

DIETARY ALOE AND GARLIC CRUDE POLYSACCHARIDES: EFFECTS ON  
GROWTH PERFORMANCE, HAEMATOLOGICAL, AND BODY COMPOSITION  
PARAMETERS OF *CLARIAS GARIEPINUS*

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## ABSTRACT

Fish health management in aquaculture is one of the main challenges across the globe (including Namibia), worsened especially by the wide adoption of intensive farming systems. Nowadays, attention is focused on the use of medicinal herbs as alternative to unsustainable pharmaceutical drugs in aquaculture. This study aimed to develop and introduce phytogenic diets made up of aloe vera (*Aloe vera*), and garlic (*Allium sativum*) crude polysaccharide extracts (separately and in mixture), which would promote growth, feed utilization, health, meat quality, and increase resistance against stress in African catfish, *Clarias gariepinus* reared in intensive aquaculture systems.

First, this study evaluated the effects of dietary *A. vera* crude polysaccharides on growth performance, feed utilization, haemato-biochemical parameters, and resistance against low water pH in African catfish fingerlings. Fish were divided into five triplicate groups before being fed feeds supplemented with control 0%, 0.5%, 1.0%, 2.0% and 4.0% *A. vera* for 60 d. Fish fed a 1.0% *A. vera* supplemented diet showed a significant increase in all growth parameters compared to the control ( $P < 0.05$ ). The protein efficiency ratio (PER) was significantly higher in fish fed 1.0% *A. vera* supplemented diet ( $1.31 \pm 0.22$ ) compared to unsupplemented fish ( $0.85 \pm 0.10$ ) and those fed 4.0% *A. vera* supplemented diet ( $0.85 \pm 0.14$ ) ( $P < 0.05$ ). The optimal dietary *A. vera* polysaccharide crude extract requirement was estimated to be 1.77% ( $y = -0.043x^2 + 0.152x + 0.593$ ,  $P = 0.045$ ) and 1.79 % *A. vera* ( $y = -2.778x^2 + 9.95x + 29.29$ ,  $P = 0.037$ ), for growth and feed utilization respectively. Overall, *A. vera* extracts improved haemato-biochemical indices in *A. vera* supplemented fish when compared to unsupplemented ones, but decreased some of the indices at the 4.0% *A. vera* level. After blood sampling, fish were subjected to a low water pH (5.2 - 5.5) challenge and survival probability was measured. Fish fed diets supplemented with 1.0%, and 2.0% *A. vera* showed higher survival probability (above 70%) throughout the challenge period compared to the control (below 70%) and those fed the 4% *A. vera* supplemented diet (below 60%).

Second, this study evaluated the effects of dietary garlic crude polysaccharide (GPE) (control 0%, 0.5%, 1.0%, 2.0%, and 4.0%) on growth, feed utilization, haematological parameters, and resistance against low water pH in African catfish juveniles. Fish fed GPE supplemented diets showed a significant improvement in all growth parameters and all feed utilization indices compared to the control ( $P < 0.05$ ). A significant increase was only observed in the red blood cells (RBC  $10^{12}/L$ ) for those fed 0.5% ( $2.01 \pm 0.07$ ), 1.0% ( $1.96 \pm 0.22$ ), and 2.0% ( $1.88 \pm 0.12$ ) and in mean corpuscular haemoglobin concentration (MCHC g/L) for those fed 0.5% ( $553.83 \pm 6.21$ ), and 1.0% ( $554.83 \pm 7.82$ ) compared to all others ( $P < 0.05$ ). After blood sampling, fish were subjected to a low water pH (5.2 - 5.5) challenge. No significant difference was observed in the cumulative survival between GPE supplemented groups and a control ( $P > 0.05$ ). The same was observed for whole body composition and organo-somatic indices. A dietary inclusion level of 1.77% ( $y = -11.89x^2 + 41.688x + 167$ ,  $P = 0.001$ ) and 1.69% of garlic ( $y = -0.056x^2 + 0.189x + 0.807$ ,  $P = 0.031$ ) was estimated as optimum for growth and feed utilization in *C. gariepinus* juvenile culture, respectively.

Finally, this study evaluated the effects of a dietary mixture of *A. vera* and *A. sativum* polysaccharides (control 0%, 0.5%, 1.0%, 2.0% and 4.0%; 1:1 ratio) on growth performance, feed utilization, haematological parameters, whole body composition, and resistance against low water pH of African catfish juveniles. Fish fed a 1.0% and 0.5% *A. vera*-*A. sativum* mixture supplemented diet presented a significant increase in all growth parameters compared to all others ( $P < 0.05$ ). Similarly, feed utilization indices significantly improved in fish fed diet supplemented with 1.0% *A. vera*-*A. sativum* mixture when compared to unsupplemented ones, and those fed 2.0% and 4.0% *A. vera*-*A. sativum* mixture ( $P < 0.05$ ). The optimum dietary *A. vera*-*A. sativum* mixture inclusion level was estimated to be 0.70% and 0.66% for growth and feed utilization respectively. *A. vera*-*A. sativum* mixture extracts improved haematological indices when compared to unsupplemented fish, but a significant increase was only observed in red blood cells (RBC) of fish fed 1.0% ( $1.92 \pm 0.01$ ) and in platelets (PLT) of fish fed 2.0% *A. vera*-*A. sativum* mixture supplemented diet ( $38.17 \pm 4.13$ ) when compared to unsupplemented ones (RBC =  $1.40 \pm 0.15$ ; PLT =  $20.66 \pm 3.75$ ) ( $P < 0.05$ ). When subjected to a low water pH (5.2 - 5.5) challenge after blood sampling, fish fed 1.0% *A.*

*vera*-*A. sativum* mixture showed the highest survival probability (above 80%) throughout the challenge period among groups. Fish fed dietary *A. vera*-*A. sativum* mixture of 2.0% ( $6.69 \pm 0.36\%$ ), followed by those fed 4.0% ( $7.18 \pm 0.24\%$ ) and 1.0% ( $7.44 \pm 0.29$ ) demonstrated a significantly lower lipid content compared to those fed a control diet ( $9.31 \pm 0.71\%$ ) ( $P < 0.05$ ).

The significance of this study is that it introduces *A. vera*, *A. sativum* crude polysaccharides extracts and their mixtures as good growth promoters, feed utilization enhancers, and good health promoters in *C. gariepinus* culture. The findings of this study encourage further studies on these herbs as potential fish growth and health management agents in the Namibian aquaculture and beyond, to ensure the application of effective products that have no harmful effects to man, animals and the environment. In addition, the study expands the existing work and knowledge on *A. vera* and *A. sativum* as medicinal herbs in aquaculture. The study recommends future studies to investigate the effects of several factors (i.e. temperature, extracts combination ratios), that could influence the performance of herbal extracts in fish for better optimization of *A. vera*, *A. sativum* crude polysaccharide extracts, and their mixture as dietary supplement in aquaculture.

**Keywords:** Aquaculture, *Clarias gariepinus*, Herbs, Immunostimulants, Stress resistance.

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## LIST OF ABBREVIATIONS

|        |                                       |
|--------|---------------------------------------|
| ACH50  | Serum alternative complement activity |
| ADG    | Average daily gain                    |
| AGR    | Absolute growth rate                  |
| ALB    | Albumin                               |
| ANOVA  | Analysis of variance                  |
| ANPU   | Apparent net protein utilization      |
| BASO   | Basophils.                            |
| BWG    | Body weight gain                      |
| BWI    | Body weight increasing                |
| CARBOX | Carboxylesterase                      |
| CAT    | Catalase                              |
| CC3    | Complement C3                         |
| CDR    | Complete randomized design            |
| CF     | Condition factor                      |
| CHOL   | Cholesterol                           |
| CL     | Chemiluminescent response             |
| CORT   | Cortisol                              |
| CP     | Crude protein                         |
| CSA    | Complement system activity            |
| DMRT   | Duncan's Multiple Range Test          |
| DO     | Dissolved oxygen                      |
| EDTA   | Ethylenediaminetetraacetic acid       |

|        |                                     |
|--------|-------------------------------------|
| EU     | Energy utilization                  |
| FCE    | Feed conversion efficiency          |
| FCR    | Feed conversion ratio               |
| FE     | Feed efficiency                     |
| FER    | Feed efficiency ratio               |
| FI     | Feed intake                         |
| FL     | Fish length                         |
| FRA    | Ferric reducing ability             |
| FW     | Final weight                        |
| GIFT   | Genetically improved farmed tilapia |
| GLOB   | Globulin                            |
| Glu    | Glucose                             |
| GPE    | Garlic polysaccharide extract       |
| GRAN   | Granulocytes                        |
| GSH-Px | Glutathione peroxidase              |
| Hb     | Haemoglobin                         |
| HCl    | Hydrochloric acid                   |
| Hct    | Haematocrits                        |
| HDL    | High-density lipoprotein            |
| HETRO  | Heterophil                          |
| HIS    | Hepatosomatic index                 |
| IgM    | Immunoglobulin M                    |
| IVL    | Intestinal villus length            |
| IVW    | Intestinal villus width             |

|                    |                                            |
|--------------------|--------------------------------------------|
| LDL                | Low-density lipoproteins                   |
| LYM                | Lymphocytes                                |
| LYZ                | Lysozyme activity                          |
| M                  | Mean                                       |
| MCH                | Mean corpuscular haemoglobin               |
| MCHC               | Mean corpuscular haemoglobin concentration |
| MCV                | Mean corpuscular volume                    |
| MDA                | Malondialdehyde                            |
| MO <sub>2</sub>    | Oxygen consumption                         |
| MON                | Monocytes                                  |
| MR                 | Metabolic rate                             |
| MS-222             | Tricaine methanesulfonate                  |
| N                  | Normality                                  |
| N/A                | Not available                              |
| NaOH               | Sodium hydroxide                           |
| NBT                | Nitroblue tetrazolium                      |
| NEU                | Neutrophils                                |
| NH <sub>3</sub> -N | Ammonia-Nitrogen                           |
| OAC                | Onavivi Aquaculture Center                 |
| PCV                | Packed cell volume                         |
| PE                 | Protein efficiency                         |
| PER                | Protein efficiency ratio                   |
| PEROX              | Peroxidase                                 |
| PHAGO              | Phagocytic activity                        |

|        |                                   |
|--------|-----------------------------------|
| PHAGOI | Phagocytic index                  |
| PHAGOR | Phagocytic ratio                  |
| ALP    | Phosphatase                       |
| PLT    | Platelets                         |
| PPV    | Protein productive value          |
| RBA    | Respiratory burst activity        |
| RBC    | Red blood cell count              |
| RDWa   | Red blood cell distribution width |
| RGR    | Relative growth rate              |
| ROS    | Reactive oxygen species           |
| SBA    | Serum bactericidal                |
| SE     | Standard error                    |
| SGR    | Specific growth rate              |
| SOD    | Superoxide dismutase              |
| SSI    | Spleen somatic index              |
| TCHO   | Total cholesterol                 |
| TG     | Triglycerides                     |
| THROM  | Thrombocytes                      |
| TP     | Total protein                     |
| VSI    | Viscerosomatic index              |
| WBC    | White blood cell count            |
| WG     | Weight gain                       |
| Wt.    | Weight                            |

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## **DEDICATION**

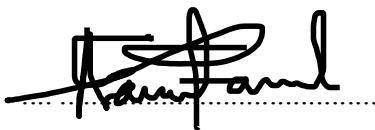
This dissertation is dedicated to my family (wife, Rebekka Shikesho-Gabriel; kids, Tangi and Tuapewa), to be an inspiration for hard work, patience, teamwork, perseverance and tolerance. It is also dedicated to my parents (Gabriel Wilhelm, and Anna Alpheus) who did not come this far in terms of education, yet they believe that education is the great equalizer.

## DECLARATION

I, Ndakalimwe Naftal Gabriel, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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Ndakalimwe Naftal Gabriel

Date 15 February 2021

## **CHAPTER ONE: INTRODUCTION**

### **1.1 General introduction**

Aquaculture is one of the fastest growing food producing sectors in the world, and in 2016, it contributed about 47% to the global seafood production (FAO 2018). Aquaculture contribution to global seafood is uneven among countries, and Asian countries have been the main contributors for many years (FAO 2014, 2016, 2018). The global success of aquaculture could be attributed to the wide adoption of intensive production systems, which are associated with higher yield as a result of higher stocking densities (Kumar and Engle 2016). However, high stocking densities could be stressful to the fish, and this could subsequently lead to several conditions such as poor growth (Gabriel and Akinrotimi 2011), poor health (Montero et al. 1999), increased susceptibility to diseases (Kibenge 2019), and in extreme cases lead to mortality (Mckenzie et al. 2012; Amal et al. 2018). Hence, good fish health management is important in intensive aquaculture systems.

In aquaculture-advanced nations, good health of farmed fish and maximization of aquaculture production is achieved by using synthetic pharmaceutical drugs such as antibiotics (Mohamed et al. 2000; Tonguthai 2000; Yulin 2000). However, the use of these drugs is considered merely production oriented and unsustainable as they are noted to cause resistance in pathogenic bacteria, environmental pollution and public health concerns (Hites et al. 2004; Cabello 2006; Gullberg et al. 2011; Liu et al. 2017). Hence, the application of synthetic drugs in aquaculture is discouraged.

Medicinal herbs possess the potential to replace synthetic pharmaceutical drugs in aquaculture. Herbs contain several biologically active metabolites with various benefits such as immune modulating (Zanuzzo et al. 2015; Yilmaz 2019), growth promoting, digestive enhancing, appetite stimulating, antioxidant enhancing, antidepressant (Zhang et al. 2010; Mahdavi et al. 2013; Reverter et al. 2014; Pu et al. 2017), and hepatoprotective effects in fish (Yilmaz et al. 2014; Gurkan et al. 2015). Other benefits associated with herbal extracts in fish include: increased resistance against pathogens (Reverter et al. 2014; Yilmaz 2019), and the sudden change in water quality parameters such as low pH (Lin and Chen 2008; Liu et al. 2016; Khan et al. 2018), high salinity (Ghehdarijani et al. 2016), and high temperature (Fazlolahzadeh et al. 2011).

The use of herbs in aquaculture could be more sustainable compared to synthetic drugs as they are locally available in most parts of the world, diverse in nature, inexpensive, and they are believed to be more biodegradable in nature (Olusola et al. 2013; Reverter et al. 2014). Therefore, medicinal herbs could be the appropriate remedies in aquaculture, if explored properly.

## **1.2 Statement of the problem**

In Namibia, aquaculture (marine and freshwater) remains one of the top priorities on the national development agenda, with most of the fish farmers (private and government) adopting the semi-intensive to intensive production systems. This sector is predominated by freshwater species (African catfish, *Clarias gariepinus*, and tilapia species), and since its inception in Namibia, it has been challenged to reap the benefits associated with

intensive aquaculture systems (Hilundwa and Teweldemedhin 2016; FAO 2019). One of the main drawbacks faced by the Namibian aquaculture freshwater fish farmers, is poor fish health management, water quality issues (including fluctuating pH), and a lack of quality fish feed fortified with essential nutrients (Rana and Abban 2012). This has partly led to poor growth performance, high fish mortality, and insignificant production outputs in intensive farming systems (i.e. Hardap, Fonteintjie, Leonardville, Uis, and Epalela aquaculture farms). Natural herbs have been recognized to possess several medicinal properties and could be appropriate remedies to maintain fish health and promote growth in intensive aquaculture systems. Namibia is endowed with a wide range of medicinal herbs (native and exotics), and although there has been an increase in the research interest in medicinal herbs in aquaculture, there is still limited information on their application in the Namibian aquaculture sector. Thus, the way forward, is to do more research contributing to the standardization of the important aspects on the application of medicinal herbs in aquaculture and to introduce this application in Namibia, and this forms the basis of the current study. The results of this study could provide insights into the benefits associated with medicinal herbs in fish to the Namibian aquaculture industry and could assist in the formulation of long-term policies that ensure a sustainable aquaculture development in Namibia and beyond.

### **1.3 Objectives of the study**

This study aimed to develop and introduce phytogenic diets made up of aloe vera (*Aloe vera*), and garlic (*Allium sativum*) crude polysaccharide extracts (separately and in mixture), which would promote growth, feed utilization, health, meat quality, and

increase resistance against stress in African catfish, *Clarias gariepinus* reared in intensive aquaculture systems.

#### 1.3.1 Specific objectives

To determine the effects of dietary *A. vera* crude polysaccharide extracts on:

- (1) Growth performance parameters i.e. weight gain (WG), specific growth rate (SGR), absolute growth rate (AGR), and organo-somatic indices in *C. gariepinus* fingerlings after sixty days of feeding.
- (2) Feed utilization parameters indices (i.e. feed intake, food conversion ratio, protein efficiency ratio, and feed efficiency ratio) in *C. gariepinus* fingerlings after sixty days of feeding.
- (3) Haematological parameters of *C. gariepinus* fingerlings after sixty days of feeding.
- (4) Serum biochemical indices i.e. alanine aminotransferase (AST) and aspartate aminotransferase (ALT), glucose (Glu), total cholesterol (TC), and triglycerol (TG) of *C. gariepinus* fingerlings after sixty days of feeding.
- (5) Whole body proximate composition of *C. gariepinus* fingerlings after sixty days of feeding.
- (6) Survival of *C. gariepinus* fingerlings at low pH after sixty days of feeding.
- (7) To estimate the optimum dietary *A. vera* crude polysaccharide extracts inclusion level in *C. gariepinus* culture.

To determine the effects of dietary *A. sativum* crude polysaccharide extracts on:

- (1) Growth performance parameters i.e. weight gain (WG), specific growth rate

(SGR), absolute growth rate, and organo-somatic indices in *C. gariepinus* juveniles after sixty days of feeding.

- (2) Feed utilization parameters indices (i.e. feed intake, food conversion ratio, protein efficiency ratio, and feed efficiency ratio) in *C. gariepinus* juveniles after sixty days of feeding.
- (3) Haematological parameters of *C. gariepinus* fingerlings after sixty days of feeding.
- (4) Whole body proximate composition of *C. gariepinus* juveniles after sixty days of feeding.
- (5) Survival of *C. gariepinus* juveniles at low pH after sixty days of feeding.
- (6) To estimate the optimum dietary *A. sativum* crude polysaccharide extracts inclusion level in *C. gariepinus* juveniles' culture.

To determine the effects of dietary *A. vera* and *A. sativum* crude polysaccharide extracts mixture on:

- (1) Growth performance parameters i.e. weight gain (WG), specific growth rate (SGR), absolute growth rate, and organo-somatic indices in *C. gariepinus* juveniles after sixty days of feeding.
- (2) Feed utilization parameters indices (i.e. feed intake, food conversion ratio, protein efficiency ratio, and feed efficiency ratio) in *C. gariepinus* juveniles after sixty days of feeding.
- (3) Haematological parameters of *C. gariepinus* juveniles after sixty days of feeding.

- (4) Whole body proximate composition of *C. gariepinus* juveniles after sixty days of feeding.
- (5) Survival of *C. gariepinus* juveniles at low pH after sixty days of feeding.

