ORIGINAL RESEARCH

Effect of dietary *Aloe vera* polysaccharides supplementation on growth performance, feed utilization, hemato-biochemical parameters, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings

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Abstract This study evaluated the effects of dietary *Aloe vera* polysaccharides on growth performance, feed utilization, hemato-biochemical parameters, and resistance against low water pH in African catfish (*Clarias gariepinus*) fingerlings. Fish were divided into five triplicate groups before being fed feeds supplemented with 0% (control), 0.5%, 1.0%, 2.0%, and 4.0% *A. vera*/kg diet for 8 weeks. Fish fed 1.0% *A. vera*/kg diet had significantly increased ($P < 0.05$) growth parameters (i.e., final weight, weight gain, absolute growth rate, and specific growth rate) compared to unsupplemented ones. Among dietary groups, significantly lower feed conversion ratio was presented in fish fed 1.0% followed by those fed 0.5, 2.0%, and 4.0% *A. vera*/kg diet ($P < 0.05$). The protein efficiency ratio was significantly higher ($P < 0.05$) in fish fed 1.0% *A. vera*/kg diet compared to unsupplemented fish and those fed 4.0% *A. vera*/kg diet, respectively. Dietary *A. vera* polysaccharide crude extracts requirement suitable for growth and feed utilization was estimated to be between 1.76 and 1.79% *A. vera*/kg diet. Overall, *A. vera* extracts had improved hemato-biochemical indices when compared to unsupplemented fish, and decreased some of the indices, especially at high dietary inclusion level (4%/kg diet). Furthermore, *A. vera*-supplemented fish had higher survival probability throughout the low water pH challenge period, except those fed 4% *A. vera*/kg diet and control diet.

Keywords Aquaculture · *Aloe vera* · *Clarias gariepinus* · Freshwater fish · Herbal extracts · Prebiotics

Introduction

Medicinal herbal extracts studies have become popular, but still novel in aquaculture and other farming sectors such as livestock and poultry, among others. The main purpose of these studies is usually to reduce and/or

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eliminate the application of synthetic chemotherapeutic drugs such as antibiotics that are normally used in intensive aquaculture production systems to maintain health of farmed fish, as they are believed to be unsustainable (Reverter et al. 2014; Gabriel et al. 2015a; Bulfon et al. 2015). The application of synthetic chemicals has created substantial problems such as development of drug resistance (Seyfried et al. 2010; Gullberg et al. 2011), toxic effects on fish, environmental pollution, and negative impacts on human health (Cabello 2006; Lim et al. 2013). Thus, their application in aquaculture is not encouraged.

Medicinal herbal extracts are potential alternatives to synthetic drugs in aquaculture as they provide useful biologically active metabolites with various benefits such as immune system modulation (Zanuzzo et al. 2015a; Yang et al. 2015), growth promotion, antioxidation enhancement, antidepressant, digestion enhancement, appetite-stimulating effects, among others (Citarasu 2010; Zahran et al. 2014; Abdel-Tawwab et al. 2010), when properly administered. Medicinal herbal extracts are also easily available and inexpensive, and tend to be more biodegradable in nature compared to synthetic drugs (Olusola et al. 2013; Reverter et al. 2014). In aquaculture, herbs could be used as a whole or part (i.e., leaves, flowers, roots, seeds, or barks) in a crude form or as extracts. The wider variety of medicinal herbs may justify their broad-spectrum medicinal properties that may act against a wide range of pathogens (Harikrishnan et al. 2011). Crude extracts from *Camellia sinensis* (Abdel-Tawwab et al. 2010), *Carum carvi* (Ahmad and Abdel-Tawwab 2011), *Cinnamomum camphora*, *Euphorbia hirta*, *Azadirachta indica*, and *Carica papaya* (Kareem et al. 2016), *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera*, and *Zingiber officinale* extracts (Immanuel et al. 2009) improved growth performances of *Oreochromis niloticus* juveniles when they were administered through diets, respectively. Similar findings were reported for *Clarias gariepinus* when their diet was dietary supplemented with *Allium sativum* peels (Thanikachalam et al. 2010) and or *Agaricus bisporus* (Harikrishnan et al. 2018), respectively. Thus, medicinal herbal extracts certainly have the potential to replace synthetic chemicals, which are used as growth promoters and immunostimulants in aquaculture.

Aloe vera is one of the many *Aloe* species that has been acclaimed to manage several health conditions in humans (Abdullah et al. 2003) and in some domesticated animals such as chickens (Akhtar et al. 2012), dogs (Altug et al. 2010), and cats (Harris et al. 1991). In humans, *A. vera* has been used directly or as extracts to cure ailments such as cuts, minor burns, eczema, inflammation (Arunkumar and Muthuselvam 2009), constipation, gastrointestinal disorders, and immune system deficiency (Boudreau and Beland 2006). A number of health benefits associated with *A. vera* have been attributed to the polysaccharides contained in the gel of the leaves (Hamman 2008). Other beneficial *A. vera* phytoconstituents include glycoprotein, amino acids, anthraquinones, antioxidants compounds, and vitamins A, E, and B_{12} (López-Cervantes et al. 2018). Besides extensive research on *A. vera* composition and its application in humans, little information exists regarding its application in aquaculture. The existing information has indicated that *A. vera* could be used as a feed supplement in aquaculture for various reasons. For instance, Mahdavi et al. (2013) reported that adding ethanolic *A. vera* powder at 0.5%/kg diet inclusion level enhanced the growth performances of the common carp, *Cyprinus carpio*. Gabriel et al. (2015a) reported the same in a GIFT strain *O. niloticus* fed 100% *A. vera* extracts. In addition, improved innate immune response after dietary *A. vera* supplementation was reported in matrinxa, *Brycon amazonicus* (Zanuzzo et al. 2015b); whiteleg shrimp, *Litopenaeus vannamei* (Trejo-Flores et al. 2016); and pacu, *Piaractus mesopotamicus* (Zanuzzo et al. 2017).

Given the potential benefits of *A. vera* extracts in aquaculture feeds, this study was designed to investigate the effects of *A. vera* polysaccharides crude extracts on the growth performance, feed utilization, some hemato-biochemical indices, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings. *C. gariepinus* was used as a model in this experiment as it is one of most important aquaculture species in Namibia and several other countries.

Materials and methods

Experimental fish and management

The study was conducted at Sam Nujoma Campus, Sam Nujoma Marine and Coastal Resources Research Centre (SANUMARC) facilities in a close aerated water system in Namibia between February and April 2018. Three hundred healthy African catfish fingerlings (300 fish) with an average body weight of 3.1 ± 0.02 g were

obtained from Onavivi Inland Aquaculture Center (OIAC), Outapi District, Namibia. They were transported in a fiberglass tank supplied with liquid oxygenated freshwater. Upon arrival at the research center, the fish were acclimated in a rectangular brown plastic tank (740 L) supplied with 500 L of freshwater at 28.9 ± 0.25 °C, pH 7.4 ± 0.32, and dissolved oxygen (DO) 4.92 ± 0.19 mg/L (Eutech instruments, model PCD 650, part of Thermo Fisher Scientific, Singapore), and a 12-h light/dark cycle was maintained. The fish were acclimated for a week, and during this period, they were fed with the control diet until apparent satiation thrice daily (09:00, 13:00, and 17:00). To maintain the water quality, two-thirds of the water in the fish holding tank was exchanged with dechlorinated freshwater of similar temperature once during that week of acclimation.

