The effect of fishwort (*Houttuynia cordata*) on skin mucosal, serum immunities, and growth performance of Nile tilapia

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Abstract

The present study addressed the possible effects of fishwort *(Houttuynia cordata)* powder (FWP) on Nile tilapia's skin mucus parameter, serum immune response, and growth performance. Three hundred twenty tilapia fingerlings (average weight of 39.06 ± 0.16 g) were divided into four treatments and fed four levels of FWP; 0, 5, 10, and 20 g kg^{-1} for 72 days. Completed randomized design of the four replications was applied and revealed that fish fed FWP significantly improved skin mucus lysozyme activity (SMLA). The highest value (P<0.05) was recorded in fish fed 10 g kg^{-1} FWP. However, no significant difference in SMLA was observed by feeding the fish 5 and 20 g kg^{-1} FWP. Significant (P<0.05) enhanced skin mucus peroxidase activity (SMPA) was observed in fish fed 10 g kg^{-1} FWP, but no significant difference in SMPA was detected between FWP supplemented diets (5 and 20 g kg^{-1} FWP) and the control group. Regarding serum immunity, dietary administration of FWP showed significantly (P<0.05) improved serum lysozyme, peroxidase, alternative complement (ACH50), and phagocytosis *vs.* the control. The highest values of serum immunity (P<0.05) were recorded in fish fed 10 g kg^{-1} FWP. However, no significance in respiratory burst activity was observed. Similarly, no significant difference in growth performance, feed conversion ratio, and survival rate was observed in fish fed FWP compared to the control. In summary, diets supplemented with FWP (10 g kg^{-1}) increased the serum and mucosal immunity; however, no FWP supplementations had effects on Nile tilapia growth and survival rate.

Keywords: *Houttuynia cordata*; Growth performance; Mucosal immunity; Serum immunity; Nile tilapia

1. Introduction

Aquaculture is one of the most significant industries that provide an affordable protein source to the world population [1]. However, due to over intensification and expansion during the last decades has resulted in the emergence of many diseases and consequence led to substantial economic losses in aquaculture [2, 3]. Previously, antibiotics and chemotherapeutics were widely used to prevent and cure diseases in aquaculture [4]. However, this has resulted in the emergence of antimicrobial resistance bacteria [2, 5], environmental threats [6], food security problems [7], and decrease human resistance to pathogens [8]. Also, during the last decades, a serious concern raised concerning the transfer of antibiotic resistance genes from animals to human via food-webs resulting in the concept of "preventive medication" [9]. In this sense, environmental practices are necessary for sustainable disease handling in aquaculture [10]. The oldest evidence of medicinal plant usage is dated back to 3.000 BC [11, 12], and according to the WHO, today 80% of the human population still depends on herbs as medicine [13, 14]. To our knowledge, the first study using herbal plants as an alternative to antimicrobials and chemotherapeutics to cure fish diseases was carried out by Harikrishnan, Balasundaram, Dharaneedharan, Moon, Kim, Kim and Heo [15]. Since then, prohibition and healing by herbal plants have gained interest due to their ecological harmlessness, easily biodegrading, non-hazardous residue, reduced toxicity, drug-resistant free, and ease of access [16, 17]. A large number of medicinal plants have promising characteristics by improving growth performance [18, 19], stimulating immune response [20-22], and controlling fish diseases [23-25].

Fishwort (*Houttuynia cordata*) - FW, is a herbal, rhizomatous, and perennial plant mostly used in Japan, Korea, China, and Southeast Asia [26]. The herb possesses a

73 little cold taste and contains detoxifying, diuretic, and other medication characteristics
74 [27]. FW has been known to contain medicinally important activities, such as
75 antileukemic [28], antitumor [29], antiviral [30], antioxidant [31], and can also be an
76 adjuvant [32]. Several studies have revealed that the bioactive compounds in FW are
77 mostly broken down into volatile constituents [26, 33], flavonoids [34, 35], organic
78 acids [36], alkaloids [37, 38], and phenols [39]. Moreover, several compounds, such as
79 inorganic salts, vitamins, and metal components, are found in FW [21]. Previous
80 investigations have been demonstrated that the antibacterial activity of FW is owing to
81 houttuynin in its volatile oil [40]. Houttuynin can be applied to synthesize sodium
82 houttuyfonate with sodium bisulfite. This sodium substance possesses a
83 good antibacterial properties against gram-positive bacteria [41] and gram-negative
84 bacteria [42]. Furthermore, FW volatile oil is a novel and selective COX-2
85 inhibitor with anti-inflammatory activity [43]. Similarly, flavonoids exert anti-tumor
86 ability [44], while chlorogenic acid and its derivatives able to exhibit antioxidant effects
87 [45]. Meng, Leung, Dong, Zhou, Jiang and Zhao [46] found that *H. cordata* consists of
88 several polyphenols, such as rutin, quercetin, hyperoside, quercitrin, and chlorogenic
89 acid, which have been proven to be responsible for the antioxidant activity. Although
90 the beneficial effects of FW have been studies in human and terrestrial animals [47-50],
91 little information regarding the effects of FW on fish and shellfish is available.
92 Information on a significant increase in transformation rate and innate immune response
93 has been reported in grass carp (*Ctenopharyngodon idella*), cobia (*Rachycentron*
94 *canadum*), and sea cucumber (*Apostichopus japonicus*), respectively [21, 51]. However,
95 to our knowledge, no information is available regarding the effects of FW on skin

mucus, serum immunity, and growth performance of Nile tilapia (*Oreochromis niloticus*).