## **1.4 Hypotheses of the study**

### **1.4.1 *Aloe vera* polysaccharides**

- (1) H<sub>0</sub>: Dietary *A. vera* crude polysaccharide extracts have no effects on the growth performance parameters i.e. weight gain (WG), specific growth rate (SGR), absolute growth rate (AGR), and organo-somatic indices in *C. gariepinus* fingerlings after sixty days of feeding.
- (2) H<sub>0</sub>: Dietary *A. vera* crude polysaccharide extracts have no effects on the feed utilization parameters indices (i.e. feed intake, food conversion ratio, protein efficiency ratio, and feed efficiency ratio) in *C. gariepinus* fingerlings after sixty days of feeding.
- (3) H<sub>0</sub>: Dietary *A. vera* crude polysaccharide extracts have no effects on the haematological parameters of *C. gariepinus* fingerlings after sixty days of feeding.
- (4) H<sub>0</sub>: Dietary *A. vera* crude polysaccharide extracts have no effects on the serum biochemical indices i.e. alanine aminotransferase (AST) and aspartate aminotransferase (ALT), glucose (Glu), total cholesterol (TC), and triglycerol (TG) of *C. gariepinus* fingerlings after sixty days of feeding.
- (5) H<sub>0</sub>: Dietary *A. vera* crude polysaccharide extracts have no effects on the whole-body proximate composition of *C. gariepinus* fingerlings after sixty days of feeding.

- (6) H<sub>0</sub>: Dietary *A. vera* crude polysaccharide extracts have no effects on the survival of *C. gariepinus* fingerlings at low pH after sixty days of feeding.

#### 1.4.2 *Allium sativum* polysaccharides

- (1) H<sub>0</sub>: Dietary *A. sativum* crude polysaccharide extracts have no effects on the growth performance parameters i.e. weight gain (WG), specific growth rate (SGR), absolute growth rate (AGR), and organo-somatic indices in *C. gariepinus* juveniles after sixty days of feeding.
- (2) H<sub>0</sub>: Dietary *A. sativum* crude polysaccharide extracts have no effects on the feed utilization parameters indices (i.e. feed intake, food conversion ratio, protein efficiency ratio, and feed efficiency ratio) in *C. gariepinus* juveniles after sixty days of feeding.
- (3) H<sub>0</sub>: Dietary *A. sativum* crude polysaccharide extracts have no effects on the haematological parameters of *C. gariepinus* juveniles after sixty days of feeding.
- (4) H<sub>0</sub>: Dietary *A. sativum* crude polysaccharide extracts have no effects on the whole-body proximate composition of *C. gariepinus* juveniles after sixty days of feeding.
- (5) H<sub>0</sub>: Dietary *A. sativum* crude polysaccharide extracts have no effects on the survival of *C. gariepinus* juveniles at low pH after sixty days of feeding.

#### 1.4.3 The combination of *A. vera* and *A. sativum* crude polysaccharides extracts

- (1) H<sub>0</sub>: Dietary *A. vera* and *A. sativum* crude polysaccharide extracts mixture has no effects on the growth performance parameters i.e. weight gain (WG), specific growth rate (SGR), absolute growth rate (AGR), and organo-somatic indices in *C. gariepinus* juveniles after sixty days of feeding.
- (2) H<sub>0</sub>: Dietary *A. vera* and *A. sativum* crude polysaccharide extracts mixture has no effects on the feed utilization parameters indices (i.e. feed intake, food conversion ratio, protein efficiency ratio, and feed efficiency ratio) in *C. gariepinus* juveniles after sixty days of feeding.
- (3) H<sub>0</sub>: Dietary *A. vera* and *A. sativum* crude polysaccharide extracts mixture has no effects on the haematological parameters of *C. gariepinus* juveniles after sixty days of feeding.
- (4) H<sub>0</sub>: Dietary *A. vera* and *A. sativum* crude polysaccharide extracts mixture has no effects on the whole-body proximate composition of *C. gariepinus* juveniles after sixty days of feeding.
- (5) H<sub>0</sub>: Dietary *A. vera* and *A. sativum* crude polysaccharide extracts mixture has no effects on the survival of *C. gariepinus* juveniles at low pH after sixty days of feeding.

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## **CHAPTER TWO: LITERATURE REVIEW**

### **2.1 Introduction**

Globally, aquaculture had an annual growth rate of 5.3% between 2001 and 2016, and is expected to increase by 37% in 2030 (FAO 2018). One of the reasons for the current success and continual growth of aquaculture sector is a wide adoption of intensive production systems, which are associated with high yield as a result of high stocking densities (Kumar and Engle 2016). However, intensive aquaculture system issues such as fish handling, fluctuation of water quality parameters, transportation and harvesting may be stressful to fish. These stress factors lead to a number of conditions including poor metabolism capacity (Herrera et al. 2015), poor meat quality (Jittinandana et al. 2003), increased susceptibility to diseases (Lara-Flore 2011; Fečkaninová et al. 2017 ), and in extreme cases to deaths (Mckenzie et al. 2012). All these constraints have made it hard for fish farmers to convert the benefits of higher production yields associated with intensive production systems into economical gains. Therefore, aquaculture is still to reach its full potential.

In an effort for fish farmers to economically benefit from intensive farming systems, they started using synthetic pharmaceutical drugs to maintain good health of farmed fish. The adoption of these drugs in aquaculture was later shown to be unsustainable, as they cause fish pathogen drug resistance, immunosuppression, environmental pollution, and accumulation of chemical residues, which is potentially hazardous to public health (Thorne 2006; Heuer et al. 2009; Bulfon et al. 2013). For this reason, many nations such

as the United States, countries in the European Union (Bulfon et al. 2013), and Asian countries (Ji et al. 2007) have a strict demand for aquatic products free from synthetic pharmaceutical drugs. Consequently, the need to replace pharmaceutical drugs with dietary supplements or ingredients or additives (immuno-stimulants) that are capable of strengthening fish health, and enhancing their growth, feed utilization ability, and ultimately ensuring safe and good quality of aquatic products from aquaculture, has become increasingly imperative.

Based on results of searching for papers using keywords of “herbal extracts and aquaculture” using Google Scholar search engine ([www.scholar.google.com](http://www.scholar.google.com)), the number of publications on herbal extracts in aquaculture have increased by 30-fold over the past two decades (Figure 2.1). From this it can be inferred that indeed these extracts have the potential to eradicate the use of synthetic pharmaceutical drugs in fish farming. Herbs provide a wide range of useful biologically active metabolites such as polysaccharides, alkaloids, flavonoids, volatile oils, organic acids, tannins, and nutrients (amino acids, carbohydrates, minerals and vitamins) (Pu et al. 2017). If properly administered, these metabolites have the ability to increase growth and feed intake (Zhang et al. 2010; Mahdavi et al. 2013; Gabriel et al. 2015), enhance antioxidants, antidepressants and modulate immunity in fish (Zanuzzo et al. 2015a), and enhance meat quality (Ma et al. 2015) (Figure 2.2). Some of the benefits of using herbs in aquaculture include the following: they are available to small-scale rural fish farmers, they are inexpensive, and they are more biodegradable in nature compared to pharmaceutical drugs (Olusola et al. 2013; Reverter et al. 2014). Nevertheless, the current challenges in using herbs in aquaculture may include: difficulties to standardize them as they are

diverse in nature (with complex chemical structures), their biological metabolites may not be consistent (Pu et al. 2017), and their modes of action are yet to be fully understood. Thus, more research is necessary to allow the full implementation of medicinal herbs in aquaculture across the globe.

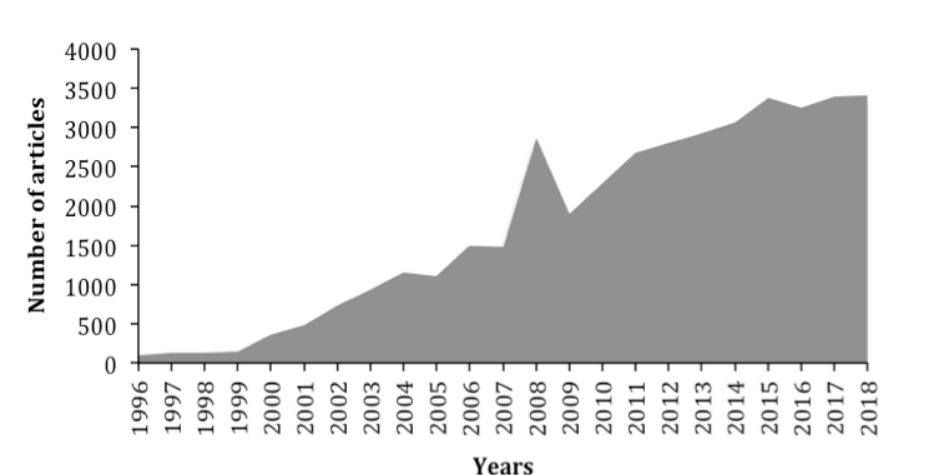


**Figure 2.1** Number of published articles about the use of plants, algae, or natural products in aquaculture (Google Scholar data).



**Figure 2.2** Herbal extracts roles and main action mechanisms when supplemented in fish (adapted from Pu et al. 2017).

Herbs could be used as a whole plant or parts (i.e. leaves, flowers, roots, seeds, or bark) in a crude form or as extracts / compounds from the whole plant or parts of the plant. For instance, crude extracts in the form of powder from *Mespilus germanica* (Hoseinifar et al. 2017), *Garcinia kola* (Dada et al. 2011) and *Camellia sinesis* (Abdel-Tawwab et al. 2010) were incorporated in fish feeds to investigate their effects on growth and health parameters in *Cyprinus carpio*, *Clarias gariepinus*, and *Oreochromis niloticus*, respectively. These herbal extracts were able to increase growth and improve health status of the studied fish compared to a control in all three cases. The same was reported in fish fed feed supplemented with *Garcinia mangostana* methanolic extracts (Soosean et al. 2010), *Stragalus polysaccharides* (Ardo et al. 2008), *Pontogammarus maeoticus* aqueous extracts (Rufchaei et al. 2017), and *Mentha piperita* ethanolic extracts (Adel et al. 2015), respectively.

The current study focused on the effects of garlic (*Allium sativum*) and aloe vera (*Aloe vera*) crude polysaccharide extracts in African catfish, *C. gariepinus*. A literature review on these extracts as feed additives and remedies in aquaculture and gaps in the existing knowledge is therefore provided in this chapter.

## **2.2 The medicinal use of garlic, *Allium sativum***

Garlic (*A. sativum*) is a perennial herb, belonging to the Liliaceae family, and is grown in temperate to subtropical regions of the world (Fritsch and Friesen 2002). It has long, green flat grass-like leaves rising from a squamous, white, and round bulb (composed of many densely packed elongated bulbs), which are the main organs consumed by

humans. This herb has been used since ancient times as a spice and a medicinal remedy for a variety of illnesses (Mirelman et al. 1987; Ebrahimi et al. 2015). It has been proven effective as a hypolipidemic (Asdag 2015), antimicrobial (Reiter et al. 2017), antihypertensive (Nandhini et al. 2018), insecticidal (El-Beih et al. 2017), hepatoprotective (Ahmed 2018), anti-inflammatory, immunomodulatory, antioxidant drug (Alam et al. 2018), and growth-promoting agent (Alagawany et al. 2016) in humans and animals. Increased growth and improved health status were reported in fish after being supplemented with garlic extracts (Al-Salahy 2002; Shalaby et al. 2006; Farahi et al. 2010; Shakya and Labh et al. 2014; Zaefarian et al. 2017). These beneficial effects of garlic in animals have been attributed to its various biological compounds including organosulfur compounds (Gabreyohannes and Gabreyohannes 2013), oil (Mousa et al. 2013), polysaccharides (Pan 2014; Chen and Huang 2019) or nutritional constituents (Josling 2005) as discussed below.

Garlic contains about 65% of water, 28% carbohydrates (fructans), 2.3% organosulfur compounds (alliin, allicin, ajoene, diallyl disulfide, diallyl trisulfide, allyl methanethiosulfinate, and S-allylcysteine), 2% protein (allinase), 1.2% free amino acids (arginine) and 1.5% fibre (Santhosha et al. 2013; Table 2.1). It also contains minerals such as calcium (24.33 mg/100g), iron (3.93 mg/100g), potassium (50.66 mg/100g), magnesium (2.63 mg/100g), and vitamins (A, B1, and C) (Josling 2005; Joo et al. 2013; Khalid et al. 2014) and about 35% polysaccharides (Pan and Wu 2014). Of the constituents of garlic, the organosulphur compounds are the most bioactive compounds, responsible for the typical pungent smell and for its medicinal properties (Macpherson et al. 2005; Bhandari 2012; Kumar et al. 2013; Lanzotti et al. 2014). These compounds

may enhance the biosynthesis of glutathione (which has antioxidant functions), and other volatile compounds with strong bioactive properties such as ajoenes (Block et al. 1993), alliin, allicin (alliin is converted to allicin by allinases, when the garlic is cut or crushed), allyl sulfide, and 1,2 vinylthiin (Bhandari 2012; Martin et al. 2016) (Figure 2.3). In addition, medicinal properties of garlic are also attributed to its phytonutrients such as vitamins, minerals, oil, and other anti-nutritional factors such as flavonoids, saponins, phenol compounds (Lanzotti et al. 2014) and polysaccharides (Pan and Wu 2014).

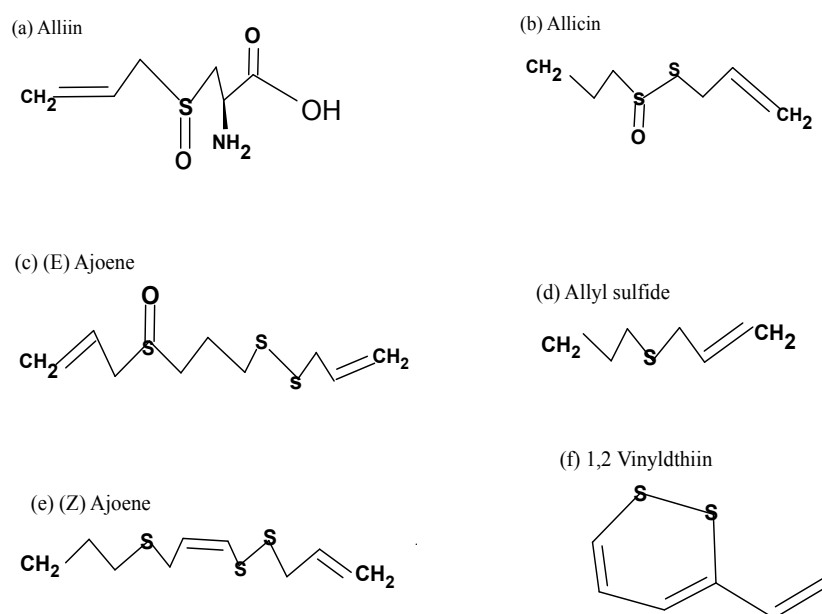


**Figure 2.3** Chemical structures of the most bioactive compounds (alliin, allicin, ajoene, allyl sulfide, and 1,2 vinylthiin from *Allium sativum* (adapted from Martin et al. 2016).

**Table 2.1** Some of the biological functions of abundant bioactive compounds found in garlic reported in organisms.

| <b>Compounds</b>  | <b>Biological effects</b>                                                          | <b>References</b>                                                                                                               |
|-------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| Alliin            | Antidiabetic<br>Antioxidant<br>Immunomodulatory<br>Antimicrobial                   | Anwar and Younus (2017)<br><br>Salman et al. (1999)<br>Rahman (2007)                                                            |
| Allicin           | Antioxidant<br>Antimicrobial<br>Immunomodulatory                                   | Nya et al. (2010)                                                                                                               |
| Essential oil     | Hepato-protective<br>Antioxidant<br>Antifungal<br>Preservative<br>Growth promoting | Liu and Xu (2007)<br>Abdel-Daim et al. (2015)<br>Chung et al. (2007)<br>Gomez-Estaca et al. (2010)<br>Hassaan and Soltan (2016) |
| Ajoene            | Antimicrobial<br>Antioxidant<br>Cardio-protective                                  | Rahman (2007)<br>Capasso (2013)                                                                                                 |
| 1,2 –Vinyldithiin | Anti-microbial<br>Anti-oxidant<br>Anti-thrombotic                                  | Higuchi et al. (2003)                                                                                                           |
| Polysaccharides   | Antioxidant<br><br>Immunomodulatory<br>Growth promoting<br>Preservative            | Pan and Wu (2014)<br>Kallel et al. (2015)<br>Li et al. (2017)<br>Yan-hua et al. (2010)<br>Kallel et al. (2015)                  |
| Saponins          | Antifungal,<br>Cholesterol lowering<br>Growth promoting                            | Matsuura (2001)<br>Ng’ambi et al. (2016)                                                                                        |

### **2.3 Previous studies on garlic extracts in aquaculture**

Garlic is one of the medicinal herbs that are broadly studied in both freshwater (Kumar et al. 2009; Nya et al. 2010; Thanikachalam et al. 2010; Millet et al. 2011; Hyun Kim et al. 2019; Onumu 2019) and marine aquaculture (Guo et al. 2012; Javadzadeh et al. 2012; Militz et al. 2014; Irkin et al. 2014; Huang et al. 2018). The effects of this herb have been investigated when used either as a 100% crude powder (Thanikachalam et al. 2010; Talpur and Ikhwanuddin 2012; Naeiji et al. 2013; Saleh et al. 2015), as solvent extracts (semi-purified) (Guo et al. 2012; Dash et al. 2014; Militz et al. 2014; Saha and Bandyopadhyay 2017; Büyükdeveci et al. 2018) or as purified extracts (Nya et al. 2010; Hassaan and Soltan 2016; Huang et al. 2018; Hyun Kim et al. 2019), with crude garlic powder being the most commonly researched form (Table 2.2). A number of the studies concisely support the beneficial effects of garlic in fish (i.e. immunomodulation, growth promotion, appetites stimulation, digestion stimulation, antioxidation, antimicrobial, antiparasitic, and appetite, hepatoprotective), and recommended further efforts to be directed at investigating purified garlic extracts for easy standardizations, and to advance in parameters of assessments to understand the mechanisms of the actions of garlic (Nya and Austin 2009, 2011; Talpur and Ikhwanuddin 2012; Zaefarian et al. 2017).

In aquaculture, garlic is typically incorporated into fish feed and administered orally, which is a common administration method of herbal extracts reported in fish studies (Reverter et al. 2014; Dawood et al. 2016). As demonstrated by Militz et al. (2013), and Hyun Kim et al. (2018) garlic extracts may also be delivered through immersion. The selection of the delivery method is mainly dictated by the purpose of garlic administration, the size of the fish and type of species, the types of extracts, and the type

of farming system (Reverter et al. 2014; Dawood et al. 2016). For instance, garlic extracts administered orally were reported to have improved growth, feed utilization, and disease resistance in Nile tilapia, *Oreochromis niloticus* (Abu-Elala et al. 2016), redbelly tilapia (Ajiboye et al. 2016), and rainbow trout, *Oncorhynchus mykiss* (Nya and Austin 2009). Immersion administration of garlic has also been reported to treat fish parasites effectively (Militz et al. 2013, 2014; Fredman et al. 2014; Hyun Kim et al. 2018).