Experimental diets and growth trial

Five iso-nitrogenous (30.6% crude protein), iso-energetic (17.36 kJ/g diet), and iso-lipid (4.39 g/kg) experimental diets were formulated to contain 28.5 (g/kg) fishmeal, 22.5 (g/kg) cowpea, 8.4 (g/kg) corn grain meal, 13.9 (g/kg) wheat flour, 22.7% pearl millet meal, 3.0% vegetable oil, and 1.0% vitamin–mineral premixes for the control (without *A. vera* polysaccharide extracts) (Table 1). For the other groups, *A. vera* crude polysaccharides dry powder (30%) was incorporated into the control feed at 0.5% (group 2), 1.0% (group 3), 2.0% (group 4), and 4.0%/kg diet (group 5). The *A. vera* crude polysaccharides powder used for this experiment was a solvent-extracted and lyophilized commercial product purchased from Ningxia SangNutrition Biotech Inc., China. This product consisted of acemannan, glucomannan, saponin, glycosides, galactan, mannose, aloin, and emodin. The dry ingredients were mixed thoroughly with water for 30 min. The resulting dough was pelleted with 2-mm die, dried at room temperature for 2 days, and then stored in airtight plastic bags until use.

The use of experimental fish was in accordance with the scientific research protocols of University of Namibia (Windhoek, Namibia) and complied with all relevant local and international animal welfare laws, guidelines, and policies. After a week of acclimation, the experimental fish (3.16 ± 0.03 g) were randomly distributed into fifteen aquaria in five triplicated groups at a stocking density of 20 fish/aquarium (0.18 m^3, supplied with 150 L of dechlorinated freshwater). A day after stocking, fish were hand-fed with the experimental diets. Fish in group 1 were fed the control diet (0% *A. vera* 30% polysaccharide powder), and others

Table 1 Ingredients proportions and composition of the basal diet (control diet) (g/100 dry matter)

Ingredients	Proportion
Fish meal (60% CP)	28.5
Cow peas (25% CP)	22.5
Corn grain (10.2% CP)	8.4
Wheat flour (11.7% CP)	13.9
Pearl millet (12.5% CP)	22.7
Vegetable oil	3
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Total	100
Proximate composition (%)	
Dry matter	91.67
Crude protein	30
Crude lipid	4.39
Ash	5.25
Gross energy (KJ/g diet)	17.36

[a]Vitamin premix (g or IU kg-premix); thiamine, 5; riboflavin, 5; niacin, 25; folic acid, retinol palmitate, 500,000IU, 1; pyridoxine, 5; cyanocobalamin, 5; cholecalciferol; 50,000IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; ascorbic acid, 10; choline chloride, 100; biotin, 0.25

[b]Mineral premix (g/kg): KH_2PO_4, 502; $MgSO_4 \cdot 7H_2O$, 162; NaCl, 49.8; $CaCO_3$, 336; Fe(II) gluconate, 10.9; $MnSO_4 \cdot H_2O$, 3.12; $ZnSO_4 \cdot 7H_2O$, 4.67; $CuSO_4 \cdot 5H_2O$, 0.62; KI, 0.16; $CoCl_2 \cdot 6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02

were fed 0.5% (group 2), 1.0% (group 3), 2.0% (group 4), and 4.0% *A. vera*/kg diet (group 5), three times a day (09:00, 13:00, and 17:00) until apparent satiation for 60 days. Dietary *A. vera* inclusion levels used in this study were adopted from our previous study (Gabriel et al. 2015a), which, however, used a 100% *A. vera* crude powder in the GIFT strain *O. niloticus*. During the feeding trial, continuous aeration, photoperiod (12-h light/dark cycle), and water exchange (60%), twice a week, were maintained. Water quality parameters such as DO, and temperature were monitored once a day, while pH and ammonia nitrogen were monitored on a weekly basis. Throughout the feeding trials, temperatures ranged from 26 to 28 °C, pH values from 6.9 to 7.3, and DO values from 4.7 to 5.4 mg/L, and ammonia nitrogen concentration was lower than 0.05 mg/L.

Evaluation of growth and feed utilization parameters

Growth performance indices were assessed in terms of weight gain (WG), final weight (FW), absolute growth rate (AGR), specific growth rate (SGR), condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI). Meanwhile, feed utilization indices were feed intake (FI), feed conversion ratio (FCR), and feed efficiency ratio (FER). Survival was expressed as percentage of the initial number of fish. After 60 days of feeding, 24 h after the last feeding, body weight and length of all the fish in each tank were measured. Liver weight and gutted weights of three fish from each replicate were recorded, respectively. For ethical reasons, before these fish were sacrificed, they were anesthetized with 100 mg MS-222, tricane methane sulfonate, Biodynamic Pty, Ltd, Namibia. In addition to account for feed intake and survival rate, the amount of feed consumed and the mortality in each replicate were both recorded throughout the experimental period. Calculations were conducted using the following formulae:

$$\text{WG (g)} = W_2 - W_1 \tag{1}$$

$$\text{SGR} \left(\%\text{day}^{-1}\right) = \left(\frac{\text{Ln}(W_2) - \text{Ln}(W_1)}{t}\right) \times 100 \tag{2}$$

$$\text{AGR} \left(\text{g day}^{-1}\right) = \left(\frac{W_2 - W_1}{t}\right) \tag{3}$$

$$\text{CF} \left(\text{g cm}^{-3}\right) = \left(\frac{W}{L^3}\right) \times 100 \tag{4}$$

$$\text{HSI} \left(\%\right) = \left(\frac{\text{liver weight}}{W}\right) \times 100 \tag{5}$$

$$\text{VSI} \left(\%\right) = \left(\frac{\text{visceral weight}}{W}\right) \times 100 \tag{6}$$

$$\text{FCR} = \frac{\text{FI}}{\text{WG}} \tag{7}$$

$$\text{FER} = \frac{\text{WG}}{\text{FI}} \tag{8}$$

$$\text{PER} = \frac{\text{WG}}{\text{crude protein intake}} \tag{9}$$

$$\text{Survival} \left(\%\right) = \left(\frac{\text{Number of survived fish}}{\text{Initial number of fish}}\right) \times 100, \tag{10}$$

where W_2 = final body weight, W_1 = initial body weight, t = trial period, W = body weight, WG = *weight gain*, and L = total body length.

Hematological–biochemical parameters

At the end of the experiment, 24 h after the last feeding, blood was collected from the caudal vein of three anesthetized (100 mg MS-222) randomly selected fish per aquarium with a 2.5-ml sterile hypodermic syringe and carefully transferred into sterile EDTA heparinized 1.5-ml tubes at room temperature. One part of each blood sample was investigated for red blood cell count per L (RBC), white blood cell count per L (WBC), hematocrits (L/L), red blood cell distribution width (RDW) (fl/cell), mean platelet counts per L, lymphocytes per L (LYM), monocytes per L (MON), mean corpuscular volume (L/cell) (MCV), and granulocytes per L (GRAN) which were determined by Coulter principle using an automatic blood cell analyzer (HESKA veterinary hematology analyzer, New Zealand). Hemoglobin, mean corpuscular hemoglobin (fmol/cell) (MCH), and mean corpuscular hemoglobin concentration (g/L) (MCHC) were assessed according to Bouguer–Lambert–Beer law using the HESKA blood cell analyzer (He et al. 2015). The samples were analyzed immediately after collection. A part of each blood sample was centrifuged at 5000 rpm, 4 °C for 10 min, and the collected serum was stored at − 20 °C for further biochemical analysis. Aspartate aminotransferase (AST) (U/L), alanine aminotransferase enzyme (ALT) (U/L), glucose (mmol/L) (GLU), total cholesterol (mmol/L) (TCHO), and triglycerol (mmol/L) were quantified by colorimetric method using Fuji DRI-Chem, auto analyzer (FDC NX 5000v v2.3) with kits supplied by DIAG Import and Export CC, South Africa.

