Administration of watermelon rind powder to Nile tilapia (*Oreochromis niloticus*) culture under biofloc system: Effect on growth performance, innate immune response, and disease resistance

Hien Van Doan[a,b,*], Chompunut Lumsangkul[a], Seyed Hossein Hoseinifar[c], Tran Quang Hung[d], Vlastimil Stejskal[d], Einar Ringø[e], Mahmoud A.O. Dawood[f], Maria Ángeles Esteban[g]

[a]Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200 Thailand

[b]Science and Technology Research Institute, Chiang Mai University 239 Huay Keaw Rd., Suthep, Muang, Chiang Mai 50200

[c]Department of Fisheries Gorgan University of Agricultural Sciences and Natural Resources Gorgan-Iran

[d]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters, Na Sádkách 1780, 370 05 České Budějovice, Czech Republic.

[e]Norwegian College of Fishery Science, Faculty of Bioscience, Fisheries and Economics, UiT The Arctic University of Norway, Tromsø, Norway

[f]Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt

[g]Fish Innate Immune System Group, Department of Cell Biology & Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain.

*Author for correspondence: Hien Van Doan. E-mail address: hien.d@cmu.ac.th

Abstract

An eight-week experiment was performed to assess the effectiveness of watermelon rind powder (WMRP) on growth efficiency, immunity, and disease resistance of Nile tilapia, *O. niloticus*. Three hundred fish (17.14 ± 0.12 g) were fed five diets; 0 (Diet 1- control), 20 g kg^{-1} WMRP (Diet 2), 40 g kg^{-1} WMRP (Diet 3), 80 g kg^{-1} WMRP (Diet 4), and 160 g kg^{-1} WMRP (Diet 5). Growth parameters, skin mucus, and serum immunities were analyzed after four and eight weeks of feeding. After eight weeks of the feeding, ten fish were used in the challenge against *Streptococcus agalactiae* over 15 days. Statistically significant enhancement ($P \leq 0.05$) of skin mucus and serum immune parameters were revealed through the WMRP feeding *vs*. control fed fish, in which the maximum ($P \leq 0.05$) enhancement of immune parameters was detected in tilapia fed the 40 g kg^{-1} WMRP diet, followed by the 20, 80, and 160 g kg^{-1} WMRP diets. Relative percent survival (RSP) in the challenge study of fish fed 20, 40, 80, and 160 g kg^{-1} WMRP was 57.14%, 76.19%, 61.90%, and 52.38%, respectively. The growth parameters were statistically ($P \leq 0.05$) enhanced in the WMRP feedings, in which the largest increase was revealed in the 40 g kg^{-1} WMRP treatment. In summary, the 40 g kg^{-1} WMRP additive increased both the growth efficiency and health status of Nile tilapia.

Keywords: Watermelon rind; Biofloc; Nile tilapia; Innate Immune; *Streptococcus agalactiae*

1. Introduction

Global aquaculture accounts for more than fifty percent of world seafood production and is accountable for the remarkable growth of protein sources for mankind (FAO, 2018). Nile tilapia is among the world's most farmed fish, owing to its robust production, adaptability, and significant commercial price (El Asely, Reda, Salah, Mahmoud, Dawood, 2020; FAO, 2018). However, the over intensified tilapia culture has induced severe stresses on the quality of cultivated water, and raised the prevalence of the infected disease, particularly bacterial infections (Nicholson, Mon-on, Jaemwimol, Tattiyapong, Surachetpong, 2020; Piamsomboon, Thanasaksiri, Murakami, Fukuda, Takano, Jantrakajorn, Wongtavatchai, 2020). This results in a high mortality rate for farmed fish and severe economic damages (Chen, Liu, Hu, 2019). Among them, *Streptococcus* spp. is one of the most frequently observed pathogens, causing significant economic losses in tilapia industry (Guangjin, Jielian, Kangming, Tingting, Huochun, Yongjie Liu, Wei, Chengping, 2016; Mishra, Nam, Gim, Lee, Jo, Kim, 2018; Xia, Lu, Chen, Cao, Gao, Wang, Liu, Zhang, Zhu, Yi, 2018). In past decades, antibiotic administration was widely implemented worldwide to inhibit and treat bacterial diseases (Rico, Oliveira, McDonough, Matser, Khatikarn, Satapornvanit, Nogueira, Soares, Domingues, Van den Brink, 2014); however, antibiotic treatments have resulted in the appearance of antimicrobial bacteria, and degradation of the cultured environment (Kraemer, Ramachandran, Perron, 2019). Therefore, safer and sustainable solutions for tilapia cultivation are needed.

The dietary inclusion of functional feed additives has recently gained much attention in aquaculture practice (Encarnação, 2016; Mohan, Ravichandran, Muralisankar, Uthayakumar, Chandirasekar, Seedevi, Abirami, Rajan, 2019). In this context, agricultural by-products present possible addition of dietary fibre, behaving as prebiotics that can be incorporated as therapeutic additives to treat diseases linked to the modification of gut microbiota (Buruiana, Gómez, Vizireanu, Garrote, 2017). Watermelon rind powder (WMRP) presents a viable choice of such products. Global watermelon production was approximately 118 million tons in 2017 (Rico, Gullón, Alonso, Yáñez, 2020). However, watermelon rinds comprise a significant portion of the entire fruit production, yet are typically unusable, and are wasted (Al-Sayed, Ahmed, 2013; Romdhane, Haddar, Ghazala, Jeddou, Helbert, Ellouz-Chaabouni, 2017). The rind possesses minerals, fats, proteins, carbohydrates, vitamins, phytochemicals, and citrulline (Maoto, Beswa, Jideani, 2019). Carbohydrates are the major substances of WMR and are considered an effective source of pectin production (Petkowicz, Vriesmann, Williams, 2017), which has been demonstrated to be a novel prebiotic (Babbar, Dejonghe, Gatti, Sforza, Kathy, 2015; Míguez, Gómez, Gullón, Gullón, Alonso, 2016). Therefore, making use of such by-products would add value to this industrial residue, as well as provide a beneficial and well needed raw material for nutraceutical industries (Gómez-García, Campos, Aguilar, Madureira, Pintado, 2020). However, research on the impacts of such a biofloc-added environment has not yet been fully investigated.

