fish\ alive \times 100$$

$$CF = [body\ weight\ (g)/\ (body\ length\ (cm)] \times 100$$

$$VSI\ (\%) = \left[viscera\ weight\ (g)/\ whole\ body\ weight\ (g)\right] \times 100$$

$$HSI\ (\%) = \left[wet\ weight\ of\ liver\ (g)/\ final\ weight\ of\ fish\ (g)\right] \times 100$$

2.5. Sample collection

2.5.1. Liver

Five fishes were randomly selected in each tank and sacrificed for its liver sample collection. Data for the body weight and liver weight were recorded individually according to each sacrificed fish. A total of 1.0 g of each liver was homogenized in 10ml buffer containing 25mM sodium phosphate buffer (pH 7.4), 0.1 mM protease inhibitor, 1.0 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM dithiothreitol (DTT) and 0.1 phenylthiourea (PTU). The sample was then homogenized using a laboratory homogeniser at 150 rpm for 2 minutes until a distributed uniform liquid was achieved. Later, the homogenates were centrifuged at 100,000 rpm (Beckman Coulter Optima L-100K Ultracentrifuge), at 4°C for 30 minutes and the supernatants were separated and later stored at -80°C for further analysis.

2.5.2. Blood and serum

Blood samples were collected randomly from five fishes in each tank through the caudal puncture. Approximately 1 ml of the blood sample was collected by using a 1-ml syringe with a needle size of 22G 1 $\frac{1}{2}$ inch from each fish.. For each individual fish, 0.5 ml of the blood was transferred to a heparinised vacutainer tube for haematological analysis (complete blood counts) and the remaining 0.5 ml was transferred into a non-heparinised serum vacutainer tube and later centrifuged at 5000 x g at 4°C for 10 min, according to the method described by Zhang et al. (2019). The five blood samples from each replicate were pooled together. The isolated serum was then stored at -20°C for further analysis.

2.6. Analytical methods

2.6.1. Proximate composition

Proximate composition of the feed ingredients (Table 2), experimental diets (Table 1) and fish muscle were determined according to the Standard Method of AOAC (2005). Crude protein was analysed by using the semi-auto Kjeldahl system (N × 6.25) (Kjeltec semi auto-analyzer). Crude lipid was determined by Soxhlet extraction method using petroleum ether as the solvent. Moisture was measured by drying the sample in an oven at 105 °C overnight and later continue in a muffle furnace for ash content at 600 °C for 6 h. The crude fibre was analyzed after acid and alkali digestion. Carbohydrate or NFE data were obtained through calculation [100 - (% crude protein + % crude lipid + % crude fiber + % crude ash)] and gross energy was determined by using the physiological values (crude protein x 23.9) + (crude lipid x 39.8) + (carbohydrate x 17.6) according to Schulz et al. (2005).

2.6.2. Antioxidant enzyme assay

Glutathione S-transferase (GST) was analysed spectrophotometrically by measuring the activity towards 1-Chloro-2,4-dinitrobenzene (CDNB) as a substrate at 340 nm as described by the method from Habig et al. (1974). Catalase activity (CAT) was measured according to the decline in H_2O_2 at 240 nm according to Claiborne (1985). Both antioxidant enzyme activities were expressed in nmol/min/mL.

2.6.3. Haematology and serum protein analysis

Complete blood counts (CBC) which include red blood cells (RBC), white blood cells (WBC), haemoglobin (HGB), haematocrit (HCT) and RBC indices namely: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were measured using automatic haematology analyser (Sysmex XN, Germany). Serum protein analysis was measured by using Advia 2400 Chemistry System (Siemens Healthineers, Germany)

2.7. Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to compare the means between individual treatments using SPSS software version 23 (SPSS Inc., Chicago IL. USA). Data were expressed as mean ± standard deviation (SD) values and differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Growth performance, feed efficiency and organosomatic indices

At the end of the 6-week trial, the effect of different EFF on growth performance and feed utilisation in red hybrid Tilapia is shown in Table 2. Precise observation of fish morphological measured bi-weekly was characterized in Figure 1. The groups treated with 2E (2 g/kg EFF) and 3E (3 g/kg EFF) had significantly higher WG and SGR compared with those fed with control although no significant difference was observed in 1E (1 g/kg EFF) group. In addition, FCR, FI and SR did not affect all the experimental diets ($P > 0.05$) throughout the feeding trial. Based on the organosomatic indices results, HSI and VSI were significantly affected by the supplementation of EFF. Groups with either high EFF (2 and 3 g/kg) and control shows significantly higher HSI values compared to 1E (1 g/kg EFF). In contrast, significantly lower VSI value was recorded in 3E (3 g/kg EFF) fed fish compared to other diets ($P < 0.05$). However, the supplementation of EFF did not influence the CF of red hybrid Tilapia during the experimental period.