In situ low pH challenge experiment

In aquaculture research, fitness and quality of animals supplemented with medicinal herbs are tested by exposing them to stresses including manipulation of water quality parameters such as pH (Li and Chen 2008; Khan et al. 2018), salinity (Ghehdarijani et al. 2016), and temperature (Fazlolahzadeh et al. 2011). Accordingly after the initial sampling, the stocking density of each five triplicated dietary groups was adjusted to 10 fish/aquarium (0.18 m^3, supplied with 50 L of dechlorinated freshwater). They were then exposed to low pH (pH 5.2–5.5) for 3 days (72 h). The water pH was adjusted by adding 4N HCl and 4N NaOH and was renewed daily, as demonstrated by (Li and Chen 2008). During this experiment pH, temperature (28 ± 1.5 °C), DO (> 4 mg/L), and NH_3–N (> 0.08 mg/L) were monitored daily. Fish mortality was recorded at three 24-h intervals, to determine the survival (%).

Statistical analysis

Data were statistically analyzed using descriptive statistics in SPSS (version 21, IMB Corp, Armonk, NY, USA). The mean values were further subjected to one-way analysis of variance (ANOVA) to study the treatment effects. Significant differences between the group means were further compared using Duncan's multiple range test (DMRT). $P < 0.05$ was considered statistically significant. Results were expressed as mean ± standard error (M ± SE). The survival (%) of fish in each low pH treatment group was estimated using Kaplan–Meier analysis (Jelkić et al. 2014); Breslow (generalized Wilcoxon), Tarone-Ware, and log-rank (Mantel–Cox) were used to determine the significant difference ($P < 0.05$) between groups at each sampling interval of the pH challenge.

Results

Growth performance and feed utilization parameters

Growth of the fish fed dietary *A. vera* polysaccharides was enhanced significantly ($P < 0.05$) compared to the unsupplemented diet (Fig. 1). Among dietary *A. vera* groups, fish fed 1.0% had the highest ($P < 0.05$) FW, WG, and AGR compared to unsupplemented ones. The latter parameters were intermediate ($P > 0.05$) in fish
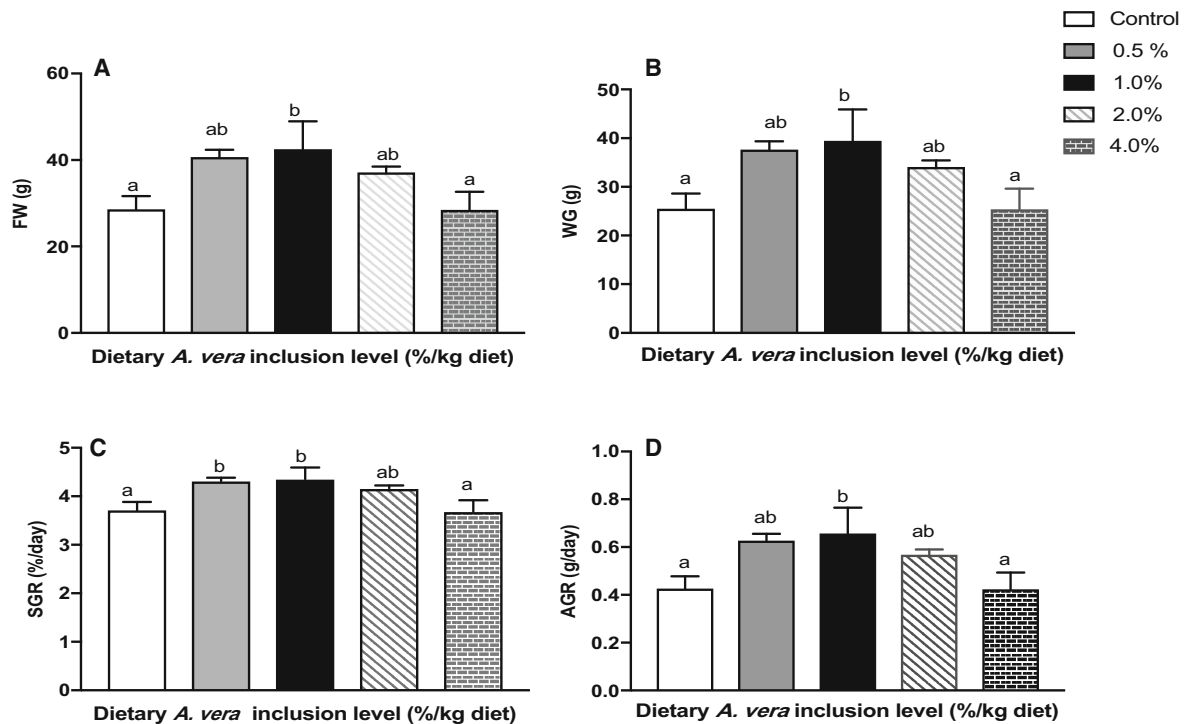
Fig. 1 FW (**A**), WG (**B**), SGR (**C**), and AGR (**D**) of African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* [1]WG = $W_2 - W_1$, SGR = [ln (W_2) − ln (W_1)/t)] × 100, AGR = ($W_2 - W_1$/t); [2]W_1 = initial weight, W_2 = final weight, t = experimental period; [3]different lower case letters denote a significant difference ($P < 0.05$) among dietary groups. [4]Values were expressed as mean ± standard error (M ± SE)

fed 0.5% and 2.0% *A. vera* polysaccharide supplemented diet. SGR was significantly higher ($P < 0.05$) in fish fed 1.0% and 0.5% *A. vera*/kg feed when compared to the control and those fed 4.0% *A. vera*/kg supplemented diet, respectively; intermediate SGR response was observed in fish fed 2.0% *A. vera*/kg feed. Based on the second-order polynomial analysis on WG ($Y = - 2.78x^2 + 9.95x$, $P = 0.035$, $R^2 = 0.37$, Fig. 4A) or FW ($Y = - 2.78x^2 + 9.95x$, $P = 0.032$, $R^2 = 0.37$ Fig. 4B), the optimum dietary *A. vera* inclusion level (%) was estimated to be 1.79%/kg feed. Dietary *A. vera* polysaccharides did not affect ($P > 0.05$) organo-somatic indices (HSI and VSI) as well as CF and survival rate (Fig. 2).

Dietary *A. vera* polysaccharides had no significant effect on fish FI when compared to unsupplemented fish; however, significantly lower FI was recorded in fish fed 4.0% *A. vera*/kg compared to those fed 2.0% (Fig. 3A). Feed utilization parameters, particularly FCR, FER, and PER, were improved in dietary *A. vera* polysaccharides-supplemented fish. Among dietary groups, significantly lower FCR was presented in fish fed 1.0% followed by those fed 0.5%, 2.0%, control, and 4.0% *A. vera*/kg feed ($P < 0.05$); and the opposite trend was recorded for FER with no significant difference. PER was significantly higher ($P < 0.05$) in fish supplemented with 1.0% *A. vera*/kg feed compared to the control and those fed 4.0% *A. vera*/kg feed. The relationships between dietary *A. vera* polysaccharide levels and either FER or FCR were expressed by the second-order polynomial regression equation as follows: FER ($Y = - 0.043x^2 + 0.152x$, $P = 0.045$, $R^2 = 0.253$, Fig. 4C) and FCR ($Y = 0.1224x^2 - 0.429x$, $P = 0.041$, Fig. 4D). Based on these equations, the optimum dietary *A. vera* 30% polysaccharide inclusion level for maximum feed utilization was 1.76%/kg feed.

