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Influences of spent coffee grounds on skin mucosal and serum immunities, disease resistance, and growth rate of Nile tilapia (*Oreochromis niloticus*) reared under biofloc system

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ABSTRACT

The study was executed to find out the potential effects spent coffee ground (SCG) on Nile tilapia's skin mucosal and serum immunities, disease prevention, and growth rate reared in a biofloc system. Nile tilapia fingerlings (average weight 15.25 ± 0.07 g) were disseminated into 15 aquaria (150 L tank^{-1}) at a density of 20 fish per aquarium and treated five diets: SCG1 (control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}) for eight weeks. A Completely Randomized Design (CRD) with three replications was applied. Growth rate, skin mucus, and serum immunities were quantified every 4 weeks; whereas the challenge study was conducted at the termination of the feeding trial. The outputs indicated that dietary incorporation of SCG give rise to the enhancement of SGR and FCR in comparison with the control, with best levels noted in fish fed SCG2 diet. Similarly, significant enhancements in skin mucosal and serum immunities were revealed in fish treated SCG2 over the control and other SCG diets. Likewise, higher survival rates against *Streptococcus agalactiae* were displayed in fish fed SCG, with the maximum level displayed in the fish treated SCG2. In conclusion, dietary supplementation of SCG2 (10 g kg^{-1}) can be potential used as immunostimulants in tilapia aquaculture.

1. Introduction

Coffee is among the most profitable crop in global market owing to the huge demand of coffee drinks [1]. International Coffee Organization stated in 2019 that world coffee production was estimated to 169.34 million 60 kg coffee bags [2]. Coffee consumption generates a considerable volume of spent coffee grounds (SCG), with such an estimated total of around 6 million tons globally [3]. The majority of SCG is generally burnt or dumped into landfills [4], even though its potential appeal for various applications, such as cosmetic, nutraceutical, and pharmaceutical [5]. SCG is rich source chlorogenic acid [6], caffeine [7], bioactive peptides [8], phenolic acids and flavonoids [9]. Therefore, the use of SCG by-product is an important topic in the drive for a circular economy.

Nile tilapia (*Oreochromis niloticus*) is an important farmed fish production, as it provides a beneficial protein source of human consumption [10,11]. Due to high market demand, over expansion of Nile tilapia occur worldwide, and cause problems such as water pollution and disease outbreaks [12–14]. The intensive use of antibiotics has resulted in increased bacterial resistance, which can then be passed on to animal and human pathogens, resulting in a massive in bacterial infections [15]. As a result, new techniques to controlling infectious diseases have

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been proposed [16,17]. In this context, the use of immunomodulators in aquaculture has been shown to improve health, growth rates, and immunological responses of cultured aquatic species [18–21]. Furthermore, one of the most important criteria for sustainable aquaculture is to offer nutritionally balanced feed at affordable costs in order to keep fish farming costs consistent [22]. Tilapia is an omnivorous fish that can consume fibers and carbohydrates in their digestive system and transform them to high-energy nutrients for their better growth [23,24]. As the result, tilapia is an excellent model fish for studying the effects of SCG on fish growth and health status.

Biofloc technology has been extensively used in farming aquaculture [25]. The standout attribute comprises a combination of microorganisms that provide feed to omnivorous species [26–28]. Recently, various researches have shown that biofloc technology positively impacts the aquaculture industry [29–31]. Therefore, it was proposed that the introduction of different prebiotics and/or probiotics proliferates the microbiota community whether in the water or in the host's intestine to inhibit the possibly harmful pathogens. Current findings have been undertaken in such a field based on this concept. Results like to indicate that the introduction of that technique to the biofloc significantly improves the water quality of water and the immunity of aquatic animals [32–34]. Research of the effects of phytogenics on aquaculture based on biofloc is a new and interdisciplinary strategy but less investigated. This study aimed to investigate the functional use of SCG on skin mucosal and serum immune responses, growth rates, and disease protection of Nile tilapia against *Streptococcus agalactiae* reared in a biofloc system.

2. Materials and methods

2.1. Preparation of feed additives

Spent coffee ground was obtained at Lanna Thai Coffee Development Center, Faculty of Agriculture, Chiang Mai University. After the arrival, they were dried in a hot air oven at 60 °C for 48 h and crushed into powder. The powder was kept in a -4 °C for further use.