Biofloc technology (BFT), used extensively in aquaculture, contains multiple microorganisms, algae, and other detritus that provide food for omnivorous farmed fish and shellfish (Khanjani, Sharifinia, 2020; Liu, Li, Wei, Zhu, Han, Jin, Yang, Xie, 2019). In recent decades, numerous studies have demonstrated biofloc technology's many beneficial impacts on water quality, production, immunological responsiveness, and disease protection in fish (Khanjani, Sharifinia, 2020; Liu, Li, Wei, Zhu, Han, Jin, Yang, Xie, 2019). Prebiotics, at the same time, play an equally significant role in fish cultivation

(Li, Tran, Ji, Sun, Wen, Li, 2019; Serradell, Torrecillas, Makol, Valdenegro, Fernández-Montero, Acosta, Izquierdo, Montero, 2020). Therefore, the introduction of prebiotics to the biofloc is thought to propagate beneficial microbiota, not just in the water, but also in the host's intestine to counteract the potentially hazardous pathogens. Recent research has been undertaken based on this hypothesis, the results of which indicated that the introduction of functional feeds into the biofloc significantly improved water quality, animal development, immunity, and survivability (Mandal, Das, 2018; Qiao, Chen, Sun, Zhang, Zhang, Li, Li, 2020). Researching of the effects of watermelon rind powder (WMRP) within the biofloc represents an innovative and interdisciplinary strategy; yet to be fully investigated. We hypothesized that the combination of watermelon rind powder and biofloc system would improve fish growth and health status. Our research, herein, aimed to examine the influence of WMRP on growth, immunity, disease resistance to *Streptococcus agalactiae* of Nile tilapia cultivated under the biofloc system.

2. Methodologies

2.1 Watermelon rind powder preparing

Watermelon was collected from a local farm. After processing, the peels were gathered and dried in an oven for 48 hours at 60ºC, crushed using a hammer mill, screened using a 100-mesh sieve, and then preserved at 4°C.

2.2 Diets preparation

The initial tilapia diet was established in the previous work of Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan (2020). Five diets, modifications of those in the previous study of Nguyen, Han, Yang, Ikeda, Eltahan, Chowdhury, Furuse (2019) were prepared with the inclusion of WMRP: 0 (Diet 1 - control), 20 g kg^{-1} WMRP (Diet 2), 40 g kg^{-1} WMRP (Diet 3), 80 g kg^{-1} WMRP, and 160 g kg^{-1} WMRP (Diet 5) given in Table 1. In the production of feed pellets, powdered feedstuffs were blended thoroughly, and soya oil and water were added to make a stiff dough. It was then pushed across an extruder to shape the pellets. The wet pellets were then gathered and dehydrated in a hot air oven at 50°C to obtain roughly ten percent moisture content, then placed in plastic bags and store at 4°C.

2.3 Trial set-up

Farm-raised mono sex fish (male) were confined and fed a completed diet (CP, 9950) for 60 days, following by a basal diet for two weeks. Twenty fish were then captured unexpectedly for health screenings, in which their physical structures, gills, and major organs were inspected. Next, 300 fish (17.14 ± 0.12 g fish^{-1}) were then captured and moved to 15 tanks (volume 150 L tank^{-1}) at a density of 20 fish tank^{-1}. The experiment was planned in a completed randomized design (CRD). The five experimental diets were distributed in triplicates to the tanks for eight weeks. Growth performance and immunity were determined every 4 weeks, and ten fish tank^{-1} were randomly selected for the challenge trial with *S. agalactiae.* The test diets were provided *ad libitum*, twice daily, at 8:30 a.m. and 4:30 p.m. Water temperature, pH, and dissolved oxygen were kept at 27.5 ± 0.8°C, 7.79 ± 0.15, and 5 mg litre^{-1}, respectively.

2.4 Managing water conditions

Parameters for water quality were measured using HI98196 meter, while total ammonia-nitrogen (TAN) was detected via HI96733 meter. The amount of the biofloc was estimated via an Imhoff cone (Khoa, Tao, Van Khanh, Hai, 2020).

2.5 Immunity analysis

2.5.1 Skin mucus preparation

Skin mucus preparation was performed in three-clove oil anesthetized fish tank^{-1}. Anesthetized fish were put inside polyethylene bags comprising 10mL of 50mM NaCl and softly rubbed for two minutes. The mixture was directly discharged into 15 mL sanitary tubes and centrifuged for ten minutes, 1500g, at 4ºC. Pipettes containing 500 µL of supernatant were stored -80ºC for future study.

2.5.2 Serum and mucus preparation

Serum was collected (3 fish tank^{-1}) following the protocol described in (Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan, 2020). Briefly, blood (1 mL) was collected via the caudal vein of each fish using a 1mL syringe and immediately released into 1.5 mL Eppendorf tubes without anticoagulant. The tubes were then incubated at room temperature for one hour, and stored in a refrigerator (4°C) for four hours. After incubation, the samples were centrifuged at 1500g for five minutes at 4 °C, and the anticipated serum was gathered using a micro-pipette and stored at - 80 °C for further evaluation.

Skin mucus was sampled from three fish and pooled in the manner employed in Hoseinifar, Sohrabi, Paknejad, Jafari, Paolucci, Van Doan (2019). Briefly, fish were anaesthetized using clove oil at a concentration of 5 mL litre^{-1}. They were then placed in polyethylene bag containing 10 ml of 50 mM NaCl (Merck, Germany), and gently rubbed in a downward motion for approximately one minute. The mucus was immediately poured into 15 mL sterile centrifuge tubes and centrifuged (1.500 × g for 10 min at 4 °C). Additionally, 1 mL of the mucus was kept in a 1.5 mL Eppendorf tube at −80 °C.

2.5.3 Leukocytes preparation

Blood leukocytes were isolated under the procedure outline in (Chung, Secombes, 1988), with several variations (Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan, 2020).

Briefly, one milliliter of blood was withdrawn from each fish, at a rate of four fish per replication, and then transferred into 15 mL tubes containing 2 mL of RPMI 1640 (Gibthai). This mixture was then carefully inserted into 15mL tubes, containing 3 mL of Histopaque (Sigma, St. Louis, MO, USA). These tubes were then centrifuged at 400 g for 30 minutes at room temperature. Upon completion, a buffy coat of leucocytes cells drifted to the top of the Histopaque was carefully collected using a Pasteur pipette, and released into sanitized 15mL tubes. After which, 6mL of phosphate buffer solution (PBS: Sigma-Aldrich, USA) was added to each tube and gently aspirated. The cells in these tubes were washed twice by centrifugation at 250g for ten minutes at room temperature to remove any residual Histopaque. The cells obtained were then re-suspended in the PBS and adjusted to the numbers of cells required to evaluate phagocytic and respiratory burst activities.

2.5.4 Serum and skin mucus lysozyme assays

Lysozyme assays were measured using the procedure (Parry, Chandan, Shahani, 1965) with some adjustments, as detailed in (Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan, 2020). Briefly, 25µL of undiluted serum and 100µL of skin mucus from each fish was loaded into 96-well plates in triplication. After this, *Micrococcus lysodeikticus* (175µL, 0.3 mg mL^{-1} in 0.1 M citrate phosphate buffer, pH 5.8) was added to each well. The contents were rapidly mixed, and any changes in turbidity were measured every 30 seconds, for five minutes, at 540nm, 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 0-20µl mL^{-1} (Sigma Aldrich, USA), and expressed as µg mL^{-1} serum.

