Fish & Shellfish Immunology 44 (2015) 504–514

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Dietary *Aloe vera* supplementation on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT)

Ndakalimwe Naftal Gabriel [a, c], Jun Qiang [a, b], Jie He [a], Xin Yu Ma [a], Mathew D. Kpundeh [a], Pao Xu [a, b, *]

[a] Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China
[b] China Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, Jiangsu, China
[c] Directorate of Aquaculture, Ministry of Fisheries and Marine Resources, Hardap, Namibia

ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form
20 February 2015
Accepted 1 March 2015
Available online 7 March 2015

Keywords:
Bacterium
Challenge
Cichlidae
Feeding
Immunity
Oreochromis
Herbs
Survival

ABSTRACT

This study investigated effects of dietary *Aloe vera* on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). Five groups were designed including a basal diet (control) and 100% *A. vera* powder incorporated in fish feed at 0.5% 1%, 2%, and 4%/kg feed, which were administered for 8 weeks. Fish fed 0.5%, 1%, and 2% *A. vera* supplemented diet significantly improved ($p < 0.05$) weight gain, absolute growth rate and specific growth rate. Feed intake significantly increased in fish fed with *A. vera* diet at 1% and 2%/kg feed. Feed efficiency ratio, feed conversion ratio, and hepatosomatic index were significantly enhanced in 4% *A. vera* supplemented fish over unsupplemented ones ($p < 0.05$). Several haemato-biochemical indices were examined before and after fish were challenged with *S. iniae* pathogen containing 7.7×10^6 CFU cells mL^{-1}. *A. vera* supplemented fish showed a significant increase ($p < 0.05$) in red blood cells, hematocrits (Hb), hemoglobin (Hb), white blood cells (WBC), neutrophils, monocytes, eosinophils, serum total protein, glucose and cortisol after challenge when compared to unsupplemented ones. Meanwhile, 4% *A. vera* supplemented fish showed a decrease ($p < 0.05$) in RBC, Hb, Ht, WBC, and mean corpuscular hemoglobin (MCH) after challenge compared to unsupplemented ones and other supplemented ones. In addition, lower mean corpuscular volume values (MCV) ($p < 0.05$) were observed in fish fed with *A. vera* diet at 2% and 4% *A. vera*/kg feed than those fed unsupplemented diet. Unchallenged fish fed 0.5%, 1%, and 2% *A. vera* showed significantly higher values ($p < 0.05$) of mean corpuscular hemoglobin concentration (MCHC) than those fed unsupplemented diet and 4% *A. vera* supplemented diet. There was a significant increase ($p < 0.05$) in the neutrophil to lymphocyte ratio (N/L) within experimental groups after challenge; N/L ratio in *A. vera* unsupplemented fish and those supplemented with *A. vera* diet at 1%/kg feed increased significantly ($p < 0.05$) throughout challenge period; while those fed 4% *A. vera* supplemented diet maintained higher values at all experimental stages among groups. There was a significant correlation ($p < 0.05$, $r = 0.53$) between N/L ratio and glucose concentration, 96 h after challenge. Aloe had no significant effect ($p > 0.05$) on the survival of the fish when compared to the control; no mortality was recorded in challenge trial. Overall, our results indicated that dietary aloe supplementation could improve growth, feed utilization, and haemato-biochemical parameters of cultured tilapia.

1. Introduction

Global food fish consumption demand is on the rise, and the need to complement capture fisheries with aquaculture is inevitable. The pressure on aquaculture to bridge the supply and demand disparity of food fish has resulted in the widely use of

* Corresponding author. Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China. Tel.: +86 510 85557959; fax: +86 510 85553304.
E-mail address: xup@ffr.cn (P. Xu).

http://dx.doi.org/10.1016/j.fsi.2015.03.002
1050-4648/

intensive fish husbandry. One of the key elements that encourage fish farmers to adopt these kinds of production systems is that, productivity per unit area is much higher as a result of higher stocking density [1]. On the contrary, high stocking density in intensive fish culture systems has led to many constraints and among them is stress, which as a consequence, results in a bunch of conditions such as, poor fish performance, alteration of physiological functions [2], poor digestion and feed utilization [3,4], increased susceptibility to diseases [5], poor fish meat quality [6], and in extreme cases lead to mortality [7,8]. The management of these stress related conditions in intensive production systems remain a challenge for the aquaculture industry [9], especially in top aquaculture producing countries (i.e. Asians). For instance, tilapia aquaculture production in Southern China was reported to have suffered a huge economic loss due to outbreaks of streptococcal infections about 5 years ago [5]. Thus, aquaculture is yet to reach its full potential.

Over the years, fish farmers have been using antibiotics and other chemotherapeutic drugs to prevent stress related conditions, especially, in intensive fish farming. The success of antibiotics in aquaculture and other farming sectors such as livestock and poultry lies in their ability to promote growth, enhance feed conversion efficiency and prevent the spreading of diseases [10]. In China, Thailand and Vietnam, both semi-intensive and intensive shrimp production were reported to rely most heavily on chemicals and biological products inputs per unit ton of harvested produce [11]. The continuous use of antibiotics and other chemicals has numerous shortcomings such as, the risk of resistant of pathogens, problem of drug residue in treated animals, impacts on human health and environmental pollution [5]. Thus, many nations around the globe have strict regulations that limit the use of antibiotics in animal farming [12]. Therefore, there is an urgent need to explore alternatives to antibiotics that could be used for better growth performance, disease control and subsequently improve production in intensive fish production systems in a sustainable manner.

Vaccines could be an alternative to prevent diseases in aquaculture; however, problems regarding inoculation and pathogen specificity obstruct their effectiveness [5]. Another plight is, there is no commercial vaccine available for many pathogenic bacterial infections including streptococcal infections [13].

Nowadays, the use of medicinal plant extracts in aquaculture for the prevention of diseases, promotion of growth and production is a novel development with potential to eradicate the use of antibiotics [14,15]. Bioactive compounds present in several medicinal plants such as, *Acalypha indica, Phyllanthus niruri, Azadirachta indica, Piper bettle, Mentha piperita* [16], *Allium sativum* and *Astragulus membraneceus* [17] reportedly enhance growth, innate immune response, and disease resistance against pathogenic bacteria in fish [16–18]. To our knowledge, at present, there is limited number of studies on the use of medicinal herbs in aquaculture; these studies concisely hinted that, herbal extracts could be indeed potential alternative to synthetic antibiotics and other chemotherapeutic drugs in fish culture.

Aloe vera (synonym: *Aloe barbadensis*) is a succulent, stemless herb, found widely distributed in tropical and subtropical regions. The genus *Aloe* comprised of more than 360 species [19] of which *A. vera* is considered to be the most popular and bioactive [20], with more than 70 biological active compounds [21]. These bioactive compounds are found in two types of exudates (bitter yellow latex and mucilaginous gel) secreted by Aloe leaves [22,23]. Aloe gel has been reported to contain wide range of polysaccharides (protein, pectin, cellulose, hemicellulose, glucomanna, acemannan and mannose derivatives), about 20 of 22 necessary amino acids required by the human body and 7 of 8 essential amino acids which the body can not synthesize, vitamins (A, B1, B2, B6, C, E and folic acid), mineral (Ca, Mg, & Na), enzymes (lipase, amylase, carboxypeptidase and more), salicylic acid, lignin, saponins, fatty acids and hormones [24,25]. Hence, *Aloe vera* medicinal properties such as antibacterial, anti-septic, anti-inflammatory, immune-modulatory effects [26], anti-oxidant, anti-cancerous properties [27], anti-mutagenic and anti-hypersensitivity [28], growth [20] and gastro-intestinal promoting effects [29] have been widely reported.

Immuno-nutritional benefits of *A. vera* have been demonstrated in several freshwater species such as rainbow trout (*Oncorhynchus mykiss*) [29–32], common carp (*Cyprinus carpio*) [33,20], *Labeo rohita* [34], *Brycon amazonicus* [35], and Sea bream (*Sparu aurata*) [36]. Taiwo et al. [37]; and Jegede [38] reported histopathological effects of *A. vera* on tilapia species. To the best of our knowledge there is no report to date on the effects of *A. vera* on growth performance, haemalogical and biochemical parameters of tilapia species, GIFT-*Oreochromis niloticus* in particular, except a recent study by Dotta et al. [39]. Therefore, the current study was executed to investigate the effects of dietary *Aloe vera* on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT).

2. Materials and methods

2.1. Experimental fish and management

375 healthy tilapia (GIFT-*O. niloticus* strain) fingerlings with an average body weight of 4.83 ± 0.01 g and average body length of 5.5 ± 0.49 cm, were obtained from the tilapia breeding center of Freshwater Fisheries Research Center (FFRC) in Wuxi, China, July 2014. The fish were transported in polythene bags filled with oxygen. Before the feeding trials, fingerlings were acclimatized in cylindrical blue plastic tanks (0.6 m^2 × 0.85 m), supplied with 300 L of de-chlorinated freshwater at 29 ± 0.33 °C, pH 8.3 ± 0.36, dissolved oxygen (DO) 6.94 ± 0.26 mgL^{-1} (YSI 650 MDS multi probe system, YSI inc. USA) under natural photoperiod, continuous aeration and water recirculating system for a week. During the adaptation period, fish were fed thrice daily (09:00; 13:00; 17:00) with a commercial diet (No. 5271, 35% crude protein, Ningbo Tech-Bank co.ltd, Yuyao city, China) until apparent satiation. 2/3 cultured water was exchanged with de-chlorinated freshwater of similar temperature to maintain the water quality.

2.2. Preparation of diets and experimental design

To study the effects of dietary *A. vera* on growth and haemato-biochemical parameters in GIFT-strain tilapia, five isonitrogenous (31.7% crude protein), isoenergetic (672 KJ g^{-1}) and isolipid (7.34%) experimental diets were formulated, of which, one was a control and four were supplemented with graded levels of a 100% commercial *A. vera* powder purchased from Jiangsu Zhe Ya Food. Co. Ltd, China. All ingredients of each diet were powdered and thoroughly blended together in a food mixer for about 40 min. After, tap water was added bit-by-bit until stiff dough resulted as required. The paste for each diet was then separately passed through a mincer with 16 mm die, resulting in strands which were gently broken into pellets while fresh, air dried at ambient temperature for 3 days and stored at 4 °C in labeled plastic lined bags until use.