2.2. Experiment diet

Five experimental diets were prepared by supplementation with SCG at 0 (SCG1), 10 (SCG2), 20 (SCG3), 40 (SCG4), and 80 (SCG5) g kg^{-1} into basal diet, which has been proven to meet the nutrient requirement of Nile tilapia [20] (Table 1).

2.3. Experiment design

Nile tilapia fingerling (male) acquired from Chiang Mai Pattana Farm were treated a commercial diet (CP, 9950) for 30 days, followed by a control diet for 2 weeks. Afterward, 300 fish (15.25 ± 0.07 g) were distributed into 15 aerated tanks (volume 150 L $tank^{-1}$) at a density of 20 fish $tank^{-1}$. Completed Randomised Designed with three replications was applied and lasted for 8 weeks. During experimental period, fish were fed treated diets up to saturation, twice daily, at 8:30 a.m. and 4:30 p.m.

2.4. Biofloc water preparation and management

Water parameters were determined via an HI98196 m (Hanna Romania), which showed 28.50 ± 0.43 °C, 7.95 ± 0.09, and 4.99 ± 0.06 mg $litre^{-1}$ for water temperature, pH, and dissolved oxygen, respectively. Flocs level in each tank was maintained at 9.55 ± 0.73 mL by adding molasses and probiotics (PondPlus – Bayer), while NH_3 was detected by HI 96733 m (Hanna, Romania) and retained at 0.12 ± 0.02 mg/L. Five percent of water was changed every week and clean water was added to maintain water quality.

Floc inoculants in each tank (150 L of water) were prepared three weeks before the trial by adding 2 g of wheat flour, 400 g salt, 5 g dolomite, and 5 g molasse. Throughout the feeding trial, the C:N ratio was retained at 15:1 via the supplementation of molasses (40% C) as a carbon source, as stated in Avnimelech [35]; after 2 h post-feeding. The C:N ratio was schematically computed based on a leftover nitrogen level in each tank and the contribution of the diet [36].

Table 1
The formulation and proximate compositions of experimental diet (g kg^{-1}).

Ingredients	Diets (g kg^{-1})				
	SCG1	SCG2	SCG3	SCG4	SCG5
Fish meal	150	150	150	150	150
Corn meal	200	200	200	200	200
Soybean meal	390	387	384	381	374
Wheat flour	70	70	70	70	70
Rice bran	150	150	150	134	106
SCG[a]	0	10	20	40	80
Cellulose	20	13	6	5	0
Soybean oil	5	5	5	5	5
Premix[b]	10	10	10	10	10
Vitamin C[c]	5	5	5	5	5
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)					
Crude protein	308.58	311.93	309.91	313.25	310.41
Crude lipid	45.53	47.76	48.66	46.14	55.37
Fibre	42.49	45.78	47.68	46.34	44.07
Ash	88.29	89.23	90.45	93.70	92.34
Dry matter	980.15	985.67	982.89	984.55	986.69
GE (cal/g)[d]	3,809	3,899	3,779	3,746	3,778

[a] SCG = Spent Coffee Ground.
[b] Vitamin and trace mineral mix supplemented as follows (IU kg^{-1} or g kg^{-1} diet): retinyl acetate 1,085,000 IU; cholecalciferol 217,000 IU; D, L-a-tocopherol acetate 0.5 g; thiamin nitrate 0.5 g; pyridoxine hydrochloride 0.5 g; niacin 3 g; folic 0.05 g; cyanocobalamin 10 g; Ca pantothenate 1 g kg-1; inositol 0.5 g; zinc 1 g; copper 0.25 g; manganese 1.32 g; iodine 0.05 g; sodium 7.85 g.
[c] Vitamin C 98% 8 g.
[d] GE = gross energy.

2.5. Innate immunological assays

2.5.1. Serum, leukocytes, and mucus preparation

Serum was collected from blood as protocol described in Van Doan, Hoseinifar, Chitmanat, Jaturasitha, Paolucci, Ashouri, Dawood and Esteban [20]. Briefly, blood samples were collected at caudal vein of three fish per tank using the 1-mL syringe. The individual collected blood sample was immediately transferred into 1.5 Eppendorf tube, and allowed to clot at room temperature for 1 h, then at 4 °C for hours. After incubation the serum from each fish was collected and kept at −80 °C until further use. The serum from individual fish was used to analyse serum immune parameters.