3.2. Oxidative stress of experimental fish

The GST activity in liver of red hybrid Tilapia was found to be significantly higher ($P < 0.05$) in 3 g/kg of EFF (38.17 nmol/min/ml)

Table 2
Growth performance of the red hybrid Tilapia treated with EPS fish feed (EFF) originated from the mycelium of *Ganoderma lucidum.*

Performance [1]	Experimental EPS feed					
Control [5]	1 g/kg	2 g/kg	3 g/kg	F	*P*-value	
Initial Weight (g/fish)	17.45 ± 1.48	16.20 ± 0.14	15.40 ± 1.41	15.65 ± 1.91	0.85	0.54
Final weight (g/fish)	28.36 ± 0.22^{a}	32.75 ± 0.35^{ab}	33.75 ± 2.21^{ab}	34.40 ±1.88^{b}	3.58	0.13
BWG(g/fish) [4]	11.87 ± 0.64^{a}	16.55 ± 0.21^{ab}	18.37 ± 0.80^{b}	18.75 ± 0.03^{b}	12.00	0.02
SGR (%/day)	1.16 ± 0.22^{a}	1.60 ± 0.11^{ab}	1.88 ± 0.06^{b}	1.89 ± 0.16^{b}	7.10	0.05
FCR	2.00 ± 0.05	1.72 ± 0.23	1.32 ± 0.06	1.31 ± 0.14	3.19	0.14
FI (g/fish)	23.78 ± 0.60	26.25 ± 0.98	24.24 ± 2.13	24.61 ± 2.60	1.25	0.12
SR (%)	100 ± 0.00	93.33 ± 6.70	100 ± 0.00	96.67 ± 3.33	3.27	0.25
Organosomatic indices [2]						
HSI (%)	1.97 ± 0.14^{b}	1.42 ± 0.51^{a}	2.27 ± 0.40^{c}	2.22 ± 0.45^{c}	5.67	0.06
VSI (%)	11.02 ± 0.99^{b}	9.75 ± 1.06^{ab}	10.71 ± 1.18^{ab}	9.42 ± 0.87^{a}	3.23	0.04
CF (%)	1.60 ± 0.19	1.51 ± 0.08	1.48 ± 0.04	1.46 ± 0.05	1.80	0.18

[3]Mean values in the same row with different superscripts are significantly different (P < 0.05)
[1] The results represent mean± SD of 15 fishes per tank (*14 fishes for control) on growth performance
[2] The results represent mean± SD of 3 fishes per tank on organosomatic indices
[4] BWG: Body Weight gain;, SGR: Specific growth rate, FCR: Feed conversion ratio, SR: Survival rate, FI: feed intake, HSI: Hepatosomatic index, VSI: Viscerosomatic index, CF: Condition factor
[5] Feed: Control = 0.0 g/kg of EPS