Tilapia is one of the most popular aquaculture fish species, and its production has increased fourfold during the last decade as its well-adapted, marketability, and stable commercial value [52]. The global tilapia production was estimated at 6.532 MT in 2018 [53] and foreseen to reach 7.3 MT tons by 2030 [54]. Thailand is one of the biggest tilapia producers (world ranking 4th) with the production of an approximately of 337,500 metric tons yearly [55], and the production were archived from river cages or irrigation canals. Fish culture in such an open environment is incredibly susceptible to stress caused by variations in water-quality and naturally occurring pathogenic bacteria [56]. The present study addresses the effect of FW on the Nile tilapia diet to access possible effects on skin mucus and serum immune response, as well as the growth performance of Nile tilapia.

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123 **2. Materials and Methods**

124 **2.1 Preparation of fishwort powder**

125 The fishwort (*Houttuynia cordata*) were gathered from a local market in Chiang Mai,
126 Thailand. The leaves and stems were dried in a hot-air oven for 48 hours at 60°C,
127 ground into fine particles, and the powder was then stored at (4°C) until use.

128 **2.2 Experimental diets**

129 The basal diet was similar to that used by Doan, Hoseinifar, Sringarm, Jaturasitha,
130 Khamlor, Dawood, Esteban, Soltani and Musthafa [19]. Pellets were produced by an
131 extruder and reserved in polyethylene bags at 4°C. Ingredients and chemical
132 compositions (g kg^{-1}) of the basal diets are given in Table 1. To prepare diets 2,3, and 4,
133 the FWP at different levels was dissolved in 70% alcohol and sprayed into the pellets,
134 and then thoroughly mixed. The obtained pellets were then coated with fish oil (Premer
135 Co., LTD), and allowed to dry at room temperature for 24 hours. The coated pellets
136 were stored at 4°C and fed to the fish within a week.

137 **2.3 Fish and experimental design**

138 Nile tilapia fingerlings were purchased from Chiang Mai Pathana Farm Co., Ltd.,
139 Chiang Mai, Thailand. Upon arrivals, were 320 tilapia (average weight = 39.06 ± 0.16
140 g) cultured in 5x5x2 m cage and fed a commercial diet, CP 9950, for two months. After
141 that, were the fish randomly distributed in 16 fiber tanks ten 500-liter tanks, 20 fish
142 $tank^{-1}$, for two weeks acclimation. During acclimation, fish were fed the experimental
143 diets *ad libitum* twice per day at 8:30 a.m. and 4:30 p.m. for 72 days. Daily feed was

144 adjusted weekly basis during the experimental trial. The experiment was based on
145 Completely Randomised Design (CRD) with four replications. The water temperature
146 was at 26 ± 1.5°C, and the pH was monitored daily and maintained at 7.1 ± 0.01. The
147 dissolved oxygen was maintained at no less than 5 ± 0.48 mg litre^{-1}.

148 **2.4 Immune parameters analysis**

149 **2.4.1 Sample preparations**

150 Fish serum was obtained from the blood of three fish per replication (9 fish per
151 treatment - group 1). Gathered blood (1 mL) from the caudal vein of each was instantly
152 poured into a 1.5 mL Eppendorf tube with no anticoagulant. The blood was allowed to
153 clot at 25°C for one hour, and then placed into a refrigerator (4°C) for four hours.
154 Afterward, the samples were centrifuged at 10,000 RPM for 15 minutes at 4°C, and the
155 anticipated serum was withdrawn via the use of a micro-pipette and kept at - 80 °C for
156 further analysis.

157 Fish leucocytes were separated from blood following the protocol of Chung and
158 Secombes (1988). Briefly, 1 mL of blood was taken from each fish (9 fish per treatment
159 – group 2) and then placed into 15 mL tube containing 2 mL of RPMI 1640 (Gibthai).
160 This blend was carefully loaded into 15 mL tubes containing 3 mL of *Histopaque*
161 (Sigma, St. Louis, MO, USA), and centrifuged at 400g for 30 minutes at 25°C. After
162 centrifugation, buffy coat of leucocytes cells drifted to the top of the Histopaque was
163 carefully withdrawn by a Pasteur pipette, and transferred into sterile 15 mL tubes. After
164 that, 6 mL of phosphate buffer solution (PBS: Sigma-Aldrich, USA) was added to each
165 tube and gently aspirated. The cells were washed twice by centrifugation at 250g for ten
166 minutes at 25°C to remove any residual *Histopaque*. The achieving cells were re-

suspended in the PBS and then adjusted to the required cell numbers for phagocytic and respiratory burst activities analysis.

Mucus was collected from the skin of 9 fish per treatment - group 3, following the method of Hoseinifar et al. (2016). The fish were anesthetized 5 mL clove oil and put into plastic bags containing 10 mL of 50 mM NaCl. Fish was then softly rubbed inside the plastic for approximately two minutes. The solution was quickly poured into a 15 mL sterile tube and centrifuged in an Eppendorf centrifuge (5810R Eppendorf, Engelsdorf, Germany) at 1.500 g at 4°C for 10 minutes. The supernatant was gathered and kept at -80°C until assay.