2.5.5 Determination of serum and mucus peroxidase

Peroxidase activity was carried out according to (Cordero, Cuesta, Meseguer, Esteban, 2016) with some minor modification (Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan (2020). Briefly, 5µL of undiluted serum or skin mucus from each fish was placed in the 96-flat-bottomed-well plates, in triplication. Then, 45µl of *Hank's Balanced Salt Solution* (without Ca^{+2} or Mg^{+2}) was added to each well. Afterward, 100µL of solution (40ml of distilled water + 10µL of H_2O_2, 30%; Sigma Aldrich + one pill of 3,3',5,5'-tetramethylbenzidine, TMB; Sigma Aldrich) was then added to each well. When the reaction color turned blue, after 30 to 60 seconds, a 50µl solution of 2M H_2SO_4 was then immediately added to each well. The optical density was then read at 450nm via a microplate reader (Synergy H1, BioTek, USA). Samples not containing serum or skin mucus were considered to be blanks. A single unit was defined as the amount which produces an absorbance change, expressed as units (U) mL^{-1} of serum or mucus following the equation: Peroxidase activity = [absorbance of the sample] – [(absorbance of blank (containing all solution without serum or mucus sample].

2.5.6 Complement activity (ACH50)

ACH50 calculations were carried out according to Yano (1992) with slight variations (Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan, 2020). Briefly, rabbit red blood cells (R-RBC) were washed with PBS by centrifugation at 3000 rpm, and in 0.01M ethylene glycol tetra-acetic acid-magnesium-gelatin veronal buffer (0.01M – EGTA-Mg-GVB) for twice. The R-RBC concentration was adjusted to $2x10^8$ cells mL^{-1} in 0.01M – EGTA-Mg-GVB buffer. Then 100 µL of the R-RBC suspension was lysed with 3.4 mL of distilled water. Hemolysate absorbance was measured at 414 nm *vs.* distilled water as a blank and was adjusted to reach 0.740.

For the ACH50 test, 100 µL of serum was diluted with 400 µL of 0.01M-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 20ºC for 1.5 hours with occasional shaking. After incubation, 3.15 mL of cold saline solution (0.85% NaCl) was placed into each tube to stop the reaction, and then the tube was centrifuged at 1600 g for 5 minutes. 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.

2.5.7 Phagocytic activity

The phagocytosis was calculated according to (Yoshida, Kitao, 1991) with slight modification, for detail see (Van Doan, Hoseinifar, Sringarm, Jaturasitha, Yuangsoi, Dawood, Esteban, Ringø, Faggio, 2019). Briefly, 200µL of leucocyte cell suspensions (2 x 10^6 cells mL^{-1}) were loaded on coverslips and incubated at room temperature for two hours. After incubation, the coverslips were washed with 3mL of RPMI-1640 to remove any non-adherent cells. Then, a solution of 200µL of fluorescence latex beads with a concentration of 2 x 10^7 of beads (mL^{-1}) (Sigma-Aldrich, USA) was placed into each coverslip and incubated again at room temperature for 1.5 hours. The coverslips were then rewashed with 3mL of RPMI- 1640 to remove any non-phagocytized bead. After washing, the coverslips were then fixed with methanol, and stained with Diff-Quik staining dye (Sigma-Aldrich, USA) for ten seconds. After staining, a wash of PBS (pH 7.4) removed any excessive stains. The washed coverslips were allowed to dry at room temperature and then attached to the slides with Permount (Merck, Germany). The number of phagocyte cells per 300 adhered cells was later counted microscopically. The phagocytic index (PI) and phagocytic rate (PR%) were calculated through the following equations: PI = (Number of phagocytized beads divided by the number of phagocytizing leukocytes) *100.

2.5.8 Respiratory burst

Respiratory burst activity was measured as described (Secomebs, 1990), with minor changes as outlined (Van Doan, Hoseinifar, Sringarm, Jaturasitha, Yuangsoi, Dawood, Esteban, Ringø, Faggio, 2019). Briefly, 175µL PBS cells suspension at a concentration of 6 x 10^6 cells mL^{-1} were loaded into the 96 well plates in triplication. Then, 25µL of nitro blue tetrazolium (NBT) at a concentration of 1mg mL^{-1} was added to each well and incubated the solution for two hours at room temperature. Later, the supernatant was carefully discarded from each well, and 125µL of 100% methanol was then added into each well for five minutes to fix the cells. After that, 125µL of 70% methanol $well^{-1}$ were added into each well, twice, for clean-up. The plates were then dried for thirty minutes at room temperature. Then, 125µL of 2N KOH and 150µL of DMSO were added to each well. Afterward, the plates were measured at 655nm via microplate-reader (Synergy H1, BioTek, USA), according to the following: Spontaneous O^{2-} production = (absorbance NBT reduction of the sample) – [(absorbance of blank (containing 125µL of 2N KOH and 150 µL with no leucocytes)].

2.6 Challenge test

Streptococcus agalactiae was isolated and prepared according to (Van Doan, Hoseinifar, Chitmanat, Jaturasitha, Paolucci, Ashouri, Dawood, Esteban, 2019). Ten tilapia of each replication were randomly used in the challenge test, and were intraperitoneally injected with 0.1mL *S. agalactiae* (10^7 CFU mL^{-1} of 0.85% saline solution) as described elsewhere (Wang, Gan, Cai, Wang, Yu, Lin, Lu, Wu, Jian, 2016).

2.7 Growth parameters

After 4 and 8 weeks feeding were growth rate and survivability measured, as described in (Doan, Hoseinifar, Jaturasitha, Dawood, Harikrishnan, 2020).

2.8 Statistical analysis

Data were measured using one-way variance analysis (ANOVA) and Duncan's Multiple Range Test) through SAS Computer Program (SAS, 2003). Various mean values ($P < 0.05$) and other measurements are shown as mean ± SD.

3. Results

3.1 Skin mucus immune responses

Fish's SMLA (skin mucus lysozyme activity) and SMPA (skin mucus peroxidase activity) fed WMRP diets are displayed in Table 2. The results evidenced that fish fed the WMRP diets significantly ($P \leq 0.05$) enhanced SMLA and SMPA four and eight weeks post-feeding, in all inclusion, except for the fish fed 160 g kg^{-1} (Table 2).The highest skin mucus immunities were revealed in the 40 g kg^{-1} WMRP, followed by the 20 and 80 g kg^{-1} WMRP diets (Table 2). No substantial discrepancies in SMLA and SMPA ($P \geq 0.05$) were displayed in fish fed the 20 and 80 g kg^{-1} WMRP diets (Table 2).

3.2 Serum immunity

The impacts of WMRP addition on serum lysozyme (SL), shown in Table 3, revealed that the 40 g kg^{-1} WMRP supplementation presented significantly higher SL over the control as well as the 20, 80, and 160 g kg^{-1} diets (Table 3). Nevertheless, no statistically significant ($P \geq 0.05$) alterations were noticed on SL between the WMRP incorporated diets; 20, 80, and 160 g kg^{-1} WMRP (Table 3). The 40 g kg^{-1} WMRP also ($P \leq 0.05$) significantly enhanced fish serum peroxidase (RB) and phagocytosis (PI), and improved ACH50 and RB (8-week post-feeding) in comparison to the other diets. No significant differences in ACH50 and RB were revealed ($P \geq 0.05$) among the 20, 80, and 160 g kg^{-1} WMRP supplemented diets (Table 3).