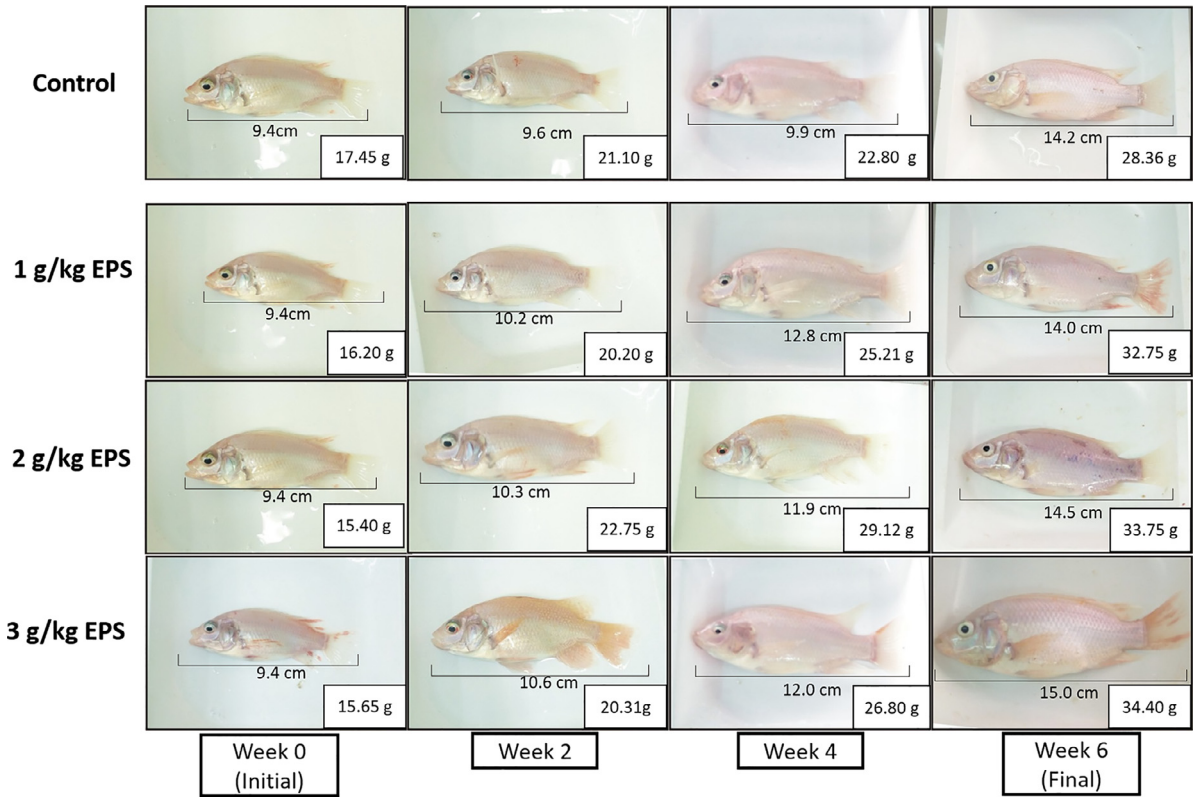

Fig. 1. Bi-weekly weight gain of red hybrid Tilapia (*Oreochromis* sp.) treated with different concentration of EPS fish feed (EFF) originated from the mycelium of *Ganoderma lucidum* in 6-week feeding trial.

(Table 3). On the other hand, no significant difference was detected in the activity of dietary 1E (1 g/kg EFF) and 2E (2 g/kg EFF) compared to control ($P > 0.05$). Similar trend was observed in 3E (3 g/kg EFF) with significantly higher CAT activity (230.51 nmol/min/ml) compared to other diets ($P < 0.05$).

3.3. Fish muscle nutritional composition

The fish muscle composition treated with EFF after 42-day of feeding trial is presented in Table 4. The data revealed that only fibre was significantly affected by EFF treatments. The fibre content of fish treated with 2 g/kg EFF is comparatively higher ($P < 0.05$) compared to other diets while control was recorded as the lowest group. Other nutrient components including crude protein, crude lipid, dry matter, ash and gross energy did not have a significant effect amongst the treatment ($P > 0.05$). In contrast, 1E (1 g/kg EFF) diet had a significantly lower level ($P < 0.05$) of carbohydrate compared to other EFF. Control EFF did not differ significantly ($P > 0.05$) compared to 2E (2 g/kg EFF) and 3E (3 g/kg EFF) in terms of carbohydrate muscle content.

3.4. Haematological indices

Haemoglobin (Hb) and RBC of red hybrid Tilapia were affected by experimental EFF and elevated significantly ($P < 0.05$) in a linear fashion

Table 3
Antioxidant responses of red hybrid Tilapia (*Oreochromis* sp.) treated with different concentration of EPS fish feed (EFF) originated from the mycelium of *Ganoderma lucidum* after 42 days.

Antioxidant enzymes (nmol/min/mg protein) [4]	Experimental EPS feed					
Control [3]	1 g/kg	2 g/kg	3 g/kg	F	*P*-value	
Glutathione-S-transferase	20.77 ± 0.99^{a}	11.18 ± 1.14^{a}	16.91 ± 1.03^{a}	38.17 ± 6.85^{b}	21.52	0.006
Catalase	117.81 ± 7.24^{a}	176.73 ± 7.99^{b}	225.90 ± 2.17^{c}	230.51 ± 13.04^{c}	75.99	0.001

[1]The results represent mean± SD of 5 fishes per tank
[2]Mean values in the same row with different superscripts are significantly different ($P < 0.05$)
[3] Feed: Control = 0.0 g/kg of EPS
[4] Specific activity of enzyme measured in nmol/min/mg protein.