#### 2.3.1 Garlic effects on growth and feed utilization of fish

The benefits of garlic extract on growth and feed utilization have been reported in different fish species in aquaculture (Table 2.2). Büyükdeveci et al. (2018) reported that *O. mykiss* fingerlings significantly increased in weight gain (WG), specific growth rate (SGR), and significantly decreased in feed conversion ratio (FCR) after being fed garlic-supplemented diets (20 g/kg) for two weeks compared to a control. Similarly, garlic supplemented diets had improved growth and feed utilization indices in *O. niloticus* (40 g/kg, 70 days) (Mabrouk et al. 2011), orange-spotted grouper, *Epinephelus coioides* (13 g/kg, 14 days) (Guo et al. 2012), sterlet sturgeon, *Acipenser ruthenus* (20-30 g/kg, 84 days) (Lee et al. 2014), European seabass, *Dicentrarchus labrax* (30 g/kg, 49 days) (Saleh et al. 2015), Caspian trout, *Salmo caspius* (20 g/kg, 6 weeks) (Zaefarian et al. 2017), *O. mykiss* (30 g/kg, 56 days) (Esmaeili et al. 2017a), Oscar, *Astronotus ocellatus* (10 g/kg, 56 days) (Saghaei et al. 2015) and sobaity seabream, *Sparidentex hasta* (10 g/kg, 56 days) (Jahanjo et al. 2018) compared to a control. Most of these studies linked the growth-enhancing and feed utilization enhancing effects of garlic to its organosulfur compounds such as allicin. Allicin has a strong stimulatory effect on olfaction and as a

result increases appetite in fish (Lee and Gao 2012). Khali et al. (2001) indicated that allicin could promote growth in fish by its ability to enhance the performance of the intestinal flora, which then improves their energy utilization capacity. Another way that allicin could improve growth in fish is by inhibiting or killing of various pathogenic bacteria, improving gastrointestinal motility, and regulating the secretion of different enzymes to improve digestion and nutrient absorption (Lee and Gao 2012). Büyükdeveci et al. (2018) supported this by reporting the improved growth performance and change in the intestinal microbiota of *O. mykiss* juveniles after being fed with diets supplemented with garlic powder for 120 days.

Some studies reported dietary garlic supplementation to have no influence on growth performance and feed utilization of neither finfish nor shellfish (Table 2.2). For instance, dietary garlic peel extracts supplemented at 5, 10, and 15 g/kg failed to significantly improve WG, SGR, and FCR of *C. gariepinus* fingerlings after 20 days administration (Thanikachalam et al. 2010). Eirna et al. 2016 reported the same in *C. gariepinus* after being fed diets supplemented with garlic peel or clove extracts at 10, 20, or 30g for 84 days. Similarly, dietary garlic had no significant effects on growth and feed utilization indices in other fish species such as *O. mykiss* (Nya and Austin 2011), barramundi, *Lates calcarifer* (Talpur and Ikwanuddin 2012), *Huso huso* (Kanani et al. 2014), cachama, *Colossoma macropomum* (Inoue et al. 2016), whiteleg shrimp, *Litopenaeus vannamei* (Labrador et al. 2016; Huang et al. 2018) compared to a control, respectively. It thus seems that growth improvement in fish following garlic supplementation is not obvious. Lee and Gao (2012) in their review on garlic in aquaculture highlighted that, duration of the experiment might be a factor contributing to poor growth and feed utilization

performance. This was demonstrated by Aly and Mohamed (2010) who reported that garlic supplemented diets had no significant effect on growth of *O. niloticus* after 30 or 60 days of feeding but a significant increase in growth was observed after 240 days. They stated that short feeding periods seemed to be unsuitable for garlic extracts to manifest their growth promoting potential in fish. However, inconsistent results exist to support this observation, as shown by Nya and Austin (2009), and Guo et al. (2012).

Other factors that could influence the effects of garlic supplementation in fish include the type of fish species, fish size, developmental stage, and garlic inclusion levels (Yang et al. 2010; Lee and Gao 2012). For example, Talpur and Ikhwanuddin (2012) reported no significant improvement in the growth and feed utilization indices of *L. calcarifer* after being fed with garlic-supplemented diets for 14 days. Guo et al. (2012) reported the opposite in *E. coioides* fed garlic-supplemented diets for the same duration.

Administering the allicin compound was reported to increase and reduce growth with increasing dosages in silberner pacu, *Colossoma barchipomum* (Xiang and Liu 2002) and allicin at 800 mg/kg caused mortality in swamp eel, *Monopterus albus* (Huang et al. 2001). Lee and Gao (2012) explained that when too much alkyl sulfide reaches the intestines of the fish, the sulfides interfere with the metabolism and suppress mitotic cell division, resulting in slow growth and even deaths. Therefore, there is still a need for adequate research to define the optimal dosage of garlic as a feed supplement for each fish species and each culture stage in different types of aquaculture production systems.

**Table 2.2** Studies testing on the effects of orally administered garlic extracts on growth performance and feed utilization indices in aquaculture.

| Type of extracts      | Dosage (g/kg diet) | Optimum dosage | Experiment duration            | Growth/ Feed utilization                                       | Scientific name       | Common name            | Initial wt.(g) | References                     |
|-----------------------|--------------------|----------------|--------------------------------|----------------------------------------------------------------|-----------------------|------------------------|----------------|--------------------------------|
| Dry powder            | 10, 15, 20         | 20             | 120 days                       | FW (>), WG (>), SGR (>)<br>FCR (<)                             | <i>O. mykiss</i>      | Rainbow trout          | 6.83-8.19      | Buyukdevci et al. (2018)       |
| Dry powder            | 30                 | N/A            | 60 days                        | FW (>), SGR (>), FCR (<)<br>PE (>)                             | <i>O. mykiss</i>      |                        | 8.26           | Esmacili et al. (2017a)        |
| Powder                | 5, 10              | N/A            | 14, 21, 28 days                | SGR (=), WG (=), gutted wt.                                    | <i>O. mykiss</i>      |                        | 14             | Nya and Austin (2009)          |
| Dry powder            | 10                 | N/A            | 56 days                        | WG (>), SGR (>), FCR (<)                                       | <i>S. hassta</i>      | Sobaitly seabream      | 3.08           | Jahanjoo et al. (2018)         |
| Dry powder            | 10, 20, 30         | 20             | 42 days                        | FW (>), SGR (>), BWI (>)                                       | <i>S. caspius</i>     | Caspian trout          | 19.94          | Zaefarian et al. (2017)        |
| Wet powder            | 15, 30, 45         | N/A            | 45 days                        | FER (=), VSI (>), HSI (>)<br>FW (=), WG (=), FL (=)<br>FCR (=) | <i>C. macropomum</i>  | Cachama                | 112.4          | Inoue et al. (2015)            |
| Peels or clove Powder | 10, 20, 30         | N/A            | 84 days                        | FW (=), WG (=), SGR (=)<br>FCR (=)                             | <i>C. gariepinus</i>  | African catfish        | 8.0            | Eima-Liza et al. (2016)        |
| Peels powder          | 5, 10, 15          | N/A            | 20 days                        | FW (=), WG (=), SGR (=)<br>FCR (=)                             | <i>C. gariepinus</i>  |                        | 8.7-8.88       | Thanikachalam et al. (2010)    |
| Powder                | 5, 10, 20, 30      | 10             | 56 days                        | WG (>), FW (>), SGR (>)<br>FCR (<)                             | <i>A. ocellatus</i>   | Oscar                  | 12.43          | Saghaei et al. (2015)          |
| Powder                | 10, 20, 30         | 30             | 49 days                        | FW (>), WG (>), SGR (>)<br>FI (<), FCR (<), PER (>)<br>PPV (>) | <i>D. labrax</i>      | European seabass       | 0.4            | Saleh et al. (2015)            |
| Powder                | 5, 10, 15          | N/A            | 14 days                        | WG (=), SGR (=), FCR (=)                                       | <i>L. calcarifer</i>  | Barramundi             | 20             | Talpur and Ikhwannuddin (2012) |
| Powder                | 13, 40             | 13             | 14 days                        | WG (>), FE (>)                                                 | <i>E. coioides</i>    | Orange-spotted grouper | N/A            | Guo et al. (2012)              |
| Powder                | 5, 10, 30          | 20-30          | 84 days                        | WG (>), FW (=), SGR (>)<br>PER (>), HSI (<)                    | <i>A. ruthenus</i>    | Sterlet sturgeon)      | 5.5            | Lee et al. (2014)              |
| Powder                | 40                 | N/A            | 84 days                        | ADG (>), SGR (>), FCR (<)<br>PPV (>), EU (>)                   | <i>O. niloticus</i>   | Nile tilapia           | 3.12           | Mabrouk et al. (2011)          |
| Powder                | 30                 | N/A            | Summer season<br>Winter season | SGR (>), BWG (>)<br>SGR (=), BWG (=)                           | <i>O. niloticus</i>   |                        | 0.8            | Aly and Mohamed (2010)         |
| Powder                | 10, 20, 30         | 30             | 60 days                        | WG (>), ADG (>), RGR (>)                                       | <i>Tilapia zillii</i> | Redbelly tilapia       | 0.09           | Ajiboye et al. (2016)          |

|         |                     |     |         |                                                                |                     |      |                           |
|---------|---------------------|-----|---------|----------------------------------------------------------------|---------------------|------|---------------------------|
| Powder  | 5                   | N/A | N/A     | FW (>), WG (>), SGR (>)<br>FCR (<), PER (>)                    | <i>O. niloticus</i> | 41.4 | Abu-Eljala et al. (2016)  |
| Powder  | 10, 20, 30, 40      | 30  | 90 days | FW (>), WG (>), SGR (>)<br>FI (>), FCR (<), FER (>)<br>PER (>) | <i>O. niloticus</i> | 7.0  | Shalaby et al. (2006)     |
| Powder  | 5, 10, 15           | 10  | 56 days | WG (>), SGR (>)                                                | <i>R. rutilus</i>   | 1.0  | Ghehdarjani et al. (2016) |
| Allicin | 0.5, 1.0            | N/A | 21 days | WG (=)                                                         | <i>L. vannamei</i>  | N/A  | Huang et al. (2018)       |
| Powder  | 20, 40, 60          | 60  | 60 days | FW (=), WG (=), FCR (<)                                        | <i>L. vannamei</i>  | 2.29 | Labrador et al. (2016)    |
| Powder  | 10                  | N/A | 60 days | DGR (=), BWG (=), SGR (=)<br>FCR (=)                           | <i>H. huso</i>      | 30   | Kanani et al. (2014)      |
| Oil     | 1ml                 | N/A | 84 days | WG (>), SGR (>), FI (>)<br>FCR (>), PER (>)                    | <i>O. niloticus</i> | 1.88 | Hassan & Soltan (2016)    |
| Powder  | 1, 5, 10            | N/A | 60 days | SGR (=), FCR (=)                                               | <i>L. rohita</i>    | 10   | Sahu et al. (2007)        |
| Powder  | 0.05, 0.1, 0.5, 1.0 | 1.0 | 14 days | WG (>), SGR (>), FCR (<)<br>PER (>)                            | <i>O. mykiss</i>    | 15   | Nya and Austin (2009)     |

Notes: SGR: Specific growth rate; WG: Weight gain; FL: Fish length; BWI: Body weight increasing; PE: Protein efficiency; ADG: Average daily gain; PPV: Protein productive value; BWG: Body weight gain; RGR: Relative growth rate; VSI: Viscerosomatic index; HSI: Hepatosomatic index; wt.: Weight; FI: Feed intake; FW: Final weight; PPV: Protein productive value; PER: Protein efficiency ratio; EU: Energy utilization, FCR: Feed conversion ratio; FI: Feed intake; FER: Feed efficiency ratio; FE: Feed efficiency; N/A: Not available; (>): Significantly increased; (<): Significantly decreased; (=): Not affected.

### 2.3.2 Garlic effects on haemato-biochemical indices of fish

Blood is the most frequently examined tissue to assess the health or physiological status of vertebrates in response to various factors such as drugs, diets, environmental changes and stress (Shalaby et al. 2006). The health status index such as oxygen carrying capacity has been directly determined by reference to primary hematological indices such as red blood cell (RBC), haemoglobin concentration (Hb), percentage of blood volume consisting of red blood cells, and haematocrits (Hct) (Houston 1997). Secondary indices, sometimes called Wintrobe indices (Urrechaga et al. 2014) can be derived from primary indices for the classification of anaemia condition such as Mean Corpuscular Volume (MCV) =  $(\text{Hct} \times 10 / \text{RBC})$ , Mean Corpuscular Haemoglobin (MCH) =  $(\text{Hb} \times 10 / \text{RBC})$ , and Mean Corpuscular Haemoglobin Concentration (MCHC) =  $(\text{Hb} \times 100 / \text{Hct})$  as demonstrated in Gabriel et al. (2015a). Other haematological indices such as white blood cell (WBC), and a number of their differential counts (i.e. leucocyte counts such as lymphocytes, neutrophils, eosinophils, monocytes, and basophils) have been assessed to measure the innate immune status of animals, especially during stressful conditions (Harikrishnan et al. 2010; Van Rijn and Reina 2010).

Positive and no effects of garlic on haematological parameters in different fish species have been reported (Table 2.3). For example, Nya and Austin (2009, 2011) observed that garlic extracts significantly stimulated erythropoiesis and leucopoiesis (i.e. increased RBC, WBC, Hct, Hb, MCV, MCH, MCHC, and differential leucocytes counts) in *O. mykiss*, which resulted in an improvement of fish health status and increased resistance against a pathogenic bacterium, *Aeromonas hydrophila*. Similarly, dietary garlic significantly increased haematological parameters of *L. rohita* (Sahu et al. 2007), *C.*

*gariepinus* (Thanikachalam et al. 2010), *D. labrax* (Saleh et al. 2015), *L. calcarifer* (Talpur and Ikhwanuddin 2012), *H. huso* (Kanani et al. 2014), common carp, *Cyprinus carpio* (Chesti et al. 2018), *O. mykiss* (Esmaeili et al. 2017b), and *S. hasta* (Jahanjoo et al. 2018). Garlic powder was also reported to have no effects on haematological parameters of *Salmo caspius* (Zaefarian et al. 2017), *D. labrax* (Yilmaz and Ergün 2012), and *O. niloticus* (Hassaan and Soltan 2016).

In addition to haematological parameters, blood serum contains numerous elements that can be used to monitor the health status of fish. For example, serum total protein (product of WBC) (Misra et al. 2006), and globulin levels (source of immunoglobulins or antibodies) (Goda 2008) in the blood reflect immune system activation (Siwicki et al. 1994). The presence of lysozymes, antimicrobial peptides and phagocytes in the blood indicates pathogens inhibiting activity (Uribe et al. 2011). Furthermore, several endogenous antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxide GSH-Px have been used to indicate cell damage from reactive oxygen species (ROS) in fish (Wu et al. 2006). Hepatoprotective activity is mainly determined by aspartate aminotransferase (AST), and alanine aminotransferase (ALT) (Cui et al. 2014), while stress can be reflected by glucose and cortisol blood content (He et al. 2015).

**Table 2.3** Tested effects of orally administered garlic extracts on haemato-biochemical indices on farmed fish species.

| Garlic products | Dosages (g/kg diet) | Duration of exposure            | Haemato-biochemical indices                                                                                                                                                                                                       | Fish species               | References                     |
|-----------------|---------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|--------------------------------|
| Crude powder    | 0.5, 1, 0           | 14 days                         | RBC (>), WBC (>), MON (>)<br>Lymph. (>), NEU. (<), THROM. (=)<br>TP (=), RBA (>), LYZ. (>).                                                                                                                                       | <i>O. mykiss</i>           | Nya and Austin (2011)          |
| Crude powder    | 0.05, 1.0, 0.5      | 14 days                         | RBC (>), WBC (>), Hct (>), Hb (=)<br>MCV (>), MCHC (>), MCH (>)<br>Lymph. (>), MON (>), NEU (>)<br>THROM (=), TP (>), ALB (=), GLOB (>)<br>PHAGOR (>), PHAGOI (=)<br>LYZ (>).                                                     | <i>O. mykiss</i>           | Nya and Austin (2009)          |
| Crude powder    | 1, 5, 10            | after every 20 days for 70 days | RBC (>), WBC (>), Hb (>), Glu (<)<br>TP (>), ALB (>), GLOB (>).                                                                                                                                                                   | <i>L. rohita</i>           | Sahu et al. (2007)             |
| Crude powder    | 10, 20, 30          | 49 days                         | RBC (=), Hb (>), PCV (=), MCV (>)<br>MCH (>), MCHC (=), WBC (>)<br>RBC (>), WBC (>), Hct (>), Hb (>)<br>LYMP. (>), MON (>), NEU (>)<br>THROM. (>), EOS (>), BASO (>)<br>Glu. (<), TP (>), ALB (>), GLOB (>)<br>RBA (>), PHGR (>). | <i>D. labrax</i>           | Saleh et al. (2015)            |
| Crude powder    | 5, 10, 15, 20       | 14 days                         | Glu. (<), TP (>), ALB (>), GLOB (>)<br>RBA (>), PHGR (>).                                                                                                                                                                         | <i>L. calcarifer</i>       | Talpur and Ikhtvanuddin (2012) |
| Oil             | 1.0 ml              | 84 days                         | Hct (=), Hb (=), RBC (=), AST (=)<br>TP (>), ALB (=), GLOB (>).                                                                                                                                                                   | <i>O. niloticus</i>        | Hassan and Soltan (2016)       |
| Paste           | 15, 30, 45          | 45 days                         | TP (=), Glu. (=), RBC (=), Hb (=), Hct (=)<br>MCV (=), MCHC (=), THROM. (=), MON (=)<br>EOS (=).<br>Glu. (<), AST (<), ALT (<).                                                                                                   | <i>Clossoma macrodonum</i> | Inoue et al. (2016)            |
| Juice           | 2mg/kg body weight  | 5 days                          | Glu. (<), AST (<), ALT (<).                                                                                                                                                                                                       | <i>Clarias lazera</i>      | Al-Shalaby 2002                |
| Peels           | 5, 10, 15           | 20 days                         | TP (>), ALB (>), GLOB (>), RBC (>), WBC (>)<br>erythrocytes (>), Hb (>), Hct (<), MCV (=)<br>MCH (=), MCHC (>), Glu. (<), TP (>)<br>AST (<), ALT (<).                                                                             | <i>C. gariepinus</i>       | Thamikaachalam et al. (2010)   |
| Crude powder    | 10, 20, 30, 40      | 90 days                         | MCH (=), MCHC (>), Glu. (<), TP (>)<br>AST (<), ALT (<).                                                                                                                                                                          | <i>O. niloticus</i>        | Shalaby et al. (2006)          |
| Crude powder    | 10                  | 60 days                         | RBC (=), Hb (=), Hct (=), MCHC (>), MCH (=)                                                                                                                                                                                       | <i>H. huso</i>             | Kanani et al. (2014)           |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |            |          | LYMP (>), NEU (>), EOS (>), MON (=)<br>ALT (=), AST (<), TP (=), ALB (<), LYZ (<)                                                                       |                   |                         |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-------------------------|
| Crude powder                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 5, 10, 15  | 245 days | Erythrocytes (>), leukocytes (>), Hb (>), PCV (<)<br>MCV (<), MCHC (>)                                                                                  | <i>C. carpio</i>  | Chesti et al. (2018)    |
| Crude powder                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 30         | 60 days  | RBC (>), Hb (>), Hct (>), WBC (>), LYZ (>)<br>TP (>), ACH50 (>).                                                                                        | <i>O. mykiss</i>  | Esmacili et al. (2017b) |
| Powder                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 10         | 56 days  | WBC (>), RBC (>), Hb (>), Hct (>), MON (=)<br>LYMP (>), NEU (>), EOS (=), TP (>), ALB (>),<br>AST (<), ALT (<), ALP (<), SOD (>), LYZ (>)<br>ACH50 (>). | <i>S. hasta</i>   | Jahanjoo et al. (2018)  |
| Crude powder                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 10, 20, 30 | 42 days  | Hb (=), Hct (=), WBC (=), RBC (=), MCV (=)<br>MCH (=), MCHC (=), Glu. (=), TP (>), ALB (=)<br>LYZ (>).                                                  | <i>S. caspius</i> | Zaefarian et al. (2017) |
| Oil Bath immersion                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 0.01, 0.02 | 9 hours  | RBC (=), Hct (=), Hb (=), MCV (=), MCH (=)<br>MCHC (=).                                                                                                 | <i>D. labrax</i>  | Yilmaz and Ergün (2012) |
| Crude powder                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 20, 40, 60 | 60 days  | RBC (<), Hb (<), Hct (<), MCH (<), Glu. (<)                                                                                                             | <i>D. labrax</i>  | Irkin et al. (2014)     |
| Notes: Hct: Hematocrits; Hb: Hemoglobin; WBC: White blood cells; MCV: Mean corpuscular volume; LYZ: Lysozyme activity; Glu: Glucose; TP: Total protein; ALB: Albumin; GLOB: Globulin; RBC: Red blood cells; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentrate; NEU: Neutrophils; LYM: Lymphocytes; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; THROM: Thrombocytes; RBA: Respiratory burst activity; MON: Monocytes; PHAGOL: Phagocytic index; (>): Significantly increased; (<): Significantly decreased; (=): Not affected; PHAGOR: phagocytic ratio; BASO: Basophils. |            |          |                                                                                                                                                         |                   |                         |

Notes: Hct: Hematocrits; Hb: Hemoglobin; WBC: White blood cells; MCV: Mean corpuscular volume; LYZ: Lysozyme activity; Glu: Glucose; TP: Total protein; ALB: Albumin; GLOB: Globulin; RBC: Red blood cells; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentrate; NEU: Neutrophils; LYM: Lymphocytes; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; THROM: Thrombocytes; RBA: Respiratory burst activity; MON: Monocytes; PHAGO: Phagocytic index; (>): Significantly increased; (<): Significantly decreased; (=): Not affected; PHAGOR: phagocytic ratio; BASO: Basophils.