Leukocyte were isolated from blood using Chung and Secombes [37], schedule with some modifications as mentioned in Van Doan, Hoseinifar, Chitmanat, Jaturasitha, Paolucci, Ashouri, Dawood and Esteban [20]. Briefly, 1 mL of blood sample from each fish was taken via caudal vein and immediately mixed with 2 mL of RPMI 1640 (Gibthai) in a 15 mL Eppendorf tube. The mixture was carefully transferred on the top of 3 mL of Histopaque (Sigma, St. Louis, MO, USA) in a 15-mL tube. The tub was then centrifuged at 400g, for 30 min at room temperature. After the centrifugation, a white buffy coat of leucocytes present on the top of the Histopaque was collected, aspirated using a Pasteur pipette, and transferred into a clean 15-ml tube. The obtained leucocytes washed three times with phosphate buffer solution (PBS) for removing residual Histopaque, and the required cell number was prepared for the phagocytosis and respiratory burst assays.

Mucus collected from fish skin (3 fish per tank) was followed the previous method of Subramanian, MacKinnon and Ross [38], with modifications stated in Van Doan, Hoseinifar, Chitmanat, Jaturasitha, Paolucci, Ashouri, Dawood and Esteban [20]. Briefly, fish were anaesthetized using clove oil at concentration of 5 mL $litre^{-1}$. They were then placed into polyethylene bag containing 10 ml of 50 mM NaCl (Merck,

Germany), and gently rubbed inside the plastic in a downward motion for approximately 1 min. Th mucus was immediately poured into 15 mL sterile centrifuge tubes, and centrifuged 1.500×*g* for 10 min at 4 °C. Then 1 mL of obtained mucus was kept in 1.5 mL Eppendorf tube at −80 °C until further use.

2.5.2. Lysozyme assays

Serum and mucus lysozyme levels were determined in accordance with method Parry, Chandan and Shahani [39] with some variations. Briefly, 25 μL of undiluted serum and 100 μL of skin mucus from each fish were loaded into 96-well plates in triplication. Micrococcus lysodeikticus (175 μL, 0.3 mg mL^{-1} in 0.1 M citrate phosphate buffer, pH 5.8) was then added to each well. The contents were rapidly mixed, and any changes in turbidity were measured every 30 s, for 5 min, at 540 nm, 25 °C, via a microplate reader. The sample's equivalent unit of activity was determined and compared with the standard curve, which was generated from the reduction of OD value *vs*. the concentration of hen egg-white lysozyme ranging from 0 to 20 μL mL^{-1} (Sigma Aldrich, USA), and expressed as μg mL^{-1} serum.

2.5.3. Peroxidase assays

Serum and skin mucus peroxidase levels were detected using protocol of Quade and Roth [40], with slight modifications. Briefly, 5 μL of serum or skin mucus was loaded into 96-well flat-bottom plates in triplicate. 45 μL of Hank's balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+} was added. After that, 100 μL of solution containing of 40 ml of distilled water, 10 μL of H_2O_2 (30% - Sigma Aldrich), 1 pill of 3,3′,5,5′-tetramethylbenzidine (TMB; Sigma Aldrich) was loaded into each well. Later, 50 μL of 2 M H2SO4 was added to stop the colour-change reaction, and the optical density was read at 450 nm in a plate reader. Standard samples without serum or skin mucus were used as blanks. One unit was defined as the amount producing an absorbance change of 1 and the activity was expressed as units (U) mg^{-1} serum or mucus proteins.

2.5.4. Phagocytic assay

Phagocytic assay was determined via the protocol of Yoshida and Kitao [41], with some variations. Briefly, 200 μL leukocyte suspensions containing of 2 × 106 cells mL^{-1} was placed on cover slip and incubated for 2 h. After incubation, RPMI 1640 was used to wash the non-attached cells, and then 200 μL of fluorescence latex beads (Sigma Aldrich - USA) solution containing of 2×10^7 of beads mL^{-1} was dropped on each cover slip and incubated for 1.5 h at room temperature (RT). Afterward, the non-phagocyte beads were washed with RPMI 1640, and the cover slips were fixed with 100% methanol and stained with Diff-Quick staining dye (Sigma Aldrich - USA) for 10 s. Excessive stain was washed with PBS (pH 7.4), and the number of phagocyte cells per 300 adhered cells was counted microscopically. The phagocytic index (PI) was determined as follows: PI = Average number of beads per cell/the number of phagocytizing cells.