2.4.2 Lysozyme activity of serum and skin mucus

Lysozyme activity of serum and mucus was determined following the protocol of Parry *et al.* (1965) and detailed in our previous study Van Doan, Hoseinifar, Sringarm, Jaturasitha, Yuangsoi, Dawood, Esteban, Ringø and Faggio [57].

2.4.3 Peroxidase activity of serum and skin mucus

The peroxidase activity of serum and skin mucus was determined by the methods of Quade and Roth [58] and Cordero, Cuesta, Meseguer and Esteban [59].

2.4.4 Phagocytosis activity

Phagocytic activity was detected as described elsewhere (Yoshida and Kitao 1991) with detail descriptions in the previous publication of Van Doan, Hoseinifar, Sringarm, Jaturasitha, Yuangsoi, Dawood, Esteban, Ringø and Faggio [57].

2.4.5 Respiratory burst

Respiratory burst activity of blood leukocytes was determined using the protocol of Secomebs (1990) with detail descriptions in the previous publication of Van Doan, Hoseinifar, Sringarm, Jaturasitha, Yuangsoi, Dawood, Esteban, Ringø and Faggio [57].

2.4.6 Alternative complement pathway activity (ACH50)

Measurement of ACH50 was following the protocol of Yano [60] with detail descriptions in the previous publication of Van Doan, Hoseinifar, Sringarm, Jaturasitha, Yuangsoi, Dawood, Esteban, Ringø and Faggio [57].

2.5 Growth performance

After 72 days of feeding, growth parameters were determined via the use of following formula: Specific growth rate (SGR %) = 100 x (ln final weight - ln initial weight)/total 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.

2.6 Statistical analysis

The data normality was checked by Kolmogorov- Smirnov test. Significant differences among treatments were determined via using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test) via the SAS Computer Program [61]. The values and other parameters are shown as means ± standard deviation.

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220 **3. Results**

221 **3.1 Mucosal immune response**

222 After 72 days of feeding, dietary inclusion of fishwort powder (FWP) resulted in the
223 enhanced ($P < 0.05$) skin mucus lysozyme activity compared to fish fed the basal diet
224 (Figure 1). The highest value ($P < 0.05$) was revealed in fish fed 10 g kg^{-1} FWP *vs*. the
225 other treatment groups. However, no significant ($P > 0.05$) difference was displayed by
226 feeding the fish 5 and 20 g kg^{-2} FWP. Significant ($P < 0.05$) increased skin mucus
227 peroxidase activity was detected in fish fed 10 g kg^{-2} FWP compared to 5, 20 g kg^{-2}
228 FWP and the control (Figure 2). Nonetheless, no significant ($P > 0.05$) differences were
229 observed in fish fed 5 and 20 g kg^{-2} FWP *vs*. the control group.

230 **3.2 Serum immune responses**

231 Variations in serum immunity activities were noticed between the control fed fish, and
232 those fed the FWP diets (Figure 3-7). Dietary enrichment of FWP led to the significant
233 improvement of serum lysozyme activity compared to the control, except the dietary
234 inclusion of 5 g kg^{-1} FWP (Figure 3). The highest value was noticed in fish fed 10 g kg^{-1}
235 FWP compared to the other treatment groups. However, no significant difference was
236 seen between fish fed 5 and 20 g kg^{-1} FWP ($P > 0.05$).

237 In terms of alternative complement activity (ACH50), fish fed with FWP diets revealed
238 higher ($P < 0.05$) ACH50 compared to the control (Figure 4). Maximum ACH50 was

displayed when fish were fed 10 g kg^{-1} FWP compared to the other supplemented and control group. However, no significant ($P > 0.05$) difference was noticed between fish fed 5 and 20 g kg^{-1} FWP. Similarly, phagocytosis (PI) and serum peroxidase activities were significantly ($P < 0.05$) improved in the fish fed the FWP diets compared to those fed the control diet, and the highest values were recorded in the administrated with 10 g kg^{-1} FWP (Figure 5 and 6). Nonetheless, no significant ($P > 0.05$) differences were revealed between 5 and 20 g kg^{-1} FWP supplemented diets, and no significant ($P > 0.05$) differences in respiratory burst activity (RB) were displayed between fish fed FWP diets and the control (Figure 7).

3.3 Growth performance

After 72 days post-feeding, dietary inclusion of FWP did not influence the specific growth rate (SGR), weight gain (WG), and final weight (FW); compared with the control treatment (Table 2). Similarly, no significant difference was present in the survival rates among treatments after eight weeks of feeding (Table 2).

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4. Discussion

The imminent development of resistant bacteria has compelled the research community to search for alternative strategies, natural healings, which be able to enhance growth parameters and immunities of aquaculture species [62, 63]. Natural immunostimulants have gained interest due to their potential favorable impacts; protection against several pathogenic bacteria, stimulation of fish immunity, lowered the risk involved with the application of antibiotics, and reducing the damage caused by toxic substances in fish [64-66]. Herbal based immunostimulants contain a variety of relevant properties, such as naturally occurring, high profitable impact on a living thing, and less harmfulness. Therefore, the present study was carried out to assess the possible effects of FWP on skin mucus and serum immunities, as well as the growth performance of Nile tilapia.