Hemato-biochemical parameters

Dietary *A. vera* polysaccharides had significant effects ($P > 0.05$) on some hematological parameters of African catfish fingerlings when compared to the unsupplemented ones (Figs. 5, 6, 7, and 8. Improved RBC (Fig. 5A), hematocrits (Fig. 5B), and hemoglobin (Fig. 5C) were observed in fish fed 0.5% and those fed 1.0% *A. vera*/kg diet, and decreased in those fed 2.0% and 4.0% *A. vera*/kg diet, respectively ($P > 0.05$); platelet
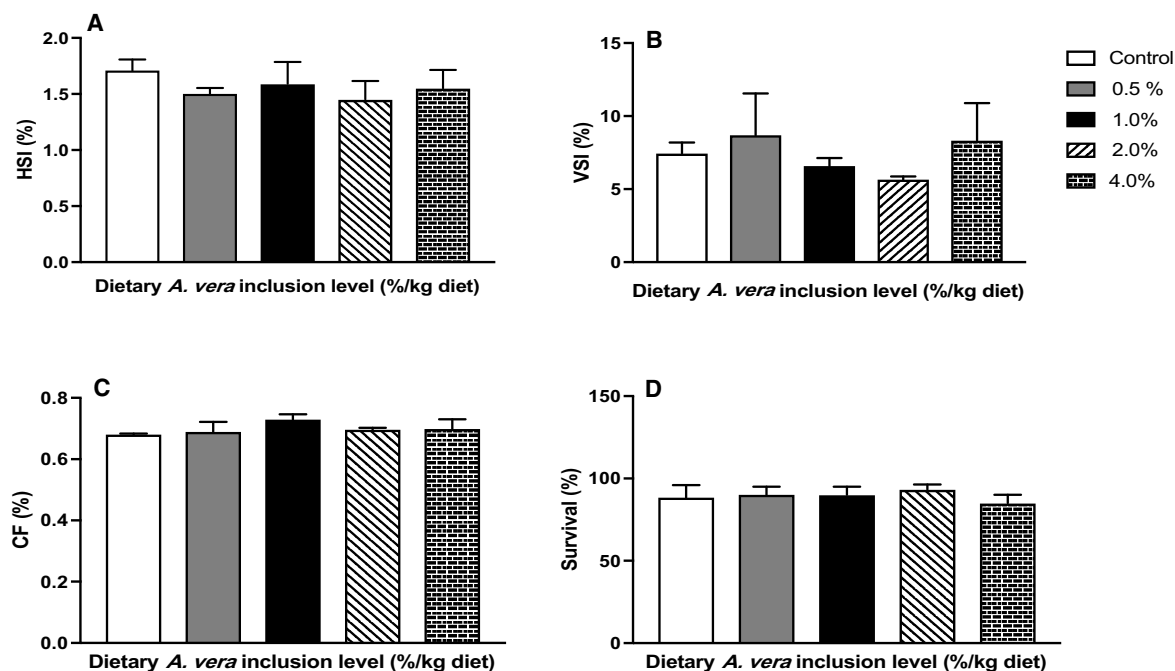
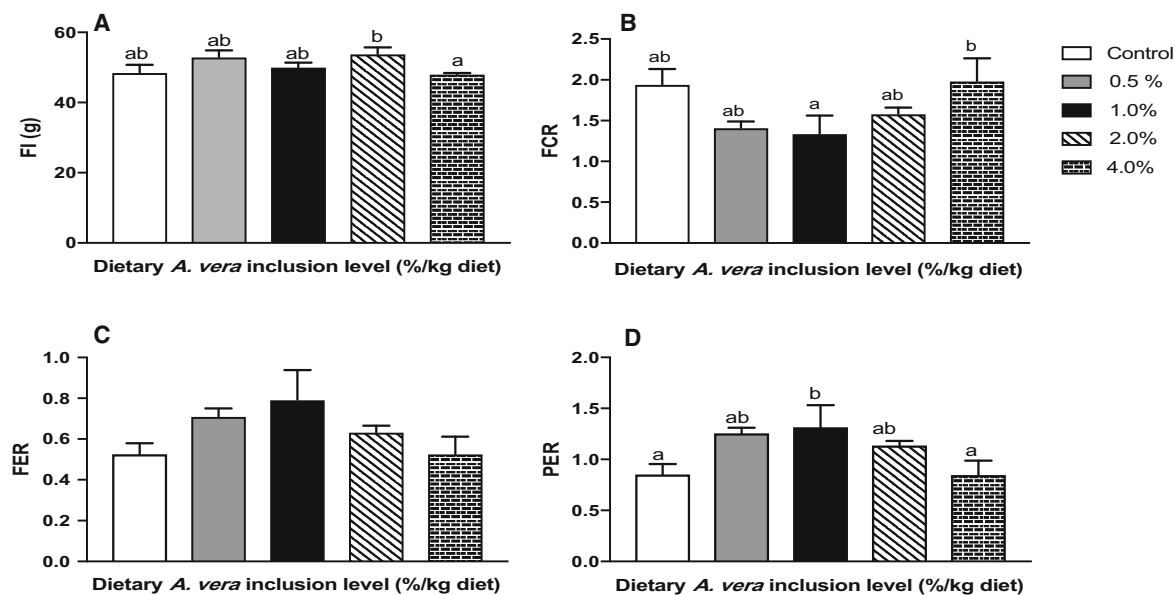**Fig. 2** HSI (**A**), VSI (**B**), CF (**C**), and survival (%) (**D**) of the African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* [1]HSI (%) = [liver weight/W] × 100, VSI (%) = [visceral weight/W] × 100, CF (%) = (W/L^3) × 100, survival (%) = [number of fish survived/initial number of fish] × 100; [2]W = fish body weight, L = fish body length; [3]values were expressed as (M ± SE), and differences among group were tested at $P < 0.05$

Fig. 3 FI (**A**), FCR (**B**), FER (**C**), and PER (**D**) of the African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* [1]FCR = FI (g)/WG (g), FER = WG (g)/FI (g), and PER = WG/feed crude protein content; [2]different lower case letters denote a significant difference ($P < 0.05$) among dietary groups. [3]Values were expressed as mean ± standard error (M ± SE)

counts (Fig. 5D) in fish fed 4.0% *A. vera*/kg diet decreased significantly when compared to those fed the control diet ($P < 0.05$).