2.5.5. Respiratory assay

Blood leucocytes respiratory burst activity was determined as the method of Secomebs [42], with slight modification. Briefly, 175 μL leukocytes solution containing of 6 × 106 cells mL−1 in PBS loaded in the 96-well plate in triplicate. Later, 25 μL of Nitro Blue Tetrazolium (NBT) at a concentration of 1 mg mL^{-1} was placed into each well and incubated at RT for 2 h. After incubation, the supernatant in each well was carefully discarded and 125 μL of 100% methanol was added to fix the adherent cells for 5 min. The supernatant was discarded again and 125 μL of 70% methanol was added to each well for washing. After washing, the plate was allowed to dry at room temperature for 30 min. 125 μL of 2 N KOH and 150 μL of DMSO were then added to each well. After adding the solution, the plate was measured at 655 nm by a microplate reader. Spontaneous O^{2-} production = (Absorbance NBT reduction of sample) − (Absorbance of blank).

2.5.6. Alternative complement pathway activity (ACH50)

ACH50 was determined using method of Yano [43], with several changes. Briefly, rabbit red blood cells (R-RBC) were washed with PBS by centrifugation at 3000 rpm, and in 0.01 M ethylene glycol tetra-acetic acid-magnesium-gelatin veronal buffer (0.01 M − EGTA-Mg-GVB) for twice. The R-RBC concentration was adjusted to 2 × 108 cells mL^{-1} in 0.01 M − EGTA-Mg-GVB buffer. Then 100 μL of the R-RBC suspension was lysed with 3.4 mL of distilled water. The absorbance of hemolysate was measured at 414 nm against distilled water as a blank and was brought to be close to 0.740.

For the ACH50 test, 100 μL of serum was diluted with 400 μL of 0.01 M-EGTA-Mg-GVB and serial two-fold dilution was conducted. The tubes were performed on ice to retard the reaction of complement until all tubes were prepared. Consequently, 100 μL of R-RBC suspension was loaded into each tube and incubated at 20 °C for 1.5 h with occasional shaking. After incubation, 3.15 mL of cold normal saline solution was placed into each tube to stop the reaction, and then the tube was centrifuged at 1600 g for 5 min. After centrifugation, 100 μL of supernatant in each dilution was loaded into 96-well plate and read at 414 nm. The degree of hemolysis was calculated by dividing the corrected absorbance 414 value by the corrected absorbance 414 of the 100% hemolysis control. The degree of hemolysis and the serum volume were plotted on a log-log paper. The volume of serum that gave 50% hemolysis was used for calculating the ACH50 using the formula: ACH50 (units/ml) = 1/K x r x ½. Where K is the amount of serum giving 50% hemolysis, r is the reciprocal of the serum dilution, and ½ is the correction factor. The assay was performed on a ½ scale of the original method.

2.6. Challenge test

S. agalactiae determination was detailed in our previous study [44]. Ten fish per tank were randomly caught and intraperitoneally injected with 0.1 mL of 0.85% saline solution containing 10^7 CFU ml^{-1} of *S. agalactiae* [45]. After injection, fish were returned to their tanks (150 L per tank) and fed different diets as following: SCG1 (control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}) for 15 days. Dead fish were removed daily, and the relative percentage of survival (RPS) was calculated following Amend [46]: RPS = (1- % mortality in vaccinated/% mortality in control) × 100.

2.7. Growth performance

Growth parameters (20 fish per tank) were calculated after 4- and 8-weeks post-feeding using equations: Weight gain (WG) = final weight (g) – initial weight (g); specific growth rate (SGR %) = 100 × (ln final weight - ln initial weight)/Duration of experiment; feed conversion ratio (FCR) = feed given (dried weight)/weight gain (wet weight); survival rate (%) = (final fish number/initial fish number) x 100.