Skin mucus is an essential constituent of the innate immune system and acts as the first line of defense versus against invasion of pathogenic bacteria [67]. It possesses a broad spectrum of innate and adaptive immune elements, which generate a physiochemical barrier to protect fish against infective bacteria [68-70]. To our knowledge, there is no data available concerning the impacts of fishwort (*Houttuynia cordata*) on fish's skin mucus immunity. However, the present study displayed that dietary inclusion of FWP resulted in notable promotes of skin mucus lysozyme and peroxidase activities. Nonetheless, significant boost skin mucosal immunity has been demonstrated in common carp (*Cyprinus carpio*) [66, 71, 72], striped catfish, *Pangasianodon hypophthalmus* [73], and Nile tilapia [19, 57], and rainbow trout [74]. In contrast, Taee, Hajimoradloo, Hoseinifar and Ahmadvand [75] indicated that no significant variations in rainbow trout's skin mucus lysozyme activity fed myrtle extracts. Based on these data, we put forward the hypothesis that dietary administration of the same

292 immunostimulants may have different effects on the immune response of different fish
293 species. It is acknowledged that fish mucosal immunity can be stimulated via the use of
294 immunostimulants [76]. As immunologic places, skin-associated lymphoid tissues
295 (SALT), gill-associated lymphoid tissues (GIALT), and gut-associated lymphoid tissues
296 (GALT) can elevate a powerful defense mechanism versus pathogens [77, 78]. At the
297 immunological site, GALT is the factory of leukocytes, plasma, as well as T and B
298 cells. These cells, in association with the epithelial, goblet, and neuroendocrine cells,
299 can create and modulate intestinal immunological reactions [79, 80]. Nevertheless, the
300 precise mode of action to which FWP stimulated skin mucus parameters requires
301 additional studies.

302 Serum immune parameters in this study displayed significant improvements following
303 72 days post-feeding with FWP. Lysozyme acts as an essential defense element, which
304 plays a crucial role in the bacteriolysis [81]. Determination of lysozyme activity is an
305 essential parameter to measure whether or not innate immune responses are enhanced
306 by immunostimulants. In the present experiment, fish fed FWP recorded a significantly
307 stimulated lysozyme activity, and our finding is following previous studies with cobia,
308 *Rachycentron canadum* fed *Plantago asiatica*, *Houttuynia cordata*, and *Mentha*
309 *haplocalyx* [51] and sea cucumber, *Apostichopus japonicus* [21]. Nonetheless, the
310 lysozyme activity is not always improved in fish after being fed with
311 immunostimulants, as some medicinal plant extractions has revealed as an
312 immunosuppressor of the non-specific immune system [82, 83]. This can be attributed
313 to the existence of several phenolic substances in the herbal extracts, which capable of
314 inhibiting the enzyme activity. Several bioactive compounds, such as flavonoids, for
315 instance, firmly link to lysozyme because of the number and position of hydrogen, as

316 well as type and position of glycosides [84]. Alternative complement activity has been
317 demonstrated to be one of the most critical means of removal of fish pathogens [85, 86].
318 Additionally, its activating as an independent alternative complement pathway may be
319 obtained via immunostimulants [87-89]. The present study revealed that dietary
320 inclusion of FWP could enhance alternative complement activity after 72 days of
321 feeding. This result is consistent with the works of Nile tilapia fed elephant's foot and
322 assam tea extracts [19, 57], rainbow trout fed *Coriandrum sativum* extract [90], striped
323 catfish, *Pangasianodon hypophthalmus* fed plant extract-based diets [73]. Fish
324 leukocytes comprise numerous phagocytosis, bactericidal, respiratory burst, and
325 peroxidase activities [80, 91-93]. Assessment of the neutrophil function is essential for
326 the evaluation of the fish's health overall [94, 95]. It is indicated, with this, that the
327 incorporation of FWP stimulated serum peroxidase activity following 72 days of
328 feeding. Similarly, significantly enhance peroxidase activity was recorded in Nile tilapia
329 fed elephant's foot, Thai ginseng, and assam tea extract [18, 19, 57], gilthead seabream,
330 *Sparus aurata* fed fenugreek seeds [96], and common carp fed bioactive substance from
331 turmeric [66]. Respiratory burst, through motivation by external agents, has been
332 demonstrated to enhance the oxidation degrees in phagocytic cells and is believed to be
333 a key element in the fish's defensive mechanisms [97, 98]. The generation of respiratory
334 burst activity and reactive oxygen metabolites by phagocytic cells are crucial elements
335 in restricting the extension of fish's diseases [92]. The present study showed that
336 respiratory burst activity was stimulated in FWP fed fish. This is in accordance with
337 earlier researches reported in gilthead sea bream, *Sparus aurata* and European sea bass,
338 *Dicentrarchus labrax* fed tetra, *Cotinus coggygria* and common mallow, *Malva*
339 *sylvestris* plant extracts [99]. Phagocytosis is an essential cellular immune system