After acclimatization periods, fish with an average body weight 4.83 ± 0.01 g were randomly distributed into 15 tanks in 5 triplicate groups at a stocking density of 25 fish/tank. The fish were hand-fed the experimental diets the next day after stocking. Group 1 was fed with a control diet (0% *A. vera* powder), and other groups were fed 0.5% *A. vera*/kg feed (Group 2), 1% *A. vera*/kg feed (Group 3), 2% *A. vera*/kg feed (Group 4) and 4% *A. vera*/kg feed (Group 5) for 60 days, 6 days a week, 3 times a day (09:00; 13:00; 17:00) until

apparent satiation. Dietary *A. vera* inclusion levels used in this study is a modification of those previously used by Mahdavi et al. [20] and Heidarieh et al. [29]. Throughout the experimental period, continuous aeration, water recirculation, pH 7.5 ± 0.19, water temperature 28±0.55 °C, Ammonia-Nitrogen free, DO 6.9 ± 0.27 mg L^{-1} and photoperiod 12 h light/dark cycle were maintained. Furthermore, 2/3 of the water in all 15 tanks was exchanged bi-weekly with de-chlorinated freshwater of similar temperature to maintain the water quality during the study.

2.3. Fish growth, survival and feed utilization performance

In this study, fish growth was assessed in terms of weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR), condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI), whereas feed utilization parameters included, food conversion ratio (FCR), Feed efficiency ratio (FER), and Feed intake (FI). In addition, survival was expressed as percentage. Accordingly, 24 h after the last experimental feeding, body weight and length of all the fish in each tank were measured. Throughout the experiment, the amount of feed consumed and mortality in each replicate was noted. Calculations were carried out using the following formulas [30].

$$\mathrm{WG\ (g)} = W_2 - W_1$$

$$\mathrm{AGR}\left(\mathrm{g\,day^{-1}}\right) = (W_2 - W_1)/t$$

$$\mathrm{SGR}\left(\%\,\mathrm{day^{-1}}\right) = [\ln(W_2) - \ln(W_1)/t] \times 100$$

$$\mathrm{CF}\left(\mathrm{g\,cm^{-3}}\right) = \left(W/L^3\right) \times 100$$

$$\mathrm{HSI}(\%) = [\text{liver weight}/W] \times 100$$

$$\mathrm{VSI}(\%) = [\text{visceral weight}/W] \times 100$$

$$\mathrm{FI(g/fish)} = \text{dry feed intake}/\text{Number of fish}$$

$$\mathrm{FCR(g/g)} = \mathrm{FI(g)}/\mathrm{WG(g)}$$

$$\mathrm{FER} = \mathrm{WG(g)}/\mathrm{FI(g)}$$

$$\mathrm{Survival}(\%) = [\text{Number of survived fish}/\text{initial number of fish}] \times 100$$

where W_2 is final weight (g), W_1 is initial weight (g), t is the feeding trial period (days), W is weight of body (g), and L is total length (cm).

2.4. Physiological performance

GIFT tilapia physiological performance was measured using hematological and biochemical parameters. 24 h after the last feeding trial, blood was collected from the caudal vein of three anesthetized fish per tank with an air-dried, heparinized (500 U sodium heparinized/ml) hypodermic syringe. The collected blood sample was divided into two portions. One portion was transferred into Eppendorf tubes with Phosphate Buffer Saline (PBS, 0.02 M, pH 7.3) solution used as a diluent to measure hematological parameters; total white blood cell count (WBC × 10^9/L), were determined in a 1:100 dilution of the blood sample in the PBS solution, red blood cell count (RBC × 10^{12}/L) in a 1:1000 dilution of the blood sample with a Neubauer hemocytometer (Mindray BC-5300, Vet, China) [40], differential leukocytes count (neutrophils, lymphocytes, monocytes and eosinophils) was determined through an indirect method using blood smear stained with May-Grunwald-Giemsa, hematocrit percentage (Ht %) was determined through the microhematocrit method, and hemoglobin (Hb) concentration was determined using cyanomethemoglobin method. Stress was assessed using neutrophils/lymphocytes count ratio (N/L) [41].

Hb, Ht, and RBC values were used to determine the following haematimetric indices: the mean corpuscular volume (MCV) = (Ht × 10)/RBC, mean corpuscular hemoglobin (MCH) = (Hb × 10)/RBC, and mean corpuscular hemoglobin concentration (MCHC) = (Hb × 100)/Ht [42].

The second portion of blood sample was also transferred in Eppendorf tubes, left to clot at 4 °C, and centrifuged at 5000 rpm, 4 °C for 10 min. The collected serum was stored at −20 °C for further biochemical analysis. Biochemical indices such as: total protein content (TP), glucose (GLU), cortisol, and lysozyme (LYS) were quantified by electrochemiluminescence method using Mindray biochemical auto analyzer (BS-400), with kits supplied by Mindray biomedical electronics Co.Ltd Shenzhen, China.

2.5. In situ Streptococcus iniae challenge

After the initial sampling, stocking density in all groups was adjusted to 15 fish per tank. A pathogenic *S. iniae* strain (CMS No.005) from Guangdong province (South China) was procured by FFRC (Wuxi, China) to be used as a stressor in this study. Following Ndong et al. [43] procedures, *S. iniae* suspension was incubated in a disodium hydrogen phosphate (Na_2HPO_4) culture media for 24 h, at 30.1 °C . After, it was collected, cleaned and suspended in a 0.85% sterile saline. The bacterium CFU cells/mL^{-1} was determined using automatic colony counter software connected to a gel-pro analyzer (WD-9413C, Beijing Liuyi instrument factory). Fish from all groups were injected with the bacterial suspension, 7.7 × 10^6 CFU cells mL^{-1}, 0.6 mL per 100 g body weight into the abdominal cavity. During this challenge, fish mortality was monitored, and blood samples were collected in a similar fashion as illustrated in section 2.4 after 48 h and 96 h respectively. The blood samples were used to determine haemato-biochemical parameters following similar procedures as in section 2.4.

2.6. Statistical analysis

In this study, all results for each parameter measured were expressed as mean ± standard error (M ± SE). Data were analyzed using one-way analysis of variance (ANOVA) with *Aloe vera* inclusion levels as a factor, using the statistical package for the social sciences (SPSS) computer software (version 22). Duncan's multiple range tests at a significant level of 95% was used to determine significant differences between treatments. Correlation between variables (i.e. glucose and N/L ratio; and cortisol and N/L ratio) was performed using Pearson's correlation coefficient in SPSS. Mean value of $P < 0.05$ was considered significant.

3. Results

3.1. Fish growth, survival and feed utilization performance

Growth and feed utilization of the fish fed dietary *A. vera* were significantly enhanced compared to the control (Table 2). WG, AGR, and SGR significantly increased ($P < 0.05$) with increase dietary *A. vera* inclusion level from 0.5% to 2%. Dietary *A. vera* did not

Table 1
Composition and proximate analysis of the basal diet (g/100 g dry matter).

Ingredients	Proportion (%)
Fish meal	10.0
Corn starch	16.8
Soybean oil	6.00
Soybean meal	20.0
Cottonseed meal	20.0
Rapeseed meal	20.0
Vitamin premix[a]	0.50
Mineral premix[b]	0.50
Choline chloride	0.50
Vit C Phosphate ester	0.20
Ca $(H_2PO_4)_2$	1.50
Cellulose	4.0
Total	100
Composition	**proximate analysis (%)**
Crude protein	31.7
Crude lipid	7.3
Gross energy (KJ/g)	672.00

[a] Vitamin premix (mg/kg dry diet): V_A 10, V_D 0.05,V_E 400, V_K 40, V_{B1} 50, V_{B2} 200,V_{B3} 500, V_{B6} 50, V_{B7} 5,V_{B11} 15, V_{B12} 011,V_C 1000, inositol 2000, choline 5000.
[b] Mineral premix (mg/kg dry diet): $FeSO_4.7H_2O$ 372, $CuSO_4.5H_2O$ 25, $ZnSO_4.7H_2O$ 120, $MnSO_4.H_2O$ 5, MgSO4 2475, NaCl 1875, KH_2PO4 1000, Ca $(H_2PO_4)_2$ 2500.

significantly affect ($p > 0.05$) the CF, however, high values were observed in dietary *A. vera* supplemented fish compared to unsupplemented ones. High VSI was observed in fish fed *A. vera* diet at 0.5%/kg feed, followed by those fed 4%, 1%, 2% *A. vera*/kg feed and unsupplemented diet respectively; there was no significant difference among the groups ($P > 0.05$). HSI increased with dietary *A. vera* inclusion level, with a significant increase ($p < 0.05$) presented in fish fed dietary *A. vera* at 4%/kg feed. Feed intake (FI) was significantly affected by dietary *A. vera* inclusion level; fish fed 4% *A. vera* supplemented diet presented poor FI ($P < 0.05$) among dietary groups, meanwhile, better FI was observed in those fed 2% and 1% *A. vera*/kg feed ($P < 0.05$). Fish fed *A. vera* supplemented diet presented better FCR and ultimately high FER values; significant differences ($p < 0.05$) were observed in fish fed 4% dietary *A. vera* when compared to unsupplemented ones. Dietary *A. vera* did not significantly affect percentage survival ($P > 0.05$) when compared to unsupplemented fish, however, percentage survival of those fed 2% *A. vera* supplemented diet was negatively affected ($P < 0.05$), while fish fed 0.5% *A. vera* supplemented diet revealed a significant increase in percentage survival among *A. vera* supplemented ones.

3.2. Hematological parameters

No significant changes ($p > 0.05$) were observed in RBCs, Hb, and Ht (Fig. 1) among experimental groups before fish were challenged with *S. iniae*. RBC, Hb and Ht in fish fed 0.5% *A. vera* supplemented diet were significantly higher ($P < 0.05$) and lower ($p < 0.05$) in those supplemented with 4% *A. vera*/kg feed, 48 h post challenge, compared to unsupplemented ones, other supplemented ones, and initial values, respectively. 4% dietary *A. vera* continued to affect ($p < 0.05$) RBCs, Hb, and Ht in a similar fashion, 96 h post challenge. An increase in RBCs, Hb, and Ht was observed in fish fed 0.5% *A. vera* supplemented diet, after 96 h post challenge, with no significant difference ($p > 0.05$) compared to unsupplemented ones.