2.8. Statistical analysis

Significant variations among diets ($P < 0.05$) were determined using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test) via the SAS Computer Program [47], and expressed as means ± standard deviation.

3. Results

After eight weeks of feeding, supplementations of SCG at 10 and 20 g kg^{-1} produced better ($P < 0.05$) mucosal lysozyme (SMLA) and peroxidase (SMPA) activities in contrast to basal diet (Table 2). The best grades were revealed in fish treated 10 g kg^{-1} (SCG2), but no significant ($P > 0.05$) difference was revealed among SCG1, SCG4, and SCG5 diets (Table 2).

SCG treated fish had considerable higher serum lysozyme (SL) level

Table 2
Skin mucus lysozyme and peroxidase activities of *O. niloticus* after 4 and 8 weeks feeding with experimental diets (n = 15, mean ± SD): SCG1 (Control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}). Different letter in a row denote significant difference ($P < 0.05$).

		SCG1	SCG2	SCG3	SCG4	SCG5
4 weeks	**SMLA**	1.57 ± 0.04^{c}	2.49 ± 0.08^{a}	1.86 ± 0.07^{b}	1.55 ± 0.03^{c}	1.57 ± 0.04^{c}
	SMPA	0.06 ± 0.008^{c}	0.12 ± 0.008^{a}	0.09 ± 0.006^{b}	0.06 ± 0.01^{c}	0.05 ± 0.008^{c}
8 weeks	**SMLA**	2.19 ± 0.07^{c}	3.22 ± 0.10^{a}	2.76 ± 0.07^{b}	2.24 ± 0.22^{c}	2.17 ± 0.15^{c}
	SMPA	0.11 ± 0.01^{c}	0.19 ± 0.008^{b}	0.16 ± 0.006^{b}	0.13 ± 0.008^{c}	0.12 ± 0.008^{c}

SMLA (μg mL^{-1}) = Skin mucus lysozyme activity.
SMPA (μg mL^{-1}) = Skin mucus peroxidase activity.

than the control (Table 3), with peaked level obtained in fish fed SCG2. Similarly, serum peroxidase (SP), alternative complement (ACH50), PI, and RB were significant higher ($P < 0.05$) in the supplemented groups (10 and 20 g SCG kg^{-1}), in contrast to the control group (Table 3), with the best levels were noticed in fish fed 10 g SCG kg^{-1}. However, no significant ($P > 0.05$) differences in these parameters were viewed in fish fed 20 and 40 g kg^{-1} SCG, as well as fish fed 80 g kg^{-1} SCG and the control diet (Table 3).

Compared to the control treatment (SCG1) (30.00% survival), the survival rates of fish fed the SCG diets were significantly ($P < 0.05$) higher; 80.0% (SCG2), 63.33% (SCG3), 60.00% (SCG4), and 56.67% for SCG5 after challenge with *S. agalactiae* (Fig. 1). The relative percent survival (RSP) was 62.50, 75.00, 58.33, and 37.50% for SCG2, SCG3, SCG4, and SCG5, respectively.

When compared to untreated group (SCG1), SCG added diets demonstrated a substantial enhancement specific growth rate (SGR), weight gain (WG), and final weight (FW), with maximal values viewed in fish fed 10 g kg^{-1} (SCG2) (Table 4). Nonetheless, no significant ($P > 0.05$) variations in SGR and WG were noticed between 20 (SCG3) and 40 (SCG4) SCG kg^{-1}, as well as between the SCG1 and SCG5 diets. The FCR was considerably ($P < 0.05$) lower in SCG2 treated diet in comparison to other diets, whereas highest ($P < 0.05$) FCR levels were viewed in the SCG1 and SCG5 diets. In terms of survivability, no impacts on survival rates ($P > 0.05$) were viewed among treatments.