component in fish [100-102]. Its function is to facilitate fish against pathogenic invasion more effectively by recognizing the present pathogens and inhibiting their scattering and development [103]. Also, in agreement with the present study, a significant enhance in phagocytic activity were detected in cobia, *Rachycentron canadum* [51]; sea cucumber, *Apostichopus japonicus* [21]; Nile tilapia [19, 57, 104], and gilthead sea bream, *Sparus aurata* and European sea bass, *Dicentrarchus labrax* [99]. Although the exact mode of actions for which *Houttuynia cordata* motivate fish's immunity is not interpreted yet, it may have been due to the existence of some biological substances. Cheng, Chan, Chan, Lin, Han, Zhou, Wan, Wang, Leung, Fung and Lau [105] indicated that pure pectic polysaccharide isolated from *H. cordata* could stimulated the excretion of interleukin-1β, tumor necrosis factor-α, macrophage inhibitory protein-1α, and macrophage inhibitory protein-1β. It also regulated on activation, normal T cell expressed and secreted in human peripheral blood mononuclear cells, which play crucial roles in the non-specific and shape the specific immunity. The study of Satthakarn, Chung, Promsong and Nittayananta [106] demonstrated that extraction from *H. cordata* could significantly induce IL-2, IL-6, IL-8, and IFN-γ and modulate oral innate immune mediators. Wan, Zheng, Liu and Yu [107] also indicated that *H. cordata* could down-regulated the serum levels of IL-6, and macrophage inflammatory protein1-α (MIP-1α) in oxaliplatin-treated rats. Recent studies indicated that polysaccharides isolated from *H. cordata* could increase the IL-10 and have displayed potent anti-complementary activity via both the classical and alternative pathways by acting on components C3 and C4 of the complement system without interfering with the coagulation system [48, 49].

Growth performance is one of the essential parameters to evaluate the effects of medicinal plants on fish. However, no significant in growth performance and survival rate was found in the present study. Similarly, Dügenci, Arda and Candan [108] revealed that herbal extracts didn't significantly influence the rainbow trout's growth. Rodríguez, Cuesta, Esteban and Meseguer [109] also indicated that medicinal fungi did not induce substantially gilthead seabream's growth performance. Recently, dietary inclusion of *P. asiatica, H. cordata,* and *M. haplocalyx* had no effects on growth performance and survival rate of cobia [51].

In conclusion, FWP inclusion can be potentially applied in tilapia culturing to trigger the skin mucosal and serum immunities.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical Approval

The study was performed in accordance with the guidelines on the use of animals for scientific purposes (Chiang Mai University Approved No. 2561/AQ-0005).

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734

Table 1 The formulation and proximate composition of the experiment (g kg^{-1})

Ingredients	Diets (g kg^{-1})
	Diet 1
Fish meal	300
Corn meal	145
Soybean meal	270
Wheat flour	60
Rice bran	150
Cellulose	30
Soybean oil	30
Premix[1]	10
Vitamin C[2]	5
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)	
Crude protein	322.06
Crude lipid	74.75
Fibre	52.48
Ash	106.68
Dry matter	817.80
GE (cal/g)[3]	4,105

[1]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.

[2]Vitamin C 98% 8 g;

[3]GE = gross energy

Table 2. Growth performances and feed utilization (mean ± SE) of tilapia after 72 days feeding with experimental diets (FWP).

	0-control	5 g kg^{-1}	10 g kg^{-1}	20 g kg^{-1}
IW (g)	39.11 ± 0.09	39.04 ± 0.08	39.11 ± 0.10	38.96 ± 0.04
FW (g)	150.92 ± 1.71	151.98 ± 1.78	156.26 ± 4.56	154.34 ± 1.52
WG (g)	111.81 ± 1.74	112.95 ± 1.80	117.15 ± 1.05	115.38 ± 0.59
SGR (%)	1.88 ± 0.02	1.89 ± 0.02	1.92 ± 0.04	1.91 ± 0.01
FCR	1.10 ± 0.05	1.26 ± 0.15	1.12 ± 0.08	1.06 ± 0.05
SR (%)	91 ± 2.64	82 ± 4.13	85 ± 3.54	93 ± 1.94
FI (g)	166.53 ± 8.48	191.46 ± 25.33	176.37 ± 17.49	162.98 ± 7.40

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

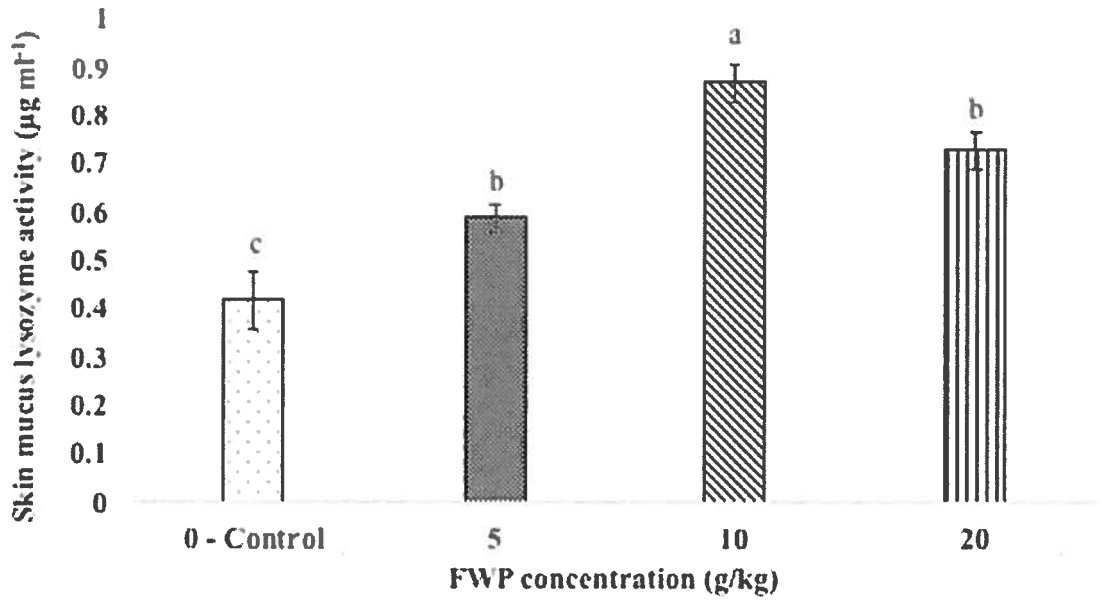

Figure 1. Skin mucus lysozyme activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

