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The Effects of *Vitex trifolia, Strobilanthes crispus* and *Aloe vera* Herbal-mixed Dietary Supplementation on Growth Performance and Disease Resistance in Red Hybrid Tilapia (*Oreochromis* sp.)

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Abstract

Herbs and herb mixtures have been used as a growth promotant in livestock and aquaculture production. The objective of the study was to evaluate the effect of a dietary herbal mix comprised of *Vitex trifolia* (VTE), *Strobilanthes crispus* (SCE) and *Aloe vera* (AVE) on the growth performance, disease resistance and histology of *Oreochromis* sp. for 60 days. The fishes were divided into i) control, infected fish, fed with normal diet and infected fishes treated with different herbal-mixed supplementation diets of ii) VTE and SCE iii) SCE and AVE iv) AVE and VTE. All experimental groups were challenged using with *Streptococcus agalactiae* (1×10^7 cfu/mL) via intraperitoneal route on day 46. On day 46th (pre-challenge) and 60th (post-challenge), five fish were randomly chosen from each tank for each experimental and control groups to blood collection. The cumulative mortality and survival rate were assessed every day. Tissues from kidney, liver and spleen were examined. The fish supplemented with herbal-mix with the combination of VTE and SCE and AVE and VTE showed improved growth performance. For haematological assays, RBC, Hb, and WBC were higher ($P<0.05$) in fish supplemented with these herb mix, while the alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly affected by mixed-herbal supplementation. Histopathological examination of the organs revealed no negative effects in tissues. In conclusion, this study suggested that methanolic extracts of herbal mix composed of *V. trifolia, S. crispus* and *A. vera* extracts were effective as growth promoters and bacterial disease treatment in *Oreochromis* sp. when supplemented in daily diet.

Keywords: *Vitex trifolia; Strobilanthes crispus; Aloe vera;* Growth performance; Red tilapia; Bacterial resistance; Herbs

Introduction

Feed additives are substances which are added in small amounts into animal feeds to serve functions other than nutrient supply [1]. These additives are known to improve growth performance through improved feed utilization, reduction of pathogenic bacteria in the gastro-intestinal tract, and production of metabolites that improve animal metabolism. Additives are also used in aquaculture for increased growth performance and reduction of mortality in fish. The most used growth promoting feed additives in animal and aqua feeds are hormones, antibiotics, ionospheres and some salts [2]. Recently, some studies showed the positive effects of dietary medicinal plants or herbs as feed additives on the fish and crayfish growth and their feed utilization [3]. Attempts to use the natural materials such as medicinal plants could be widely accepted as feed additives to enhance feed utilization and aquaculture production [4].

It is a well-known fact about medicinal or herb benefit as feed additives for humans and animals, such antimicrobial and health procuress [5]. However mixed herbs are also options to overcome disease problem, because using single plant may compliment certain shortcomings such as inadequacy of nutrient and phytochemical properties. Moreover, World Health Organization encourages the usage of medicinal plants or herbs to substitute or minimize the use of chemicals through the global trend of going back to the nature. However, there is no report on herbal-mixed extracts in dietary supplementation for *Oreochromis* sp. on growth, haematological response and diseases resistance against *Streptococcus agalactiae*.

Materials and Methods

Preparation of herbs and methanolic extraction

Vitex trifolia, Aloe vera and *Strobilanthes crispus* were obtained from University Agriculture Park, University Putra Malaysia, Selangor, Malaysia. Fresh healthy leaves, stems and including flowers were collected in morning and washed under running tap water to remove dirt particles. They were allowed to dry in a forced-draught oven at a temperature of 65°C for 48 h. They were then chopped into small pieces and ground into powder using mechanical grinder (Panasonic, MY333). The powdered plant materials were kept in airtight bottles at room temperature prior to extraction. Methanol extracts were prepared by adding 100 g of plants powder into 1000 mL of 70% of solvents and agitated for 72 h at ambient temperature in a shaking incubator. Then, the extracts were filtered through 11 µm membrane filter paper (Whatman No. 1). After that, the extracts were evaporated to dryness using rotary evaporator at 40°C. The methanol extracts were dissolved

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again in 70% methanol to make a 0.5 g/mL stock solution and stored at -20°C until further use.