The effects of garlic extract on blood biochemical parameters in fish have also been documented in aquaculture. For instance, dietary garlic crude powder supplementation was reported to have enhanced serum total protein, globulin, phagocytic ratio, and lysozyme activity in *O. mykiss* (Nya and Austin 2009). The same type of extract was reported to have significantly increased serum total protein, albumin, and globulin content in *L. rohita* (Sahu et al. 2007). Similar results were reported in *L. calcarifer* (Talpur and Ikhwanuddin 2012), *C. gariepinus* (Thanikachalam et al. 2010), *O. mykiss* (Esmacili et al. 2017b), *S. hasta* (Jahanjoo et al. 2018), and *S. caspius* (Zaefarian et al. 2017) after being fed diets supplemented with garlic crude powder. Similarly, dietary garlic oil extracts were reported to have significantly increased serum total protein, and globulin content in *O. niloticus* after 84 days administration (Hassan and Soltan 2016). Contradicting findings were also reported. For example, dietary garlic crude powder supplementation significantly decreased albumin and lysozyme activity and had no effect on the serum total protein in *H. huso* (Kanani et al. 2014). This was supported by Inoue et al. (2016) after feeding *C. macropomum* fingerlings with diets supplemented with garlic paste for 45 days.

The improvement of the health status of fish following garlic administration is mainly attributed to allicin (Khalil et al. 2001). Although there is no clear explanation on the mode of action of these compounds, the assumption is that they improve the overall health of the animal by promoting the performance of the intestinal flora, which then translates into improved feed digestion, nutrient absorption, energy utilization and growth (Diab et al. 2008; Büyükdeveci et al. 2018). In addition, since garlic contains other valuable compounds ranging from nutritional compounds (free amino acids,

minerals, oil, vitamins) (Joo et al. 2013; Santhosha et al. 2013) to anti-nutritional factors (polysaccharides, flavonoids, saponins) (Lanzotti et al. 2014; Pan and Wu 2014), more studies are required to establish the effects of different compounds of garlic on the health of different fish species.

In aquaculture research, fitness and quality of animals supplemented with medicinal herbs is tested by subjecting them to stressors such as manipulated water quality parameters, because fish can be sensitive to a sudden change in water quality (Lin and Chen 2008; Ndubuisi et al. 2015). For instance, a change in the water pH (low or high) may interfere with the osmoregulation and respiration in the fish, which may be stressful and deadly to the fish (Laurent et al. 2000). Khan et al. (2018) reported that *L. rohita* fingerlings that were fed a diet supplemented with muli bamboo (*Melocanna baccifera*) leave extracts had a higher resistance against low pH compared to the unsupplemented fish. The same phenomenon was reported in *O. mykiss* (Fazlolahzadeh et al. 2011), and *R. rutilus* (Ghehdarijani et al. 2016) after being fed with *A. sativum* supplemented diets, and subjected to high temperature and salinity, respectively. Therefore, these findings encourage for more studies to investigate the resistance effects of herbal extracts in fish against poor water quality parameters, especially in the Namibian aquaculture where water quality is one of the main causes of fish mortality.

Fish have also been exposed to pathogenic bacteria (Manaf et al. 2016; Zanzuzo et al. 2017), transport stress (Zanzuzo et al. 2012), overcrowding (Xie et al. 2008), and parasites (Fridman et al. 2014; Hyun et al. 2019). Herbal extracts supplemented fish have been presented to show high resistance against physiological stress when compared

to unsupplemented ones. Studies on resistance of fish against stress include measuring of the stress and immune parameters, and survival rate during pre and post-challenge. For example, Sahu et al. (2007) reported that garlic extracts enhanced immune response (increased WBC, serum total protein, albumin and globulin), reduced stress (lower glucose level), and increased survivability of *L. rohita* after *A. hydrophila* challenge (Table 2.3). Similarly, introducing the *A. hydrophila* had no adverse effects in *O. mykiss* (Nya and Austin 2009, 2011), *O. niloticus* (Aly and Mohamed 2009), and *C. gariepinus* (Thanikachalam et al. 2010) supplemented with garlic. Garlic extracts in the diet were reported to reduce mortality and enhance immune response in *L. calcarifer* (Talpur and Ikhwanuddin 2012), and in *L. vannamei* (Huang et al. 2018) when challenged with a pathogenic bacterium, *Vibrio harveyi*. Meanwhile, hepatoprotective effects (lower AST and ALT) of garlic were reported in *Clarias lazera* (Al-Shalahy 2002), *H. huso* (Kanani et al. 2014), and *S. hasta* (Jahanjoo et al. 2018).

Garlic has also been reported to treat a number of parasitic infections in aquaculture. Lower infestation of *Cyrtocaryon irritans* parasite was reported in *Poecilia reticulata* after garlic administration (Hyun et al. 2019). Similarly, garlic reduced infestation of *Anacanthorus spathulatus* parasite in *C. macropomum* (Inoue et al. 2016). Garlic extracts also reportedly reduced infestation of *Gyrodactylus turnbulli*, *Dactylogyrus sp.* in *P. reticulata* (Schelkle et al. 2013; Fridman et al. 2014), and *Neobenedenia sp.* parasite infestation in *L. calcarifer* (Militz et al. 2013). Thus, it appears that garlic (*A. sativum*) has the ability to increase resistance in fish against several stressors associated with culture systems.

## 2.4 The medicinal use of *Aloe vera*

*Aloe vera* synonym *Aloe barbadensis* Miller is a stemless, drought-resistant cactus-like herb, which is one of the > 400 species in the genus *Aloe* belonging to the Liliaceae family that is indigenous to South Africa, but now is found widely distributed in hot and dry climate regions of the world (Reynold and Dweck 1999). Among *Aloe* species, *A. vera* is the most researched, commercially important *Aloe* species, and used as a traditional medicine of many nations across the globe (Foster and Samman 2011; Maan et al. 2018).

*Aloe vera* is mainly characterized by its high water content (99 - 99.5%) (Hamman 2008), while the remaining 0.5 - 1.0% content contains about 70 biologically active compounds such as water-soluble and fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenol compounds, and organic acids (Gupta and Malhotra 2012; Radha and Laxmipriya 2015). These bioactive compounds are found either in the gel (inner transparent mucilaginous jelly like tissues), latex (middle layer) and/or rind (outer thick layer) part of the *A. vera* leaf (Figure 2.4). Active ingredients in *A. vera* are found in the gel such as the polysaccharides (glucomannans, xylose, rhamnose, galactose and arabinose) vitamins (A, C, E, B1, B2, B12, niacin, choline and folic acid), minerals (Potassium, chloride, sodium, calcium, magnesium, copper, zinc, chromium and iron), enzymes (catalase, amylase, oxidase, cellulase, lipase and carboxypeptidase), amino acids, and anthraquinones (aloin A and aloin B) (Ahlawat and Khatkar 2011; Radha and Laxmipriya 2015). Anthraquinones can be found in the latex part of the leaf (Hamman 2008). *A. vera*'s rich composition justifies its widely acclaimed pharmacological properties such as anti-inflammatory properties (Hutter et al.

1996), antioxidant properties, immune boosting properties (Im et al. 2005; Tai-Ni et al. 2005; Budai et al. 2013), wound healing properties (Reynold and Dweck 1999; Tarameshloo et al. 2012), intestinal absorption enhancement (Carrien et al. 2013) and hepatoprotective effects (Rahimifard et al. 2013).

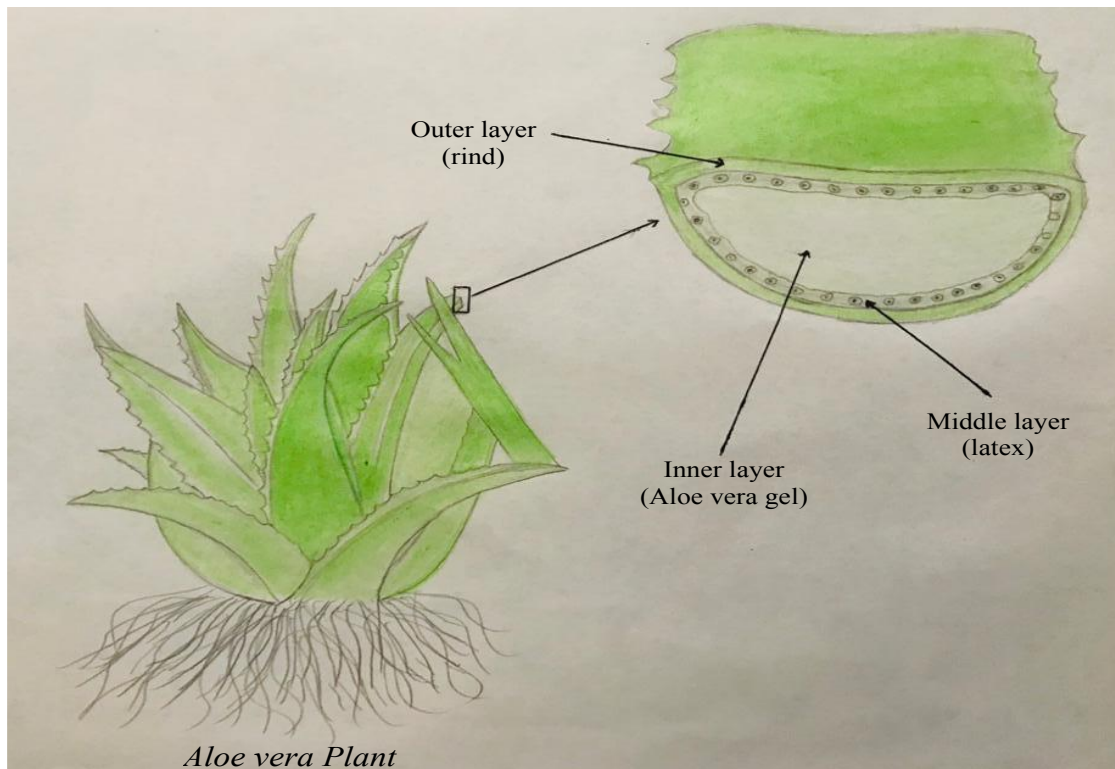


**Figure 2.4** *Aloe vera* plant and its leaf cross-sectional view adapted from Boudreau and Beland (2006).

**Table 2.4** Bioactive ingredients in the *Aloe vera* leaf gel and latex, adapted from Gupta and Malhotra (2012).

| Class                                                                                                | Compounds                                                                                                                                                                                                         |
|------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Vitamins B1, B2, B6, C, A ( $\beta\beta$ -carotene), choline, folic acid, $\alpha\alpha$ -tocopherol |                                                                                                                                                                                                                   |
| Enzymes                                                                                              | Alkaline phosphatase, amylase, carboxypeptidase, catalase, bradikininase, cyclooxygenase, peroxidase, carboxy-peptidase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, superoxide dismutase.  |
| Anthraquinones / Anthrones                                                                           | Aloe-emodin, aloetic-acid, anthranol, aloin A and B (together known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid.                                                                                  |
| Inorganic compounds                                                                                  | Calcium, chlorine, chromium, copper, iron, magnesium, manganese, selenium, zinc, potassium, phosphorus, and sodium.                                                                                               |
| Carbohydrates                                                                                        | Pure mannan, acetylated mannan, acetylated glucomannan (acemannan), galactan, Glucogalactomannan, galactogalacturan, galactoglucoarabinomannan, arabinogalactan, pectic substance, xylan, cellulose.              |
| Saccharides                                                                                          | Mannose, glucose, L-rhamnose, aldopentose.                                                                                                                                                                        |
| Organic compounds and lipids                                                                         | Arachidonic acid, $\gamma\gamma$ -linolenic acid, steroids (campesterol, cholesterol, $\beta\beta$ -sitosterol), triterpenoid, triglycerides, gibberellin, lignins, potassium sorbate, salicylic acid, uric acid. |
| Chromones                                                                                            | 8-C-glucosyl-(2'-O-cinnamoyl)-7-O-methylaloeol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-noreugenin, isoaloesin D, isorabaichromone.                                                                              |
| Non-essential amino acids                                                                            | Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine.                                                                                                         |
| Essential amino acids                                                                                | Lysine, methionine, phenylalanine, proline, threonine, and valine.                                                                                                                                                |

## 2.5 Previous studies on *Aloe vera* extracts in aquaculture

In humans, *A. vera* research has already moved from the experimental level to the application level (i.e. *A. vera* is used in cosmetics, immunostimulants and food additives). In agriculture, including aquaculture, *A. vera* research has not yet fully transcended into the applications. In fact, a study by Kim et al. (1999) on the nonspecific immune response and disease resistance effects of *A. vera* in rockfish (*Sebastes schlegeli*) is the first in testing the effects of *A. vera* in aquaculture. Research interest on *A. vera* in aquaculture only increased in the late 2000s. Several studies have investigated different *A. vera* extracts such as crude extracts powder (Zanuzzo et al. 2012, 2015a, 2015b; Gabriel et al. 2015a, 2015b), gel (Golestan et al. 2015; Soltanizadeh and Mousavinejad 2015; Mehrabi et al. 2019), solvent extracts (Mahdavi et al. 2013), nanoparticles (Sharif et al. 2017), and aloin (Srivastava et al. 2018) in fish for different purposes. All these studies concluded that *A. vera* used as a medicinal herb has the potential to promote growth, immunity, disease resistance, anti-oxidation and anti-stress in fish, and can be used as a preservative and as a sex reversal agent in aquaculture. However, there is still little information regarding the application of *A. vera* as a medicinal herb in aquaculture, for *C. gariepinus* culture, and no herbal feed additives tested in aquaculture in Namibia has been reported.

### 2.5.1 *Aloe vera* effects on fish growth and feed utilization parameters

Several studies have reported the beneficial effects of different *A. vera* extracts on growth and feed utilization efficiency in various fish species (see Table 2.5). *A. vera* leaf paste (10 g/kg diet) significantly improved growth performance parameters i.e. FW, WG, SGR as well as feed utilization indices (FCR, PER, and apparent net protein

utilization, ANPU) in *C. gariepinus* fingerling after 84 days of administration (Adegbesan et al. 2018). A significant improvement in the same parameters was reported in *C. carpio* juveniles after being fed a diet supplemented with ethanolic extracts of *A. vera* (25 g/kg diet) for a period of 56 days (Mahdavi et al. 2013). Alishahi and Abdy (2013) reported a significant improvement in growth and feed utilization parameters after feeding *C. carpio* juveniles with a diet supplemented with *A. vera* gel (5 g/kg). *A. vera* gel (1.0 to 10 g/kg diet) also increased growth and feed utilization indices in *O. mykiss* juveniles after 42 days of administration (Heidarieh et al. 2013). Diets supplemented with *A. vera* (10 to 20 g/kg diet) also reported to have significantly improved growth and feed utilization efficiency in Genetically Improved Farmed Tilapia (GIFT), *O. niloticus* (Gabriel et al. 2015a). This indicates that *A. vera* extracts could be utilized to enhance growth and production in farmed fish.

Dietary *A. vera* extracts were also been reported to exert no influence on the growth in fish. For example, Zanuzzo et al. (2015a) reported that *A. vera* crude powder supplemented diets (5 g/kg basal diet) failed to significantly increase the WG and SSI (spleen somatic index) of *O. mykiss* after 42 days of feeding. Similarly, 2 mg *A. vera* powder/kg diet had no influence on WG and SGR of goldfish (*Carassius aurata*) after 30 days administration (Palermo et al. 2013). However, these studies only used a single *A. vera* inclusion level, despite the observations that *A. vera* extracts affect the growth of fish in a dose-dependent fashion (Gabriel et al. 2015a; Sharif et al. 2017; Mehrabi et al. 2019). The duration of the experiment, 30 days as designed by Palermo et al. (2013) could be another shortfall because the minimum duration of rearing fish with the aim to understand the growth performance effects of a feed ingredient is about 60 days (Jobling

2012). Thus, adequate rearing periods, and multiple dietary inclusion levels are some of the factors to be considered in optimizing herbal extracts as growth promoters in aquaculture.

Improved growth in fish following *A. vera* supplementation could be a result of several factors, including the compounds present in the leaves of the plant such as the complex polysaccharides and the phenolic compounds (anti-nutritional) (Hamman 2008; Radha and Laxmipriya 2015). Growth-promoting effects of medicinal herbal extracts in fish have been mainly attributed to their polysaccharides (Chen et al. 2003; Tremaroli and Backed 2012; Zahran et al. 2014). Polysaccharides possess the ability to sustain the homeostasis of the fish gut microbial community as well as their health (Tremaroli and Backed 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 subsequently improves feed digestibility and availability of nutrients from feedstuffs, and shortens the feed transit time, which might have a beneficial influence on digestive enzymes (Patel and Srinivasan 2004) and also minimizes the amount of feed substrate available for proliferation of pathogenic bacteria (Citarasu 2010). In support of this premise, Gabriel et al. (2017) showed that 100% *A. vera* extracts significantly increased amylase, trypsin and lipase activities in GIFT-tilapia. To strengthen the conclusion that the growth promoting effects of *A. vera* are attributed to their polysaccharides, there is a need to further study these type of *A. vera* extracts in farmed fish, similar to the reported *Astragalus* polysaccharides growth effects in *O. niloticus* (Ardo et al. 2008; Zahran et al. 2014). This also formed the basis of the present study.