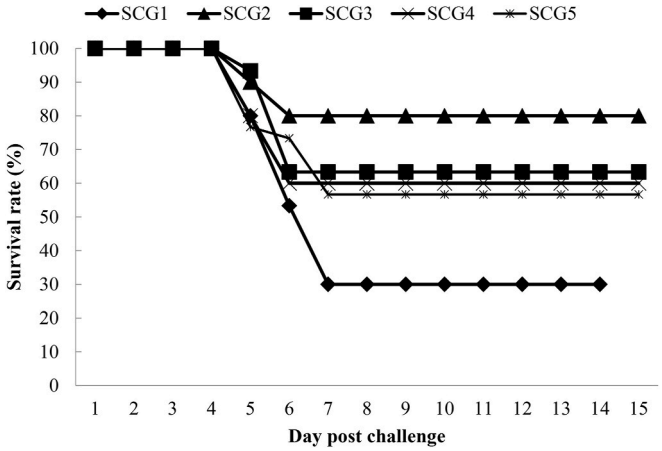

Fig. 1. Survival rate of *O. niloticus* after feeding with experimental diets (n = 30): SCG1 (Control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}).

4. Discussion

Skin mucus is an essential element in fish immune system [48,49], which known as the first layer of the defense system with many biological substances [50,51]. The results of present study demonstrated that SCG administrated diets boosted skin mucosal immune response. The findings are in accordance with those of earlier works European sea bass (*Dicentrarchus labrax*) [52]; common carp (*Cyprinus carpio*) [53,54]; Nile tilapia (*Oreochromis niloticus*) [55–57], and zebrafish (*Danio rerio*) [58];

In terms of serum immunological measures, the current work demonstrated that SCG administrated diets could boost these parameters. Numerous latest evidences have demonstrated that consuming agricultural by-products could enhance serum immunological parameters in gibel carp (*Carassius auratus* gibelio) [59]; barramundi (*Lates calcarifer*) [60]; gilthead seabream (*Sparus aurata*) [61]; Nile tilapia (*O. niloticus*) [55–57]; European seabass (*Dicentrarchus labrax*) [62], and common carp (*Cyprinus carpio*) [54]. Though its mode of action behind SCG immunomodulatory properties is unknown, it could be due to the presence of biological activities, such as galactomannans and arabinogalactans [63–65], and as a potential source of prebiotic [64,66,67]. Moreover, Choi and Koh [68] indicated that SCG is high in caffeine, gallic acid, protocatechuic acid, chlorogenic acid, kahweol, and cafestol, which are renowned to stimulate immune system [68–70]. López-Barrera, Vázquez-Sánchez, Loarca-Piña and Campos-Vega [71] revealed that SCG, rich in fiber, can be fermented by colon beneficial micro-organisms producing short chain fatty acid (SCFAs) with the capability to inhibit inflammation. The immunological stimulatory characteristic of functional feed additives is widely acknowledged to be

Table 3
Serum immunity of *O. niloticus* after 4 and 8 weeks feeding with experimental diets (n = 15, mean ± SD): SCG1 (Control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}). Different letter in a row denote significant difference ($P < 0.05$).

		SCG1	SCG2	SCG3	SCG4	SCG5
4 weeks	**SL**	3.88 ± 0.16^{c}	7.20 ± 0.15^{a}	6.15 ± 0.25^{b}	6.03 ± 0.20^{b}	4.09 ± 0.34^{c}
	SP	0.09 ± 0.01^{c}	0.18 ± 0.005^{a}	0.17 ± 0.006^{a}	0.14 ± 0.008^{b}	0.09 ± 0.006^{c}
	ACH50	146.70 ± 11.56^{c}	205.82 ± 7.38^{a}	179.87 ± 4.82^{ab}	154.32 ± 6.36^{bc}	146.95 ± 6.67^{c}
	PI	1.94 ± 0.08^{c}	2.74 ± 0.11^{a}	2.29 ± 0.05^{b}	2.03 ± 0.08^{bc}	1.86 ± 0.08^{c}
	RB	0.06 ± 0.008^{c}	0.12 ± 0.008^{a}	0.09 ± 0.005^{b}	0.07 ± 0.003^{bc}	0.05 ± 0.008^{c}
8 weeks	**SL**	6.66 ± 0.34^{c}	9.37 ± 0.30^{a}	9.02 ± 0.15^{a}	7.80 ± 0.05^{b}	6.89 ± 0.42^{bc}
	SP	0.18 ± 0.008^{c}	0.26 ± 0.008^{a}	0.24 ± 0.009^{a}	0.22 ± 0.01^{b}	0.19 ± 0.003^{c}
	ACH50	200.23 ± 5.59^{c}	300.68 ± 10.13^{a}	264.34 ± 10.49^{b}	212.67 ± 7.63^{c}	196.57 ± 17.21^{c}
	PI	2.67 ± 0.09^{c}	3.69 ± 0.08^{b}	3.25 ± 0.11^{b}	2.74 ± 0.06^{c}	2.69 ± 0.10^{c}
	RB	0.13 ± 0.01^{c}	0.20 ± 0.008^{a}	0.17 ± 0.006^{b}	0.12 ± 0.008^{c}	0.12 ± 0.006^{c}