Table 4
Fish muscle composition of red hybrid Tilapia (*Oreochromis* sp.) treated with different concentration of EPS fish feed (EFF) originated from the mycelium of *Ganoderma lucidum* after 42 days.

Components (%)	Experimental fish feed [3,4]					
Control [5]	1 g/kg	2 g/kg	3 g/kg	F	*P*-value	
Crude protein	81.87 ± 1.73	88.70 ± 2.01	83.80 ± 3.13	83.21 ± 1.47	3.75	0.12
Crude lipid	1.50 ± 0.09	1.68 ± 0.11	1.37 ± 0.25	1.45 ± 0.33	0.72	0.59
Crude fiber	0.03 ± 0.00^{a}	0.20 ± 0.00^{bc}	0.22 ± 0.08^{c}	0.06 ± 0.06^{ab}	7.18	0.04
Dry matter (wet weight basis)	24.20 ± 0.14	24.40 ± 0.11	25.40 ± 0.20	25.70 ± 0.12	0.42	0.53
Ash	6.60 ± 0.41	6.93 ± 0.63	5.90 ± 0.89	6.55 ± 1.06	0.79	0.56
Carbohydrate [1]	10.00 ± 1.32	2.50 ± 2.38	8.70 ± 3.66	8.73 ± 2.89	3.14	0.15
Gross energy (kJ/g) [2]	21.93 ± 0.22	22.52 ± 0.26	22.11 ± 0.20	22.00 ± 0.03	3.73	0.12

[1] Carbohydrate % = 100 – (crude protein % + crude lipid % + ash % + crude fiber %).
[2] Gross energy was calculated as 23.9 kJ/g, 39.8 kJ/g, and 17.6 kJ/g for protein, lipid, and carbohydrate, respectively
[3] The result represents the mean ± SD of 3 fishes per tank
[4] Mean values in the same row with different superscripts are significantly different ($P < 0.05$).
[5] Feed: Control = 0.0 g/kg of EPS

Table 5
Haematological parameters of red hybrid Tilapia (*Oreochromis* sp.) treated with different concentration of EPS fish feed (EFF) originated from the mycelium of *Ganoderma lucidum* after 42 days.

Parameters[3]	Experimental diets[1,2]					
Control[4]	1 g/kg	2 g/kg	3 g/kg	F	*P*-value	
HGB (g/dl)	5.75 ± 0.70^{a}	6.98 ± 0.10^{b}	7.08 ± 0.51^{b}	7.43 ± 0.22^{b}	10.18	0.001
HCT (%)	30.00 ± 3.46^{a}	36.00 ± 1.41^{b}	39.00 ± 2.31^{b}	37.50 ± 0.58^{b}	12.66	0.001
RBC (10^{6}/mm^{3})	2.04 ± 0.29^{a}	2.47 ± 0.14^{b}	2.57 ± 0.24^{b}	2.69 ± 0.02^{b}	7.92	0.004
WBC (10^{3}/mm^{3})	133.88 ± 27.84	155.20 ± 6.64	161.63 ± 36.34	176.65 ± 21.99	1.92	0.18
MCV (fl)	148.00 ± 3.46^{bc}	145.75 ± 4.35^{b}	152.50 ± 4.04^{c}	139.50 ± 1.29^{a}	9.57	0.002
MCH (pg)	28.20 ± 0.68	28.28 ± 0.94	27.83 ± 4.52	27.65 ± 0.62	0.07	0.98
MCHC (g/L)	190.75 ± 2.22	193.75 ± 4.35	182.25 ± 24.84	198.50 ± 2.89	1.15	0.37
Serum total protein (g/dl)	3.20 ± 0.12	2.75 ± 0.40	3.23 ± 0.10	3.03 ± 0.26	3.01	0.07

[1] The result represents mean ± SD of 5 fishes per tank
[2] Mean values in the same row with different superscripts are significantly different ($p< 0.05$).
[3] HGB = Haemoglobin; HCT = hematocrit; RBC = red blood cell; WBC = white blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.
[4] Feed: Control = 0.0 g/kg of EPS

as the supplementation level EFF increased from 0 (control) to 3 g/kg (Table 5). Similarly, a significant higher level of HCT was observed in experimental diets compared to control. White blood cell (WBC) count, MCH and MCHC values of fish did not vary significantly ($P > 0.05$) among different treatments, albeit discernible improvement was seen in WBC as the EFF increased. Nevertheless, the MCV was significantly lower in high EFF (3E, 3 g/kg) than other diets ($P < 0.05$). For biochemical serum total protein, no significant differences were observed between all treatments.