3.3 Challenge test

Survival rates were substantially ($P \leq 0.05$) enhanced in all supplemented WMRP groups: 66.7% (Diet 2), 83.3% (Diet 3), 70.0% (Diet 4), and 60.0% (Diet 5) *vs*. control fed fish 30.0% (Fig. 1). The relative percent survival (RSP) was 57.1%, 76.2%, 61.9%, and 52.48% in the 20, 40, 80, and 160 g kg^{-1} WMRP, respectively (Fig. 1). The results, shown in Figure 1, again demonstrated the superior benefits of the fish fed 40 g kg^{-1} WMRP diet, in which the highest RPS and the greatest resistance against *S. agalactiae* were found.

3.4 Growth performance

Fish fed the WMRP diets for eight weeks showed substantially ($P \leq 0.05$) increased final weight (FW), weight gain (WG), and specific growth rate (SGR) *vs*. the control (Table 4). The best growth rate was illustrated in the fish administrated with 40 g kg^{-1} WMRP diet, followed by the 20, 80, and 160 g kg^{-1} WMRP diets. Nevertheless, the results did not significantly ($P \geq 0.05$) vary among the 20, 80, and 160 g kg^{-1} WMRP fed fish. The 40 g kg^{-1} WMRP fed fish also presented the lowest FCR, on which the remaining groups, again, showed no significant differences. Survival rate was not affected within the groups (Table 4).

4. Discussion

The secretion of mucus from the fish skin is the first defensive response during stress and outbreaks (Brinchmann, 2016; Kulczykowska, 2019; Reverter, Tapissier-Bontemps, Lecchini, Banaigs, Sasal, 2018). Skin mucus is abundant with several immune responses, including lysozyme, peroxidase, and bactericidal activities (Dash, Das, Samal, Thatoi, 2018; Dawood, 2016; Pietrzak, Mazurkiewicz, Slawinska, 2020). In the current study, the increased lysozyme and peroxidase activities in the skin mucus were markedly displayed through the inclusion of WMRP in the tilapia diets. Significant increases skin mucus were also observed in convict cichlid, *Amatitlania nigrofasciata* (Hoseinifar, Jahazi, Nikdehghan, Van Doan, Volpe, Paolucci, 2020) and common carp, *Cyprinus carpio* L. fed polyphenols from agricultural by-products; rainbow trout (*Oncorhynchus mykiss*) fed olive (*Olea europea* L.) waste (Hoseinifar, Shakouri, Yousefi, Van Doan, Shafiei, Yousefi, Mazandarani, Torfi Mozanzadeh, Tulino, Faggio, 2020); common carp, *Cyprinus carpio* fed turmeric and white-button mushroom powder (Giri, Sukumaran, Park, 2019; Hoseinifar, Khodadadian Zou, Paknejad, Hajimoradloo, Van Doan, 2019), and in yellowfin seabream, *Acanthopagrus latus* fed taurine (Dehghani, Oujifard, Mozanzadeh, Morshedi, Bagheri, 2020). Along with enhanced mucosal immune response, the WMRP additives also improved the serum immunity. The results were similar to previous studies involving the application of several functional supplements in tilapia diets (Srichaiyo, Tongsiri, Hoseinifar, Dawood, Esteban, Ringø, Van Doan, 2020; Srichaiyo, Tongsiri, Hoseinifar, Dawood, Jaturasitha, Esteban, Ringø, Van Doan, 2020).

Although no former studies of the impact of WMRP on aquatic animals have been conducted, the results of the present study confirm the importance of WMRP as a functional ingredient in Nile tilapia diet. WMRP is a rich source of β-carotene and vitamin C, which are associated with local intestinal immunity, and acts as immunostimulants with antioxidative factors (Tarazona-Díaz, Viegas, Moldao-Martins, Aguayo, 2011). WMR also contains high levels of lycopene essential amino acids, known as citrulline (Alagbe, 2018; Tarazona-Díaz, Viegas, Moldao-Martins, Aguayo, 2011), which is an effective hydroxyl radical scavenger and powerful antioxidant (Ginguay, Regazzetti, Laprevote, Moinard, De Bandt, Cynober, Billard, Allinquant, Dutar, 2019). The *de novo* synthesis of citrulline in the small intestine of rats transformed 83% of citrulline to arginine as a non-essential amino acid in the kidney (Marini, Stoll, Didelija, Burrin, 2012). Arginine is a crucial amino acid that plays a key role in reproductive, pulmonary, renal, gastrointestinal, hepatic, and immune systems, as well as in the ability to cure wounds (Tarazona-Díaz, Viegas, Moldao-Martins, Aguayo, 2011; Wu, Bazer, Davis, Kim, Li, Marc Rhoads, Carey Satterfield, Smith, Spencer, Yin, 2009).

S. agalactiae severely impacts aquaculture activities and causes massive economic damage around the world (Amal, Saad, Zahrah, Zulkafli, 2015; Mishra, Nam, Gim, Lee, Jo, Kim, 2018; Sukhavachana, Tongyoo, Massault, McMillan, Leungnaruemitchai, Poompuang, 2020). The effective defense of fish against Streptococcus infection is also one of the key goals of today's fish farming practices. Significant enhance disease resistance via agricultural and industrial by-products have been proved in various fish species, such as olive flounder, *Paralichthys olivaceus* fed citrus by-products fermented and fermented tuna by-product meal (Lee, Kim, Song, Oh, Cha, Jeong, Heo, Kim, Lee, 2013; Oncul, Aya, Hamidoghli, Won, Lee, Han, Bai, 2019); Nile tilapia, *O. niloticus* fed orange peels derived pectin, corncob derived xylooligosaccharides, and spent mushroom substrate crude glucan (Chirapongsatonkul, Mueangkan, Wattitum, U-taynapun, 2019; Doan, Hoseinifar, Elumalai, Tongsiri, Chitmanat, Jaturasitha, Doolgindachbaporn, 2018; Van Doan, Hoseinifar, Faggio, Chitmanat, Mai, Jaturasitha, Ringø, 2018; Van Doan, Hoseinifar, Naraballobh, Jaturasitha, Tongsiri, Chitmanat, Ringø, 2019); barramundi, *Lates calcarifer* fed tuna hydrolysate in poultry by-product meal (Siddik, Howieson, Fotedar, 2019). The present findings demonstrate the protective abilities of WMRP in Nile tilapia against *S. agalactiae*. The elevated antimicrobial efficacy against *E. coli* following by *B. cereus* and *S. aureus* expose to watermelon rind extracts was proved (Kumar, Mehta, Malav, Kumar Chatli, Rathour, Kumar Verma, 2018). In a previous study, Cemaluk (2015) demonstrated that watermelon rind extracts aided in the protection against ten pathogenic bacteria, creating greater inhibition zones in *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Likewise, El Zawawy (2015) determined that watermelon peel extract with phenolics, carotenoids, saponins, flavonoids, and tannins properties significantly improved the defense against bacterial infections. Don (2018) has recently demonstrated that watermelon rind extract was capable of inhibiting *Staphylococcus aureus*, *E. coli*, and *Salmonella typhi* infections.