Furthermore, mean corpuscular volume (Fig. 6A) and mean corpuscular hemoglobin per cell (Fig. 6B) showed no significant differences ($P > 0.05$) between feeding groups (Fig. 6). Mean corpuscular hemoglobin
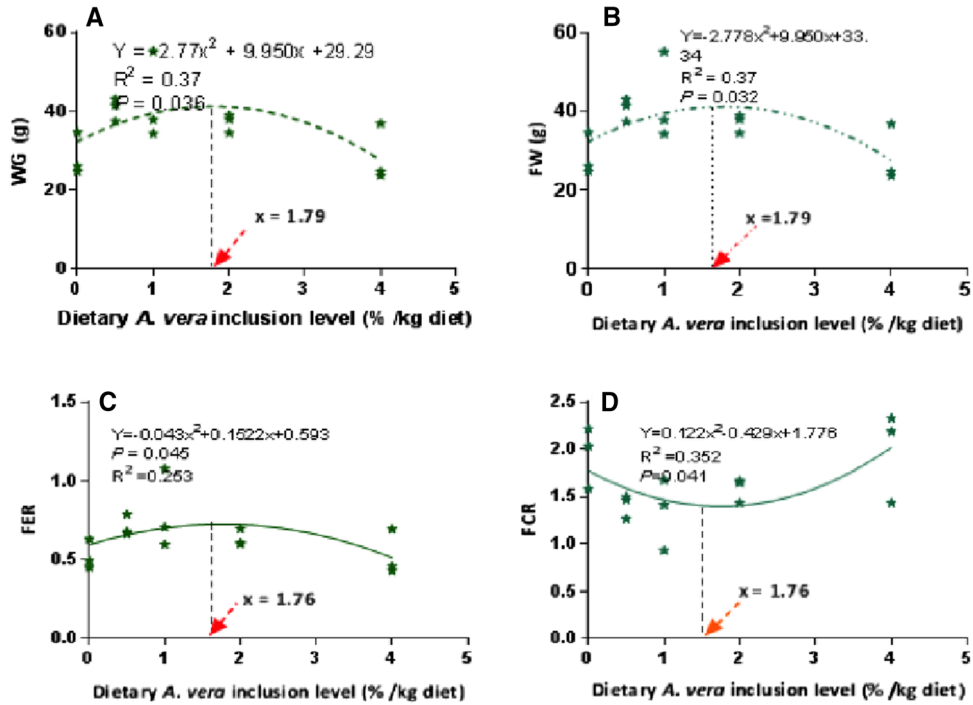
Fig. 4 Relationships between dietary *A. vera* 30% polysaccharide crude extracts levels and either WG (**A**), FW (**B**), FER (**C**) or FCR (**D**) of African catfish (*C. gariepinus*) fingerlings
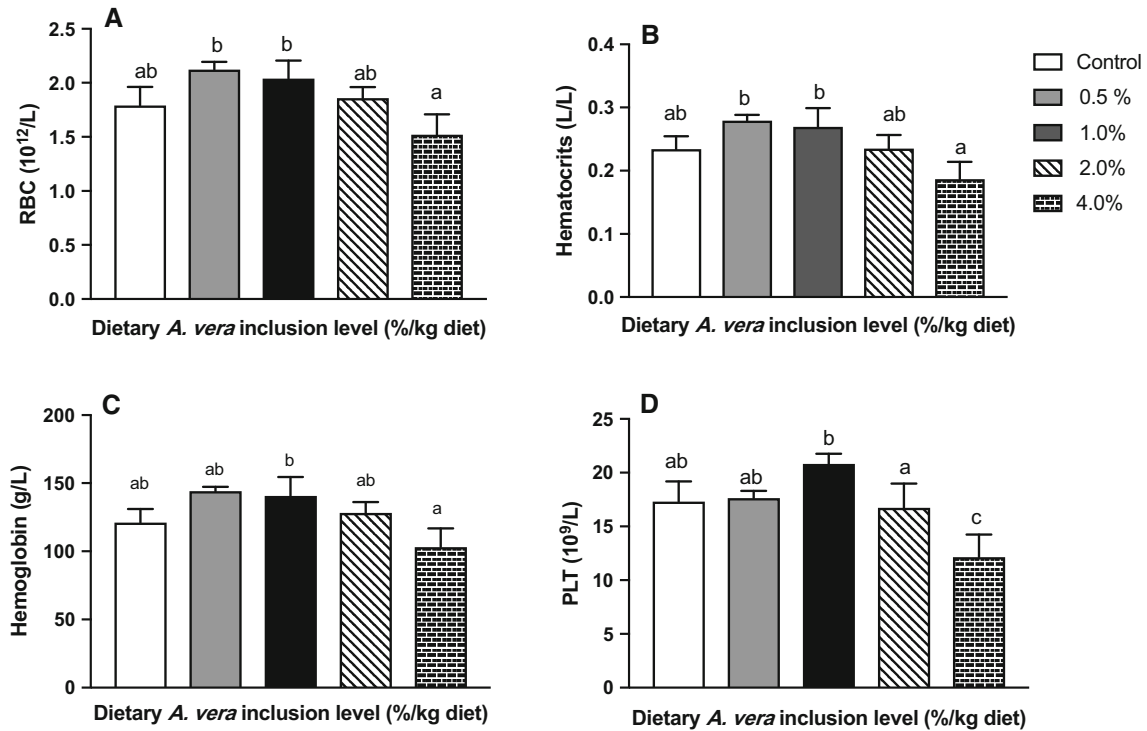

Fig. 5 Red blood cells (RBC) (**A**), hematocrits (**B**), hemoglobin (**C**), platelets (PLT) (**D**) of African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* Different lower case letters denote a significant difference ($P < 0.05$) among dietary groups. Values were expressed as mean ± standard error (M ± SE)
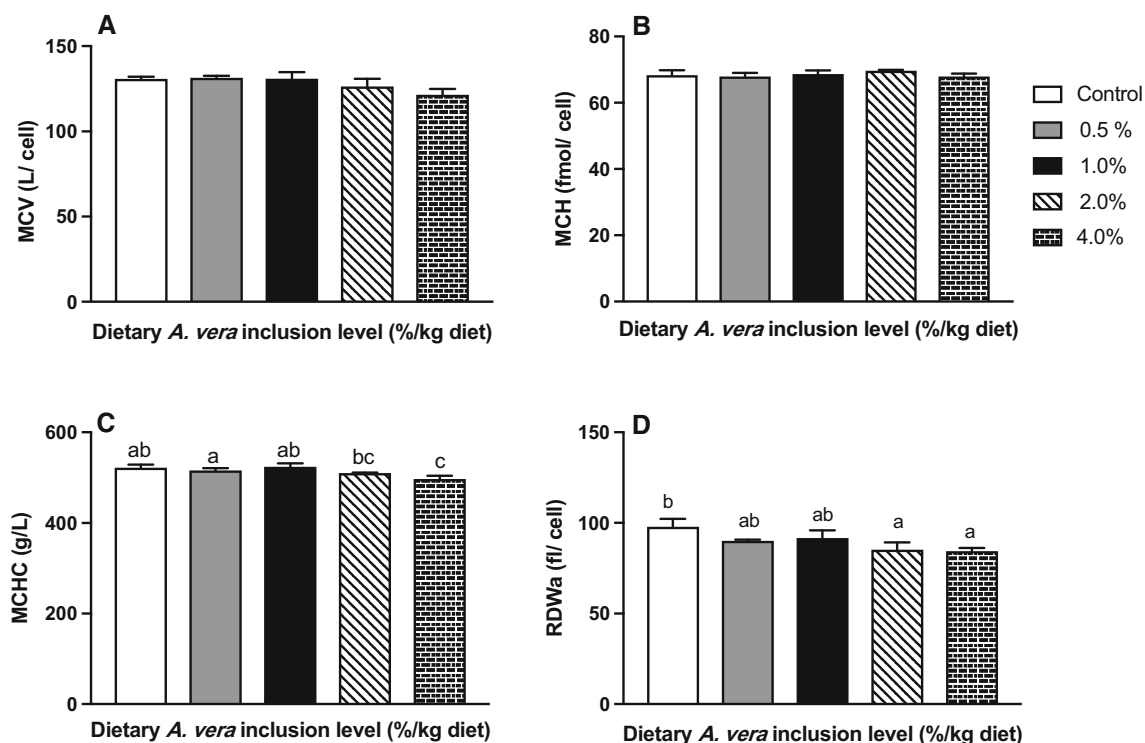
Fig. 6 Mean corpuscular volume (MCV) (**A**), mean corpuscular hemoglobin (MCH) (**B**), mean corpuscular hemoglobin concentration (MCHC) (**C**), red blood cell distribution width (RDW) (**D**) of African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* [1]Different lower case letters denote a significant difference ($P < 0.05$) among dietary groups. [2]Values were expressed as mean ± standard error (M ± SE)

concentration was the same for the control, 0.5%, 1% and 2%, but decreased significantly ($P < 0.05$, Fig. 6C) in fish fed 4.0% *A. vera*/kg diet when compared to those fed the control diet, 0.5% and 1.0% *A. vera*/kg diet. Fish fed 4.0% and 2.0% *A. vera*/kg diet had significantly lower red blood cell distribution width ($P < 0.05$) compared to the control.