Diets and experimental design

The experiments were conducted at an Aquatic Animal Health Unit, Faculty of Veterinary Medicine, University Putra Malaysia. *Oreochromis* sp. fry were obtained from a local supplier. They were allowed two weeks for adaptation before they were allotted to the different dietary treatments. During the adaptation period they were given a commercial starter diet fed to satiation twice a day. After the adaptation period, 390 fishes (weighing about 120 ± 0.5 g) were randomly distributed into 100 L tanks (10 fishes per tank) containing aerated recirculated freshwater. Each treatment had three replicates and the fish were fed over a period of 60 days. A non-stop aeration to maintain the dissolved oxygen to the optimal level was provided. All fish were fed twice daily at 4% of body weight and the daily ration was adjusted accordingly. The feed consumption in each aquarium was recorded daily. Dead fishes from each aquarium were collected daily and weighed.

In this experiment, a commercial starter diet was used as a basal diet. The dietary treatments were basal diet supplemented with three extracts from herbs, namely, *V. trifolia* (VTE), *A. vera* (AVE) and *S. crispus* (SCE). The control diet does not contain any herbal extract. A control diet was formulated according to NRC [6] recommendation and contained 35% crude protein and 3493 kcal of digestible energy kg^{-1} of dietary dry matter (DM). Additional three diets were formulated as commercial diet supplemented with 3.5g VTE/kg DM + 3.5 g SCE/kg DM (VTE and SCE), 3.5g VTE/kg DM + 3.5g AVE/kg DM (VTE and AVE) and 3.5g AVE/kg DM + 3.5 g SCE/kg DM (AVE and SCE) which prepared. Distilled deionized water was added into each diet and mixed [7]. The diets were air-dried at ambient temperature for 72 h, packed in air-tight containers, labelled and stored.

Growth performance

Fish were weighed at the beginning and at the end of the experiment, and the growth performance and feed utilization were assessed on weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) [8].

Challenge assay

After feeding the fish with VTE, AVE and SCE for 45 days, the fish in subgroup will be tested by challenge test. The challenge was made by intraperitoneal injection with 0.1/ml suspensions of *S. agalactiae* in 0.9% (w/v) saline containing 10^7 cells/mL. The percentage survival in each treatment was calculated as previously described by Akinwole AO, et al. [9] using the following modified formulae:

$$\text{Survival rate \%} = \frac{\text{Total no. of fish stocked} - \text{No. of fish died}}{\text{Total no. of fish stocked}} \times 100$$

Haematological assessment

On day 46th (pre-challenge) and 60th (post-challenge), five fish were randomly chosen from each tank for each experimental and control groups and were anaesthetized with tricane methane sulfate (MS222) at 150 mg/L prior to blood collection after 24 h of final feeding for. The blood samples were collected by puncturing the caudal vein by using a 25G X 1 syringe and transferred into lithium heparin tubes. The collected blood samples were immediately subjected to haematological analysis. Evaluation of the haemogram involves the determination of the Red Blood Count (RBC), Haematocrit (Hct), Hemoglobin concentration (Hb), White Blood Cell Count (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC). The blood enzyme measurement in Alkaline phosphatase (ALP), Alkaline transeaminase (ALT) and Aspartate transeaminase (AST). An automatic blood enzyme analyzer (Hitachi 704) was used for the following determinations: Alkaline phosphatase (ALP, U/L), Alkaline transeaminase (ALT, U/L) and Aspartate transeaminase (AST, U/L). The apparatus is based upon dry chemical technology and colorimetric reaction. Kits obtained PLIVA- Lachema and DIALAB®, were used for the determination of all indices. Also for controls, same kits were used. The hematological and biochemical parameters are expressed in international units (SI).

Histopathological assessment

For histology, fish were euthanized with MS-222 and liver, kidney, spleen and brain were immediately collected. Tissues were fixed in 10% buffered formalin for at least 24 h and agitated in incubator shaker. Afterward, the tissues were transferred into new buffered formalin solution. The fixed tissues were processed in automated tissue processor. Then, the tissues were embedded in paraffin block for easy to store and handle. Sectioning using microtome to produce very thin, 4 µm sections that are placed on a microscope slide ready for staining. Finally, the tissues were stained with haematoxylin and eosin and observed under light microscope. The histologic examination to detect any possible pathologic changes resulting from local or systemic disease

Statistical analysis

Data (initial weight, final weight, weight gain, initial length, final length, total length, SGR, FCR, SR and haematological indices) were analyzed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test [10]. Significance was tested at 5% level and all statistical analyses were carried out using the SPSS Version 21.