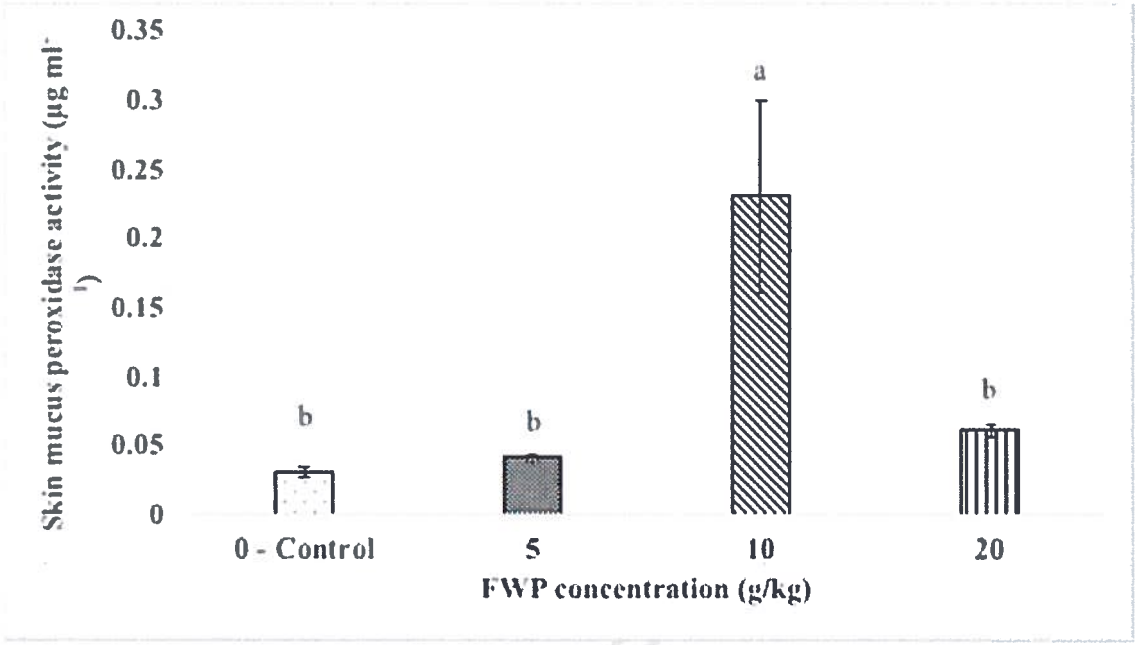

Figure 2. Skin mucus peroxidase activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

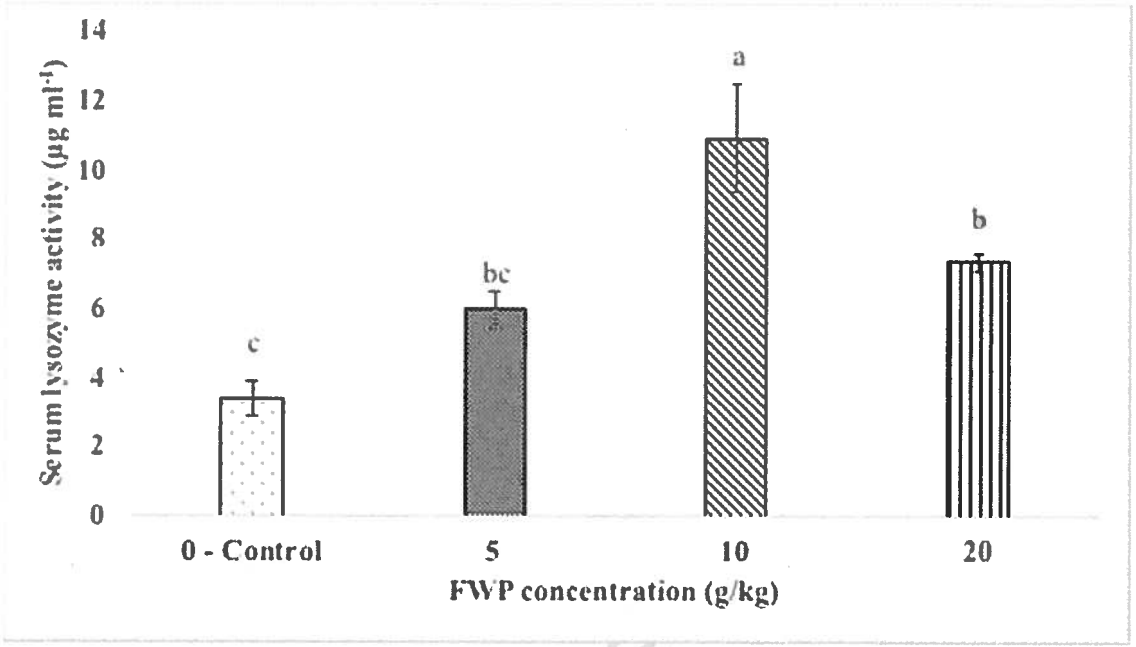

Figure 3. Serum lysozyme activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