Meanwhile, mean corpuscular volume (MCV) significantly decreased ($p < 0.05$) in unchallenged fish fed 2% and 4% dietary *A. vera* compared to unsupplemented ones; no significant changes ($p > 0.05$) were observed in challenged fish (Fig. 1). Unchallenged fish fed *A. vera* supplemented diet did not show significant differences ($p > 0.05$) in mean corpuscular hemoglobin (MCH) compared to unsupplemented ones, except an increase ($p < 0.05$) observed in those fed 0.5% and 1% over 4% *A. vera* supplemented ones (Fig. 1). 48 h post challenge, MCH decreased significantly ($p < 0.05$) in 4% *A. vera* supplemented fish compared to unsupplemented ones and other supplemented ones. After 96 h challenge, MCH increased significantly ($p < 0.05$) in 1% *A. vera* supplemented fish over 2% supplemented ones. MCHC increased significantly ($p < 0.05$) between 0.5% and 2% *A. vera* supplemented unchallenged fish compared to unsupplemented ones and those fed 4% *A. vera* supplemented diet; MCHC in fish fed 4% *A. vera* supplemented diet continued to decrease significantly ($p < 0.05$) over 2% supplemented ones, 48 h post challenge (Fig. 1).

Dietary *A. vera* supplemented fish did not show significant changes in WBCs, neutrophils, lymphocytes, monocytes, eosinophils, and neutrophils/lymphocytes count ratio (N/L) before challenge compared to *A. vera* unsupplemented ones (Fig. 2). WBC count was significantly higher ($p < 0.05$) in fish fed 0.5% dietary *A. vera* and lower ($p < 0.05$) in those received 4% *A. vera*/kg feed compared to *A. vera* unsupplemented ones and those in other *A. vera* supplemented groups, 48 h post challenge. WBCs remained higher ($p < 0.05$) in fish fed 0.5% *A. vera* supplemented diet and lower ($p < 0.05$) in those fed with *A. vera* diet at 4%/kg feed, 96 h post challenge. Accordingly, WBCs in challenged fish fed 4% *A. vera* supplemented diet were significantly lower ($P < 0.05$) when compared to pre-challenge value.

Neutrophils relative proportion (%) increased significantly ($p < 0.05$) in all experimental groups, except in fish fed 2% *A. vera* supplemented diet, 48 h after challenge; higher values were

Table 2
Growth performance and feed utilization of GIFT strain tilapia; *O. niloticus* fed dietary *Aloe vera* for 60 days.

		Dietary *Aloe vera* (%) (g/100 g dry matter			
Parameters	Control	0.5	1	2	4
WG	57.85 ± 2.67^a	64.80 ± 1.54^b	64.91 ± 2.10^b	67.49 ± 0.72^b	58.30 ± 1.30^a
SGR	4.27 ± 0.65^a	4.45 ± 0.33^b	4.44 ± 0.05^b	4.51 ± 0.02^b	4.28 ± 0.03^a
AGR	0.96 ± 0.04^a	1.08 ± 0.03^b	1.08 ± 0.04^b	1.12 ± 0.01^b	0.97 ± 0.02^a
VSI	17.00 ± 3.05	18.74 ± 0.87	18.33 ± 1.20	17.66 ± 1.78	18.58 ± 1.29
HIS	1.05 ± 0.15^a	1.09 ± 0.04^a	1.15 ± 0.05^{ab}	1.20 ± 0.03^{ab}	1.40 ± 0.05^b
CF	2.67 ± 0.25	2.64 ± 0.03	2.74 ± 0.11	2.75 ± 0.10	3.10 ± 0.14
FI	74.91 ± 2.30^b	78.80 ± 1.30^{bc}	80.84 ± 1.87^{cd}	84.93 ± 1.08^d	68.97 ± 0.50^a
FCR	1.30 ± 0.03^b	1.22 ± 0.04^{ab}	1.25 ± 0.03^{ab}	1.26 ± 0.01^{ab}	1.18 ± 0.02^a
FER	0.77 ± 0.02^a	0.82 ± 0.03^{ab}	0.80 ± 0.03^{ab}	0.79 ± 0.01^{ab}	0.85 ± 0.02^b

[a]Data are expressed as mean ± standard error (M ± SE). Values with different superscript letters in the same row are significantly different ($P < 0.05$) from the control. Where, WG = weight gain, SGR = specific growth rate, AGR = absolute growth rate, CF = condition factor, HIS = hepatosomatic index, VSI = viscerosomatic index, FI = Feed intake, FCR = food conversion ratio, and FER = Feed efficiency ratio.

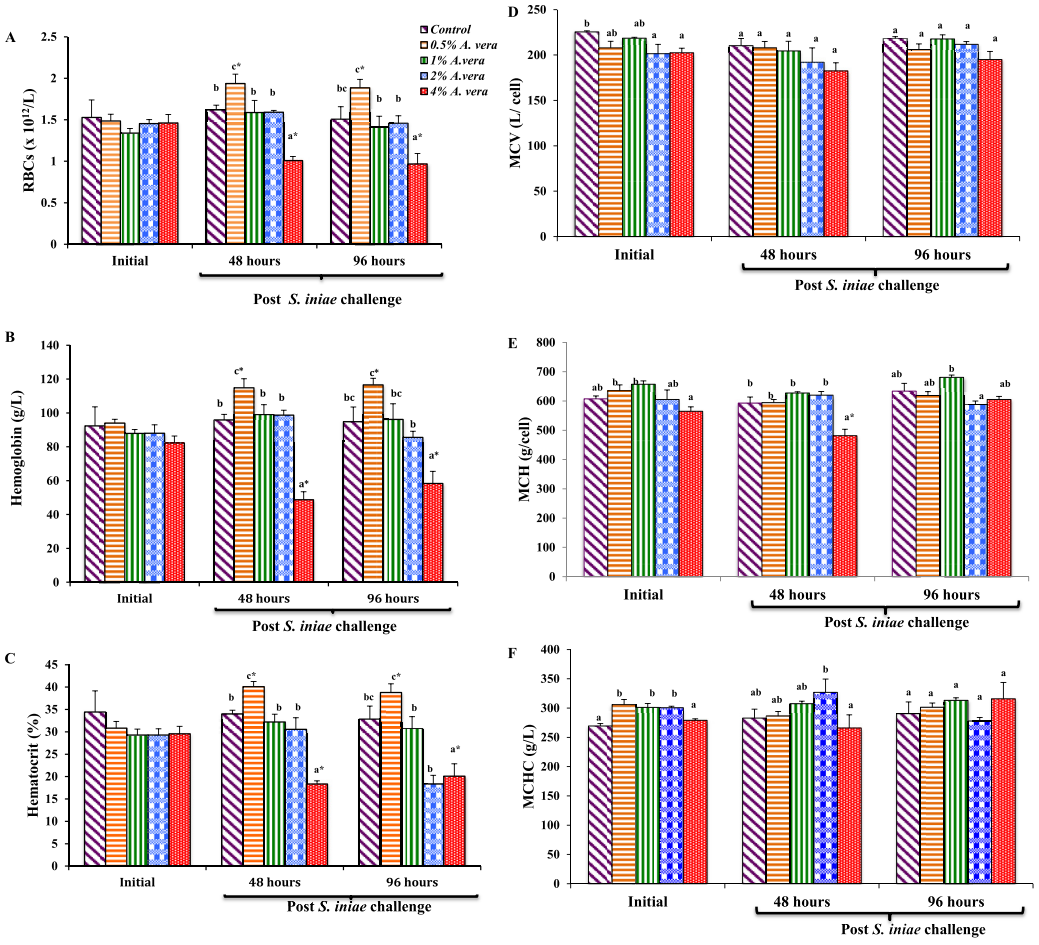

Fig. 1. RBCs (**A**), Hb (**B**), Ht (**C**), MCV (**D**), MCH (**E**), and MCHC (**F**) of Gift-*O. niloticus* fed four *Aloe vera* supplemented diets (0.5, 1, 2, 4%/kg feed) and unsupplemented diet at initial sampling (pre-infection), 48 h and 96 h after *S. iniae* challenge. Different lower case letters denote a significant difference ($p < 0.05$) between *A. vera* unsupplemented fish and *A. vera* supplemented fish at respective post infection stages, while asterisks (*) indicate a significant difference ($p < 0.05$) between a particular post infection period and initial values, within groups. Values are expressed as M ± SE.

observed in dietary *A. vera* supplemented fish compared to unsupplemented ones. Neutrophils in 1% *A. vera* supplemented fish and unsupplemented ones remained significantly higher ($p < 0.05$) after 96 h challenge. Contrarily, lymphocytes decreased significantly ($p < 0.05$) in all experimental groups throughout challenge period when compared to pre challenge values, within groups, except fish fed 4% *A. vera* supplemented diet. Monocytes fluctuated during challenge period; they decreased after 48 h challenge, with significant decrease ($p < 0.05$) observed in fish fed 2% dietary *A. vera* compared to pre challenge value; and increased within groups after 96 h challenge, but no significant increase ($p > 0.05$) was presented. Eosinophils increased significantly ($p < 0.05$) within all experimental groups after 48 h challenge compared to pre challenge values, except in fish fed 4% *A. vera* supplemented diet; after 96 h challenge significant increase within groups was only observed in fish fed 1% and 2% dietary *A. vera* when compared to initial values, respectively. Meanwhile, N/L ratio in unchallenged fish fed 2% and 0.5% dietary *A. vera* was lower and higher in those fed 4% compared to *A. vera* unsupplemented ones. 48 h post challenge, N/L ratio increased significantly ($p < 0.05$) in fish fed 1% dietary *A. vera* followed by 0.5% dietary and *A. vera* unsupplemented diet compared to initial values. After 96 h challenge, N/L ratio remained significantly higher ($p < 0.05$) in *A. vera* unsupplemented fish and those fed 1% *A. vera* supplemented diet, when compared to pre challenge values, respectively. N/L ratio also remained higher in fish fed with *A. vera* diet at 4%/kg feed throughout challenge trial among groups.