The primary goal of aquaculture is to achieve the highest growth rate and the best feed efficiency. To achieve this aim, aquaculture professionals have established several techniques that facilitate rapid-growth output through feed additives and growth boosters (Hernández, Romero, Gonzalez-Stegmaier, Dantagnan, 2016; Katya, Yun, Park, Lee, Yoo, Bai, 2014). In the present study, improved growth performance and feed efficiency (FCR) parameters of Nile tilapia fed WMRP and reared under biofloc conditions was revealed. In accordance with our results, improved growth performance was observed in

African sharptooth catfish, *Clarias gariepinus* fed *Citrullus lanatus* seed meal (Tiamiyu, Okomoda, Agbese, 2015); Nile tilapia, *O. niloticus* fed melon seed peel (Iheanacho, Ikwo, Igweze, Ogueji, 2018), and in African catfish, *Heterobranchus bidorsalis* fed watermelon syrup booster (Nwanevu, Sokari, Isitor, Orlu, Ogolo, Ebere, 2019). The WMRP additive proved to be a nutritious ingredient with enhanced prosperities, capable of increasing the palatability of the diets, which in turn, enhanced the feed intake (Nobakht, Gorbanalinejad, 2017). Furthermore, WMRP may increase the potential role of the intestinal microbiota in fish digestion, and to facilitate the absorption of the nutrients through its intestinal barriers.

Biofloc technology (BT) is based on the principles of recycled waste, established to enhance water quality, minimize water consumption, and waste generation. BT also plays a vital role in reducing feed utilization and promotes growth, immunity, and disease resistance of farmed fish and shellfish (Bossier, Ekasari, 2017; García-Ríos, Miranda-Baeza, Coelho-Emerenciano, Huerta-Rábago, Osuna-Amarillas, 2019; Khanjani, Sharifinia, 2020; Liu, Li, Wei, Zhu, Han, Jin, Yang, Xie, 2019). Several biological substances are found in biofloc, such as microbial associated molecular patterns (MAMPs), essential fatty acids, carotenoids, free amino acids, chlorophylls (Ekasari, Hanif Azhar, Surawidjaja, Nuryati, De Schryver, Bossier, 2014), trace minerals (Tacon, Cody, Conquest, Divakaran, Forster, Decamp, 2002), vitamin C (Ju, Forster, Conquest, Dominy, Kuo, David Horgen, 2008), and poly-β-hydroxybutyrate (PHB) (Qiao, Chen, Sun, Zhang, Zhang, Li, Li, 2020). These compounds provide significant impacts on aquatic animals, such as enhanced antioxidant status, growth, reproduction, immunity, and disease resistance. The recently studied integration of biofloc with feed supplements has received significant acclaim as a novel approach for sustainable aquaculture.

Significant improvements in growth performance, immune response, and disease resistance have been reported in fish and shellfish treated in biofloc environment combined with various functional feed additives, such as Pacific white shrimp (Rodrigues, Bolívar, Legarda, Guimarães, Guertler, do Espírito Santo, Mouriño, Seiffert, Fracalossi, do Nascimento Vieira, 2018); Nile tilapia (Doan, Hoseinifar, Elumalai, Tongsiri, Chitmanat, Jaturasitha, Doolgindachbaporn, 2018; Van Doan, Hoseinifar, Naraballobh, Jaturasitha, Tongsiri, Chitmanat, Ringø, 2019), and gibel carp, *Carassius auratus gibelio* (Qiao, Chen, Sun, Zhang, Zhang, Li, Li, 2020). Similar results were observed in the present study of Nile tilapia fed WMRP within the biofloc system. The improvements in growth performance, immune response, and disease resistance may be due to the bioactive compound present in WMRP, which contains a high amount of pectin. Pectin, considered to be a novel prebiotic (Chung, Meijerink, Zeuner, Holck, Louis, Meyer, Wells, Flint, Duncan, 2017; Khorasani, Shojaosadati, 2017), has been shown to include the positive effects of the beneficial bacteria present in biofloc, which improve fish production and health status.

In conclusion, the supplementation of WMRP in diets fed to tilapia reared in biofloc conditions exhibited improved humoral and skin mucus immunity, as well as increased growth performance. WMRP represents an alternative, environmentally friendly concept to increase the resistance of Nile tilapia to *S. agalactiae* infection.

Acknowledgements

The researchers wish to show their sincere gratitude to the Thailand Research Fund for its financial aid (Grant No. MRG6180291). This research work was partially supported by Chiang Mai University. The study was also financially supported by NAZV project (QK1810296), Czech Republic.

Ethical Approval

Animal use protocol was followed the guideline of Chiang Mai University (No. 2561/AQ-0004).

References

Al-Sayed, H.M., Ahmed, A.R., 2013. Utilization of watermelon rinds and sharlyn melon peels as a natural source of dietary fiber and antioxidants in cake. Annals of Agricultural Sciences. 58, 83-95.

Alagbe, J., 2018. Performance and haemato-biochemical parameters of weaner rabbits fed diets supplemented with dried water melon peel (Rind) meal. Journal of Dairy and Veterinary Science.

Amal, M.N.A., Saad, M.Z., Zahrah, A.S., Zulkafli, A.R., 2015. Water quality influences the presence of *Streptococcus agalactiae* in cage cultured red hybrid tilapia, *Oreochromis niloticus* × *Oreochromis mossambicus*. Aquaculture Research. 46, 313-323.

Babbar, N., Dejonghe, W., Gatti, M., Sforza, S., Kathy, E., 2015. Pectic oligosaccharides from agricultural by-products: production, characterization and health benefits. Critical Reviews in Biotechnology. 0, 1-13.

Bossier, P., Ekasari, J., 2017. Biofloc technology application in aquaculture to support sustainable development goals. Microbial Biotechnology. 10, 1012-1016.

Brinchmann, M.F., 2016. Immune relevant molecules identified in the skin mucus of fish using -omics technologies. Molecular BioSystems. 12, 2056-2063.

Buruiana, C.-T., Gómez, B., Vizireanu, C., Garrote, G., 2017. Manufacture and evaluation of xylooligosaccharides from corn stover as emerging prebiotic candidates for human health. LWT - Food Science and Technology. 77, 449-459.

Cemaluk, C.E.A., 2015. Comparative investigation of the antibacterial and antifungal potentials of the extracts of watermelon (*Citrullus lanatus*) rind and seed. European Journal of Medicinal Plants, 1-7.

Chen, S.-W., Liu, C.-H., Hu, S.-Y., 2019. Dietary administration of probiotic *Paenibacillus ehimensis* NPUST1 with bacteriocin-like activity improves growth performance and immunity against *Aeromonas hydrophila* and *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology. 84, 695-703.

Chirapongsatonkul, N., Mueangkan, N., Wattitum, S., U-taynapun, K., 2019. Comparative evaluation of the immune responses and disease resistance of Nile tilapia (*Oreochromis niloticus*) induced by yeast β-glucan and crude glucan derived from mycelium in the spent mushroom substrate of *Schizophyllum commune*. Aquaculture Reports. 15, 100205.