4. Discussion

The emergence of bacterial antibiotic-resistance due to their excessive use has stimulated research and development to elucidate alternative eco-friendly compounds from fungal products. In addition to been considered inexpensive, safe and biodegradable, use of fungal polysaccharides (such as β-glucans) are unlikely to influence bacterial resistance to front-line antibiotics that is reached a crisis-point, according to the World Health Organisation (Reverter et al., 2014; WHO, 2019). One of the most promising medicinal mushroom, *G. lucidum,* has demonstrated potential for human and animal use by way of improved immune function: however, these bioactives have not been developed or tested as potentially essential aquaculture-based protein for farmed fish. Moreover, recent adjacent findings have shown that exopolysacharides, extracted from the same *Ganoderma lucidum,* is considered as non-toxic, as demonstrated by use zebrafish embryo toxicity (ZFET) assay: thus, inferring it's safe use in aquaculture feed (Taufek et al., 2020).

Aligned with previous findings, the results of this preliminary study revealed that administration of EPS from the mycelium of *G. lucidum* improved growth performance and feed efficiency, antioxidant activities and haematological indices in red hybrid Tilapia. To the best of our knowledge, this constitutes the first study to examine the performance

of EPS from the fermented mycelium of *G. lucidum* as a feed supplement for red hybrid Tilapia. Previous published studies were limited to using mature fruiting bodies of *G. lucidum* for animal feed trials including fish (*Ctenopharyngodon Idella*) (Chithra et al., 2016), prawn (Mohan et al., 2016) and poultry (Bederska-Łojewska et al., 2017; Khan et al., 2019). Previous study by Chitra et al, (2016) indicated that administration of 1.0g/kg of *Ganoderma lucidum* polysaccharides regulate better performance of grass carp. Hence in the current study, the concentration of EPS was observed at 1, 2 and 3 g/kg diet. This study demonstrated that the supplementation of 2 to 3 g/kg EPS in red hybrid Tilapia diet provide better growth when observed in terms of WG, SGR and organosomatic indices of HSI. Moreover, improved FCR values were also observed in these supplemented diets when compared to control, which demonstrates the ability of EPS to promote fish growth.

Antioxidant activity has been regarded as an essential measure to detect fish well-being. Glutathione S-transferase (GST) plays a critical role in protecting cells in terms of toxicity of xenobiotics electrophiles and from a foreign contaminants perspective (Dasari et al., 2018). In aquatic animals, significant alteration of GST activities will reflect the metabolic disturbance and cell damage in specific organs (Mohan et al., 2015; Raji et al., 2018). The increasing level of GST in any material implies that it may contain a compound that could trigger the biotransformation of xenobiotics. Our findings suggest that incorporation of EPS did not differ significantly in between control, 1E and 2E although 1E diet exhibited numerically lower activities than other treatments.

As the first line of defence mechanism, catalase (CAT) is correlated with an increasing concentration of H_2O_2 (Wilhelm Filho et al., 2005). In contrast to GST, high CAT activity was observed in EPS supplemented groups when compared to control, whereby group fed with 3E shows significantly higher activity than the others. This might corresponds to the previous study by Wu et al. (2016) which reported the presence of ß-1,3/1,6 glucan polysaccharides in cell wall of *G.lucidum*. The CAT activity trend is in accordance to the growth performance analysis where high SGR and WG, as well as numerically low FCR value, was observed in the group fed with 3E, which might be attributed to high metabolic rate (Ahmed et al., 2017; Taufek et al., 2016). Ahmed et al. (2017) investigated the effect of hot water extract (HWE) of the waste mushroom-stalk (*Pleurotus sp.*) on CAT activity of tilapia. They found a significant increase in CAT activity in 0.5% and 1% of HWE when compared to control, which is contributed by the presence of EPS. Oxidative stress response findings reported in this current study will inform new innovation in terms of fish supplement. Consequently, there are pressing opportunities that include potential immediate issues in fish farming from farm to fork, that focues on reduced environmental stress conditions.