Results and Discussion

Table 1 shows the growth performance of *Oreochromis* sp. fed diets supplemented with herbal-mix for 60 days. The fingerling with average 12g and initial length 12 cm used were used to determine the beneficial effect of the chosen mixed herb.

Means with the same superscript in rows were not significantly different at $P<0.05$. Values in parentheses are standard errors of means. SGR = specific growth rate, FCR = feed conversion ratio

Herbal-mixed supplementation showed improved growth performance in fish fed with the combination of VTE and SCE and AVE and VTE as compared to the control group. Final weight and body weight gain, showed increased significantly ($P< 0.05$) in the all mixed-herbal compared to the control group. AVE and VTE showed the most significant growth compared to other fed mixed herbs combination. The lowest fish growth was obtained in AVE and SCE diet. Moreover, fish fed on diets containing AVE and VTE and VTE and SCE showed the optimum FCR with 1.16 and 1.10 respectively, whereas fish fed with control group produced higher FCR, 1.33. The total length was significantly different between treatments except with the AVE and SCE. However, for SGR the growth performances resulted with AVE and SCE and VTE and SCE combination was significantly lower ($P>0.05$) compared to the control group.

Only AVE and VTE showed promising result with significantly

	Diets			
	C	AVE & SCE	AVE & VTE	VTE & SCE
Initial weight (g)	12.07 ± 0.65^{a}	12.06 ± 0.40^{a}	12.09 ± 0.05^{a}	12.08 ± 0.65^{a}
Final weight (g)	19.34 ± 0.09^{a}	21.59 ± 0.67^{b}	24.58 ± 0.08^{d}	24.19 ± 0.73^{c}
Weight gain (g)	60.32 ± 0.93^{a}	78.97 ± 0.04^{b}	103.29 ± 0.61^{d}	100.27 ± 0.52^{c}
Initial length (cm)	11.95 ± 0.00^{a}	11.78 ± 0.75^{a}	12.00 ± 2.50^{a}	12.10 ± 2.5^{a}
Final length	13.12 ± 0.08^{a}	13.11 ± 0.53^{a}	15.55 ± 3.23^{b}	15.08 ± 3.75^{b}
Specific Growth Rate (SGR) (%)	10.90 ± 0.04^{b}	10.10 ± 0.06^{a}	11.00 ± 0.04^{b}	10.71 ± 0.20^{b}
Feed Conversion Ratio (FCR)	1.33 ± 0.00^{d}	1.27 ± 0.01^{c}	1.16 ± 0.00^{b}	1.10 ± 0.00^{a}
Survival (%)	90.00 ± 0.00^{a}	97.50 ± 2.5^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}

Table 1: Mean growth performance and feed utilization of *Oreochromis* sp. fingerlings fed commercial diet supplemented with herbal-mix diets for 60 days.