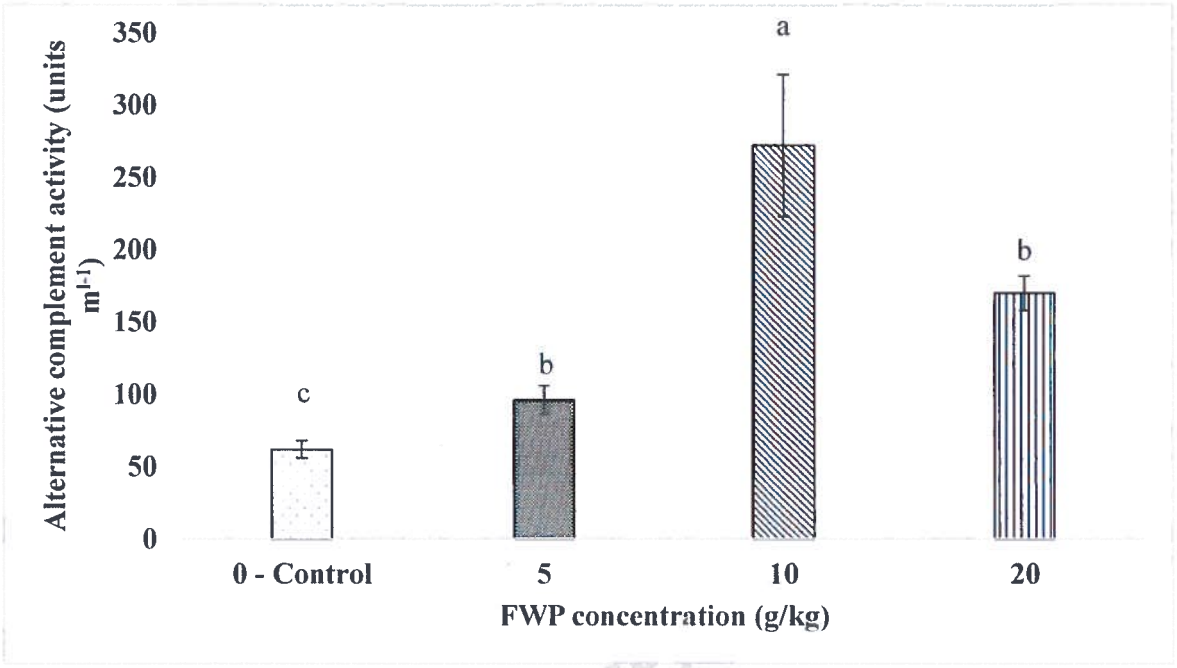

Figure 4. Alternative complement activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

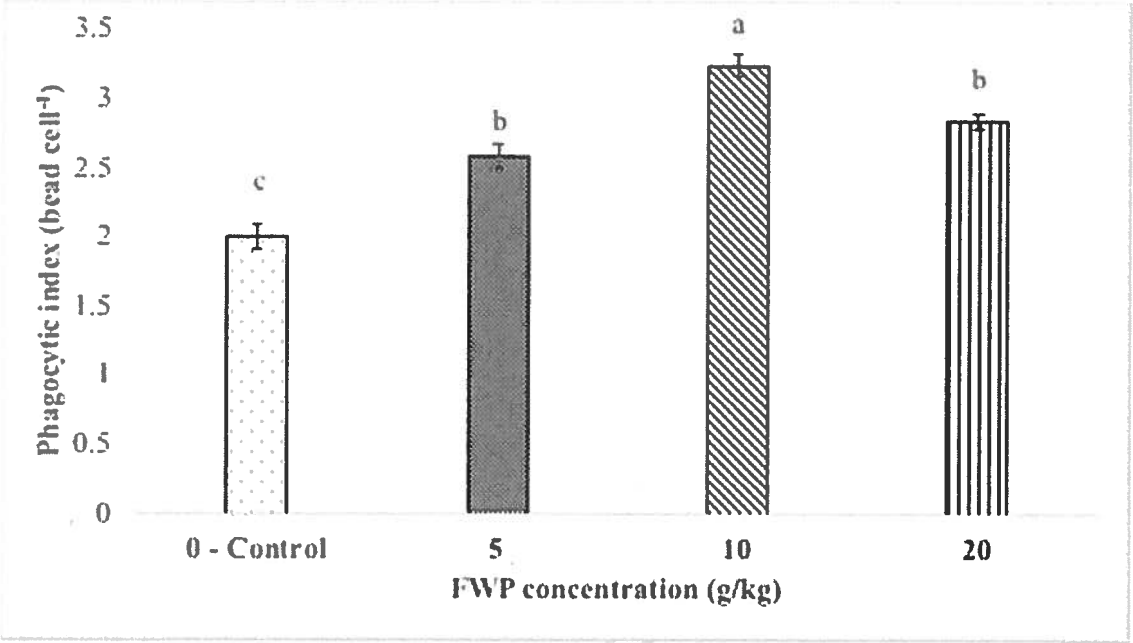

Figure 5. Phagocytosis activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