White blood cell counts (Fig. 7A), lymphocyte counts (Fig. 7B), and monocyte counts (Fig. 7C) increased in fish supplemented with 0.5% and 1.0% *A. vera*/kg diet, and decreased in fish fed 2.0% and 4.0%/kg diet when compared to those fed the control diet ($P > 0.05$, Fig. 3). Only a significant decrease ($P < 0.05$) in granulocyte counts was observed in fish fed 4.0% *A. vera*/kg diet ($P < 0.05$) when compared to the unsupplemented ones (Fig. 7D).

Dietary *A. vera* polysaccharides had significant effects ($P < 0.05$) on biochemical parameters (Fig. 8). AST and ALT were significantly lower in fish supplemented with 0.5% and 1.0% *A. vera*/kg diet compared to the control and 4% treatment group ($P < 0.05$). Though not significantly different ($P > 0.05$), lower glucose level was observed in *A. vera*-supplemented fish between groups, especially in those fed 0.5% followed by 1.0%, 2.0%, and 4.0% *A. vera*/kg diet. Similarly, TCHO and TG levels were not significantly different ($P > 0.05$) in supplemented fish when compared to unsupplemented ones; however, somewhat lower levels were observed in *A. vera*-supplemented fish (Fig. 8).

Low pH challenge experiment

Low pH had a significant effect on fish survival at 24-h, 48-h, and 72-h post-challenge, based on Breslow (generalized Wilcoxon), Tarone-Ware, and log-rank (Mantel–Cox) tests ($P < 0.05$), respectively (Fig. 9). Fish fed 4.0% *A. vera*/kg diet followed by those fed a control diet had the lowest survival probability throughout the challenge period. Meanwhile, 24-h and 48-h post-challenge, the highest survival probability was observed in fish fed 2.0% followed by those fed 1.0%, and then 0.5% *A. vera*/kg diet; 72-h post-challenge, higher survival probability was observed in fish fed 1.0% followed by those fed 2.0% and then 0.5% *A. vera*/kg diet.
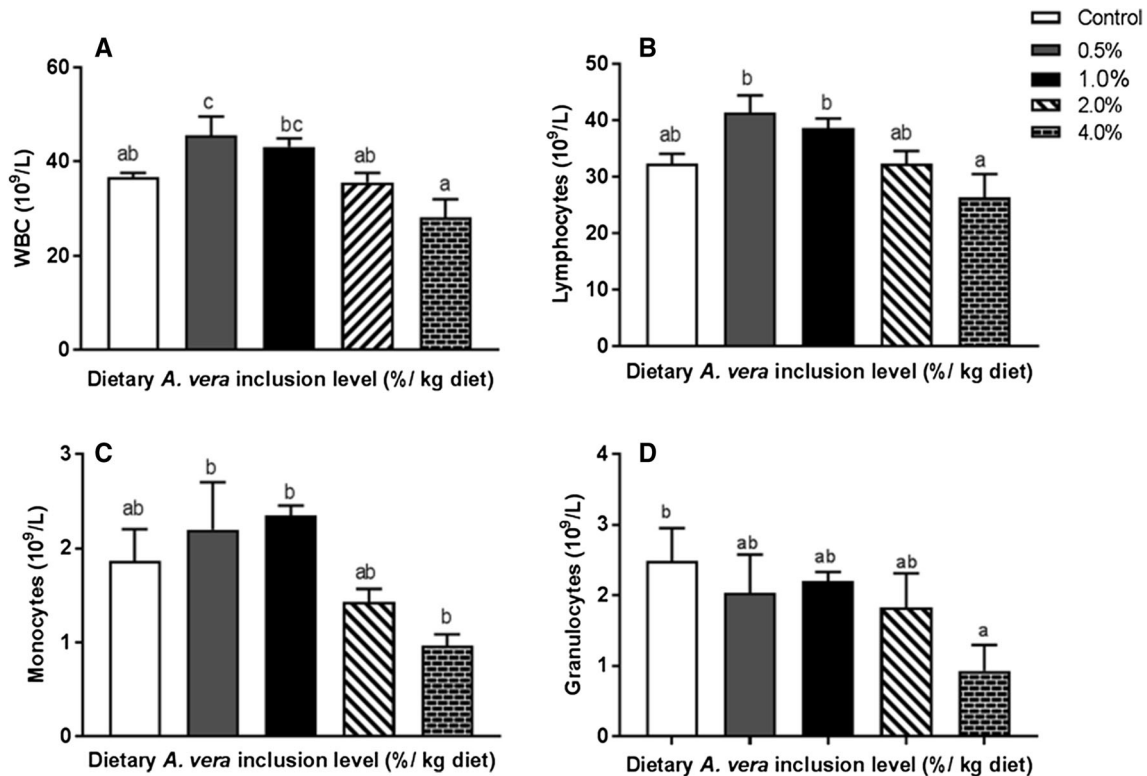
Fig. 7 White blood cells (WBC) (**A**), lymphocytes (**B**), monocytes (**C**), granulocytes (**D**) of African catfish (*C. gariepinus*) fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* [1]Different lower case letters denote a significant difference ($P < 0.05$) among dietary groups. [2]Values were expressed as mean ± standard error (M ± SE)

Discussion

In the present study, growth performance indices (WG, SGR, FW, and AGR) and feed utilization (FI, FCR, FER, and PER) were enhanced in *A. vera* polysaccharides-enriched diets as compared to those fed the control diet with optimum inclusion level estimated to be 1.79% *A. vera*/kg diet. Similarly, a recent study reported that dietary *A. vera* leave paste at 1.0% effectively improved growth performance and nutrient utilization of cultured *C. gariepinus* fingerlings (Ibidunni et al. 2018). In addition, dietary *A. vera* 100% powder at an inclusion level between 0.5% and 2.0%/kg diet was able to significantly enhance growth and feed utilization performance in GIFT *O. niloticus* strain (Gabriel et al. 2015a). Similar performance was reported in *Cyprinus carpio* juveniles when fish were fed diets supplemented with ethanolic *A. vera* extracts at 0.5% and 2.5% *A. vera*/kg, respectively (Mahdavi et al. 2013). *A. vera* gel extracts supplemented diet at an inclusion level as lower as 0.1% could also effectively enhance growth performance in *O. mykiss* (Heidarieh et al. 2013). Differences in dietary *A. vera* inclusion levels suitable for growth and feed utilization reported in previous studies including this study could mainly be due to different types of extracts (i.e., crude powder, gel, solvent extracted among others), different fish species, and different rearing conditions. Hence, more studies with well-defined constituents are required for standardization and better comparison.