**Table 2.5** The effects of orally administered *A. vera* extracts on fish growth performance and feed utilization indices in aquaculture.

| Types of <i>A. vera</i> extracts | Dosages (g/kg diet) | Optimum dosage/s | Experiment duration | Growth/ Feed utilization                                                 | Fish species                                | initial wt.(g) | References                                     |
|----------------------------------|---------------------|------------------|---------------------|--------------------------------------------------------------------------|---------------------------------------------|----------------|------------------------------------------------|
| Gel                              | 0.1, 1.0, 10,       | 1.0- 10          | 42 days             | SGR (>), WG (>), IVL (>)<br>IVW (>), FCR (<)                             | <i>O. mykiss</i>                            | 50.3           | Heidarieh et al. (2013)                        |
| Ethanollic extracts              | 1, 5, 25            | 5-25             | 56 days             | FW (>), SGR (>), FL (>)<br>PE (>), FCR (<), FCE (>)                      | <i>C. carpio</i>                            | 29.74          | Mahdavi et al. (2013)                          |
| Gel                              | 0.5, 1.0, 2.0       | N/A              | 56 days             | FW (=), SGR (=), WG (=)<br>RGR (=), CF (=), FCR (=)                      | <i>O. mykiss</i>                            | 9.5            | Golestan et al. (2015)                         |
| Gel                              | 1, 5, 10            | 5                | 60 days             | GR (>), SGR (>), FCR (<)<br>FW (>), SGR (>), WG (>)                      | <i>C. carpio</i>                            | 45             | Alishahi and Abdy (2013)                       |
| Leave paste                      | 10, 20, 30          | 10               | 84 days             | FI (>), FCR (>), PER (<)<br>ANPU (>)                                     | <i>C. gariepinus</i>                        | 2.33           | Adegbesan et al. (2018)                        |
| Powder Nanoparticles             | 5, 10, 15           | N/A<br>10-15     | 42 days<br>60 days  | WG (=), SSI (=),<br>FW (>), FL (>), WG (>),<br>SGR (>), FCR (<), PER (>) | <i>O. mykiss</i><br><i>Acipenser baerii</i> | 133.9<br>10.95 | Zanuzzo et al. (2015a)<br>Sharif et al. (2017) |
| Aqueous crude extracts           | 5, 10, 15           | 15               | 56 days             | WG (>), SGR (>), FCR (>)                                                 | <i>O. mykiss</i>                            | 10.89          | Mehrabi et al. (2019)                          |
| Powder                           | 2mg/g diet (2%)     | N/A              | 30 days             | WG (=), SGR (=)                                                          | <i>C. auratus</i>                           | 4-7cm length   | Palermo et al. (2013)                          |
| Powder                           | 5, 10, 20, 40       | 10-20            | 60 days             | WG (>), SGR (>), AGR (>)<br>FI (<), VSI (=), HSI (=)<br>FCR (<), FER (>) | GIFT- <i>O. niloticus</i>                   | 4.83           | Gabriel et al. (2015a)                         |

Notes: SGR: Specific growth rate; WG: Weight gain; FL: Fish length; IVL: Intestinal villus length; FCE: Feed conversion efficiency; RGR: Relative growth rate; CF: condition factor; VSI: Viscerosomatic index; HSI: Hepatosomatic index; wt: Weight; FI: feed intake; FW: Final weight; ANPU: Apparent net protein utilization protein; PER: Protein efficiency ratio; FCR: Feed conversion ratio; FI: Feed intake; FER: Feed efficiency ratio; (-): Not available; (>): Significantly increased; (<): Significantly decreased; (=): Not affected.

### 2.5.2 *Aloe vera* effects on fish haemato-biochemical indices (Table 2.6)

In addition to promoting growth, different *A. vera* extracts were reported to significantly improve immune indices and to a certain extent improve specific immune responses in fish (Table 2.6). Mehrabi et al. (2019) reported that dietary *A. vera* extracts were able to significantly increase haematological parameters (RBC, WBC, Hct, and Hb) as well as some blood serum elements (the total protein, albumin, globulin, respiratory burst activity, and lysozymes), and complement system activity in *O. mykiss* fingerlings after being fed these extracts for 56 days. Aloin (*A. vera* extract) was reported to significantly improve innate immune response (increased lysozyme activity), and antioxidant activity (increased catalase activity) in *L. rohita* seven days after a 1 mg aloin/kg fish body weight injection. *A. vera* extracts such as emodin in *L. rohita* (Devi et al. 2019), aqueous extracts in *C. carpio* (Alishahi et al. 2010), and pacu (*Piaractus mesopotamus*) (Zanuzzo et al. 2017), crude powder in *O. mykiss* (Zanuzzo et al. 2015a, 2015b), *C. auratus* (Palermo et al. 2013) and GIFT-*O. niloticus* (Gabriel et al. 2015a, 2015b) were also reported to significantly improve the overall health status of each species, respectively. Thus, *A. vera* extracts have the potential to be used as immuno-stimulants in aquaculture.

The enhancement of haematological indices in fish following supplementation of *A. vera* extracts in previous studies signifies the ability of *A. vera* to stimulate erythropoiesis (generation of mature red blood cells), hence increase the oxygen carrying capacity and strengthen the body to better tolerate physiological stress. The erythropoietin effects of *A. vera* extracts in haematopoietic cells of bone marrow have been reported by Iji et al. (2010). The assumption is that these effects could be due to vitamins (beta carotene, C,

E, B<sub>12</sub>, riboflavin, thiamine, and folic acid), minerals (calcium, chromium, copper, selenium, manganese, potassium, sodium, and zinc), essential and non-essential amino acids present in *A. vera* that are essential for the synthesis of haemoglobin as demonstrated by Kayode (2017). Erythropoiesis and leukopoiesis (formation of white blood cells in bone marrow) has also been attributed to polysaccharides present in *A. vera* leaves by Ni et al. (2004), Chow et al. (2005), Im et al. (2005), and Hamman (2008). Some immuno-modulating effects were linked to lectins, which are glycoproteins found in *A. vera* gel (Reynold and Dweck 1999). In addition to the innate immune responses, *A. vera* extracts have been also reported to evoke specific immune responses in fish. For instance, Alishahi et al. (2010) reported that 0.5% dietary *A. vera* increased serum bactericidal activity and immunoglobulin M (IgM) antibody levels in *C. carpio* infected with *A. hydrophila*. All of these studies are indicators that dietary supplementation of *A. vera* extracts can improve the health status of fish, and as a result, produce animals with high resistance against physiological stresses.

*Aloe vera* has been reported to increase the resistance of fish against different physical and biological stressors. For instance, *A. vera* (0.2 mg/L of water) was reported to significantly increase the innate immune response (respiratory burst activity) post transport stress in *B. amazonicus* (Zanuzzo et al. 2012). Gabriel et al. (2015a) reported that GIFT *O. niloticus* juveniles fed a diet supplemented with *A. vera* powder presented a significantly lower glucose level, cortisol level and neutrophil/lymphocyte ratio than unsupplemented fish during a pathogenic bacterium (*Streptococcus inae*) challenge. The same was reported when *A. vera* supplemented *Oreochromis sp.* (Manaf et al. 2016), and *P. mesopotamicus* (Zanuzzo et al. 2017) were subjected to pathogenic bacteria, *S.*

*agalactiae*, and *A. hydrophila*, respectively. Dietary *A. vera* supplementation also increased the survival probability in *C. carpio* (Abdy et al. 2017), and *O. mykiss* (Heidarieh et al. 2013; Mehrabi et al. 2019), post *A. hydrophila*, *S. agalactiae*, and *Saprolegnia parasitica* (parasite) exposure. Indeed, this is an indication that *A. vera* has the ability to prevent disease outbreaks and prevent negative responses of fish to other types of stresses in aquaculture.

On the other hand, medicinal herbs have been reported to be harmful to fish and even deadly at high dosages (Palanisamy et al. 2011). Anaemia and tissue necrosis are some of the negative effects of *A. vera* so far reported in fish following dietary supplementation by Gabriel et al. (2015a) and Taiwo et al. (2005), respectively. Spermatogenic dysfunction, decreased central nervous system activity, and a reduced red blood cell count was observed in mice supplemented with *A. vera* extracts (Boudreau et al. 2013). The side effects of herbal extracts such as anaemia in animals was assumed to be a result of their ability to disrupt erythropoiesis, haemosynthesis and osmoregulation by Cope (2005). *A. vera* adverse effects such as haematuria (presence of red blood cells in the urine), metabolic acidosis (acid-base imbalance in the blood), malabsorption (inability to absorb nutrients and fluids), and electrolyte disturbance (mineral imbalance) in animals have also been reported (Mulle-Lissner 1993; Beuers 1991). These negative effects may explain the poor haemato-biochemical parameters and decrease in growth as observed in some of the previous studies (Boudreau et al. 2013; Gabriel et al. 2015a; Zanuzzo et al. 2015a; Adegbesan et al. 2018) (Table 2.5, 2.6). Hence, an upper limit in dosage of *A. vera* supplementation is crucial in enhancing immune responses as well as resistance against physiological stressors.

**Table 2.6** Effects of *A. vera* extracts on haemato-biochemical indices of some of the farmed fish species.

| Aloe products | Delivery  | Dosages (g/kg diet)                               | Duration of exposure | Haemato-biochemical indices           | Fish species                  | References               |
|---------------|-----------|---------------------------------------------------|----------------------|---------------------------------------|-------------------------------|--------------------------|
| Gel           | oral      | 0.5, 1.0, 2.0                                     | 56 days              | FRA (>), MDA (<)                      | <i>O. mykiss</i>              | Golestani et al. (2015)  |
| Crude powder  | immersion | 0.02, 0.2, 2.0 mg/L                               | 9hrs                 | Glu. (=), RBA (>),                    | <i>B. amazonicus</i>          | Zanuzzo et al. (2012)    |
| Gel           | oral      | 1.0, 5.0, 10.0                                    | 60 days              | SBA (<), NBT (>), CSA, TP (>)         | <i>C. carpio</i>              | Alishahi and Abdy (2013) |
|               |           |                                                   |                      | IgM (>), PCV (>), Hb (>), MCV (=)     |                               |                          |
|               |           |                                                   |                      | MCHC (=), MCH (=), RBC (>), HETR (=)  |                               |                          |
|               |           |                                                   |                      | LYMP (=), MON (=), EOS (=), LYZ (>)   |                               |                          |
|               |           |                                                   |                      | PCV (>), Hb (>), RBC (>), MCH (=)     |                               |                          |
| Leave paste   | oral      | 10.0, 20.0, 30.0                                  | 84 days              | MCV (=), MCHC (=), WBC (>)            | <i>C. gariepinus</i>          | Adegbesan et al. (2018)  |
|               |           |                                                   |                      | NEU (<), LYMP. (>), EOS (<)           |                               |                          |
|               |           |                                                   |                      | MON (=), BASO (>).                    |                               |                          |
|               |           |                                                   |                      | ACH50 (>), LYZ (>), BCIDAL (<)        |                               |                          |
| Gel           | injection | 100ul added 5ml TSB<br>0.1ml injected into a fish | 42 days              |                                       | <i>C. carpio</i>              | Aby et al. (2017)        |
| Gel           | oral      | 5.0                                               | 56 days              | CL (>), PHAGO (>)                     | <i>Paralichthys olivaceus</i> | Kim et al. (2002)        |
| Powder        | oral      | 5.0                                               | 42 days              | RBA (=), LYZ (=), CSA (=)             | <i>O. mykiss</i>              | Zanuzzo et al. (2015a)   |
|               |           | 4.0                                               | 28 days              | MR (=), MO2 (8% lower), CORT (=)      |                               | Zanuzzo et al. (2015b)   |
| Aloin         | injection | 1mg/body wt.                                      | 7 days               | LYZ (>), Protease (>), CARBOX. (>)    | <i>L. rohita</i>              | Srivastava et al. (2018) |
|               |           |                                                   |                      | ALP (>) AP (>)                        |                               |                          |
|               |           |                                                   |                      | CAT (>), PEROX (>)                    |                               |                          |
| Aqueous       | oral      | 0.5, 1.0, 2.0                                     | 10 days              | RBA (>), Glu. (<), NBT (>), LYZ (>)   | <i>P. mesopotamicus</i>       | Zanuzzo et al. (2017)    |
| Extracts      |           | (w/w)                                             |                      | CSA (>)                               |                               |                          |
| Aqueous       | oral      | 5.0                                               | 42 days              | WBC (>), IgM (>), LYZ (>), BCIDAL (>) | <i>C. carpio</i>              | Alishahi et al. (2010)   |
| Extracts      |           |                                                   |                      | RBC (=), PVC (=), CSA (=)             |                               |                          |
| Aloe-emodin   | oral      | 0.001, 0.005, 0.01,                               | days interval        | ALB (>), GLOB (>), PHAGO (>), RBA (>) | <i>L. rohita</i>              | Devi et al. (2019)       |
|               |           | (mg/kg diet)                                      | (14, 28, 42, 56)     | CC3 (>), Lzy. Act. (>)                |                               |                          |
| Aqueous       | oral      | 5, 10, 15                                         | 56 days              | RBC (=), WBC (>), Hct (>), Hb (>)     | <i>O. mykiss</i>              | Mehrabi et al. (2019)    |
| Extracts      |           |                                                   |                      | TP (>), ALB (>), GLOB (>), RBA (>)    |                               |                          |
|               |           |                                                   |                      | LYZ (>), CSA (>).                     |                               |                          |
| Crude powder  | oral      | 2mg/g diet                                        | 30 days              | CHOL (<), LDL (=), HDL (=), TG (=)    | <i>C. auratus</i>             | Palermo et al. (2013)    |
| Crude powder  | oral      | 5, 10, 20, 40                                     | 60 days              | WBC (>), RBC (>), Hb (>), Hct (>),    | <i>O. niloticus</i>           | Gabriel et al. (2015a)   |
|               |           |                                                   |                      | MON (=), LYMP (<), NEU (>), EOS (>).  |                               |                          |

| Crude powder                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | oral | 5, 10, 20, 40 | 60 days | TCHO (<), TG (<), LDL (=), HDL (>)<br>CAT (>), GSH-Px (>), SOD (=), | <i>O. niloticus</i> | Gabriel et al. (2015b) |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|---------------|---------|---------------------------------------------------------------------|---------------------|------------------------|
| Notes: FRA: Ferric reducing ability; CAT: Catalase; MDA: Malondialdehyde; SBA: Serum bactericidal; CL: Chemiluminescent response; CORT: Cortisol; TG: Triglycerides; TCHO: Total cholesterol; CHOL: Cholesterol; NBT: Nitroblue tetrazolium; CSA: Complement system activity; Hct: Hematocrit; Hb: Hemoglobin; WBC: White blood cells; MCV: Mean corpuscular volume; SOD: Superoxide dismutase; LYZ: Lysozyme activity; GSH-Px: Glutathione peroxidase; Glu: Glucose; MR: Metabolic rate; MO <sub>2</sub> : Oxygen consumption; HDL: High-density lipoprotein; LDL = Low-density lipoproteins; PEROX = Peroxidase; CARBOX = Carboxylesterase; ALP = Alkaline phosphatase; AP = Acid phosphatase; IgM = Immunoglobulin M; PCV: Packed cell volume; HETR: Heterophil; ACH50: Serum alternative complement activity; TP: Total protein; CC3: Complement C3; ALB: Albumin; GLOB: Globulin; RBC: Red blood cells; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentrate; NEU: Neutrophils; LYMP: Lymphocytes; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PHAGO: Phagocytic activity; RBA: Respiratory burst activity; MON: Monocytes; (>): Significantly increased; (<): Significantly decreased; (=): Not affected; BASO: Basophils. |      |               |         |                                                                     |                     |                        |

## 2.6 Gaps in the existing knowledge and the way forward

This review has provided substantial evidence that garlic (*A. sativum*) and *A. vera* possess the ability to promote growth, feed utilization, overall health and resistance against different types of stressors in farmed fish. However, *A. sativum* and *A. vera* were only studied in some fish species, and sometimes inadequate doses or time periods were tested. Studies on the effects of these herbs on African catfish, *C. gariepinus* are rarely reported. For example, this is the first study to report the resistance response of *C. gariepinus* against low water pH following *A. vera* and *A. sativum* extracts dietary supplementation (separately and in mixture).

These herbs contain several beneficial nutrients, and it is therefore important to isolate (purify), characterize, and quantify the effective compounds for easy optimization as recommended by Bulfon et al. (2013). There is a lack of knowledge on the toxicological reports, stability of the herbal extracts in the aquatic environment, digestibility in fish and product quality, which would also assist in determining the optimum inclusion levels of the herbs in question. Furthermore, the explanation of the true mechanisms of the activity of herbal extracts in aquatic animals is lacking, because fish have different mechanisms for the metabolism and immunization of these compounds (Kong et al. 2007). Thus more studies including nutrigenomic and metabolomic studies (*in vivo* and *in vitro*) are required to optimize and safely apply herbal products (*A. sativum* and *A. vera*) in fish farming.

From this review, crude extracts of *A. vera* or *A. sativum* were the most studied types of extracts used as supplement for fish, but little has been reported for African catfish, *C.*

*gariepinus* (Thanikachalam et al. 2010; Eina-Liza et al. 2016; Adegbesan et al. 2018). It is for this reason that the current study was designed to investigate the effects of dietary *Aloe vera* and garlic (*A. sativum*) crude polysaccharide extracts on the growth performance, feed utilization, whole body composition, haematological parameters and the resistance against low water pH in African catfish, *C. gariepinus*.

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**CHAPTER THREE: EFFECT OF DIETARY *ALOE VERA* CRUDE  
POLYSACCHARIDES SUPPLEMENTATION ON GROWTH  
PERFORMANCE, FEED UTILIZATION, HAEMATO-  
BIOCHEMICAL PARAMETERS, AND SURVIVAL AT LOW PH IN  
AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FINGERLINGS**

**Abstract**

This chapter evaluated the effects of dietary *Aloe vera* crude polysaccharides on growth performance, feed utilization, haemato-biochemical parameters and resistance against low water pH in African catfish (*Clarias gariepinus*) fingerlings. Fish (initial weight,  $3.1 \pm 0.02$  g) 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* for 60 d. Fish fed 1.0% *A. vera* had a significant increase in all growth parameters compared to unsupplemented ones ( $P < 0.05$ ). Among dietary groups, a significantly lower feed conversion ratio was presented in fish fed 1.0% ( $1.34 \pm 0.22$ ) compared to those fed 4.0% *A. vera* supplemented diet ( $1.99 \pm 0.278$ ) ( $P < 0.05$ ). The protein efficiency ratio was significantly higher in fish fed 1.0% *A. vera* supplemented diet ( $1.31 \pm 0.21$ ) compared to unsupplemented fish ( $0.85 \pm 0.10$ ) and those fed 4.0% *A. vera* supplemented diet ( $0.85 \pm 0.14$ ) ( $P < 0.05$ ). The optimal dietary *A. vera* polysaccharide crude extract requirement was estimated to be 1.79% ( $y = -2.778x^2 + 9.95x + 29.29$ ,  $P = 0.037$ ), and 1.77% *A. vera* ( $y = -0.04x^2 + 0.15x + 0.593$ ,  $P = 0.045$ ), for weight gain and feed efficiency ratio, respectively. Overall, *A. vera* extracts improved haemato-biochemical indices when compared to unsupplemented fish, and decreased some of the indices at the highest dietary inclusion level (4%). Fish fed 1.0%, and 2% *A. vera* supplemented fish showed a higher survival probability (above 70%) throughout the low water pH challenge (5.2 - 5.5) period than the control diet (below 70%), and those fed 4% *A. vera* supplemented diet (below 60%). Thus, *A. vera* polysaccharides are recommended to be used as feed supplements in *C. gariepinus* fingerlings culture.