SL = Serum lysozyme activity (μg mL^{-1}); SP = Serum peroxidase activity (μg mL^{-1}); ACH50 = Alternative complement activity (units mL^{-1}); PI = Phagocytosis activity (bead $cell^{-1}$); RB = Respiratory burst activity (OD655).

Table 4
Growth performances and feed utilization (n = 300, mean ± SD) of *O. niloticus* after 4 and 8 weeks feeding with experimental diets: SCG1 (Control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}). Different letter in a row denote significant difference ($P < 0.05$).

	SCG	SCG2	SCG3	SCG4	SCG5
IW (g)	15.23 ± 0.13	15.25 ± 0.09	15.25 ± 0.08	15.23 ± 0.14	15.30 ± 0.09
FW (g)					
4 weeks	45.93 ± 0.42^{d}	48.73 ± 0.10^{a}	47.23 ± 0.17^{b}	47.05 ± 0.35^{bc}	46.07 ± 0.25^{dc}
8 weeks	79.05 ± 1.36^{c}	94.95 ± 0.42^{a}	85.53 ± 0.47^{b}	82.42 ± 1.53^{bc}	79.98 ± 0.90^{c}
WG (g)					
4 weeks	30.70 ± 0.34^{c}	33.48 ± 0.43^{a}	31.98 ± 0.15 ^{b}	31.82 ± 0.30 ^{b}	30.77 ± 0.25^{c}
8 weeks	63.82 ± 1.32^{c}	79.69 ± 2.49^{a}	70.28 ± 0.45^{b}	67.19 ± 0.51^{bc}	64.68 ± 0.90^{c}
SGR (%)					
4 weeks	3.68 ± 0.02^{c}	3.87 ± 0.01^{a}	3.77 ± 0.01^{b}	3.76 ± 0.01^{b}	3.68 ± 0.02^{c}
8 weeks	2.75 ± 0.03^{c}	3.05 ± 0.01^{a}	2.88 ± 0.01^{b}	2.81 ± 0.03^{bc}	2.76 ± 0.02^{c}
FCR					
4 weeks	1.12 ± 0.01^{a}	1.00 ± 0.02^{c}	1.05 ± 0.04^{b}	1.05 ± 0.01^{b}	1.11 ± 0.02^{a}
8 weeks	1.18 ± 0.01^{a}	1.08 ± 0.01^{c}	1.13 ± 0.02^{b}	1.14 ± 0.01^{b}	1.18 ± 0.01^{a}
SR (%)					
4 weeks	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
8 weeks	91.68 ± 1.44	95.00 ± 2.50	95.00 ± 1.33	95.00 ± 1.33	93.33 ± 1.44

FW (g) = Final weight; WG (g) = Weight gain; SGR (%) = Specific growth rate; FCR = Feed conversion ratio; SR (%) = Survival rate.

due to SCFAs generation [72]. SCFAs influence non-specific immunity by attaching to GPR43, a protein coupled receptor found primarily on immune system and inflammatory cells [73–75]. Surprisingly, increasing the amount of SCG administrated showed no effect on these immunological markers. This seems likely because of the fact that greater levels of immunostimulants generally result in immunosuppressive, but the specific explanation for this is still unknown [76].

S. agalactiae has a serious influence on fish production and results in significant economic losses around the world [77–79]. The present results indicated that after injection with *S. agalactiae*, SCG administrated diets had higher survival rate than the control. Higher survival in 10 g kg^{-1} fed group may be as a result of the augmentation of immune parameters. SCG extracts could inhibit *Aeromonas hydrophila* in carp [80]. SCG contains a wide range of biological active substances with antimicrobial and antifungal activities [81]. In addition, biological substances in coffee by-products, including chlorogenic acid and caffeine are known to inhibit *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes*, and yeast (*Candida albicans*) [82,83]. Furthermore, they demonstrated strong antioxidant potential using various mechanisms and showed high antibacterial against *Phoma violacea* and *Cladosporium cladosporioides* [84].