Day	Diets							
	C		AVE&SCE		AVE&VTE		VTE&SCE	
	45 (Pre-challenge)	60 (Post-challenge)	45 (Pre-challenge)	60 (Post-challenge)	45 (Pre-challenge)	60 (Post-challenge)	45 (Pre-challenge)	60 (Pre-challenge)
RBC (x 10^{12}/L)	1.18 ± 0.01^{a}	1.38 ± 0.02^{b,c}	1.29 ± 0.01^{b}	2.10 ± 0.06^{d}	1.66 ± 0.01^{c}	2.24 ± 0.01^{e}	1.51 ± 0.05^{c}	2.05 ± 0.01^{d}
Hb (g/L)	66.2 ± 5.30^{b}	62.8 ± 1.40^{a,b}	57 ± 1.40^{a}	86.65 ± 0.75^{c}	66.95 ± 2.35^{a}	90.9 ± 0.20^{c}	66.8 ± 1.30^{a}	83.8 ± 1.00^{c}
WBC (x 10^{9}/L)	1.29 ± 0.05^{a}	6.80 ± 0.01^{c}	3.49 ± 0.32^{b}	15.95 ± 0.55^{e}	3.32 ± 0.12^{b}	16.00 ± 0.50^{e}	3.35 ± 0.13^{b}	11.52 ± 0.02^{d}
PCV (L/L)	0.17 ± 0.020^{a}	0.20 ± 0.01^{a,b}	0.17 ± 0.03^{a}	0.23 ± 0.01^{b}	0.15 ± 0.04^{a}	0.23 ± 0.01^{b}	0.18 ± 0.01^{a,b}	0.24 ± 0.01^{b}
MCV (fL)	108.5 ± 0.50^{a,b}	107.5 ± 1.50^{b}	106.5 ± 1.00^{a}	105.5 ± 1.50^{a}	119.5 ± 0.50^{c}	107.5 ± 0.50^{b}	115.5 ± 2.50^{b}	112.5 ± 1.00^{a,b}
MCHC (g/L)	401.5 ± 1.50^{e}	370.5 ± 0.50^{b}	405 ± 2.00^{f}	393 ± 2.50^{c}	395 ± 2.50^{e}	375 ± 1.00^{c,d}	399 ± 2.00^{d,e}	355.5 ± 0.50^{a}
Thrombocyte (x 10^{9}/L)	10.05 ± 0.05^{a}	16.7 ± 1.40^{b}	9.45 ± 0.35^{a}	16.65 ± 0.95^{b}	10.75 ± 0.05^{a}	10.35 ± 0.15^{a}	9.25 ± 0.05a	35.8 ± 1.00^{c}
ALT (U/L)	43.45 ± 0.95^{g}	38.85 ± 0.95^{f}	21.75 ±1.15^{c}	12.5 ± 0.20^{a}	32.5 ± 1.20^{d}	21.85 ± 0.45^{c}	35.1 ± 0.10^{e}	15.95 ± 0.45^{b}
AST (U/L)	43.00 ± 1.00^{b,c}	42.00 ± 2.00^{b}	47.5 ± 0.50^{e}	46.00 ± 1.00^{d,e}	37.00 ± 1.00^{a}	44.00 ± 1.00^{b,c,d}	40.50 ± 0.50^{a,b}	46.00 ± 1.00^{d,e}
ALP (U/L)	302.4 ± 2.30^{f}	335.65 ± 2.45^{g}	303.9 ±1.60^{f}	247.85 ± 2.45^{e}	239.65 ±1.05^{d}	154.2 ± 1.40^{a}	222.95 ± 0.05^{c}	204.3 ± 2.1^{b}

Table 2: Haematological characteristics of *Oreochromis* sp. fed experimental diets with herbal-mixed.

higher (P<0.05) compared to the control group. Fish survival rate after the diseases challenge ranges from 90% to 100% with significant difference (P<0.05) among all treatments including control treatment.

Means in a given rows with the same superscript letter were not significantly different at P<0.05. Values in parentheses are standard errors of means. PCV = packed cell volume, Hb = haemoglobin, WBC = white blood cell count, RBC = red blood cell count, TC = thrombocyte count, MCV = mean corpuscular volume, MCHC = mean corpuscular haemoglobin concentration, P. protein = plasma protein, ALT = alanine aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase

Table 2 shows haematological parameters pre and post challenge after 45 days of continuous feeding after the diseases challenge. RBC, Hb, and WBC showed higher significant difference (P<0.05) compared to control treatment. In the other hand, PCV did not show any significant difference among all the tested treatments. As for MCV and MCHC results, all mixed herbs showed significant different (P<0.05) except in AVEandSCE. Thrombocyte showed no significant difference in all treatments after feeding with experimental diets. Level of RBC increased after post-challenge for all treatments. Hb level of AVE and SCE, AVE and VTE, VTE and SCE and control at day 60 were 86.65 ± 0.75 g/dL, 90.9 ± 0.2 g/dL 83.8 ± 1.00 g/dL and 62.8 ± 1.4 g/dL respectively, showed that tremendous increase statistical was reported (P<0.05) from data at day 46 (Hb: 57 ± 1.4 g/dL; 66.95 ± 2.35 g/dL; 66.8 ± 1.3 g/dL and 66.2 ± 5.3 g/dL). The WBC post challenge were significantly increased (P <0.05) at day 60 with a higher 16.00 ± 0.50 cells/mm^{3} exhibited by AVE and VTE. Likewise, after the infection, the haematological indices like PCV, MCV (Table 2) resulted with no significant difference (P>0.05). On the other hand, MCHC results on days 60 were significantly difference (P<0.05) compared to the control group. The thrombocyte showed no significant difference only with AVE and SCE treatment.