Chung, S., Secombes, C.J., 1988. Analysis of events occurring within teleost macrophages during the respiratory burst. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 89, 539-544.

Chung, W.S.F., Meijerink, M., Zeuner, B., Holck, J., Louis, P., Meyer, A.S., Wells, J.M., Flint, H.J., Duncan, S.H., 2017. Prebiotic potential of pectin and pectic oligosaccharides to promote anti-inflammatory commensal bacteria in the human colon. FEMS microbiology ecology. 93, fix127.

Cordero, H., Cuesta, A., Meseguer, J., Esteban, M.A., 2016. Changes in the levels of humoral immune activities after storage of gilthead seabream (*Sparus aurata*) skin mucus. Fish and Shellfish Immunology. 58, 500-507.

Dash, S., Das, S.K., Samal, J., Thatoi, H.N., 2018. Epidermal mucus, a major determinant in fish health: a review. Iran J Vet Res. 19, 72-81.

Dawood, M.A.O., 2016. Effect of various feed additives on the performance of aquatic animals. Kagoshima University.

Dehghani, R., Oujifard, A., Mozanzadeh, M.T., Morshedi, V., Bagheri, D., 2020. Effects of dietary taurine on growth performance, antioxidant status, digestive enzymes activities and skin mucosal immune responses in yellowfin seabream, *Acanthopagrus latus*. Aquaculture. 517, 734795.

Doan, H.V., Hoseinifar, S.H., Jaturasitha, S., Dawood, M.A.O., Harikrishnan, R., 2020. The effects of berberine powder supplementation on growth performance, skin mucus immune response, serum immunity, and disease resistance of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture. 520, 734927.

Doan, H.V., Hoseinifar, S.H., Elumalai, P., Tongsiri, S., Chitmanat, C., Jaturasitha, S., Doolgindachbaporn, S., 2018. Effects of orange peels derived pectin on innate immune response, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticu*s) cultured under indoor biofloc system. Fish & Shellfish Immunology. 80, 56-62.

Don, P., 2018. Preliminary study of the antimicrobial effect of watermelon bark (Endocarp or Rind on *S. thphi, E. coli* and *S. aureus*.

Ekasari, J., Hanif Azhar, M., Surawidjaja, E.H., Nuryati, S., De Schryver, P., Bossier, P., 2014. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish & Shellfish Immunology. 41, 332-339.

El Asely, A.M., Reda, R.M., Salah, A.S., Mahmoud, M.A., Dawood, M.A.O., 2020. Overall performances of Nile tilapia (*Oreochromis niloticus*) associated with using vegetable oil sources under suboptimal temperature. Aquaculture Nutrition. n/a.

El Zawawy, N.A., 2015. Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. International Journal Current Microbioogyl Aplication Science. 4, 298-309.

Encarnação, P., 2016. 5 - Functional feed additives in aquaculture feeds. in: Nates, S.F. (Ed.), Aquafeed Formulation. Academic Press, San Diego, pp. 217-237.

FAO, 2018. The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. FAO Rome, Italy.

García-Ríos, L., Miranda-Baeza, A., Coelho-Emerenciano, M.G., Huerta-Rábago, J.A., Osuna-Amarillas, P., 2019. Biofloc technology (BFT) applied to tilapia fingerlings production using different carbon sources: Emphasis on commercial applications. Aquaculture. 502, 26-31.

Ginguay, A., Regazzetti, A., Laprevote, O., Moinard, C., De Bandt, J.-P., Cynober, L., Billard, J.-M., Allinquant, B., Dutar, P., 2019. Citrulline prevents age-related LTP decline in old rats. Scientific Reports. 9, 20138.

Giri, S.S., Sukumaran, V., Park, S.C., 2019. Effects of bioactive substance from turmeric on growth, skin mucosal immunity and antioxidant factors in common carp, *Cyprinus carpio*. Fish & Shellfish Immunology. 92, 612-620.

Gómez-García, R., Campos, D.A., Aguilar, C.N., Madureira, A.R., Pintado, M., 2020. Valorization of melon fruit (*Cucumis melo* L.) by-products: Phytochemical and Biofunctional properties with Emphasis on Recent Trends and Advances. Trends in Food Science & Technology. 99, 507-519.

Guangjin, L., Jielian, Z., Kangming, C., Tingting, G., Huochun, Y., Yongjie Liu, Wei, Z., Chengping, L., 2016. Development of *Streptococcus agalactiae* vaccines for tilapia. Diseases of Aquatic Organisms. 122, 163.

Hernández, A.J., Romero, A., Gonzalez-Stegmaier, R., Dantagnan, P., 2016. The effects of supplemented diets with a phytopharmaceutical preparation from herbal and macroalgal origin on disease resistance in rainbow trout against Piscirickettsia salmonis. Aquaculture. 454, 109-117.

Hoseinifar, S.H., Khodadadian Zou, H., Paknejad, H., Hajimoradloo, A., Van Doan, H., 2019. Effects of dietary white-button mushroom powder on mucosal immunity, antioxidant defence, and growth of common carp (*Cyprinus carpio*). Aquaculture. 501, 448-454.

Hoseinifar, S.H., Sohrabi, A., Paknejad, H., Jafari, V., Paolucci, M., Van Doan, H., 2019. Enrichment of common carp (*Cyprinus carpio*) fingerlings diet with *Psidium guajava*: The effects on cutaneous mucosal and serum immune parameters and immune related genes expression. Fish & Shellfish Immunology. 86, 688-694.

Hoseinifar, S.H., Jahazi, M.A., Nikdehghan, N., Van Doan, H., Volpe, M.G., Paolucci, M., 2020. Effects of dietary polyphenols from agricultural by-products on mucosal and humoral immune and antioxidant responses of convict cichlid (*Amatitlania nigrofasciata*). Aquaculture. 517, 734790.

Hoseinifar, S.H., Shakouri, M., Yousefi, S., Van Doan, H., Shafiei, S., Yousefi, M., Mazandarani, M., Torfi Mozanzadeh, M., Tulino, M.G., Faggio, C., 2020. Humoral and skin mucosal immune parameters, intestinal immune related genes expression and antioxidant defense in rainbow trout (*Oncorhynchus mykiss*) fed olive (*Olea europea* L.) waste. Fish & Shellfish Immunology. 100, 171-178.

Iheanacho, S.C., Ikwo, T.K., Igweze, N.O., Ogueji, E.O., 2018. Effect of different dietary inclusion levels of melon seed (*Citrullus lanatus*) peel on growth, haematology and histology of *Oroechromis niloticus* juvenile. Turkish Journal of Fisheries and Aquatic Sciences. 18, 377-384.

Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., Kuo, W.C., David Horgen, F., 2008. Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquaculture Research. 39, 118-133.

Katya, K., Yun, Y.-h., Park, G., Lee, J.-Y., Yoo, G., Bai, S.C., 2014. Evaluation of the Efficacy of Fermented By-product of Mushroom, Pleurotus ostreatus, as a Fish Meal Replacer in Juvenile Amur Catfish, Silurus asotus: Effects on Growth, Serological Characteristics and Immune Responses. Asian-Australasian Journal of Animal Sciences. 27, 1478-1486.