3.3. Blood biochemical parameters

3.3.1. Total protein

Dietary *A. vera* significantly influenced ($p < 0.05$) fish serum total protein (TP) content before and after challenge with reference to control (Fig. 3). Before challenge, TP content gradually increased significantly ($p < 0.05$) in 0.5% and 2% *A. vera* supplemented fish, and decreased in those fed with dietary *A. vera* at 4%/kg feed. After 48 h challenge, a further gradual increase in TP was observed in fish fed with *A. vera* diet between 0.5% and 2%/kg feed, with no significant difference ($p > 0.05$) observed, and decreased significantly in fish fed with dietary *A. vera* at 4%/kg feed over those fed with *A. vera* unsupplemented diet, 1% and 2% *A. vera*/kg feed. Similarly, TP content was higher in fish fed with *A. vera* diet at 0.5%, 1% and 2% *A. vera*/kg feed and significantly lower ($p < 0.05$) in those fed with 4% *A. vera*/kg feed, 96 h post challenge. TP content in fish fed *A. vera* unsupplemented diet, 0.5%, 1%, and 2% *A. vera*/kg feed significantly increased ($p < 0.05$) after 96 h challenge when compared with pre-challenge values, respectively.

3.3.2. Lysozyme activity

There was no significant difference ($p > 0.05$) in serum lysozyme activity between dietary *A. vera* supplemented fish and unsupplemented ones, before and after infection (Fig. 3). However, higher lysozyme activities were observed in *A. vera* supplemented fish compared to unsupplemented ones. Lysozyme activities were

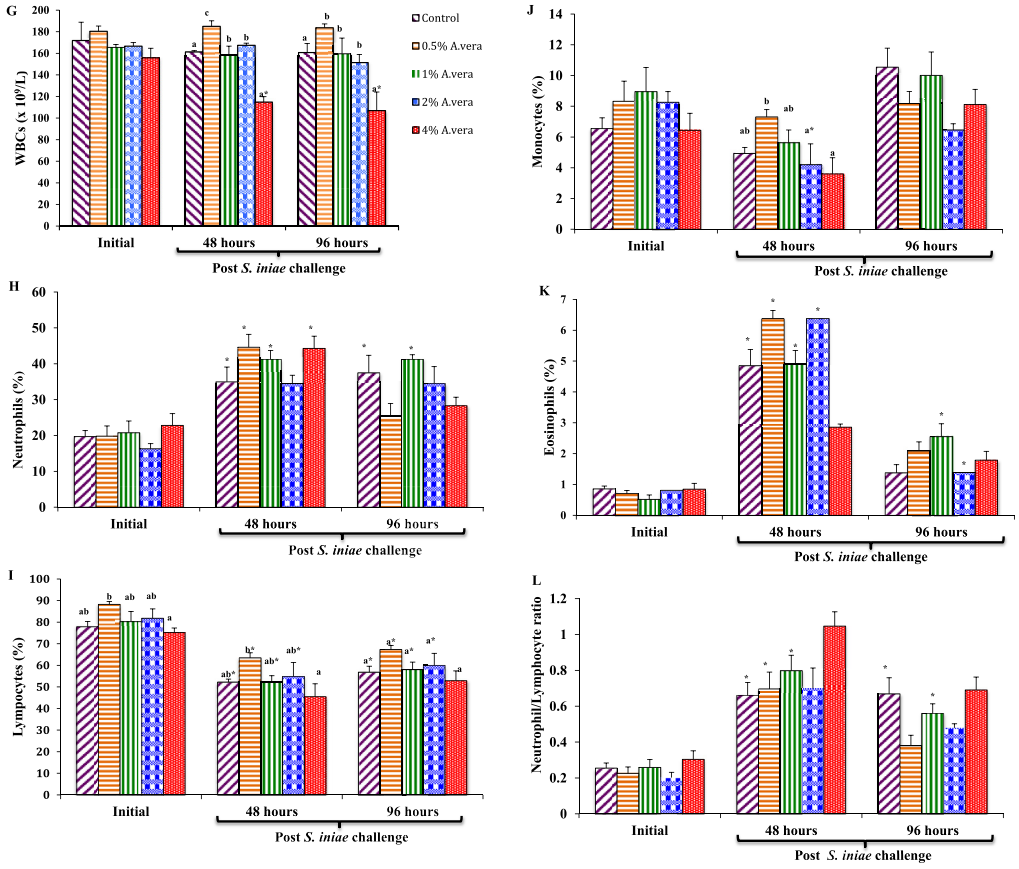

Fig. 2. WBCs (**G**), neutrophils (**H**), lymphocytes (**I**), monocytes (**J**), and eosinophils (**K**); and neutrophil to lymphocytes count ratio (NLCR) (**L**) of GIFT *O. niloticus* fed *Aloe vera* powder supplemented diet at different levels before and after *S. iniae* challenge. Different superscript letters at initial, 48 h and 96 h post infection stage indicate a significant difference ($p < 0.05$) between *A. vera* unsupplemented fish and *A. vera* supplemented ones, respectively. Asterisks (*) indicate a significant difference ($p < 0.05$) between a particular post infection period and initial values, within groups. Values are expressed as M ± SE.

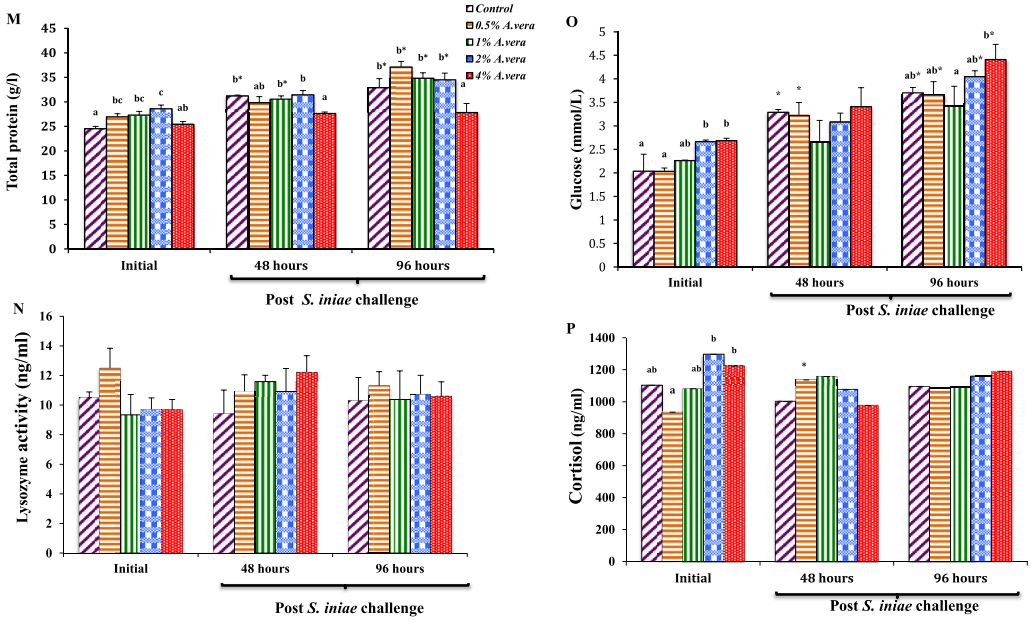

Fig. 3. Serum total protein content (**M**), lysozyme activity **(N**), glucose (**O**) and cortisol concentration (**P**) in GIFT-*O. niloticus* fed *Aloe vera* powder supplemented diet at different levels, before and after *S. iniae* challenge. Values are expressed as M ± SE. Different superscript letters at initial, 48 h, and 96 h post infection indicate a significant difference ($p < 0.05$) between *A. vera* unsupplemented fish and *A. vera* supplemented ones, respectively. Asterisks (*) indicate a significant difference ($p < 0.05$) between a particular post infection period and initial values, within groups.

higher in fish fed with *A. vera* diet at 0.5%/kg feed, before challenge and 96 h post challenge. Meanwhile, fish fed 4% *A. vera* supplemented diet presented higher lysozyme activities, followed by those fed 1%, 0.5% and 2% *A. vera*/kg feed, 48 h post challenge. There was also no significant difference ($p > 0.05$) observed within post challenge groups when compared to pre-challenge ones, respectively.

3.3.3. Glucose concentration

There was a significant ($p < 0.05$) change in the serum glucose concentration of fish fed *A. vera* supplemented diets with reference to control, pre and post–challenge (Fig. 3). Before challenge, glucose concentration increased significantly ($p < 0.05$) in fish fed with *A. vera* diet at 2% and 4%/kg feed; the lowest glucose concentration was observed in fish fed *A. vera* diet at 0.5%/kg feed, but there was no significant difference ($p > 0.05$) over unsupplemented ones. No significant difference ($p > 0.05$) was observed in glucose concentration between test groups, 48 and 96 h post challenge. However, high and low glucose concentration values were observed in *A. vera* supplemented fish over unsupplemented ones, post challenge. The lowest glucose concentration value showed in fish supplemented with 1% *A. vera*/kg feed, while the highest was observed in those fed with *A. vera* at 4%/kg feed with reference to unsupplemented ones and those fed other *A. vera* supplemented diets, throughout post challenge trial. After 48 h challenge, only *A. vera* unsupplemented fish and those fed with *A. vera* at 0.5%/kg feed showed a significant increase ($p < 0.05$) in glucose when compared to their initial values. Meanwhile, fish in all test groups except those fed 1% *A. vera*/kg feed presented a significant increase ($p < 0.05$) in glucose after 96 h challenge when compared to those in pre-challenge groups, respectively.

Furthermore, glucose concentration was found to correlated with N/L ratio before and after challenge (Table 3); a positive correlation ($r = 0.22$, $p > 0.05$) was found before challenge; a negative correlation ($r = -0.49$, $P > 0.05$) was observed 48 h post challenge; and a significant positive correlation ($r = 0.53$, $P < 0.05$) was present 96 h post challenge.