**Keywords:** Aquaculture, *Claria gariepinus*, Herbs, Immunostimulants, Stress resistance.

### 3.1 Introduction

Studies on medicinal herbal extracts studies have become popular, but still novel in aquaculture and other farming sectors such as livestock and poultry, amongst others. The main purpose of these studies was usually to reduce and/or 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 these drugs are believed to be unsustainable (Reverter et al. 2014; Bulfon et al. 2015; Gabriel et al. 2015a). The application of synthetic chemicals has created substantial problems such as the 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, and appetite stimulating effects, amongst others (Abdel-Tawwab et al. 2010; Citarasu 2010; Zahra et al. 2014), when properly administered. Medicinal herbal extracts are also more easily available, less expensive, 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 in 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*, *Euphora hirta*, *Azadirachta indica*, and *Carica papaya* (Kareem et al. 2016), *Cynodon daetylon*, *Aegle marmelos*, *Withania somnifera* and *Zingber officinale* (Immanuel et al. 2009) improved growth performance of *Oreochromis niloticus* juveniles when they were administered through diets. Similar findings were reported for *Clarias gariepinus* when they were fed diets supplemented with *Allium sativum* peels (Thanikachalam et al. 2010) and or *Agaricus bisporus* (Harikrishna et al. 2018), respectively. Thus, medicinal herbal extracts certainly have the potential to replace synthetic pharmaceutical drugs, 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). Several 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<sub>12</sub> (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 dietary inclusion level enhanced the growth performances of the common carp, *Cyprinus carpio*. Gabriel et al. (2015a) reported the same in GIFT-tilapia strain, *O. niloticus* after being fed *A. vera* crude extracts. In addition, improved innate immune response after dietary *A. vera* supplementation was reported in matrinx, *Brycon amazonicus* (Zanuzzo et al. 2015b) and whiteleg shrimp, *Litopenaeus vannamei* (Trejaflores et al. 2016), and pacu, *Piaractus mesopotumicus* (Zanuzzo et al. 2017) (Tables 2.5, 2.6).

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

### **3.2 Materials and methods**

#### **3.2.1 Experimental fish and management**

The experiment was conducted at the University of Namibia's Sam Nujoma Campus, Sam Nujoma Marine and Coastal Resources Research Centre (SANUMARC) facilities in a close aerated water system between February and April 2018. Three hundred healthy African catfish fingerlings (mean body weight of  $3.1 \pm 0.02$  g) were obtained from the Onavivi Inland Aquaculture Center (OIAC), Outapi, Namibia. The fish were transported to the laboratory facilities 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 a temperature of  $28.9 \pm 0.25$  °C, a pH of  $7.4 \pm 0.32$ , and dissolved oxygen (DO) concentration of  $4.92 \pm 0.19$  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 to laboratory conditions for one week, and during this period, they were fed with the control diet (Table 3.1) until apparent satiation thrice daily (09:00; 13:00; 17:00). To maintain good water quality, two-thirds of the water in the fish holding tank was exchanged with de-chlorinated freshwater of similar temperature once during the week of acclimation.

### 3.2.2 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 fishmeal (28.5 g/kg), cowpea (22.5 g/kg), corn grain meal (8.4 g/kg), wheat flour (13.9 g/kg), pearl millet meal (22.7%), vegetable oil (3.0%), and vitamin-mineral premixes (1.0%) for the control (diet 1, without *A. vera* polysaccharide extracts) (Table 3.1). This feed formula was adapted from that of Onavivi aquaculture feed manufacturing plant. For the other groups, *A. vera* crude polysaccharides dry powder (30%) was incorporated into the control feed at 0.5% (diet 2), 1.0% (diet 3), 2.0%, (diet 4), 4.0% (diet 5) (Table 3.1). 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 two mm die, dried at room temperature for two days, and then stored in airtight plastic bags until use.

**Table 3.1** Formulation and composition of the experimental diets (g/100 g dry matter).

| Ingredients                      | Dietary groups |               |                |                |               |
|----------------------------------|----------------|---------------|----------------|----------------|---------------|
|                                  | 1              | 2             | 3              | 4              | 5             |
| Fish meal (60% CP)               | 28.50          | 28.50         | 28.50          | 28.50          | 28.50         |
| Cow peas (25% CP)                | 22.50          | 22.50         | 22.50          | 22.50          | 22.50         |
| Corn grain (10.2% CP)            | 8.40           | 8.40          | 8.40           | 8.40           | 8.40          |
| Wheat flour (11.7% CP)           | 13.90          | 13.90         | 13.90          | 13.90          | 13.90         |
| Pearl millet (12.5% CP)          | 22.70          | 22.70         | 22.70          | 22.70          | 22.70         |
| Vegetable oil                    | 3.00           | 3.00          | 3.00           | 3.00           | 3.00          |
| Vitamin premix <sup>a</sup>      | 0.50           | 0.50          | 0.50           | 0.50           | 0.50          |
| Mineral premix <sup>b</sup>      | 0.50           | 0.50          | 0.50           | 0.50           | 0.50          |
| <b>Total</b>                     | <b>100 .00</b> | <b>100.00</b> | <b>100 .00</b> | <b>100 .00</b> | <b>100.00</b> |
| <i>Aloe vera</i>                 | 0.00           | 0.50          | 1.0 0          | 2.00           | 4.00          |
| <u>Proximate composition (%)</u> |                |               |                |                |               |
| Dry matter                       | 91.67          | 91.69         | 91.69          | 91.71          | 91.73         |
| Crude protein                    | 30.60          | 30.41         | 30. 55         | 30.58          | 30.49         |
| Crude lipid                      | 4.39           | 4.41          | 4.37           | 4.35           | 4.33          |
| Ash                              | 5.25           | 5.27          | 5.27           | 5.26           | 5.27          |
| Gross energy (KJ/g diet)         | 17.36          | 17.38         | 17.40          | 17.39          | 17.40         |

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

<sup>b</sup>Mineral premix (g kg<sup>-1</sup>): KH<sub>2</sub>PO<sub>4</sub>, 502; MgSO<sub>4</sub>. 7H<sub>2</sub>O, 162; NaCl, 49.8; CaCO<sub>3</sub>, 336; Fe (II) gluconate, 10.9; MnSO<sub>4</sub>.H<sub>2</sub>O, 3.12; ZnSO<sub>4</sub>. 7H<sub>2</sub>O, 4.67; CuSO<sub>4</sub>. 5H<sub>2</sub>O, 0.62; KI, 0.16; CoCl<sub>2</sub>. 6H<sub>2</sub>O, 0.08; ammonium molybdate, 0.06; NaSeO<sub>3</sub>, 0.02.

The use of experimental fish in the investigation 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 (see Appendix D, for ethical clearance certificate). After acclimation, the experimental fish ( $3.16 \pm 0.03$  g) were randomly distributed into fifteen aquaria in five triplicated groups at a stocking density of 20 fish /aquarium ( $0.18 \text{ m}^3$ , supplied with 150 L of dechlorinated freshwater). A day after stocking, fish were hand fed with the experimental diets. Fish in dietary group 1 were fed the control diet (0% *A. vera* 30% polysaccharide powder), and others were fed *A. vera* supplemented diet (diet 2, 3, 4, and 5), three times daily (09:00; 13:00; 17:00) until apparent satiation for 60 d. Dietary *A. vera* inclusion levels used in this study were adopted from Gabriel et al. (2015a), which however used a 100% *A. vera* crude powder in the genetically improved farmed tilapia (GIFT)-strain *O. niloticus*. During the feeding trial, a continuous aeration, a photoperiod of a 12-h light/dark cycle, and water exchange (60%) twice a week were maintained. Levels of dissolved oxygen (DO) concentration, and water temperature were recorded once a day, while pH and ammonia nitrogen were recorded on a weekly basis (Eutech instruments, model PCD 650, Thermo Fisher Scientific, Singapore).

### 3.2.3 Evaluation of growth and feed utilization parameters

Growth performance indices were assessed in terms of weight gain (WG), final weight (FW) (fish body weight after 60 d), 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 consumed after 60 d), feed conversion ratio (FCR), feed efficiency ratio (FER), and protein efficiency

ratio (PER). Survival was expressed as percentage of the number of fish survived after 60 days of the feeding trial. After 60 d of feeding, 24h after the last feeding, body weight and length of all the fish in each tank were measured. Liver weight and eviscerated weights of three fish from each replicate were recorded after 60 d. For ethical reasons, before these fish were sacrificed, they were anaesthetized 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 (NRC 1993):

$$(1) WG (g) = W_2 - W_1$$

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

$$(3) AGR (gday^{-1}) = \left( \frac{W_2 - W_1}{t} \right)$$

$$(4) CF (gcm^{-3}) = \left( \frac{W}{L^3} \right) \times 100$$

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

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

$$(7) FCR = \frac{FI}{WG}$$

$$(8) FER = \frac{WG}{FI}$$

$$(9) PER = \frac{WG}{\text{crude protein intake (g)}}$$

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

Where,  $W_2$  = final body weight (g),  $W_1$  = initial body weight (g),  $t$  = trial period (d),  $W$  = body weight (g),  $WG$  = weight gain (g), and  $L$  = total body length (cm), FI = feed intake (g).

#### 3.2.4 Haematological-biochemical parameters

At the end of the experiment, 24 h after the last feeding, blood was collected from the caudal vein of three anaesthetized (with 100 mg of tricaine methanesulfonate, 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), haematocrits volume (HCT) (L/L), red blood cell distribution width (RDW) (fl/cell), mean platelet count (PLT) per L, lymphocytes per litre (LYM), monocytes per litre (MON), mean corpuscular volume (L/cell) (MCV) and granulocytes per litre (GRAN). All these were determined by Coulter principle using an automatic blood cell analyzer (HESKA veterinary hematology analyzer, New Zealand). Haemoglobin (HGB), mean corpuscular haemoglobin (fmol/cell) (MCH), and mean corpuscular haemoglobin 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 analysed 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 analyser (FDC NX 5000v v2.3) with kits supplied by DIAG Import and Export CC, South Africa. All the blood tests were carried out at Swakopmund veterinary clinic laboratory, Namibia.

### 3.2.5 Proximate body composition analysis.

Three gutted fish were collected from each replicate and stored at -20 °C for proximate composition analysis (moisture, crude protein, crude lipid, and ash). Moisture content was determined by oven drying at 105°C, until constant weight and expressed as a percentage:

$$(11) \text{ moisture (\%)} = (\text{wet weight} - \text{dry weight}) / (\text{sample weight}) \times 100.$$

Crude protein (nitrogen x 6.25) determined by the Kjeldahl method (Kjeltec 8200, Foss Analytic Co., Ltd., China) was expressed as a percentage. Crude lipid (%) was determined by ether extraction system (Foss, Soxtec, 2043, Foss Scino, Co., Ltd) and expressed as: % lipid = (weight of residue /weight of the sample taken x 100). Ash (%) was determined by burning the dry samples at 560°C for 5h.

### 3.2.6 In situ low pH challenge experiment

The optimum water pH for *C. gariepinus* range between 7 - 8, and deviation (low or high pH) may be deadly to the fish, especially the younger ones (Ndubuisi et al. 2015).

After the growth trial and blood sampling, the stocking density of each five triplicated dietary groups was adjusted to 10 fish / aquarium (0.18m<sup>3</sup>, supplied with 50 L of dechlorinated freshwater). They were then all exposed to low pH (pH 5.2 -5.5) for three days (72 h). The water pH was adjusted by adding 4N HCl and 4N NaOH, and was renewed daily, as demonstrated by Lin and Chen (2008). During this experiment pH, temperature (28 ±1.5°C), DO (> 4 mg/L), and NH<sub>3</sub>-N (> 0.08 mg/L) were monitored daily. Fish mortality was recorded at three 24 h intervals, to determine the survival (%).

### 3.2.7 Statistical analyses

Collected data were statistically analyzed statistics in SPSS® software (version 21, IMB Corp, Armonk, NY, USA). Normality and homogeneity of variance were confirmed using Kolmogorov-Smirnov and Levene's test, respectively. All recorded variables were expressed as mean ± standard error. The mean values were further subjected to one-way analysis of variance (ANOVA) to study the treatment effects at significant level of 95% ( $P = 0.05$ ). The second order polynomial regression model ( $y = b_0 + b_1x + b_2x^2$ , where,  $y$  = maximum WG or FER,  $x$  = optimum inclusion level) (Zeitoun et al. 1976) was used to estimate the optimum dietary *A. vera* polysaccharide extracts requirement in *C. gariepinus* fingerlings. Significant differences between the group means were further compared using Duncan's Multiple Range Test (DMRT). 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 in survival ( $P < 0.05$ ) between groups at each sampling interval of the pH challenge.

### 3.3 Results

#### 3.3.1 Growth performance and feed utilization parameters

Throughout the feeding trials, water temperatures ranged from 26 to 28 °C, pH values from 6.9 to 7.3, and DO concentration from 4.7 to 5.4 mg/L, and ammonia nitrogen concentration was lower than 0.05 mg/L throughout. Among dietary *A. vera* groups, fish fed 1.0% had the highest FW ( $42.48 \pm 6.47$  g), WG ( $39.44 \pm 6.47$  g), and AGR ( $0.66 \pm 0.11$  g) compared to unsupplemented ones (FW =  $28.57 \pm 3.09$  g; WG =  $25.52 \pm 3.09$ ; AGR =  $0.43 \pm 0.05$  g), and those fed 4.0% ( $P < 0.05$ ) (Figure 3.1). These parameters were intermediate in fish fed 0.5% (FW =  $40.67 \pm 1.69$  g; WG =  $37.63 \pm 1.69$  g; AGR =  $0.62 \pm 0.03$  g), and 2.0% *A. vera* polysaccharide supplemented diet (FW =  $37.13 \pm 1.34$  g; WG =  $34.08 \pm 1.34$  g; AGR =  $0.57 \pm 0.02$  g) ( $P > 0.05$ ). SGR was significantly higher in fish fed 1.0% ( $4.35 \pm 0.24\%$ ) and 0.5% *A. vera* supplemented feed ( $4.31 \pm 0.07\%$ ) when compared to the control and those fed 4.0% *A. vera* supplemented diet ( $3.69 \pm 0.23\%$ ) ( $P < 0.05$ ). An intermediate SGR ( $4.16 \pm 0.06\%$ ) response was observed in fish fed 2.0% *A. vera* supplemented feed. Dietary *A. vera* polysaccharides did not affect organo-somatic indices (HSI and VSI) or CF and survival rate ( $P > 0.05$ ) (Table 3.2).

Dietary *A. vera* polysaccharides had no significant effect on fish FI when compared to unsupplemented fish ( $P > 0.05$ ), however a significantly lower FI was recorded in fish fed 4.0% *A. vera* supplemented diet ( $48.11 \pm 0.28$  g) compared to those fed 2.0% ( $53.85 \pm 1.85$ ) ( $P < 0.05$ ) (Figure 3.2A). Among dietary groups, a significantly lower FCR was presented in fish fed 1.0% ( $1.34 \pm 0.22$ ) compared to those fed 4.0% *A. vera* supplemented feed ( $1.99 \pm 0.278$ ) ( $P < 0.05$ ); and the opposite trend was recorded for

FER (figure 3.2C). Protein efficiency ratio was significantly higher in fish supplemented with 1.0% *A. vera* supplemented feed ( $1.31 \pm 0.21$ ) compared to the control and those fed 4.0% *A. vera* supplemented feed ( $0.85 \pm 0.21$ ) ( $P < 0.05$ ). The optimum dietary *A. vera* inclusion level (%) was estimated to be 1.79% ( $y = -2.78x^2 + 9.95x$ ,  $P = 0.035$ ,  $R^2 = 0.37$ ), and 1.77 % *A. vera* ( $y = -0.043x^2 + 0.152x$ ,  $P = 0.045$ ,  $R^2 = 0.253$ ) (Figures 3.1, 3.2).

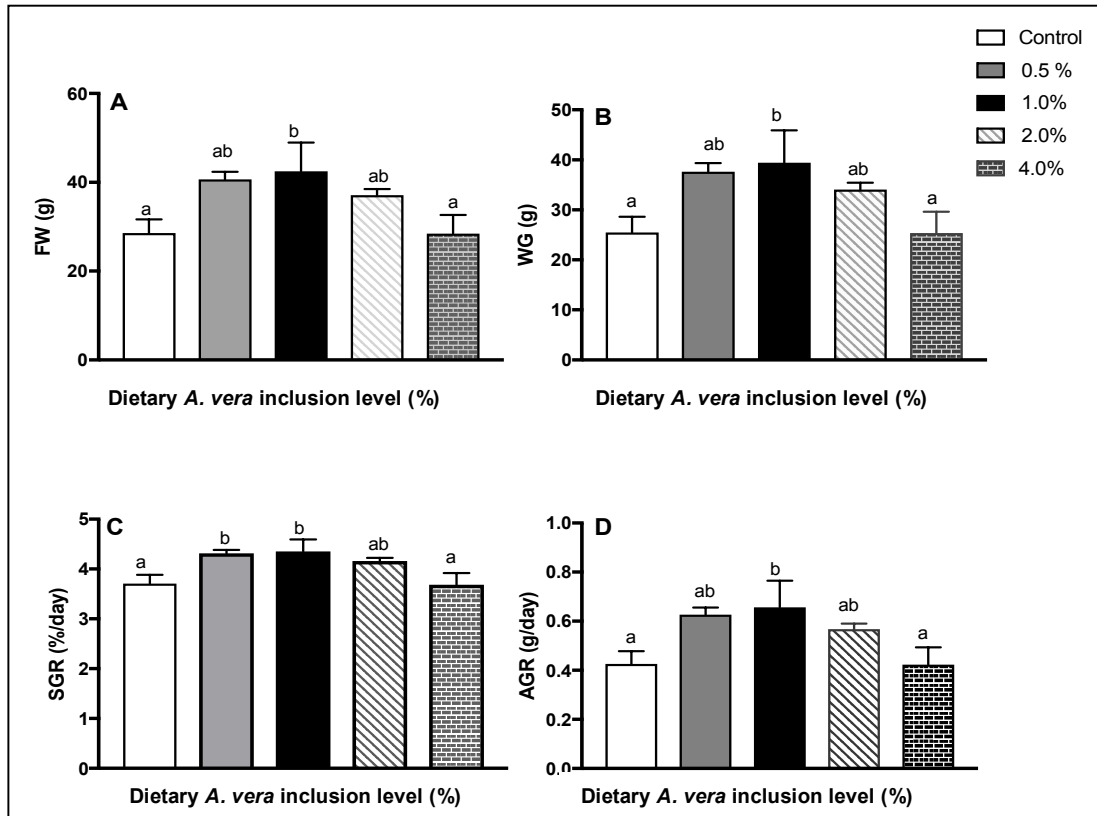


**Figure 3.1** Final weight (FW) (A), weight gain (WG) (B), specific growth rate (SGR) (C), and absolute growth rate (AGR) (D) of African catfish, *C. gariepinus* fingerlings fed four *A. vera* crude polysaccharide extracts supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference ( $P < 0.05$ ) among dietary groups; Values were expressed as mean  $\pm$  standard error; WG:  $y = -2.78x^2 + 9.95x$ ,  $P = 0.035$ ,  $R^2 = 0.37$  (second order polynomial regression model).

**Table 3.2** Organo-somatic indices, condition factor, and survival (%) of the African catfish, *C. gariepinus* fingerlings fed four *A. vera* crude polysaccharide extracts supplemented diets and a control for 60 d.

| <u>Parameters</u> | <u>Dietary <i>A. vera</i> inclusion level (%)</u> |                           |                           |                           |                           |
|-------------------|---------------------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                   | Control                                           | 0.5                       | 1.0                       | 2.0                       | 4.0                       |
| VSI               | 7.46 ± 0.75 <sup>a</sup>                          | 8.68 ± 2.86 <sup>a</sup>  | 6.57 ± 0.54 <sup>a</sup>  | 5.64 ± 0.22 <sup>a</sup>  | 8.33 ± 2.75 <sup>a</sup>  |
| HSI               | 1.71 ± 0.01 <sup>a</sup>                          | 1.50 ± 0.05 <sup>a</sup>  | 1.59 ± 0.19 <sup>a</sup>  | 1.45 ± 0.16 <sup>a</sup>  | 1.55 ± 0.16 <sup>a</sup>  |
| CF                | 0.68 ± 0.00 <sup>a</sup>                          | 0.69 ± 0.03 <sup>a</sup>  | 0.73 ± 0.17 <sup>a</sup>  | 0.70 ± 0.01 <sup>a</sup>  | 0.70 ± 0.01 <sup>a</sup>  |
| Survival          | 88.33 ± 4.41 <sup>a</sup>                         | 90.00 ± 2.89 <sup>a</sup> | 90.00 ± 2.89 <sup>a</sup> | 93.33 ± 1.67 <sup>a</sup> | 85.00 ± 2.87 <sup>a</sup> |

Data are expressed as mean ± standard error (M ± SE). Values with different superscript letters in the same row are not significantly different ( $P > 0.05$ ) from the control. Where VSI = viscerosomatic index, HSI = hepatosomatic index, and CF = condition factor.