Khanjani, M.H., Sharifinia, M., 2020. Biofloc technology as a promising tool to improve aquaculture production. Reviews in Aquaculture. n/a.

Khoa, T.N.D., Tao, C.T., Van Khanh, L., Hai, T.N., 2020. Super-intensive culture of white leg shrimp (*Litopenaeus vannamei*) in outdoor biofloc systems with different sunlight exposure levels: Emphasis on commercial applications. Aquaculture. 524, 735277.

Khorasani, A.C., Shojaosadati, S.A., 2017. Pectin-non-starch nanofibers biocomposites as novel gastrointestinal-resistant prebiotics. International Journal of Biological Macromolecules. 94, 131-144.

Kraemer, S.A., Ramachandran, A., Perron, G.G., 2019. Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy. Microorganisms. 7, 180.

Kulczykowska, E., 2019. Stress Response System in the Fish Skin—Welfare Measures Revisited. Frontiers in Physiology. 10.

Kumar, P., Mehta, N., Malav, O.P., Kumar Chatli, M., Rathour, M., Kumar Verma, A., 2018. Antioxidant and antimicrobial efficacy of watermelon rind extract (WMRE) in aerobically packaged pork patties stored under refrigeration temperature (4±1°C). Journal of Food Processing and Preservation. 42.

Lee, B.J., Kim, S.S., Song, J.W., Oh, D.H., Cha, J.H., Jeong, J.B., Heo, M.S., Kim, K.W., Lee, K.J., 2013. Effects of dietary supplementation of citrus by-products fermented with a probiotic microbe on growth performance, innate immunity and disease resistance against *Edwardsiella tarda* in juvenile olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel). Journal of Fish Diseases. 36, 617-628.

Li, Z., Tran, N.T., Ji, P., Sun, Z., Wen, X., Li, S., 2019. Effects of prebiotic mixtures on growth performance, intestinal microbiota and immune response in juvenile chu's croaker, *Nibea coibor*. Fish & Shellfish Immunology. 89, 564-573.

Liu, H., Li, H., Wei, H., Zhu, X., Han, D., Jin, J., Yang, Y., Xie, S., 2019. Biofloc formation improves water quality and fish yield in a freshwater pond aquaculture system. Aquaculture. 506, 256-269.

Mandal, A., Das, S.K., 2018. Comparative efficacy of neem (*Azadirachta indica*) and non-neem supplemented biofloc media in controlling the harmful luminescent bacteria in natural pond culture of *Litopenaeus vannaemei*. Aquaculture. 492, 157-163.

Maoto, M.M., Beswa, D., Jideani, A.I.O., 2019. Watermelon as a potential fruit snack. International Journal of Food Properties. 22, 355-370.

Marini, J.C., Stoll, B., Didelija, I.C., Burrin, D.G., 2012. *De novo* synthesis is the main source of ornithine for citrulline production in neonatal pigs. American journal of physiology. Endocrinology and metabolism. 303, E1348-E1353.

Míguez, B., Gómez, B., Gullón, P., Gullón, B., Alonso, J.L., 2016. Pectic Oligosaccharides and Other Emerging Prebiotics. in: Rao, V., Rao, L.G. (Eds.), Probiotics and Prebiotics in Human Nutrition and Health. InTech, Rijeka, pp. Ch. 15.

Mishra, A., Nam, G.-H., Gim, J.-A., Lee, H.-E., Jo, A., Kim, H.-S., 2018. Current Challenges of Streptococcus Infection and Effective Molecular, Cellular, and Environmental Control Methods in Aquaculture. Molecules and cells. 41, 495-505.

Mohan, K., Ravichandran, S., Muralisankar, T., Uthayakumar, V., Chandirasekar, R., Seedevi, P., Abirami, R.G., Rajan, D.K., 2019. Application of marine-derived polysaccharides as immunostimulants in aquaculture: A review of current knowledge and further perspectives. Fish & Shellfish Immunology. 86, 1177-1193.

Nguyen, L.T., Han, G., Yang, H., Ikeda, H., Eltahan, H.M., Chowdhury, V.S., Furuse, M., 2019. Dried Watermelon Rind Mash Diet Increases Plasma L-Citrulline Level in Chicks. The Journal of Poultry Science. 56, 65-70.

Nicholson, P., Mon-on, N., Jaemwimol, P., Tattiyapong, P., Surachetpong, W., 2020. Coinfection of tilapia lake virus and *Aeromonas hydrophila* synergistically increased mortality and worsened the disease severity in tilapia (*Oreochromis* spp.). Aquaculture. 520, 734746.

Nobakht, A., Gorbanalinejad, V., 2017. The effects of different levels of watermelon skin with and without enzyme, on performance, carcass traits, blood biochemical parameters and immune status of broilers. Iranian Journal of Animal Science Research. 9, Pe57-Pe72.

Nwanevu, E., Sokari, G., Isitor, G., Orlu, E., Ogolo, S., Ebere, N., 2019. Effect of Locally Formulated Watermelon and Moringa Syrup Booster on the Growth Performance of *Heterobranchus bidorsalis* Fingerlings. Journal of Applied Life Sciences International, 1-12.

Oncul, F.O., Aya, F.A., Hamidoghli, A., Won, S., Lee, G., Han, K.R., Bai, S.C., 2019. Effects of the dietary Fermented tuna by-product meal on growth, blood parameters, nonspecific immune response, and disease resistance in juvenile olive flounder, *Paralichthys olivaceus*. Journal of the World Aquaculture Society. 50, 65-77.

Parry, R.M., Jr., Chandan, R.C., Shahani, K.M., 1965. A rapid and sensitive assay of muramidase. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.). 119, 384-386.

Petkowicz, C.L.O., Vriesmann, L.C., Williams, P.A., 2017. Pectins from food waste: Extraction, characterization and properties of watermelon rind pectin. Food Hydrocolloids. 65, 57-67.

Piamsomboon, P., Thanasaksiri, K., Murakami, A., Fukuda, K., Takano, R., Jantrakajorn, S., Wongtavatchai, J., 2020. Streptococcosis in freshwater farmed seabass *Lates calcarifer* and its virulence in Nile tilapia *Oreochromis niloticus*. Aquaculture. 523, 735189.

Pietrzak, E., Mazurkiewicz, J., Slawinska, A., 2020. Innate Immune Responses of Skin Mucosa in Common Carp (*Cyprinus Carpio*) Fed a Diet Supplemented with Galactooligosaccharides. Animals. 10, 438.

Qiao, G., Chen, P., Sun, Q., Zhang, M., Zhang, J., Li, Z., Li, Q., 2020. Poly-β-hydroxybutyrate (PHB) in bioflocs alters intestinal microbial community structure, immune-related gene expression and early Cyprinid herpesvirus 2 replication in gibel carp (*Carassius auratus gibelio*). Fish & Shellfish Immunology. 97, 72-82.