3.3.4. Cortisol level

Dietary *A. vera* did not significantly affect ($p > 0.05$) cortisol level over control, before and after challenge (Fig. 3). Unchallenged fish fed 0.5% *A. vera* supplemented diet showed the lowest cortisol concentration among test groups, and showed a significant difference when compared to those fed with dietary *A. vera* at 2% and 4%/kg feed. Higher cortisol concentration was observed in fish fed with *A. vera* diet at 1%/kg feed, while low concentration was observed in those fed 4% dietary *A. vera*, 48 h post challenge. Furthermore, a significant increase ($p < 0.05$) in cortisol concentration was observed in fish fed 0.5% *A. vera* supplemented diet, 48 h post challenge over initial value. After 96 h challenge, cortisol level increased gradually with increase in *A. vera* dietary doses, with the lowest concentration observed in fish fed 0.5% *A. vera* supplemented diet.

Table 3
Correlation between glucose level and neutrophil/lymphocytes count ratio (N/L), and cortisol level and N/L before and after *S. iniae* challenge.

		S. iniae challenge	
Parameters	Initial (r-values)	48 h (r-values)	96 h (r-values)
N/L and glucose level	0.22	−0.49	0.53*
N/L and cortisol level	0.15	−0.22	0.44

[a]Values with an asterisk (*) are significantly different ($p < 0.05$). r = correlation coefficient.

Moreover, cortisol and N/L ratio were found to correlate before and after challenge (Table 3); a positive correlation ($r = 0.15$, $p > 0.05$) was found before challenge; a negative correlation ($r = -0.22$, $p > 0.05$) was observed 48 h post challenge; and a positive correlation ($r = 0.44$, $p > 0.05$) was presented 96 h after challenge.

4. Discussion

Aloe vera is as old as civilization and throughout history it has been used as a popular folk medicine. Recently, it's potential to serve as an alternative growth promoter, anti-stressor, immunostimulant, appetizer and digestion stimulant have been reported in fish farming [33,29] and terrestrial animals such as poultry [44,45].

The current study demonstrated that inclusion of *A. vera* extracts in tilapia diet markedly affects feed utilization, and ultimately growth performance and resistance against *S. iniae* compared to the control. From the results (Table 1) feed utilization parameters such as FCR and FER were enhanced with increased dietary *A. vera* inclusion levels, except feed intake (FI), which seemed to have a favorable range between 0.5% and 2% *A. vera*/kg feed; this consequently affected WG, SGR and AGR in the similar fashion. Fish fed 4% dietary *A. vera* had a markedly low feed intake among test groups, however they could efficiently utilize their feed better, resulting in improved organo-somatic indices (VSI and HIS), condition factor, and somewhat high WG, SGR, and AGR values when compared to *A. vera* unsupplemented fish. Our results are in accordance with the findings of Mahdavi et al. [20] who reported that ethanolic dietary *A. vera* inclusion levels at 0.5% and 2.5% were able to significantly improve growth performance and nutrient utilization in common carp after been fed for two months. Heidarieh et al. [29] showed that *A. vera* inclusion level of 0.1% and 1% increased growth performance in rainbow trout (*O. mykiss*). Similar findings were also reported in broiler chickens [45]. The wide range of immuno-nutrional ingredients such as: proteins, lipids, vitamins, enzymes, minerals, sugar, lignin, saponin and salicylic acids [24,25] in *A. vera* supplemented diet could have enhanced the growth performance of the fish. Besides, the polysaccharides molecules such as acemannan present in *A. vera* leaves are believed to possess prebiotic properties [46–48]. Prebiotics are non-digestible feed ingredients that beneficially affect the host by stimulating growth or activity of one or a limited number of bacterial species already resident in the gut and thus improving host health [49]. The improvement in feed utilization and growth performance could be associated with increased nutrient digestibility, absorption and assimilation capacity, through improved digestive enzymes and healthy intestinal microflora fostered by the supplemented *A. vera.* A study by Heidarieh et al. [29] reported that *A. vera* improved gastrointestinal morphology of *O. mykiss* by increasing intestinal villus length and intestinal surface area, for increased food digestion and absorption capacity of the gut.

Furthermore, other interesting findings on feed utilization (FCR and FER) and growth performance (WG and SGR) in other tilapia species following administration of medicinal herbs; *A. sativum* [10,50], *Astragalus membranaceus* [51] *and Echinacea purpurea* extracts [50] have been reported, respectively. However, contradicting results have been reported too. For example, Dongmeza et al. [51] reported that diets supplemented with *Moringa oleifera* extracts significantly reduced feed intake, which subsequently led to a significant decrease in growth performance in Nile tilapia compared to the unsupplemented ones. Similarly, Afuang et al. [52] showed that fish fed methanolic moringa extracts at a level of 102 g/kg of feed caused a 17.5% reduction in weight gain compared to control. It has been reported that bioactive compounds such as saponins and tannins in most medicinal herbs may be toxic to

animals especially at high concentration [53]; they may present a bitter taste that might act as feed deterrents [51,53,54], and this may consequently affect growth. This phenomenon may partly explain the significant reduction in feed intake observed in fish supplemented with 4% *A. vera*/kg feed in the present study; contrary to the findings of Dongmeza et al. [51] and Afuang et al. [52], 4% *A. vera*/kg feed in the present study did not negatively affect growth, but rather enhanced it, this dosage was probably not too high enough to cause significant physiological changes that could have led to poor growth performance in GIFT tilapia. Therefore, our results (Table 1) revealed that dietary *A. vera* inclusion levels between 0.5% and 4% supported growth performance and feed utilization in GIFT-*O. niloticus*.

Blood parametric tests (hematological and biochemical) have been adopted in aquaculture as an important tool to assess the health status of fish. Hematological parameters including RBC, Hb, Ht and their derivative indices such as MCV, MCH, and MCHC are particularly known to indicate erythrocyte status and oxygen carrying capability in fish [55]. WBC and a number of leukocytes together with biochemical parameters such as serum proteins, lysozyme and cortisol are some of the elements that play a crucial role in fish innate immune response especially during stressful conditions (e.g. infections, dietary imbalance, high stocking density, and environmental stressors) [56]. In addition to serum glucose and cortisol concentration, neutrophils to lymphocytes ratio has been proven to be a valuable index to measure stress in invertebrates [41,57]. Studies have demonstrated that several plants such as *Nigella sativa* [58], *A. sativum* [59], *Zingiber officinale* [60], *Pedalium murex* [61], *Melissa officinalis* [31], and *A. indica* [62] are able to enhance some of these blood parameters, which is a denotation that plant extracts can indeed improve the health and immune system of fish.

In the present study, unchallenged fish fed *A. vera* supplemented diet did not show significant changes in hematological parameters compared to unsupplemented ones, except MCV which decreased significantly in fish fed 2% and 4% *A. vera*/kg feed, and MCH increased significantly in fish fed 0.5% and 1% over those fed 4% *A. vera*/kg feed. Significant changes in hematological parameters of dietary *A. vera* fed fish over unsupplemented ones were only noticed after fish were challenged with *S. iniae*. The results of the present study revealed that, after challenge, hematological parameters such as RBC, Hb, and Ht of fish fed 0.5% *A. vera* supplemented diet seemed to be more enhanced than of those fed unsupplemented diet. Similar to our pre-challenged hematological results, dietary *A. vera* in other studies elicited the same effects in Nile tilapia [39], and rainbow trout (*O. mykiss*) [30]. Accordingly, post challenge hematological responses of the present study correspond with the results obtained by Alishahi et al. [33] who reported that supplementation of 0.5% *A. vera* crude extracts in common carp diet could significantly increase their RBC, and packed cell volume (PCV or Ht), after *Aeromonas hydrophila* infection. Farahi et al. [31] reported that 1% *A. vera* inclusion level increased hematological parameters in *O. mykiss* with no challenge imposed. The improvement in RBC, Hb, Ht, MCH and MCHC indices of fish fed *A. vera* supplemented diets in this study may signify the ability of *A. vera* to stimulate erythropoiesis, therefore increasing the capacity of oxygen transport and strengthening of defense mechanisms against physiological stress. The erythropoietin effects of *A. vera* extract on hemopoetic cells in the bone marrow have been reported [63]. The increase in MCH as demonstrated in this study could have been attributed to essential vitamins such as riboflavin, thiamine and folic acid; and essential and non-essential amino acids in *A. vera*, which are primarily required for hemoglobin synthesis [64]. Polysaccharides in *A. vera* gel have been also associated with increased erythropoiesis and subsequently MCV [65].

On the other hand, herbal extracts have been reported to cause anemia condition in fish [66,67] and to a certain extent even death [68]. They are assumed to do this by disrupting erythropoiesis, haemosynthesis and osmoregulatory functions or by increasing erythrocyte destruction in hematopoietic organs [69]. *A. vera* in particular, has been reported to cause adverse effects including hematuria, metabolic acidosis, malabsorption [70], and electrolyte disturbances (hypokalaemia, hypocalcaemia) in animals at a particular dosage [71]. In addition, Taiwo et al. [38] reported that *A. vera* dietary inclusion level as high as 50 ppm caused tissue necrosis, hypoxia, gill, heart, liver, and kidney damage in tilapia, and also caused severe normocytic normochromic anemia in rats. In the same line, the current study observed a significant decrease in MCH, RBC, Hb, and Ht in fish fed 4% *A. vera* supplemented diet after challenge, which can be classified as hypochromic microcytic anemia condition as reported by Palanisamy et al. [66]. In this study, microcytic anemia condition was also observed in fish fed 2% and 4% *A. vera* supplemented diet before challenge as demonstrated by a significant decrease in MCV. High *A. vera* dosage particularly at 4% dietary inclusion level could have been harmful to GIFT tilapia, resulting in hemolysis, minerals and vitamins deficiency, decreased Hb and RBC synthesis, and ultimately hypochromic microcytic anemia condition after challenge as previous reported in *Channa striata* exposed to *Cleistanthus collinus* extracts [66]. Therefore, this inclusion level may lead to vulnerable fish with weakened defense mechanisms against physiological stress; it may not be appropriate in GIFT tilapia culture.