On the other hand, dietary *A. vera* extracts have been reported to have no influence on the growth of some fish. The same inclusion levels (0.1% and 1.0% *A. vera*/kg diet) that were concluded to have increased growth in *O. mykiss* (Heidarieh et al. 2013) have been reported to have no effect on growth of the same fish species (Farahi et al. 2012; Golestan et al. 2015). In the present study, growth and feed utilization promoting effects diminished with increased dietary *A. vera* inclusion level, which is consistent with our previous study where 100% *A. vera* crude extract was used (Gabriel et al. 2015a), and other herbs such as *Zingiber officinale* (Vahedi et al. 2017) and *Foeniculum vulgare* (Sotoudeh and Yeganeh 2017).
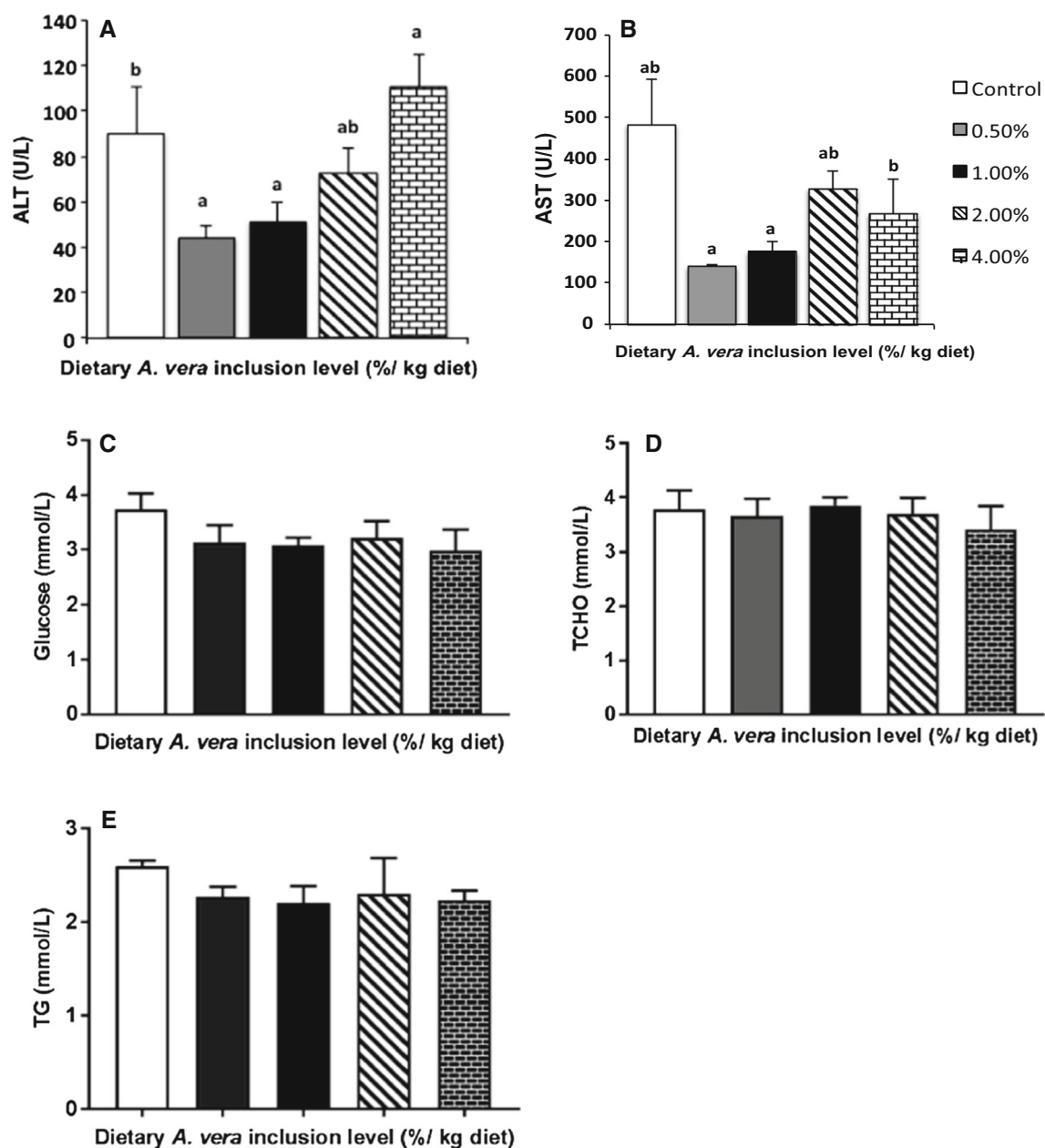
Fig. 8 Serum alanine aminotransferase enzyme (ALT) (**A**), aspartate aminotransferase (AST) (**B**), glucose (**C**), total cholesterol (TCHO) (**D**), and triglycerol (TG) (**E**) of African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days. *Notes* [1]Different lower case letters denote a significant difference ($P < 0.05$) among dietary groups. [2]Values were expressed as mean ± standard error (M ± SE)

Furthermore, effects of *A. vera* extracts on the growth of *C. gariepinus* fingerlings reported in this study may be attributed to several factors, either by *A. vera* nutritional factors present in the leaves or its anti-nutritional factors such as complex polysaccharides and phenolic compounds (Hamman 2008; Radha and Laxmipriya 2014). Growth-promoting effects of medicinal herbal extracts in animals have been mainly attributed to polysaccharides (Chen et al. 2003; Tremaroli and Backhed 2012; Zahran et al. 2014). Polysaccharides are known to act as prebiotic that have been shown to have the ability to sustain the homeostasis of gut microbial community as well as the host health (Tremaroli and Backhed 2012), either by reducing the bacterial and viral infection (Chen et al. 2003) or by directly affecting pathogenic gut microflora (Sohn et al. 2000; Citarasu 2010; Yu et al. 2018). This as a result improves feed digestibility and availability of nutrients from feedstuffs, and shortens the feed transit time, which might have beneficial influence on
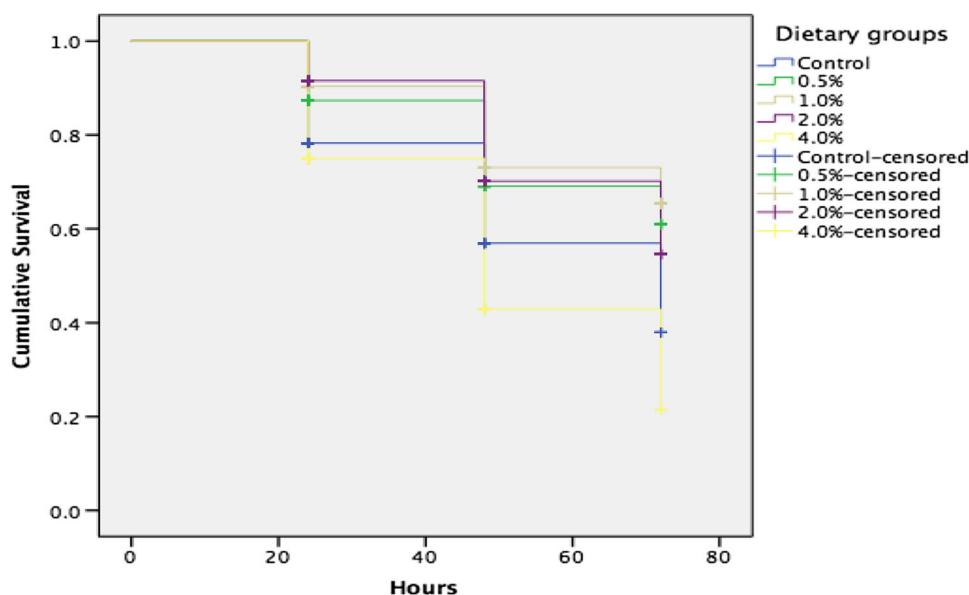
Fig. 9 Low pH challenge cumulative survival of African catfish (*C. gariepinus*) fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets (0.5, 1.0, 2.0, and 4.0%/kg diets) and unsupplemented diet for 60 days

digestive enzymes (Platel and Srinivasan 2004) as well as minimizing the amount of feed substrate available for proliferation of pathogenic bacteria (Citarasu 2010). Feed digestibility enhancement in fish following herbal extract administration was supported by our previous study (Gabriel et al. 2017), which reported that 100% *A. vera* extracts had significantly increased amylase, trypsin, and lipase activities in GIFT. The same herb was also reported to have improved gastrointestinal morphology of *O. mykiss* by increasing intestinal villi lengths and intestinal surface area for increased feed digestion and absorption capacity of the gut (Heidarieh et al. 2013). In the same line, dietary *Astragalus* polysaccharides were also reported to increase amylase activity in *O. niloticus* and this correlated with its growth-promoting effects (Zahran et al. 2014).