The biochemical parameter of ALT and ALP were significantly affected by mixed-herbal supplementation. Pre-challenge of ALT showed significant difference in all treatments, compared to significant decreases at post challenge. However at the pre-challenge of ALP revealed only AVE and SCE with no significantly difference compared to control. Then at the post-challenge all mixed herb showed decreases in ALP value, however control treatment showed significant increases. There was a significantly (P<0.05) decrease in ALT and ALP post-challenge, compared with control. The AST showed significant different among all treatment, while control treatment did not show any significant difference at pre and post challenge. Post-challenge for AVE and VTE showed no significant difference compared to control.

Discussion

Streptococcosis is considered as the most overwhelming disease, causing massive death of fishes and is responsible for heavy economic losses. The use of antibiotics is the most effective way of treating a Streptococcosis disease. However, treating microbial infections in fish and shrimp larvae involves dissolving higher quantities of broad spectrum of chemotherapeutic agents in the culture medium [11]. The disadvantage of this method is the requirement of large amount of expensive drugs, which are used and discharged in the environment posing risk to the animals and human health. As an alternative, antimicrobial characteristic from natural source are used instead of synthetic antibiotic drugs. Several herbs have been tested for their growth promoting activity in aquatic animals [12,13]. In this study, combinations of selected herbs were subjected to the growth and health assessment of the tested fish, *Oreochromis* sp. The fish were fed with

mixed-herbal of VTE and SCE, AVE and VTE, and AVE and SCE at the concentrations of 7 g/kg diet, showed significantly improved in *Oreochromis* sp. growth and survival rate compared to control treatments. The final weight and body weight gain of the mixed-herbal supplemented, showed significant increase compared to control fed. This is supported by [14] study, who founded the feed additives from botanical extracts were able to enhance the digestibility or utilization efficiency of nutrients, including exogenous enzymes, stimulators of enzyme secretion, aid the digestive process by improving absorption, mobilization and transport of nutrients.

The most promising growth performances were exhibited by AVE and VTE, followed by VTE and SCE and AVE and SCE respectively. These results supported by [12], showed that significantly increase of the growth and survival rate during stressed conditions of *Penaeus monodon* [15] fed enriched Artemia with five herbal combination (*Hygrophila spinosa*, *Withania somnifera*, *Zingiber officinalis*, *Solanum trilobatum*, *Andrographis paniculata*, and *Psoralea corylifolia*) was reported.

Furthermore Ji SC, et al. [16] reported that other than the usage of different single herbal extracts (*Massa medicata*, *Crataegi fructus*, *Artemisia capillaries* and *Cnidium officinale*) or the mixture of the herbs also promoted growth and enhanced some non-specific immunity indicators in red sea bream (*Pagrus major)*. AVE and VTE showed the most significant growth compared with other fed mixed herbs combination. The lowest fish growth was obtained in AVE and SCE diet. The phytochemical profile of VTE consists of the tannins, flavonoid and glysocide [17]. In contrast, AVE [18] and SCE [19] contain alkaloids, tannins and flavonoids. This phytochemical profile may be the reason for the improved growth performances in *Oreochromis* sp. as indicated in this study. This is consistent with [20], who identified the treatment with mixed-herbal formulations may confer with synergistic, potentiate the agonistic/antagonistic pharmacological activity. Synergism is generally dependent on the number of extracts, the dose and the various active compound combinations such as phenolic, flavonoids, alkaloids, terpenoids and tannins.