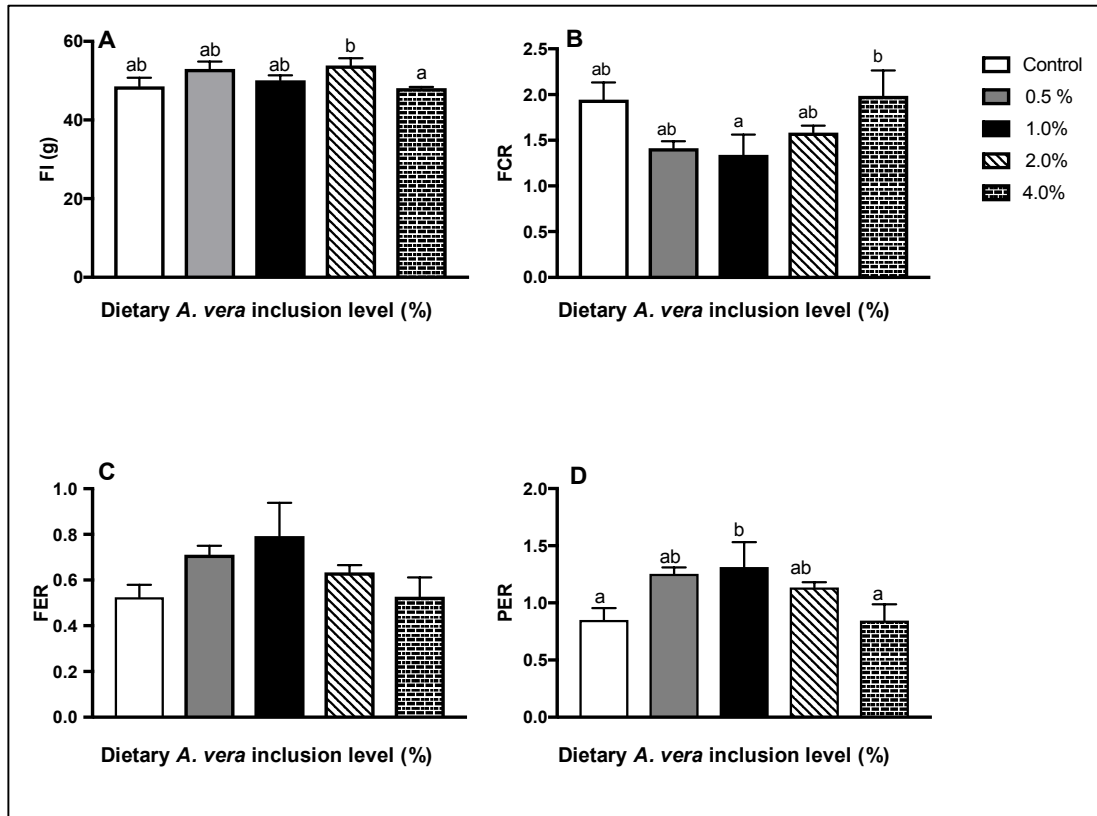


**Figure 3.2** Feed intake (FI) (A), feed conversion ratio (FCR) (B), feed efficiency ratio (FER) (C), and protein efficiency ratio (PER) (D) of the African catfish, *C. gariepinus* fingerlings fed four *A. vera* crude polysaccharide extracts supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference ( $P < 0.05$ ) among dietary groups; Values were expressed as mean  $\pm$  standard error; FER:  $y = -0.043x^2 + 0.152x$ ,  $P = 0.045$ ,  $R^2 = 0.253$  (second order polynomial regression model).

### 3.3.2 Haemato-biochemical parameters

Dietary *A. vera* polysaccharides had significant effects on some haematological parameters of African catfish fingerlings when compared to the unsupplemented ones ( $P < 0.05$ ) (Figure 3.3, 3.4, 3.5, and 3.6). No significant differences in RBC counts ( $10^{12}/L$ )

(Figure 3.3A), HCT (L/L) (Figure 3.3B), and HGB levels (g/L) (Figure 3.3C) were observed between all dietary groups ( $P > 0.05$ ). Platelet counts ( $10^9/L$ ) (Figure 3.4D) in fish fed 4.0% *A. vera* supplemented diet ( $12.17 \pm 2.08$ ) decreased significantly among dietary groups ( $P < 0.05$ ).

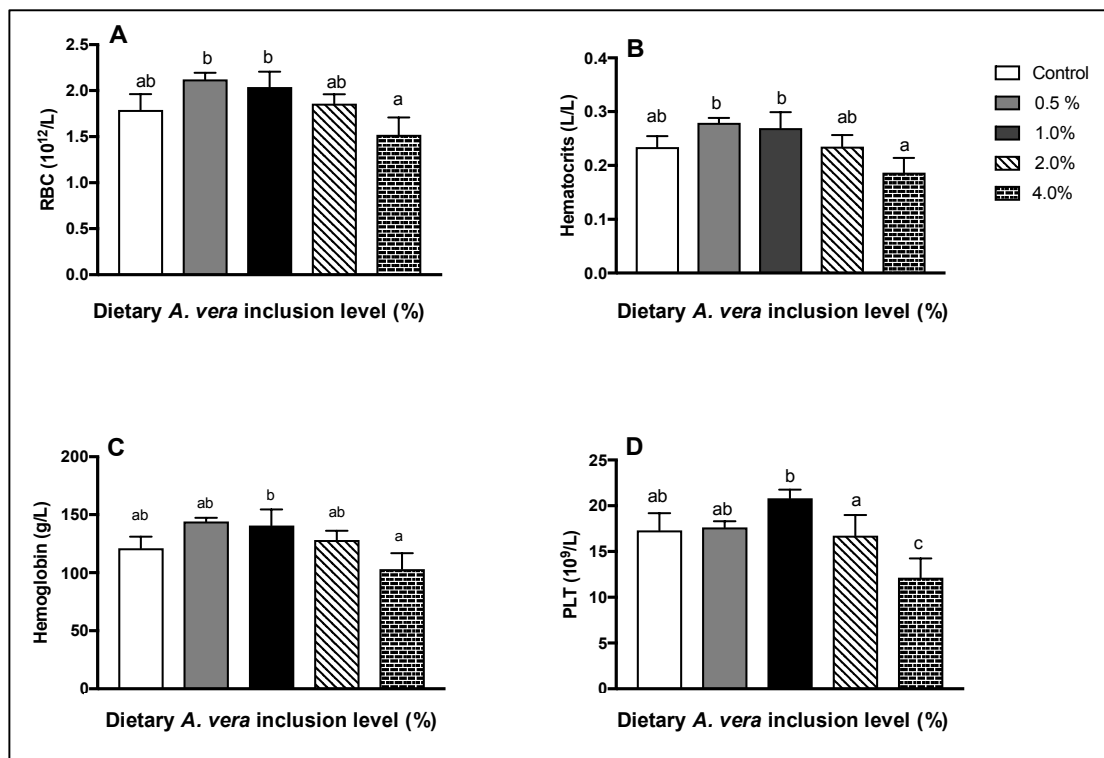


**Figure 3.3** Red blood cell counts (RBC) (A), hematocrit levels (B), Hemoglobin concentration (C), and platelet counts (PLT) (D) of African catfish, *C. gariepinus* fingerlings fed four *A. vera* crude polysaccharide extracts supplemented diets and unsupplemented diet (control) for 60 d. Different lower case letters denote a significant

difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

Mean corpuscular volume (L/cell) (Figure 3.4A) and mean corpuscular hemoglobin per cell (Figure 3.4B) showed no significant differences between feeding groups ( $P > 0.05$ ) (Figure 3.4). Mean corpuscular hemoglobin concentration was the same for the control, 0.5%, 1% and 2%, but decreased significantly in fish fed 4.0% *A. vera* supplemented diet ( $121.50 \pm 3.46$ ) when compared to those fed the control diet, 0.5% ( $131.55 \pm 1.14$ ) and 1.0% *A. vera* supplemented diet ( $131.08 \pm 3.77$ ) ( $P < 0.05$ ) (Figure 3.4C). Fish fed 4.0% ( $84.40 \pm 1.74$ ) and 2.0% *A. vera* supplemented diet ( $85.20 \pm 3.99$ ) had significantly lower red blood cell distribution width compared to the control ( $97.92 \pm 4.44$ ) ( $P < 0.05$ ) (Figure 3.4D).

White blood cell counts (Figure 3.5A), lymphocyte counts (Figure 3.5B) and monocyte counts (Figure 3.5C) all showed no significant differences dietary groups ( $P > 0.05$ ). A significant decrease in granulocyte counts ( $10^9/L$ ) was only observed in fish fed 4.0% *A. vera* supplemented diet ( $0.93 \pm 0.37$ ) when compared to the unsupplemented ones ( $2.48 \pm 0.47$ ) ( $P < 0.05$ ) (Figure 3.5D).

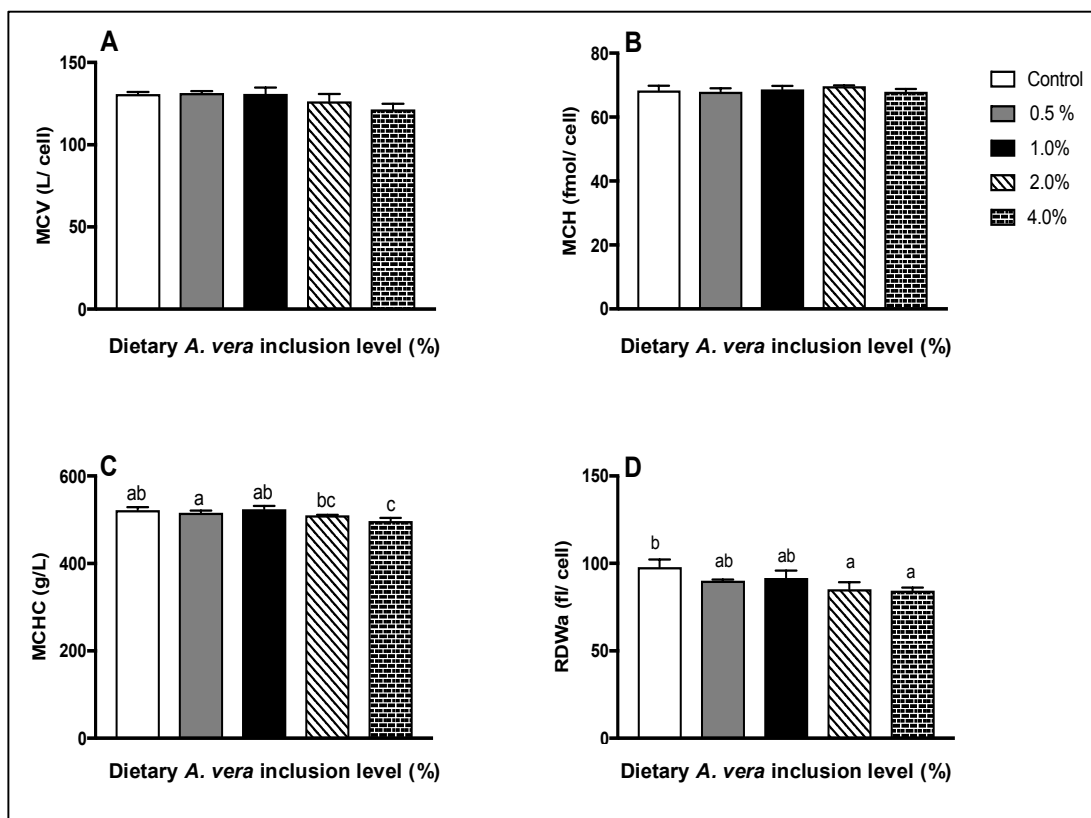


**Figure 3.4** Mean corpuscular volume (MCV) (A), mean corpuscular hemoglobin (MCH) (B), mean corpuscular hemoglobin concentration (MCHC) (C), and red blood cell distribution width (RDWc) (D) of African catfish, *C. gariepinus* fingerlings fed four *A. vera* crude polysaccharide extracts supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

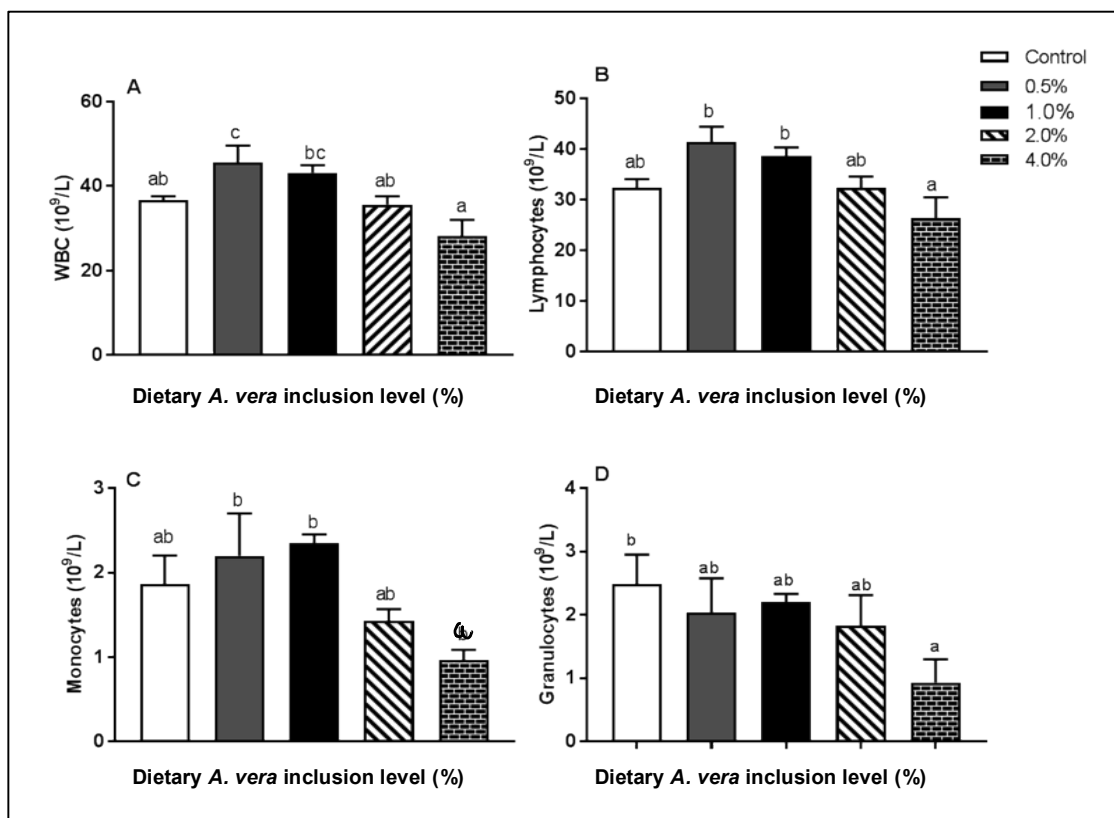


**Figure 3.5** White blood cell counts (WBC) (A), lymphocyte counts (B), monocyte counts (C), granulocyte counts (D) of African catfish, *C. gariepinus* fed four *A. vera* crude polysaccharide extracts supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

Dietary *A. vera* polysaccharides had significant effects on biochemical parameters ( $P < 0.05$ ) (Figure 3.6). AST and ALT concentrations were significantly lower in fish fed with 0.5% (AST =  $140.17 \pm 4.09$ ; ALT =  $44.83 \pm 5.52$ ) and 1.0% *A. vera* supplemented diets (AST =  $176.83 \pm 23.94$ ; ALT =  $51.33 \pm 8.29$ ) compared to the control (AST =  $483.83 \pm 90.81$ ; ALT =  $90.00 \pm 20.42$ ) and the 4% treatment group AST =  $268.50 \pm 82.77$ ; ALT =  $110.67 \pm 16.21$ ) ( $P < 0.05$ ). No significant differences in glucose levels

were observed in *A. vera* supplemented fish compared to the control ( $P > 0.05$ ). Similarly, TCHO and TG levels were not significantly different in supplemented fish when compared to unsupplemented ones ( $P > 0.05$ ).

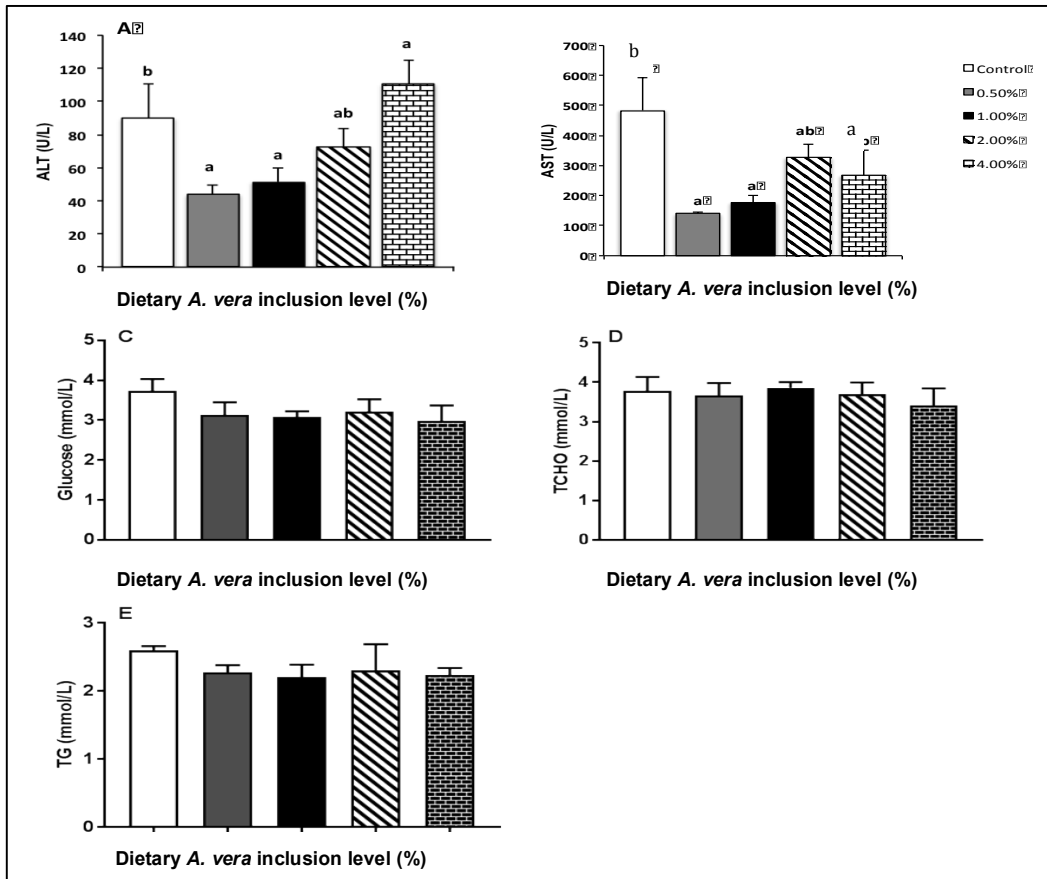


**Figure 3.6** Serum alanine aminotransferase enzyme concentration (ALT) (A), aspartate aminotransferase concentration (AST) (B), glucose level (C), total cholesterol (TCHO) (D), and triglycerol level (TG) (E) of African catfish, *C. gariepinus* fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

### 3.3.3 Proximate body composition

There was no significant difference in the protein, ash, and lipid content of fish among dietary groups ( $P > 0.05$ ) (Table 3.3).