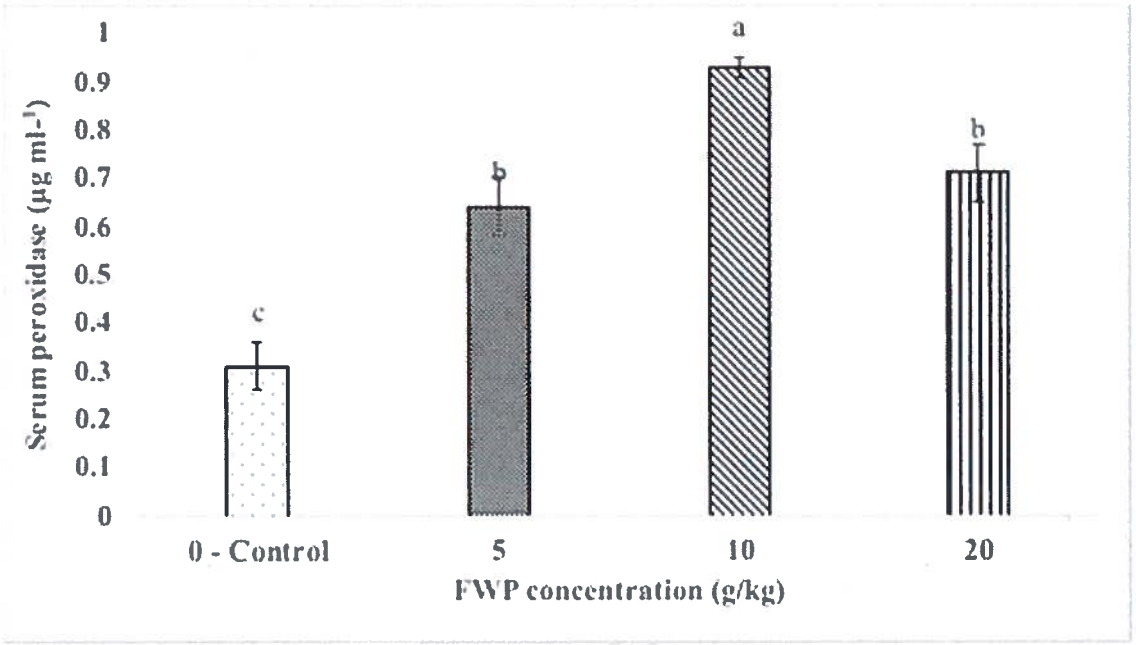

Figure 6. Serum peroxidase activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

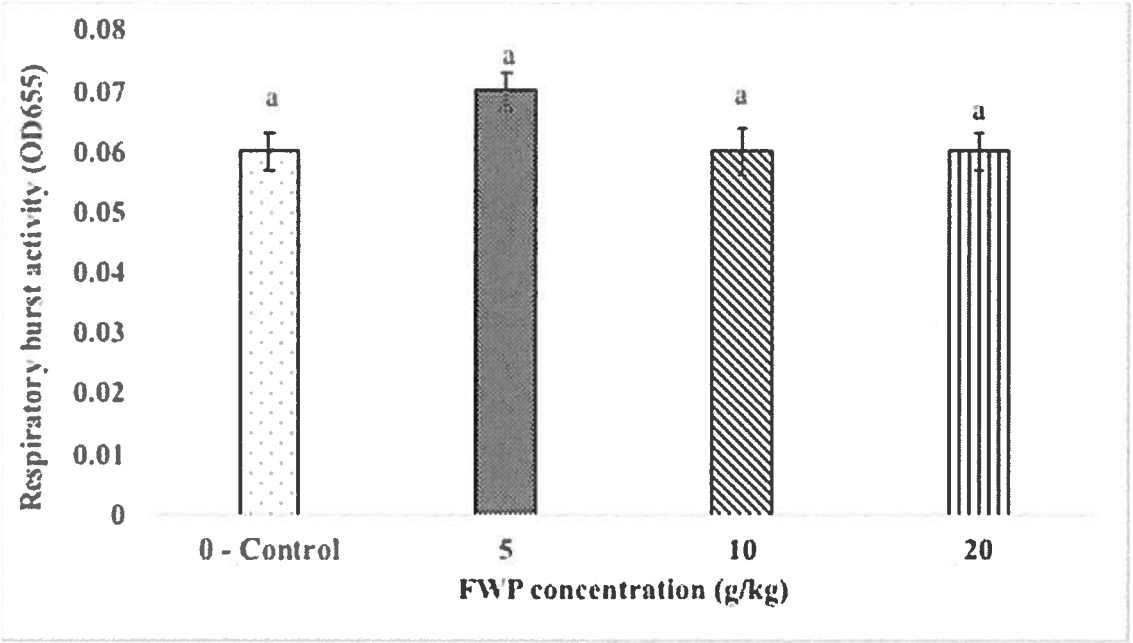

Figure 7. Respiratory burst activity of *O. niloticus* after 72 days feeding trial fed different concentrations of dietary fishwort powder (FWP) (mean ± S.E., n=4): Diet 1 (0 - control), Diet 2 (5 g kg^{-1} FWP), Diet 3 (10 g kg^{-1} FWP), and Diet 4 (20 g kg^{-1} FWP). Columns sharing the same superscript letter are not significantly different ($P<0.05$) (by Duncan's Multiple Range Test).

Highlights

Dietary inclusion of 10 g kg^{-1} FWP significantly stimulated skin mucosal immunity of Nile tilapia, *O. niloticus*

Significant enhances serum immunity were found in Nile tilapia fed 10 g kg^{-1} FWP

No significant increase in growth performance and survival rate was observed in Nile tilapia fed FWP