Reverter, M., Tapissier-Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2018. Biological and ecological roles of external fish mucus: a review. Fishes. 3, 41.

Rico, A., Oliveira, R., McDonough, S., Matser, A., Khatikarn, J., Satapornvanit, K., Nogueira, A.J.A., Soares, A.M.V.M., Domingues, I., Van den Brink, P.J., 2014. Use, fate and ecological risks of antibiotics applied in tilapia cage farming in Thailand. Environmental Pollution. 191, 8-16.

Rico, X., Gullón, B., Alonso, J.L., Yáñez, R., 2020. Recovery of high value-added compounds from pineapple, melon, watermelon and pumpkin processing by-products: An overview. Food Research International. 132, 109086.

Rodrigues, M.S., Bolívar, N., Legarda, E.C., Guimarães, A.M., Guertler, C., do Espírito Santo, C.M., Mouriño, J.L.P., Seiffert, W.Q., Fracalossi, D.M., do Nascimento Vieira, F., 2018. Mannoprotein dietary supplementation for Pacific white shrimp raised in biofloc systems. Aquaculture. 488, 90-95.

Romdhane, M.B., Haddar, A., Ghazala, I., Jeddou, K.B., Helbert, C.B., Ellouz-Chaabouni, S., 2017. Optimization of polysaccharides extraction from watermelon rinds: Structure, functional and biological activities. Food Chemistry. 216, 355-364.

SAS, 2003. SAS Institute Inc, SAS Campus Drive, Cary, NC USA 27513-2414.

Secomebs, C.J., 1990. Isolation of salmonid macrophage and analysis of their killing ability. in: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., Van Muiswinkel, W.B. (Eds.), Techniques in Fish Immunology. SOS Publication, Fair Haven, NJ, pp. 137-152.

Serradell, A., Torrecillas, S., Makol, A., Valdenegro, V., Fernández-Montero, A., Acosta, F., Izquierdo, M.S., Montero, D., 2020. Prebiotics and phytogenics functional additives in low fish meal and fish oil based diets for European sea bass (*Dicentrarchus labrax*): Effects on stress and immune responses. Fish & Shellfish Immunology. 100, 219-229.

Siddik, M.A.B., Howieson, J., Fotedar, R., 2019. Beneficial effects of tuna hydrolysate in poultry by-product meal diets on growth, immune response, intestinal health and disease resistance to *Vibrio harveyi* in juvenile barramundi, *Lates calcarifer*. Fish & Shellfish Immunology. 89, 61-70.

Srichaiyo, N., Tongsiri, S., Hoseinifar, S.H., Dawood, M.A.O., Esteban, M.Á., Ringø, E., Van Doan, H., 2020. The effect of fishwort (*Houttuynia cordata*) on skin mucosal, serum immunities, and growth performance of Nile tilapia. Fish & Shellfish Immunology. 98, 193-200.

Srichaiyo, N., Tongsiri, S., Hoseinifar, S.H., Dawood, M.A.O., Jaturasitha, S., Esteban, M.Á., Ringø, E., Van Doan, H., 2020. The effects gotu kola (*Centella asiatica*) powder on growth performance, skin mucus, and serum immunity of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture Reports. 16, 100239.

Sukhavachana, S., Tongyoo, P., Massault, C., McMillan, N., Leungnaruemitchai, A., Poompuang, S., 2020. Genome-wide association study and genomic prediction for resistance against *Streptococcus agalactiae* in hybrid red tilapia (*Oreochromis* spp.). Aquaculture, 735297.

Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. 8, 121-137.

Tarazona-Díaz, M.P., Viegas, J., Moldao-Martins, M., Aguayo, E., 2011. Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. Journal of the Science of Food and Agriculture. 91, 805-812.

Tiamiyu, L.O., Okomoda, V.T.O., Agbese, V.E., 2015. Growth performance of *Clarias gariepinus* fingerlings fed Citrullus lanatus seed meal as a replacement for soybean meal. Journal of Aquaculture engineering and Fisheries research. 1, 49-56.

Van Doan, H., Hoseinifar, S.H., Faggio, C., Chitmanat, C., Mai, N.T., Jaturasitha, S., Ringø, E., 2018. Effects of corncob derived xylooligosaccharide on innate immune response, disease resistance, and growth performance in Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture. 495, 786-793.

Van Doan, H., Hoseinifar, S.H., Naraballobh, W., Jaturasitha, S., Tongsiri, S., Chitmanat, C., Ringø, E., 2019. Dietary inclusion of Orange peels derived pectin and *Lactobacillus plantarum* for Nile tilapia (*Oreochromis niloticus*) cultured under indoor biofloc systems. Aquaculture. 508, 98-105.

Van Doan, H., Hoseinifar, S.H., Chitmanat, C., Jaturasitha, S., Paolucci, M., Ashouri, G., Dawood, M.A.O., Esteban, M.Á., 2019. The effects of Thai ginseng, *Boesenbergia rotunda* powder on mucosal and serum immunity, disease resistance, and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture, 734388.

Van Doan, H., Hoseinifar, S.H., Sringarm, K., Jaturasitha, S., Yuangsoi, B., Dawood, M.A.O., Esteban, M.Á., Ringø, E., Faggio, C., 2019. Effects of Assam tea extract on growth, skin mucus, serum immunity and disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Streptococcus agalactiae*. Fish & Shellfish Immunology. 93, 428-435.

Wang, B., Gan, Z., Cai, S., Wang, Z., Yu, D., Lin, Z., Lu, Y., Wu, Z., Jian, J., 2016. Comprehensive identification and profiling of Nile tilapia (*Oreochromis niloticus*) microRNAs response to *Streptococcus agalactiae* infection through high-throughput sequencing. Fish & Shellfish Immunology. 54, 93-106.

Wu, G., Bazer, F.W., Davis, T.A., Kim, S.W., Li, P., Marc Rhoads, J., Carey Satterfield, M., Smith, S.B., Spencer, T.E., Yin, Y., 2009. Arginine metabolism and nutrition in growth, health and disease. Amino Acids. 37, 153-168.

Xia, Y., Lu, M., Chen, G., Cao, J., Gao, F., Wang, M., Liu, Z., Zhang, D., Zhu, H., Yi, M., 2018. Effects of dietary *Lactobacillus rhamnosus* JCM1136 and *Lactococcus lactis subsp. lactis* JCM5805 on the growth, intestinal microbiota, morphology, immune response and disease resistance of juvenile Nile tilapia, *Oreochromis niloticus*. Fish & Shellfish Immunology. 76, 368-379.

Yano, T., 1992. Assay of hemolytic complement activity. in: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Hattari, S.C., Rowley, A.F. (Eds.), Techniques in Fish Immunology. SOS Publications, New Jersey, pp. 131-141.

Yoshida, T., Kitao, T., 1991. The Opsonic Effect of Specific Immune Serum on the Phagocytic and Chemiluminescent Response in Rainbow Trout, *Oncorhynchus mykiss* Phagocytes. Fish Pathology. 26, 29-33.