Furthermore, the present study observed: increased WBC, lymphopenia (decreased lymphocytes), neutrophilia (increased neutrophils), eosinophilia (increased eosinophils) and monocytosis (increased monocytes) after fish were challenged with *S. iniae* bacterium. Sebastião et al. [72] reported a neutrophilia response in Nile tilapia after *Flavobacterium columinare* challenge, similar to our study; they also simultaneously reported an increase in circulating lymphocyte counts, which contradict our findings. Moreover, Ali and Ansari [73] reported a synchronized neutrophilia and lymphopenia response in common carp after monogenean (flukes) challenge, which is in agreement with our findings. Lymphopenia response is reported to be a strategy that allows lymphocytes to move to sites of greatest potential for pathogens entry into the body such as epithelia of gills, skin and intestines; this help fish to mitigate infection from foreign pathogens [74,75]. Simultaneously, the circulating neutrophils are increased to attack pathogens that enter the body [74,75].

The leukocytosis response observed in fish fed *A. vera* supplemented diet compared to unsupplemented ones in the current study is an indication that *A. vera* have the ability to stimulate leucopoiesis, thus strengthening the body's ability to eliminate unwanted foreign pathogens such as bacteria, fungi and viruses. Acemannan molecule present in aloe gel is believed to trigger the body to produce disease fighting white blood cell, particularly macrophages [65]. In addition, *A. vera* has been reported to enhance phagocytic activity in fish especially during infection; it is unfortunate that the present study did not determine phagocytic or respiratory burst activities, however, high phagocytic and respiratory burst activities were reported in *O. mykiss* fed 1% *A. vera* supplemented diet [30]. Interestingly, Alishahi et al. [33] reported that 0.5% dietary *A. vera* increased serum bactericidal activity and IgM antibody levels in common carp infected with *A. hydrophila*, which is an indication that oral administration of *A. vera* may provide some non-specific and specific immune response in fish.

Certain herbal extracts have been reported to increase serum total protein and globulin content in fish, which is an indication of immune system activation [76]. The present study indicated that serum total protein in dietary *A. vera* supplemented fish

significantly increased before and after challenge; inclusion level from 0.5% to 2% *A. vera* tended to be more effective. This increase may be partly due to the destruction of RBCs and resultant release of cell content into the blood stream [77], especially during bacterial challenge, or due to an increase in WBC, which is a main source of serum protein production [62]. Serum total protein content findings obtained in this work could not agree with the recent findings by Dotta et al. [39] who reported that 0.5% *A. vera* supplemented diet failed to significantly increase total protein in unchallenged *O. niloticus*. Our results partly support the findings of Alishahi et al. [33] who reported that 0.5% *A. vera* inclusion level significantly enhanced total serum protein and globulin in *C. carpio*, before and after *A. hydrophila* challenge. Similar findings were also reported in *O. mykiss* fed 1% *A. vera* supplemented diet [30]. In addition to *A. vera*, several medicinal herbs have been reported to significantly enhance serum total protein content in different fish species such as; *Mucuna pruriens* in *L. rohita* [61], *N. sativa* and nettle (Quercetin) extract in rainbow trout [78], and *A. indica* in *Lates calcarifer* [79].

Furthermore, herbal extracts have been reported to increase lysozyme concentration in fish, especially during stress. Lysozyme is an important enzyme in the blood that actively lyses bacterial cell wall (peptidoglycan), and it is known to act as an opsonin and activate the complement system as well as phagocytes [80]. In the present study serum lysozyme concentration in *A. vera* supplemented fish was somewhat high in pre and post challenge stages when compared to the unsupplemented ones. The findings of the present study agree with Dotta et al. [39] who used *A. vera* in *O. niloticus*, and Hwang et al. [81] who used green tea extract in *S. schlegeli*. Remarkable results on lysozyme activities followed by administration of *A. membranaceus* in *O. niloticus* [82], *Sophora flavescens* in GIFT-*O. niloticus* [5], *N. sativa* in *O. mykiss* [78] and *A. indica* in *L. calcarifer* [79] were reported. High lysozyme concentration coupled with increased WBC, neutrophilia, monocytosis, eosinophilia, increased total serum protein and lymphopenia in peripheral blood observed in *A. vera* supplemented fish in the present study may be an indication that *A. vera* extracts could have strengthened the fish innate immune system during the course of the 60 days administration, leading to a positive immune response against *S. iniae*.

An increase in the N/L ratio observed in the current study is a sign that indeed these animals (GIFT tilapia) were experiencing physiological stress [83]; sometimes classified as stress leukogram [84] and had responded by altering their immune strategy to fight this infection. N/L ratio tended to correlate with both glucose and cortisol concentration; a significant positive correlation was found between N/L ratio and glucose, 96 h after challenge. Fish fed *A. vera* unsupplemented diet and those fed 1% *A. vera*/kg feed remained significantly stressed throughout challenge trial, while those fed with dietary *A. vera* at 4% *A. vera*/kg feed presented a high level of stress among groups throughout the study, as demonstrated by higher N/L ratio values. Fish fed 0.5% *A. vera* supplemented diet seemed to cope better with stress among groups, as demonstrated by the lower N/L ratio values; probably, they could effectively strategize their immune defense mechanisms (e.g. glucocorticoids, neutrophilia, lymphopenia) to minimize the chances of infection and subsequent immune challenge, and ultimately return life supporting physiological systems to homeostatic equilibrium, as assumed by Van Rijn and Reina [57] who subjected sharks to capture as a stress. Flavonoids, folic acid, and ascorbic acid present in *A. vera* leaves are known to act as antioxidants, which detoxify and eliminate highly unstable and reactive molecules; free radicals, which have the tendency to attack and damage normal cells of the body and cause a variety of health related problems [85]. This may help animals to easily recover from external stresses. On the other hand, fish fed 4% *A. vera* supplemented diet were anemic before challenge as discussed earlier in the current study; this condition resulted in animals of poor energy metabolism ability (as demonstrated by high glucose concentration), and vulnerable animals that could hardly cope with stress. These results could hardly be related to previous studies, because no study has investigated these parameters (N/L ratio, blood cortisol and glucose) in tilapia fed dietary *A. vera*.

Meanwhile, Xie et al. [86] reported that, both blood glucose and cortisol increased in *C. carpio var. Jian* fed anthraquinone extracts, after acute crowding stress. Abdel-Tawwab et al. [87] and Hwang et al. [81] also reported that blood glucose increased in *O. niloticus* and *Sebastes schegeli* fed green tea extracts, respectively. On the contrary *P. murex* [61], *A. sativum* [88], and *A. indica* [79] revealed hypoglycemic and anti-hyperglycemic effects in *L. rohita, L. calcarifer,* and *O. niloticus,* respectively.

The improvement of health indicative parameters in fish by medicinal plant extracts owes it to their abundant bioactive compounds [89,90]. The modes of action for many bioactive compounds to initiate immune responses are yet unknown, however Raa [91] expounded that probably, they interact with specific receptors on cells surface and promote the expression of intracellular genes encoding for antimicrobial molecules. This was further supported by Picchetti et al. [36] who reported that, Aloe extract of 1.2 mg/mL, acted as a powerful immunostimulant in lipopolysaccharide (LPS), and activated *Sparus aurata* fibroblast SAF-1 cells, inducing a synergic effect on interconnected genes that involved in different aspects of the immune responses. In addition, glycoprotein molecules present in *A. vera* have been reported to be effective in healing wound by promoting cell proliferation, new blood capillaries (angiogenesis), regeneration of tissues and increase blood supply and oxygen, particularly to the damaged cells or tissues [92]. Aloe polysaccharides have been also reported to contain anti diabetic properties (hypoglycemic response) [93]. These *A. vera* attributes highlighted above maybe responsible for the improvement of haemato-biochemical indices presented in the current study especially after fish were infected with *S. iniae* bacterium.

Furthermore, no mortality was recorded during bacterial infection; meanwhile, some haemato-biochemical indices were negatively affected with an increase in dietary *A. vera* inclusion levels. Makker et al. [85] suggested that most beneficial herbal bioactive compounds such as saponins could be toxic to cold-blooded organism including fish at particular concentrations. Hence, administration of higher herbal extract doses to fish may suppress their immune system [53], overdose for a long time may reduce their effectiveness [94], and regular consumption may lead to overstimulation of the immune system which hinders the normal metabolic activities of the fish [79]. In the present study, the expression of the immune system suppression or overstimulation could be linked with a 4% dietary *A. vera* inclusion level. Our findings agree with Ojha et al. [61] who showed that higher dietary *Pedalium murex* ethanolic extract inclusion levels decreased haemalogical indices, growth and feed utilization parameters of *L. rohita*. Overall, the current study demonstrated health growth and feed utilization modulator effects of dietary *A. vera* in GIFT-*O. niloticus*.

5. Conclusion

The present study demonstrated that *A. vera* powder extract supplemented diet has positive effects in improving growth, feed utilization, and health parameters of GIFT-*O. niloticus* fingerlings. *A. vera* extracts can be used to replace synthetic antibiotics, growth promoter, appetizer, stimulator, anti-depressant, and immunostimulant for Nile tilapia. This information is important in order to

optimize the use of Aloe in large scale and its feasibility to fish industry; thus, further similar studies are deemed necessary.

Acknowledgment

The authors are grateful for the financial support jointly provided by Nanjing Government –Nanjing Agricultural University (NJG-NAU, No. 2013NJ0906), and Namibian Government as a scholarship awarded to Ndakalimwe N Gabriel, which made this work possible. They also would like to thank Wuxi fisheries college of NAU, department of biotechnology, China, for providing facilities for the research work.