Moreover, fish fed on diets containing AVE and VTE and VTE and SCE showed the optimum FCR with 1.16 and 1.10 respectively, whereas fish fed with control diet produced higher FCR, 1.33. This is also supported by [21], with study on Nile tilapia supplemented with a mixture of herbal extracts, resulting in natural emulsifying agents and co-factors of digestion for better feed conversion and protein efficiency. However, for SGR data with AVE and SCE and VTE and SCE combination showed significantly lower ($P>0.05$) than compared to the control group. Nevertheless, AVE and VTE were able to show promising result with significantly higher ($P< 0.05$) than compared to the control group. This also suggested that the correct doses and formulas to ensure the safety and efficacy of the mixed-herb, other than the optimum ratio of each single herb also must be clearly specified [12,22,23]. However, the concentration of herb incorporated does not significantly influence the growth and survival rate supported by [24]. The right proportion is important when using mixed-herbs as feed additives. Fish survival after the diseases challenge range from 90% to 100% with significant difference ($P< 0.05$) among all treatments including control treatment. Ponpornpisit A, et al. [25] fed a Chinese herbal mix, known as C-UPIII, to the guppies (*Lebistes reticulata*) and observed an improved survival rate in fish infected with *Tetrahymena pyriformis*. Furthermore, the addition of a mixture of Chinese herbs (*Rheum officinale, Andrographis paniculata*, *Isatis indigotica*, *Lonicera japonica*) to the feed of crucian carp resulted in increased phagocytosis [26], suggesting better microbial resistance. Moreover, the herbal-mix boosted the immune system in fish and shrimps against pathogenic infections. In cases where disease outbreaks are cyclical and could be predicted, immunostimulants may be used in anticipation of events to elevate the nonspecific defence mechanism, and thus prevent losses from diseases. It has been proven that immunostimulant herbal extracts as feed additives such as *Cynodon dactylon*, *Phyllanthus niruri*, *Tridax procumbens*, *Zingiber officinalis*, *Ocimum sanctum*, *W. somnifera and Myristica fragrans* have improved the immune system in grouper *E. tauvina*, while also at the same time controlling *V. harveyi* [23,27].

The haematological and biochemical profiles of pre and post challenge infected groups (Table 2) were compared with the control group. RBC, Hb, and WBC showed higher significant difference ($P<0.05$) compare to control treatment. However, Nya EJ, et al. [28] reported that rainbow trout *Oncorhynchus mykiss* infected with*V.A. hydrophila*, leading to increase of WBC and causing lead to anaemia, attributed to increased destruction, loss, or suppression of RBCs. However, in the present study, RBC and WBC showed significant differences at pre and post challenge showing the capability of the feed additive in bacteria disease resistance. This is also supported with the increase levels of WBC in *A. hydrophila* infected goldfish [29]. Further, by enhancing the WBC levels, the herbal treatment may have a positive role in increasing goldfish immunity against *A. hydrophila.* The increase in RBC levels, which reflects increases in derived indices for the infected group, may be caused by the production of younger, immature RBCs with less Hb content in green chromide infected with epizootic ulcerative syndrome [30].

In contrast, mixed herbal treatment should be used with caution since it also leads to physiological disruptions. For example, the ethanolic leaf extract induces an increase in total and differential leukocyte counts in rats [31], an increase in RBC counts, a reduction in RBC membrane thickening, and an increase in membrane fluidity [32]. For MCV, after the infection, the haematological indices were resulted with no significantly difference ($P>0.05$). Although no significant differences were observed, it could be concluded that infected fry suffered from hypochromic microcytic anaemia [33]. It clearly suggested that a hemodilution mechanism has occurred. The MCV gave an indication of the status or size of the RBC and reflected an abnormal or normal cell division during erythropoiesis [34].

On the other hand, MCHC results on days 60 showed there were significant differences ($P<0.05$) compared to the control group. The significant decrease in the MCHC in this study was an indication of erythrocytes swelling and/or due to a decrease in haemoglobin synthesis in control group [33]. Buckley JA, et al. [35] reported that prolonged reduction in haemoglobin content was deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed. The biochemical parameter of ALT and ALP were significantly affected by mixed-herbal supplementation. Pre-challenge, ALT showed significant different in all treatment, compared to significant decreases at post challenge. However at the pre- challenge, ALP revealed only AVE and SCE with no significantly difference compared to the control group. Then at the post challenge all mixed herb showed decreases in ALP value, however control treatment showed a significant increases. There was a significant ($P<0.05$) decrease in ALT and ALP post challenge, compared to the control group. The AST, showed significant difference among all treatments, with control treatment not showing any significant difference at pre and post challenge. Post challenge, AVE and VTE showed no significant difference compared to the control group.

Conclusion

In conclusion, the combination of AVE and VTE is the most significantly improved growth of *Oreochromis* sp. juveniles and also reduces the mortalities post challenge with *S. agalactiae*. The present results suggested that the mixed herbal supplementation feeds are responsible for maintaining the haematological and biochemical parameters to normal condition and further improve to trigger the immune system of the innate immunity of *Oreochromis* sp. against *S. agalactiae* when supplemented with 7g/kg DM of mixed herbal. However, the exact mechanism of inducing the blood parameter recoveries and innate immune response using modern molecular techniques should be applied to ensure that the species of mixed herbal supplementation feeds used in aquaculture are correctly identified, for quality assurance as well as safety.

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