In the present study, hematological parameters (i.e., RBC, HCT, HGB, MCV, MCH, MCHC, RDW, WBC, lymphocytes, and granulocytes) were somewhat higher in dietary *A. vera*-supplemented fish, and the optimum inclusion levels seemed to range between 0.5% and 2.0%/kg diet. Poor hematological immune indices were presented in fish fed 4.0% *A. vera*/kg diet. This corresponds with the results obtained by Ibidunni et al. (2018) which revealed that hematological parameters of *C. gariepinus* fingerlings were enhanced after been fed 1.0%, 2.0%, and 3.0% *A. vera* leaf paste/kg for 12 week, respectively. 100% *A. vera* dietary supplementation was reported to enhance innate immune parameters in GIFT *O. niloticus*, especially after being stressed with *Streptococcus iniae* pathogenic bacterium; similarly, inclusion levels between 0.5% and 2.0%/kg diet appeared to be effective, and fish supplemented with 4.0% *A. vera*/kg diet responded poorly and, thus, classified as microcytic anemic (Gabriel et al. 2015a). In the study by Abdy et al. (2017), *C. carpio* were vaccinated with heat-killed *Aeromonas hydrophila* and in one group *A. vera* gel was used as adjuvant during this vaccination. In a challenge experiment thereafter, a higher immune response was observed in fish which were vaccinated with the *A. vera* adjuvant compared to the response in groups with no or a different adjuvant.

The sign of enhancement of hematological indices in fish following supplementation of *A. vera* extracts in this study and in previous related studies may signify the ability of *A. vera* to stimulate erythropoiesis, and hence increase the oxygen-carrying capacity and strengthening of defense mechanism against physiological stress. The erythropoietin effects of *A. vera* extracts in hemotopoietic cells of bone marrow have been reported (Iji et al. 2010). The assumption is that these effects could be due to vitamins such as beta carotene, C, E, B_{12}, riboflavin, thiamine, and folic acid, minerals (calcium, chromium, copper, selenium, manganese, potassium, sodium, and zinc essential and nonessential amino acids) present in *A. vera* that are essential for the synthesis of hemoglobin as demonstrated in Kayode (2016). Erythropoiesis has also been attributed to polysaccharides present in *A. vera* leaves (Ni et al. 2004).

The increased leukocytes presented in *A. vera*-supplemented fish and high resistance against low pH is an indication that this herb has the ability to stimulate leucopoiesis (formation of WBC or leukocytes), thus

strengthening the body's ability to fight against stressors. A number of studies have indicated that *A. vera* immuno-modulating activities including stimulation of leukocyte formation could be accredited to the presence of polysaccharides (Chow et al. 2005; Im et al. 2005), especially acemannan (Hamman 2008). Some immuno-modulating effects were linked to lectins, which are glycoprotein found in *A. vera* gel (Reynolds and Dweck 1999). In addition to innate immune response, *A. vera* extracts have been also reported to evoke specific immune response in fish. For instance, Alishahi et al. (2010) reported that 0.5% dietary *A. vera* had increased serum bactericidal activity and IgM antibody levels in *C. carpio*-infected *A. hydrophila*. This is an indication that dietary supplementation of *A. vera* extract may improve the health status of the fish and, as a result, produce animals with high resistance against stresses associated with culture conditions such as low water pH as demonstrated in the present study.

On the contrary, medicinal herbs have been reported to be harmful in fish and even deadly, especially at high dosages (Palanisamy et al. 2011). To the best of our knowledge, anemia (Gabriel et al. 2015a) and tissue necrosis (Taiwo et al. 2005) are the *A. vera* negative effects so far reported in fish following dietary supplementation. However, spermatogenic dysfunction, decreased central nervous system activity, and also reduced red blood cells were observed in mice supplemented with *A. vera* extract (Boudreau et al. 2013). Furthermore, herbal extracts' side effects such as anemia in animals have been assumed to be a result of their ability to disrupt erythropoiesis, hemosynthesis, and osmoregulation functions or by increasing erythrocyte destruction in hematopoietic organs (Cope 2005). *A. vera* adverse effects such as hematuria, metabolic acidosis, malabsorption (Müller-Lissner 1993), and electrolyte disturbance in animals (Beuers et al. 1991) have been reported long ago. This may partly explain poor hematological parameters observed in fish fed 4.0% *A. vera*/kg diet in this study. Hence, an upper limit is crucial in enhancing hematological indices as well as resistance against stressors in fish. In this study, inclusion levels between 0.5 and 2.0% *A. vera*/kg appeared to be appropriate.

In addition to hematological indices, *A. vera* extracts have been reported to enhance a wide range of enzyme activity in the blood serum in fish (Gabriel et al. 2015a, b; Zodape 2010), chicken (Ojiezeh and Ophori 2015; Fallah 2014), and mice (Cui et al. 2014). Enzyme activities, such as for AST and ALT, aid in the diagnosis of liver disease (Zodape 2010). 100% *A. vera* crude powder was reported to protect GIFT *O. niloticus* juveniles from liver damage against *Streptococcus iniae* pathogenic bacterium, and the optimum dosage was estimated to be less than or equal to 2.79%/kg diet (Gabriel et al. 2015b). In the same line, the present study observed that ALT and AST levels were lower in *A. vera*-supplemented fish compared to unsupplemented ones, especially in those fed between 0.5 and 1.0%/kg diet. This is an indication that *A. vera* at a particular dosage can effectively enhance hepatoprotective activity in fish under culture conditions as similarly demonstrated by Zodape (2010) in *Labeo rohita*.

Glucose content is one of the parameters that are used in fish studies to assess their stress status (He et al. 2015). In this study, glucose levels were somewhat lower in *A. vera*-supplemented fish, an indication that they were less stressed as demonstrated by (He et al. 2015). Similar results were reported when *A. vera* extracts were supplemented in GIFT *O. niloticus* diets at inclusion levels of 0.5% and 2.0%/kg diet (Gabriel et al. 2015a). Furthermore, the present study also observed lower TG and TCHO levels in *A. vera*-supplemented fish when compared to those fed a control diet (but not significant). The same was reported in our previous study (Gabriel et al. 2015b). This signifies antioxidant and hepatoprotective properties of *A. vera*, which have been reported to promote lipid metabolism, efficient protein accumulation, and better growth in animals (Ji et al. 2007).

Improved hematological parameters, antihyperlipidemic, antihyperglycemia, and enhancement of hepatoprotective enzymes in fish by *A. vera* owes it to its bioactive compounds (Radha and Laxmipriya 2014; Rajasekaran et al. 2005). Studies linking bioactive compounds to their effects in fish are limited. However, in rats, isolated phytosterols, namely lophenol, and cycloartenol were reported to elicit the ability to induce downregulation of fatty acid oxidation in the liver, which favors the reduction in intra-abdominal fat and improvement in hyperlipidemia (Misawa et al. 2012) and glycemia (Dana et al. 2012). *A. vera* polysaccharides, namely glycan, had showed a significant free radical scavenging and antioxidant activity in vitro and protective effects in hydrogen peroxide-induced PC12 cells (Wu et al. 2006). The ability for *A. vera* polysaccharides to increase the bioavailability of vitamin C and E (Vinson et al. 2005) is also another way of improving the body's natural antioxidant system as well as cellular damage as these vitamins play a role as strong antioxidant agents as explained in Gabriel et al. (2015b). Hence, these *A. vera* attributes could be responsible with the improved lipid profile status and hepatoprotective enzymes presented in this study.

Conclusion

This study demonstrated that *A. vera* polysaccharides crude powder extracts supplemented feed has growth, feed utilization, and hepatoprotective effects in African catfish (*C. gariepinus*) fingerlings. This extract can indeed be used to replace synthetic growth promoters, appetizer, stimulator, and feed digesting enhancer, and the optimal inclusion level is considered to be between 1.76 and 1.79% *A. vera*/kg diet. To fully optimize *A. vera* extracts as dietary supplement in aquaculture, further similar and extended studies are deemed important.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest.

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