Boosted growth performance is one of the most primarily goals in the aquaculture practice. Significant enhance growth performance and feed utilization was displayed in this study. The findings were in accordance with earlier researches reported in Nile tilapia (*O. niloticus*) [55,85] and gilthead seabream (*Sparus aurata*) [61]. Non-protein energy sources in the diet, including carbohydrates and lipids, can help animals retain and grow by lowering protein catabolism for energy [76,86]. The improvement may be due to bioactive compound presence in SCG. It is renowned that SCG contains high amount of amino acids, enzymes, polysaccharides, vitamins, and minerals [87–89]. Additionally, SCG possesses galacto-oligosaccharides and mannan-oligosaccharides, implies that they may have prebiotic property to certain beneficial microorganism, such as bifidobacteria [90,91]. It has been used as substitute feedstuff for domestic animals. Xu, Cai, Zhang and Ogawa [92] indicated that dietary inclusion of coffee spent at 100 and 200 g kg^{-1} decreased dry matter (DM), protein, fiber and energy utilization of male sheep. Choi, Rim, Na and Lee [93] also revealed that fermentation of SCG by microorganisms had the potential to improve the raw feedstuff quality in sheep. More recently, de Otálora, Ruiz, Goiri, Rey, Atxaerandio, San Martin, Orive, Iñarra, Zufia, Urkiza and García-Rodríguez [94] showed SCG incorporation up to 100 g kg^{-1} could improve performance and digestibility of Latxa dairy ewes. However, higher level of SCG cause significant lower growth rate and higher feed conversion ratio. When coffee beans are processed before being used, non-enzymatic reaction between amino acids and reducing sugar will arise, hence reducing the nutrient digestion [95]. Therefore, this could be a in which higher level of GCS supplementation in this study resulted in lower growth performance and feed utilization.

Biofloc technology aids in the reduction of feed consumption while also promoting cultured animals' development, immune response, and disease protection [28,96–98]. Earlier researches have shown that combing biofloc with different feed additives significantly improved production rate, immunology, and disease protection of different aquatic animals [55–57,99–101]. SCG has been demonstrated as a potential prebiotic [64,66,67]. Kishawy, Sewid, Nada, Kamel, El-Mandrawy, Abdelhakim, El-Murr, Nahhas, Hozzein and Ibrahim [101] reported that dietary inclusion of prebiotic source in the biofloc system led to increase lactic acid bacterial count, modulate immune response, enhance resistance against *Aeromonas hydrophila,* as well as and improve survival and growth of Nile tilapia. SCG is also a source of carbon [102–104]. The inclusion of an exterior carbon source enabled heterotrophic bacteria to ingest inorganic nitrogen by altering the water's C:N ratio, leading to greater bacterial protein biomass for fish and improved water quality [105]. In addition, it has been reported that SCG can potentially enhance wastewater treatment because of respiratory activity of microorganisms [106,107].

In short, the current findings clearly shown that SCG (10 g kg^{-1}) SCG does have the possibility being applied as growth enhancement, immunomodulatory, and disease protection in Nile tilapia culture. Future study with lower amount of SCG (less than 10 g kg^{-1}) should be conducted.

Author contributions

Hien Van Doan: Conceptual, conduct experiment, and manuscript preparation. **Chompunut Lumsangkul:** Sample collection. **Seyed Hossein Hoseinifar:** Data curation. **Sanchai Jaturasitha:** Statistical analysis. Hung Quang **Tran:** Data computation. **Yaowaluk Chanbang:** Spent coffee ground preparation. **Einar Ringø:** Final approval. **Vlastimil Stejskal:** Manuscript correction. All authors have read and agreed to the published version of the manuscript.

Ethical approval

Animal use protocol was followed the guideline of Chiang Mai University (No. AQ010/2563[02/2563-06-11).

Declaration of competing interest

No competing interests are reported.

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