References

[1] K.A. Basha, R.P. Raman, K.P. Prasad, K. Kumar, E. Nilavan, S. Kumar, Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton), Fish Shellfish Immunol 35 (2013) 1433–1441.
[2] U.U. Gabriel, A.O. Akinrotimi, Management of stress in fish for sustainable aquaculture development, Researcher 3 (2011) 28–38.
[3] N.W. Pankhurst, S.L. Ludke, H.R. King, R.E. Peter, The relationship between acute stress, food intake, endocrine status and life history stage in juvenile farmed Atlantic salmon, *Salmon salar*, Aquaculture 275 (2008) 311–318.
[4] G.A. Santos, J.W. Schrama, R.E.P. Mamauag, J.H.W.M. Rombout, J.A.J. Verreth, Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrarchus labrax*): the combined effects of fish crowding and water quality deterioration, Aquaculture 299 (2010) 73–80.
[5] Y.R. Wu, Q.-F. Gong, W.-W. Liang, M. Chen, R.J. He, Effect of *Sophora flavescens* on non-specific immune response of tilapia (GIFT- *Oreochromis niloticus*) and disease resistance against *Streptococcus agalactiae*, Fish Shellfish Immunol 34 (2013) 220–227.
[6] S. Jittinandana, P.B. Kenney, S.D. Slider, P. Mazik, J. Bebak-Williams, J.A. Hankins, Effect of fish attributes and handling stress on quality of smoked artic char fillets, J Food Sci 68 (2003) 57–63.
[7] O.A. Akinrotimi, U.U. Gabriel, P.E. Anyanwu, A.O. Anyanwu, Influence of sex, acclimation methods and period on hematology of *Sarotherodon melanatheron*, Res J Biol Sci 2 (2007) 348–352.
[8] D.J. McKenzie, E. Höglund, A. Dupont-Prinet, B.K. Larsen, P.V. Skov, P.B. Pedersen, et al., Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout, Aquaculture 338 (2012) 216–222.
[9] M. Haghighi, S.M. Rohani, The effects of powdered ginger (*Zingiber officinale*) on the haematological and immunological parameters of rainbow trout *Oncorhynchus mykiss*, JMPHTR 1 (2013) 8–12.
[10] M.A. Shalaby, Y.A. Khattab, R.A.M. Abdel, Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*), J Venom Anim Toxins Incl Trop Dis 12 (2006) 172–201.
[11] A. Rico, M.T. Phu, K. Satapornvanit, J. Min, A.M. Shahabuddin, P.J.G. Henriksson, et al., Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia, Aquaculture 412 (2013) 231–243.
[12] J.J. Dibner, J.D. Richards, Antibiotic growth promoters in aquaculture: history and mode of action, Poult Sci 84 (2005) 634–643.
[13] W. Agrew, A.C. Barnes, *Streptococcus inae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination, Vet Microbiol 122 (2007) 1–15.
[14] H.M. Ahmad, M. Abdel-Tawwab, The use of caraway seed meal as a feed additive in fish diets: growth performance, feed utilization and whole-body composition of Nile tilapia, *Oreochromis niloticus* (L) fingerlings, Aquaculture 314 (2011) 110–114.
[15] K.M. Bairwa, K.J. Jakhar, Y. Satyanarayana, D. Reddy, Animal and plant originated immunostimulants used in aquaculture, J Nat Prod Plant Res 2 (2012) 397–400.
[16] L. Ardo, G. Yin, P. Xu, L. Varadi, G. Szigeti, Z. Jeney, et al., Chinese herbs (*Astragulus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*, Aquaculture 275 (2008) 26–33.
[17] T. Jegede, Effect of garlic (*Allium sativum*) on growth, nutrient utilization, resistance and survival of *Tilapia zillii* (Gervais 1852) fingerlings, J Agri Sci 4 (2012) 269–274.
[18] E. Zahran, E. Risha, F. Abdelhamid, A.H. Mahgoub, T. Ibrahim, Effects of dietary *Astragulus* polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes and intestinal morphology of Nile tilapia (*Oreochromis niloticus*), Fish Shellfish Immunol 38 (2014) 149–157.
[19] M. Saljooghianpour, A.T. Javaran, Identification of phytochemical components of Aloe plantlets by gas chromatophy-mass spectrometry, Afri J Biotech 12 (2013) 6876–6880.
[20] M. Mahdavi, A. Hajimoradloo, R. Ghorbani, Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*), World J Med Sci 9 (2013) 55–60.
[21] L. Langmead, R.J. Makins, D.S. Rampton, Anti-inflammatory effects of *Aloe vera* gel in human colorectal mucosa in vitro, Aliment Pharmacol Ther 19 (2004) 521–527.
[22] G. Kaithwas, K. Dubey, K.K. Phillai, Effects of *Aloe vera* (*Aloe bardensis* Miller) gel on doxorubicin-induced myocardial oxidative stress and calcium overload in albino rats, Indian J Experi Bio 49 (2011) 260–268.
[23] K. Thu, Y.Y. Mon, A.T. Khaing, O.M. Tun, Study on phytochemical properties antimicrobial activity and cytotoxicity of *Aloe vera* L, World Acad Sci Eng Techno 77 (2013) 5–28.
[24] A. Surjushe, R. Vasani, D.G. Saple, Aloe vera: a short review, Indian J Dematol 54 (2008) 163–166.
[25] D.A. Adesuyi, A.O. Awosanya, F.B. Adaramola, A.L. Omeonu, Nutritional and phytochemical screening of *Aloe barbadensis*, Curr Res J Bio Sci 4 (2012) 4–9.
[26] M. Poorfarid, J.H. Karimi, F. Houshmand, The effect of *Aloe vera* sap on progesterone, estrogen and gonadotropin in female rats, J Jahrom Univ Med Sci 10 (2013) 6–10.
[27] R.N. Nwaoguikpe, W. Braide, T.I.N. Ezejiofor, The effect of *Aloe vera* plant (Aloe bardensis) extracts on sickle cell blood (hbss), Afri J Food Sci Techno 1 (2010) 58–63.
[28] S. Snezana, Anti-genotoxic effect of *Aloe vera* gel on the mutagenic action of ethylsmethanesulfonate, Arch Biol Sci 59 (2007) 223–226.
[29] M. Heidarieh, R.A. Mirvaghefi, A. Sepahil, N. Sheikhzadel, A.M. Shahbazfar, M. Akbari, Effects of dietary *Aloe vera* on growth performance, skin and gastrointestinal morphology in rainbow trout (*Oncorhynchus mykiss*), Turk J Fish Aquat Sci 3 (2013) 367–373.
[30] M. Haghighi, S.M. Rohani, H. Pourmoghim, T. Toliat, M. Samadi, M. Tavoli, et al., Haemato-immunological indices in rainbow trout (*Oncorhynchus mykiss*) fry fed with *Aloe vera* extract supplemented feed, J Coast life Med 2 (2014) 350–356.
[31] A. Farahi, M. Kasiri, M. Sudagar, I.M. Soleimani, S.M.J. Zorriehzahra, Effect of dietary supplementation of Melisa officialis and *Aloe vera* on haematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*), Online J Anim Feed Resour 1 (2012) 1–5.
[32] F.S. Zanuzzo, E.C. Urbinati, M.L. Rise, J.R. Hall, G.W. Nash, A.K. Camperl, *Aeromonas salmonicida* induced gene expression in *Aloe vera* fed stealhead trout, *Oncorhynchus mykiss* (Walbaum), Aquaculture 435 (2015) 1–9.
[33] M. Alishahi, M.M. Ranjbar, M. Ghorbanpour, R. Peyghan, M. Mesbah, J.M. Razi, Effects of dietary Aloe vera on some specific and none specific immunity in the common carp (*Cyprinus carpio*), Int J Vet Res 4 (2010) 189–195.
[34] G.V. Zodape, Effect of *Aloe vera* juice on toxicity induced by metal (chromium) in *Labeo rohita* (Hamilton) 6 (2010) 1788–1793.
[35] F.S. Zanuzzo, J.D. Biller-Takahashi, E.C. Urbinati, Effect of *Aloe vera* extract on the improvement of the respiratory activity of leukocytes of matrinxa during the transport stress, Rev Bras Zootec 41 (2010) 2299–2302.
[36] S. Picchietti, C. Bernini, M.C. Belardinelli, E. Ovidi, A.R. Taddei, L. Guerra, et al., Immune modulatory effects of *Aloe barborescens* extract on the piscine SAF-1 cell line, Fish shellfish Immunol 34 (2013) 1335–1344.
[37] V.O. Taiwo, O.A. Olukuncle, I.C. Ozor, T. Oyejobia, Consumption of aqueous extract of raw *Aloe vera* leaves: histopathological and biochemical studies in rat and tilapia, Afr J Biomed Res 8 (2005) 169–178.
[38] T. Jegede, Effects of *Aloe vera* (Liliaceae) on the gonad development in Nile tilapia (*Oreochromis niloticus*) (Linnaeus 1758), in: K. Fitzsimmons, L. Liping (Eds.), Proceedings of the 9th international symposiums on tilapia aquaculture, 2011, pp. 222–227.
[39] G. Dotta, A.L.J. de Andrade, T.L.E. Gonçalves, A. Brum, J.J. Mattos, M. Maraschin, et al., Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural extracts of propolis and *Aloe barbadensis*, Fish Shellfish Immunol 39 (2014) 280–284.
[40] J. Qiang, H. Yang, H. Wang, M.D. Kpundeh, P. Xu, Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, *Oreochromis niloticus* (L.) and mortality under *Streptococcus iniae* infection, Fish Shellfish Immunol 34 (2013) 8–16.
[41] K.A. Davis, D.L. Maney, J.C. Maerz, The use of leukocyte profile to measure stress in vertebrates: a review ecologists, Funct Ecol 22 (2008) 760–772.
[42] S.G. Telli, T.J.M. Ranzani-Paiva, C.D. Dias de, R.F. Sussel, M.C. Ishikawa, L. Tachibana, Dietary administration of Bacillus subtillis on hemamatology and non-specific immunity of Nile tilapia *Oreochromis niloticus* raised at different stocking densities, Fish Shellfish Immunol 39 (2014) 305–311.
[43] D.G. Ndong, Y.Y. Chen, Y.H. Lin, B. Vaseeharan, J.C. Chen, The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures, Fish Shellfish Immunol 22 (2007) 686–694.
[44] C. Mehala, M. Moorthy, Production performance of broilers fed with *Aloe vera* and *Curcuma longa* (Turmeric), Int J Poult 7 (2008) 852–856.
[45] S.A. Bolu, T.O. Babalola, N. Elelu, R.N. Ahmed, S.A. Oyetunde, P.F. Ademola, et al., Effects of supplemental *Aloe vera* gel in drinking water on some performance histology, hematology, serum constituents and growth of turkey poults challenged with *Escherichia coli*, Wudpecker J Agric Res 2 (2013) 223–229.
[46] M. Ahmed, F. Hussain, Chemical composition and biochemical activity of *Aloe vera* (*Aloe barbadensis* Miller) leaves, IJCBS 3 (2013) 29–33.