Fish fillet is the edible part of the fish that is expected to contain high nutritional value. Consequently, the nutritional composition of fish fillet was addressed in this study with a particular role on investigating the influence of EPS supplementation on key determinants. Specifically, high protein content was observed in all EPS-supplemented fish. This contrasts to previous studies that focused on red tilapia body composition where fish were fed a diet supplemented by 10% with mushroom stalk meal (*Pleurotus sajor-caju*) (Abdul Rahman Jabir et al., 2012). Other researchers have also reported that the lipid content of fish fed the experimental diets (1.37 – 1.68 %) were higher than Nile tilapia that were fed supplemented diets containing red algae (*Gracilaria arcuata*) (Younis et al., 2018).

Haematological parameters are essential tools in understanding the physiological status of experimental fish. Overall, the HGB, HCT, and RBC had significant effects on all EPS group as compared to control, whereby the highest level was detected in 3E group. It was observed that increasing RBC is correlated with the increasing trend of HGB and HCT due to their synergistic linkage (Audu et al., 2014). Also, EPS-treated groups produced higher WBC despite no significant differences were found amongst treatments, which corresponds to the trend of RBC as immunity indicator. A similar trend was observed in the study made by Chitsaz et al. (2018) during supplementation of a medicinal mushrooms extract *(Lentinula edodes,)* in great sturgeon juvenile (*Huso huso*). Increasing in RBC and WBC values were also reported by Mohan et al. (2019b) in shrimp fed with exopolysaccharides. Red blood cell indices are mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) which used to diagnose the level of anaemia (Şahan, Duman, 2010). The maturation of red blood cells determined the value of MCV and MCH. The unstable trend was observed in MCV of fish in all treatments suggesting the presence of immature red blood cells which have a larger cell volume and lower haemoglobin (Carvalho, Fernandes, 2006).

The findings of this present study correlate well with the trend towards the use of prebiotics and probiotics in feed supplements for aquaculture production (Akhter et al., 2015; Carballo et al., 2019; Dimitroglou et al., 2011; Galankis et al., 2020). This also reflects a trend towards the use of natural processes for disease mitigation in aquaculture and adjacent food production processes for intensive sustainability (Rowan, 2019). Use of probiotics and prebiotics are normally non-digestable molecules that selectively modulate the intestinal micobiome by promoting indigenous microbial populations and by preventing pathogen proliferation (Carballo et al., 2019). Recent findings have demonstrated that certain carbohydrates (exopoly and oligosaccharides) as promising prebiotics for animal and human health including beta-glucans that are fermented in the gut resulting in enhanced production of short chain fatty acids (SCFA) such as formate, acetate, proponate and butyrate (Carballo et al., 2019; Mahdhi et al., 2020; Morrison, Preston, 2016). These secondary metabolites also modulate production and release of cytokines, chemokines and leukocyte recruitment for health benefits (Morrison, Preston, 2016). Use of beta-glucans derived from medicinal fungi, as described in this present study, shows potential as an immune-priming feed supplement for aquaculture production. However, future studies focusing on interaction of beta-glucans derived from medicinal fungi with gut microbiota in fish is merited.

Conclusion

EPS fish-feed formulation, based on the mycelium of *Ganoderma lucidum,* exerted potent benefits for red hybrid Tilapia growth performance and improved antioxidant activity of GST and CAT enzymes as well as haematological indices of RBC, HCT and HGB. Nutritional muscle composition was minorly affected by EFF supplementation. Findings from this preliminary study highlight the potential benefits of using EFF in fish feed supplementation to enhance the health and immunity of Tilapia and other farmed fish along with adjacent scale-up studies for commercial aquaculture deployment. This timely study has significant implications for the smart intensification of aquaculture through innovative steps in feed formulation, which will provide vital future food for meeting the pressing dietary needs of our growing global populations. Developing sustaining and disruptive technologies in the the food sector will also enable pivoting of society beyond recession created by ongoing COVID-19 (Rowan and Galanakis, 2020) through provision of safe and nutritious supply chain that includes health benefits (Galanakis et al., 2021). Transnational modelling of key socio-economic drivers informing sustainable development of marine industry, enabled by life cycle assessment of products and services (Ruiz-Salmón et al., 2020), will inform policy that includes Europe's Green New Deal (Rowan and Galanakis, 2020). This transdisciplinary study has significant implications for the smart sustainable intensification of aquaculture through innovative steps in feed formulation, which will provide future safe nutritious food to meet pressing needs for our growing global populations.

Declaration of Competing Interest

The authors declare that they have no competing conflict of interest and the authors alone are responsible for the content and writing of the paper.

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