[47] M. Xue, X.S. Meng, Review on research progress and prosperous of immune activities of bioactive polysaccharide, J Tradit Chin Vet Med 3 (1996) 15–18.
[48] E.N. Xia, Q.H. Cheng, Isolation, analysis and bioactivities of *Tremella fruciformis* fruit body polysaccharides, Acta Mycol Sin 7 (1998) 166–174.
[49] G.R. Gibson, M.B. Robertfroid, Dietary modulation of human colonic microbiota, introducing the concept of prebiotics, J Nutr 125 (1995) 1401–1412.
[50] S.M. Aly, M.F. Mohamed, *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*), J Physiol An N 94 (2010) 31–39.
[51] E. Dongmeza, P. Siddhuraju, G. Francis, K. Becker, Effects of dehydrated methanol extracts of moringa (*Moringa oleifera* Lamm.) leaves and three of its fraction on growth performance and feed assimilation in Nile tilapia (*Oreochromis niloticus*), Aquaculture 261 (2006) 407–422.
[52] W. Afuang, P. Siddhuraju, K. Becker, Comparative nutritional of raw, methanol extracted residues and methanol extracts of (*Moringa oleifera* Lamm) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L), Aquac Res 34 (2003) 1147–1159.
[53] S. Sakai, Current research status of fish immunostimulants, Aquaculture 172 (1999) 9–63.
[54] M. Al-Owafeir, The effect of dietary saponin and tannin on growth performance and digestion in *Oreochromis niloticus* and *Clarias gariepinus* [PhD thesis], Institute of Agriculture, University of Stiriling, UK, 1999, p. 220.
[55] A.H. Houston, Review: are the classical haematological variables acceptable indicators of fish? Trans Am Fish Soc 126 (1997) 879–894.
[56] R.J. Roberts, The pathophysiology and systematic pathology of teleost, in: R.J. Robert (Ed.), Fish pathology, Bailliere Tindal, London, 1978, pp. 55–91.
[57] A.J. Van Rijn, D.R. Reina, Distribution of leukocytes as indicators of stress in the Australian swellshark, *Cephaloscyllium laticeps*, Fish Shellfish Immunol 29 (2010) 534–538.
[58] M. Dorucu, S. Ozesen Colak, U. Ispir, B. Altinterim, Y. Celayir, The effect of black cumin seeds, *Nigella sativa*, on the immune response of rainbow trout, *Oncorhynchus mykiss*, Mediterr Aquac J 2 (2009) 1–7.
[59] E.J. Nya, B. Austin, Use of garlic, *Allium sativum* to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Wallbaum), J Fish Dis 32 (2009) 963–970.
[60] E.J. Nya, B. Austin, Use of dietary ginger, *Zingiber officinale* Roscoe, as an Immunostimulant to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Wallbaum), J Fish Dis 32 (2009) 971–977.
[61] L.M. Ojha, K.N. Chadha, P.V. Saini, S. Damroy, P.C. Gupta, B.P. Savant, Effect of ethanolic extract of *Pedalium murex* on growth and haematological parameters of *Labeo rohita*, Proc Natl Acad Sci India Sect B Biol Sci 84 (2014) 997–1003.
[62] C.K. Misra, B.K. Das, S.C. Mukherjee, P.K. Meher, The immunomodulatory effects of tuftsin on the non-specific immune system of Indian Major carp, *Labeo rohita*, Fish Shellfish Immunol 20 (2006) 728–738.
[63] O.T. Iji, A.A. Oyagbemi, O.I. Azeez, Assessment of chronic administration of *Aloe vera* gel on haematology, plasma biochemistry, lipid profiles and erythrocyte osmotic resistance in Wistar rats, Nig J Physiol Sci 25 (2010) 107–113.
[64] H.J. Hamman, Composition and application of *Aloe vera* leaf gel, Molecules 13 (2008) 1599–1616.
[65] A.A. Channa, I.H. Qazi, A.S. Soomro, A.H. Shah, J.A. Gandahi, A.R. Korejo, et al., Effect of oral supplementation of *Aloe vera* extract on haematology indices and immune cells of blood in rabbit, Afr J Pharm Pharmacol 8 (2014) 497–501.
[66] P. Palanisamy, G. Sasikala, D. Mallikaraj, N. Bhuvaneshwari, G.M. Natarajan, Haematological changes of fresh water food fish, *Channa striata* on exposure to *Cleistanthus collinus* suicidal plant extract, Res J Pharm Biol Chem Sci 2 (2011) 812–816.
[67] O.K. Adeyemo, Haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus*, Afr J Biochem Res 8 (2005) 179–183.
[68] A.G. Heath, Water pollution and fish physiology, CRC Press Increase, Boca Raton, Florida, 1987.
[69] F. Jenkins, J. Smith, B. Rajanna, U. Shameem, K. Umadevi, V. Sandhya, et al., Effect of sub-lethal concentrations of endosulfan on hematological and serum biochemical parameters in the carp *Cyprinus carpio*, Bull Environ Contam Toxicol 70 (2003) 993–997.
[70] S.A. Muller-Lissner, Adverse effects of laxatives: facts and fiction, Pharmacol 47 (1993) 138–145, http://dx.doi.org/10.1159/000139853.
[71] U. Beuers, U. Spengler, G.R. Pape, Hepatitis after chronic abuse of senna, Lancet 337 (1991) 372–373.
[72] F.A. Sebastião, D. Nomura, R. Sakabe, F. Pilarski, Hematology and productive performance of Nile tilapia (*Oreochromis niloticus*) naturally infected with *Flavobacterium columinare*, Braz J Microbiol 42 (2011) 282–289.
[73] H. Ali, K.K. Ansari, Comparison of haematological and biochemical indices in healthy and monogenean infected common carp, *Cyprinus carpio*, Ann Biol Res 3 (2012) 1843–1846.
[74] S.E. Wendelaar Bonga, The stress response in fish, Physiol Rev 77 (1997) 591–625.
[75] F.S. Dhabhar, A.H. Miller, M. Stein, B.S. McEwen, R.L. Spencer, Diurnal and acute stress-induced changes in distribution of peripheral blood leukocyte subpopulations, Brain Behav Immun 8 (1994) 66–79.
[76] A.K. Siwicki, D.P. Anderson, G.L. Rumsey, Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis, Vet Immunol Immunopathol 41 (1994) 125–139.
[77] R. Harikrishnan, C. Balasundaram, M. Heo, Supplementation diet containing probiotics, herbal and azadirachtin on hematological and biochemical changes in *Cirrhina mrigala* against *Aphanomyces invadans*, J Fish Aqua 4 (2010) 1–11.
[78] E. Awad, D. Austin, R.A. Lyndon, Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), Aquaculture 388–391 (2013) 193–197.
[79] D.A. Tapur, M. Ikhwanuddin, *Azadirachta indica* (Neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *lates calcifer* challenged with *Vibrio harvey*, Fish Shellfish Immunol 34 (2013) 254–264.
[80] B. Magnadottir, Innate immunity of fish (Overview), Fish Shellfish Immunol 20 (2006) 137–151.
[81] J.-H. Hwang, S.-W. Lee, S.-J. Rha, H.-S. Yoon, E.-S. Park, H.-K. Han, et al., Dietary green tea extract improves growth performance, body composition and stress recovery in the juvenile black rockfish *Sebastes schegeli*, Aquacult Int 21 (2013) 525–538.
[82] G. Yin, G. Jeney, T. Racz, P. Xu, X. Jun, Z. Jeney, Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*, Aquaculture 253 (2006) 39–47.
[83] T.W. Campbell, C.K. Ellis, Avian and exotic animal hematology and cytology, 3rd ed., Blackwell Publishing, Iowa, 2007.
[84] E. Bloemena, S. Weinreich, P.T.A. Schelleken, The influence of prednisolone on the recirculation of peripheral blood lymphocytes in vivo, Clin Exp Immunol 80 (1990) 460–466.
[85] J. Raa, The use of immunomodulatory substances in fish and shellfish farming, Rev Fish Sci 4 (1996) 229–288.
[86] J. Xie, B. Liu, Q. Zhou, Y. Su, Y. He, L. Pan, et al., Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on crowding stress response and growth of common carp *Cyprinus carpio var. Jian*, Aquaculture 281 (2008) 5–11.
[87] M. Abdel-Tawwab, H.A. Mohammad, E.A.S. Medhat, F.M.S. Saleh, Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* L., against *Aeromonas hydrophila* infection, J World Aquac Soc 41 (S2) (2010) 203–213.
[88] M.A.A. Metwally, Effects of garlic (*Allium sativum*) on some antioxidant activities in tilapia nilotica (*Oreochromis niloticus*), World J Fish Mar Sci 1 (1) (2009) 56–64.
[89] A.M.A.S. Goda, Effect of dietary ginseng herb (Ginsana_G115) supplementation on growth, feed utilization, and haematological indices of Nile tilapia, *Oreochromis niloticus* (L), fingerlings, J World Aquacult Soc 39 (2008) 205–214.
[90] D. Stauth, Studies force new view on biology of flavonoid, Oregon State University, USA, 2007. http://www.eurekalert.org/pub_releases/2007-03/0su-sfn030507.php.
[91] R.H. Davis, G.M. Leitner, J.M. Russo, M.E. Byrne, Wound healing. Oral and tropical activity of *Aloe vera*, J Am Pediatr MedAssoc 79 (1989) 559–562.
[92] H.P.S. Makkar, G. Francis, K. Becker, Bioactivity of phytochemicals in some lesser known plants and their effects and potential application in livestock and aquaculture production systems, Animal 1 (2007) 1371–1391.
[93] A. Yagi, Y. Sato, Y. Miwa, A. Kabbash, S. Moustafa, K.A. Shimomura, et al., Ribosomal DNA sequence analysis of different geographically distributed *Aloe vera* plants: comparison with clonally regenerated plants, Saudi Pharm J 14 (3–4) (2006) 208–211.
[94] R. Harikrishnan, C. Balasundaram, M.S. Heo, Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish, Aquaculture 317 (2011) 1–15.