**Table 3.3** Whole body composition parameters of African catfish, *C. gariepinus* fingerlings fed four *A. vera* 30% polysaccharide extracts supplemented diets and un-supplemented diet for 60 d.

| Parameters   | Dietary <i>A. vera</i> inclusion level (%) |                           |                           |                           |                            |
|--------------|--------------------------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
|              | Control                                    | 0.5                       | 1.0                       | 2.0                       | 4.0                        |
| Moisture (%) | 71.36 ± 0.30 <sup>a</sup>                  | 71.39 ± 0.15 <sup>a</sup> | 70.68 ± 0.59 <sup>a</sup> | 70.73 ± 0.57 <sup>a</sup> | 72.83 ± 0.17 <sup>a</sup>  |
| Protein (%)  | 73.13 ± 2.59 <sup>a</sup>                  | 74.57 ± 1.90 <sup>a</sup> | 74.55 ± 1.45 <sup>a</sup> | 76.77 ± 1.59 <sup>a</sup> | 74.97 ± 1.210 <sup>a</sup> |
| Lipid (%)    | 6.13 ± 0.80 <sup>a</sup>                   | 6.05 ± 0.53 <sup>a</sup>  | 5.93 ± 0.61 <sup>a</sup>  | 5.89 ± 0.36 <sup>a</sup>  | 5.56 ± 0.68 <sup>a</sup>   |
| Ash (%)      | 9.12 ± 1.12 <sup>a</sup>                   | 8.63 ± 1.23 <sup>a</sup>  | 8.15 ± 0.98 <sup>a</sup>  | 9.30 ± 1.10 <sup>a</sup>  | 9.24 ± 1.12 <sup>a</sup>   |

Values (Mean ± Standard Error, M±SE) within the same row with the same superscripts letters are not significantly different ( $P > 0.05$ ).

### 3.3.4 Low pH challenge experiment

Low water pH had a significant effect on fish survival at 24h, 48h, and 72 h post challenge, based on Breslow (generalized Wilcoxon), Tarone-ware, and log rank (Mantel-cox) tests ( $P < 0.05$ ) (Figure 3.7). Fish fed 4.0% *A. vera* supplemented diet (below 60%) followed by those fed a control diet (below 70%) 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 those fed a 0.5% *A. vera* supplemented diet (above 70%). At 72-h post

challenge, a higher survival probability was observed in fish fed 1.0% followed by those fed 2.0% and then 0.5% *A. vera* supplemented diet.

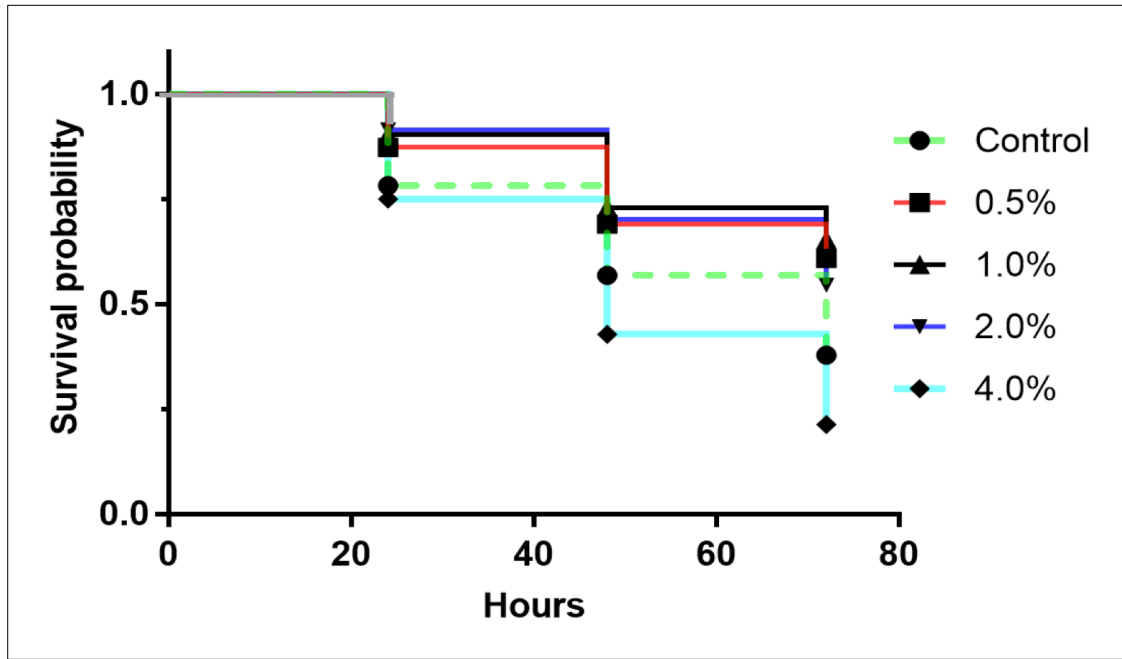


**Figure 3.7** Kaplan-Meier: low pH challenge survival probability (after every 24 h for 72 h) of African catfish, *C. gariepinus* fingerlings fed four *A. vera* 30% polysaccharide crude extracts supplemented diets and an unsupplemented diet (control) for 60 d.

### 3.4 Discussion

All growth performance indices (WG, SGR, FW, and AGR) and some feed utilization parameters (FCR, and PER) of African catfish fingerlings were enhanced in *A. vera* polysaccharides 1.0% enriched diets as compared to those fed the control diet, with optimum inclusion level for feed utilization and growth performance estimated to be between 1.77 and 1.79% *A. vera*. Similarly, a recent study reported that dietary *A. vera*

leaf 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% was able to significantly enhance growth and feed utilization performance in GIFT-*O. niloticus* strains (Gabriel et al. 2015a). Similar performances were reported in *Cyprinus carpio* juveniles when fish were fed diets supplemented with ethanolic *A. vera* extracts at 0.5% and 2.5% *A. vera* (Mahdavi et al. 2013). *Aloe vera* gel extracts supplemented diets 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 between all the studies including the present study could 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 comparisons.

Dietary *A. vera* extracts have been reported to have no influence on the growth of some fish. The inclusion levels of 0.1% and 1.0% *A. vera* 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 a previous study where 100% *A. vera* crude extract was used (Gabriel et al. 2015a), and also in studies where other herbs such as *Zingiber officinale* (Vahedi et al. 2017) and *Foeniculum vulgare* (Sotoudeh and Yeganeh 2017) were used.

Effects of *A. vera* extracts on the growth of *C. gariepinus* fingerlings may be attributed to several factors; either *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, 2015). Growth-promoting effects of medicinal herbal extracts in animals have been mainly attributed to polysaccharides (Chen et al. 2003; Tremaroli and Bäckhed 2012; Zahran et al. 2014). These compounds are known to act as prebiotics that have the ability to sustain the homeostasis of the gut microbial community as well as the host's health (Tremaroli and Bäckhed 2012), either by reducing the bacterial and viral infection levels (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 feed, and shortens the feed transit time, which might have a beneficial influence on digestive enzymes (Platel and Srinivasan 2004). It also minimizes 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 a previous study by Gabriel et al. (2017), which reported that 100% *A. vera* extracts had significantly increased amylase, trypsin and lipase activities in GIFT-tilapia. The same herb was also reported to have improved the gastrointestinal morphology of *O. mykiss* by increasing intestinal villi lengths and the 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 addition, Ji et al. (2009) has explained that, growth in fish fed dietary herbal extracts could also be as a result of their ability to promote lipid metabolism, which spares

proteins for growth, and leads to the repression of lipid accumulation. As a result, fish muscle quality (high protein, and low lipid concentrations) would be improved (Lee and Gao, 2012), as demonstrated in the current study.

In the present study, haematological parameters (i.e. RBC, HCT, HGB, MCV, MCH, MCHC, RDW, WBC, lymphocytes and granulocytes) were higher in dietary *A. vera* supplemented fish than in the control, and the optimum inclusion levels for haematological parameters seemed to range between 0.5% and 2.0%. Poor haematological immune indices were presented in fish fed 4.0% *A. vera*. This corresponds with the results obtained by Iidunni et al. (2018), which revealed that haematological parameters of *C. gariepinus* fingerlings were enhanced after being fed 1.0%, 2.0%, and 3.0% *A. vera* leaf paste for 12 weeks (84 days), respectively. One hundred percent *A. vera* dietary supplementation was reported to enhance innate immune parameters in GIFT-tilapia, *O. niloticus* especially after being stressed with *Streptococcus iniae* pathogenic bacterium. Similar to the present study, inclusion levels between 0.5% and 2.0% appeared to be effective, and fish supplemented with 4.0% *A. vera* responded poorly, thus, classified as microcytic anaemic (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 haematological 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, hence increase the oxygen carrying capacity and strengthen the defense mechanism against physiological stress. The erythropoietin effects of *A. vera* extracts in hemopoetic cells of bone marrow have been reported by Iji et al. (2010). The assumption is that these effects could be due to vitamins such as beta carotene, C, E, B<sub>12</sub>, riboflavin, thiamine, and folic acid, minerals (calcium, chromium, copper, selenium, manganese, potassium, sodium, and zinc), essential and non-essential amino acids present in *A. vera* that are essential for the synthesis of haemoglobin as demonstrated by Kayode (2017). Erythropoiesis has also been attributed to polysaccharides present in *A. vera* leaves (Ni et al. 2004).

The increased leukocyte counts 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 glycoproteins found in *A. vera* gel (Reynold 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* 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.

Medicinal herbs have been reported to be harmful in fish and even deadly at high dosages (Palanisamy et al. 2011). So far, anaemia (Gabriel et al. 2015a) and tissue necrosis (Taiwo et al. 2005) are the only *A. vera* negative effects reported in fish following dietary supplementation. However, spermatogenic dysfunction, decreased central nervous system activity, and also reduced red blood cell counts was observed in mice supplemented with *A. vera* extracts (Boudreau et al. 2013). Furthermore, the side effects of herbal extracts such as anaemia in animals has been assumed to be a result of their ability to disrupt erythropoiesis, haemosynthesis and osmoregulation functions or to increase erythrocyte destruction in haematopoietic organs (Cope 2005). *A. vera* adverse effects such as hematuria, metabolic acidosis, malabsorption (Mulle-Lissner 1993), and electrolyte disturbance in animals (Beuers 1991) have been reported long ago. This may partly explain the poor haematological parameters observed in fish fed 4.0% *A. vera* /kg diet in the present study. Hence, an upper limit is crucial in enhancing haematological indices as well as resistance against stressors in fish. In this study, inclusion levels between 0.5% and 1.0% *A. vera* appeared to be optimal for all parameters tested.

In addition to haematological 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; 2015b; Zodape 2010), chicken (Ojiezeh and Ophori 2015; Fallah 2014), and mice (Cui et al.

2014). Enzyme activity such as for AST and ALT aid in the diagnosis of liver disease (Zopade 2010). One hundred percent *A. vera* crude powder were reported to protect GIFT-tilapia, *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% (Gabriel et al. 2015b). In the same line, the present study observed that ALT and AST levels were lower in 0.5% and 1.0% *A. vera* supplemented fish compared to unsupplemented ones. This is an indication that *A. vera* at a particular dosage can effectively enhance hepatoprotective activity in fish under culture conditions as also demonstrated by Zodape (2010) in *Lebeo rohita*.

Glucose content is one of the parameters that is used in fish studies to assess their stress status (He et al. 2015). In the present study, dietary *A. vera* supplementation had no effect on the fish glucose levels when compared to the unsupplemented ones. Similar results were reported when *A. vera* extracts were supplemented in GIFT-tilapia, *O. niloticus* diets at inclusion levels of 0.5% and 2.0% (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 a previous study by 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 growth in animals (Ji et al. 2007).

The effects of *A. vera* in fish are reported to have been attributed to its bioactive compounds (Radha et al. 2015; Rajasekaran et al. 2005). Studies linking bioactive compounds in *A. vera* to their effects in fish are limited. However, in rats, isolated

phytosterols namely lophenol, and cycloarthanol were reported to elicit the ability to induce regulation of fatty acid oxidation in the liver, which favours the reduction of intra-abdominal fat and improvement of hyperlipidemia (Misawa et al. 2012) and glycaemia (Dana et al. 2012). An *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 reduce cellular damage as these vitamins play a role as strong antioxidant agents as explained by Gabriel et al. (2015b). Hence, these *A. vera* attributes could be responsible for the improved lipid profile status and hepatoprotective enzymes of fish fed *A. vera* supplements compared to the control presented in this study.

In conclusion, this experiment demonstrated that *A. vera* polysaccharides crude powder extracts supplemented feed has growth, feed utilization, hepatoprotective, and low water pH resistance effects in African catfish, *C. gariepinus* fingerlings. This extract presents the potential and option to be used as growth promoters, appetizer, stimulator, feed digesting enhancer, and anti-stress agents in *C. gariepinus* culture, and the optimal inclusion level is considered to be between 1.77% and 1.79% *A. vera*. To fully optimize *A. vera* extracts as dietary supplement in aquaculture, future studies need to adopt multivariate designs to understand the possible influence of multiples factors (i.e. include different fish sizes, different duration of exposure, and different temperatures as factors) on the growth and health effects of *A. vera* in fish. The effects of dietary *A. vera*

on fish gut microbial communities also need to be studied to help understand its mechanisms of action.

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**CHAPTER FOUR: DIETARY GARLIC (*ALLIUM SATIVUM*)  
SUPPLEMENTATION EFFECT ON GROWTH,  
HAEMATOLOGICAL PARAMETERS, WHOLE BODY  
COMPOSITION AND SURVIVAL AT LOW PH IN AFRICAN  
CATFISH (*CLARIAS GARIEPINUS*) JUVENILES**

**Abstract**

This chapter reports the potential effects of dietary garlic (*Allium sativum*) crude polysaccharide extracts (GPE) (0% (control) 0.5%, 1.0%, 2.0%, and 4.0%) on growth, haematological parameters, whole body composition, and resistance against low pH in African catfish, *Clarias gariepinus* juveniles. Fish (initial weight,  $12.28 \pm 1.26$  g) fed GPE supplemented diets had a significant increase in growth parameters compared to those fed a control diet ( $P < 0.05$ ). Similarly, feed utilization indices were significantly improved in GPE supplemented fish compared to the control ( $P < 0.05$ ). For haematological indices, a significant increase was observed in the red blood cell counts (RBC) ( $10^{12}/L$ ) of fish fed 0.5% ( $2.01 \pm 0.07$ ), 1.0% ( $1.96 \pm 0.22$ ), and 2.0% ( $1.88 \pm 0.12$ ), and in mean corpuscular haemoglobin concentration (MCHC) (g/L) for those fed 0.5% ( $553.83 \pm 6.21$ ), and 1.0% ( $554.83 \pm 7.82$ ) compared to those fed a control diet (RBC =  $1.36 \pm 0.11$ ; MCHC =  $534.67 \pm 1.83$ ) ( $P < 0.05$ ). No significant difference was observed in the survival probability among dietary groups following a challenge with low pH (5.2 – 5.5) ( $P > 0.05$ ). Similarly, no significant difference between groups was presented in the whole body composition and organo-somatic indices ( $P > 0.05$ ). The optimal dietary inclusion level was estimated at 1.69% ( $y = -0.056x^2 + 0.189x + 0.81$ ,  $R^2 = 0.52$ ,  $P = 0.031$ ) and 1.77% ( $y = -11.89x^2 + 41.69x + 167$ ,  $R^2 = 0.767$ ,  $P = 0.001$ ) of garlic for feed utilization and growth in *C. gariepinus* juvenile culture, respectively. *Allium sativum* polysaccharides are recommended as feed supplements in *C. gariepinus* juveniles' culture.

**Keywords:** Aquaculture, *Clarias gariepinus*, Immunostimulants, Plant extracts, Stress resistance.

#### 4.1 Introduction

In aquaculture, garlic is one of the most researched herbs with most of the research supporting its ability to stimulate growth (Lee et al. 2012; Büyükdeveci et al. 2018), enhance feed utilization parameters (i.e. low feed conversion ratio, and high feed efficiency ratio (Guo et al. 2012; Mehrim et al. 2014), improve non-specific immune responses (Thanikachalam et al. 2010; Talpur and Ikhwanuddin 2012), increase disease resistance (Guo et al. 2012; Talpur & Ikhwanuddin 2012), prevent parasite infections (Militz et al. 2013) and maintain meat quality (Öz 2018) in fish. These studies have investigated a wide range of garlic extracts in different fish species ranging from 100% crude powder, solvent extracted semi-purified extracts to purified extracts, with 100% crude extracts (powder) being the commonly researched form of extracts (Table 2.2). For instance, 100% garlic extracts were investigated in *Oncorhynchus mykiss* (Öz 2018), *Dicentrarchus labrax* (Saleh et al. 2015; Irkin and Yigit 2016), *Clarias gariepinus* (Eirna-Liza et al. 2016) and *Oreochromis niloticus* (Shalaby et al. 2006). Other garlic extracts studied in aquaculture include aqueous extracts (Guo et al. 2012; Fridman et al. 2014; Büyükdeveci et al. 2018), garlic oil (Hassaan and Soltan 2016), ethanolic extracts (Lee et al. 2012), garlic peels (Thanikachalam et al. 2010; Eirna-Liza et al. 2016), and allicin aqueous extracts (purified) (Militz et al. 2013).

Garlic has been reported to be rich in polysaccharides (about 77% of its dry weight) (Koch and Lawson 1996). Polysaccharides are non-digestible feed ingredients that promote growth of beneficial gastrointestinal microbiota and depress the growth of pathogenic microbiota (Song et al. 2014). This has been demonstrated by several researchers in aquaculture. For example, *Astragalus* sp. polysaccharides reportedly

enhanced growth performance, immunological parameters, digestive enzyme activity and intestinal morphology (i.e. increased villi length) in *O. niloticus* (Zahran et al. 2014). In part, the same was demonstrated in *Sparus aurata* after being fed fructooligosaccharides (Guerreiro et al. 2016a), and *Channa striata* fed  $\beta$ -glucan, galactose-oligosaccharides, and mannaoligosaccharides (Munir et al. 2018), and in *Diplodus sargus* fed diets supplemented with xylooligosaccharides, small chain fructooligosaccharides, and galactoseoligosaccharides (Guerreiro et al. 2016b). Although garlic is extensively researched in aquaculture, no study has explored the effects of its crude polysaccharide extracts in fish as a feed additive. This is therefore the first study to investigate the effect of dietary garlic (*Allium sativum*) crude polysaccharide supplementation on growth, haematological parameters, whole body composition, and resistance against low pH in African catfish, *C. gariepinus* juveniles.

## **4.2 Materials and methods**

### **4.2.1 Fish**

African catfish, *C. gariepinus* juveniles (weight  $12.28 \pm 1.26$  g) were obtained from a government fish farm, Onavivi Aquaculture Center (OAC), Outapi, Namibia. These animals were managed in the same manner as described in chapter 3, section 3.2.1.

### **4.2.2 Feeding regimes**

To formulate five dietary groups, garlic crude polysaccharide extracts (GPE) (commercial product from Shaanxi Fuheng Biotechnology, Co. Ltd. China) were supplemented to a basal diet (with 31.5 crude protein, 16.12 kJ/g diet energy, and 4.91%

lipid) (Table 4.1) at 0%, 0.5%, 1.0%, 2.0%, and 4.0% inclusion levels. All dry ingredients were mechanically mixed with oil and then water was added until a dough was observed. Each diet was then passed through a mincer. The resulting strands were shadow-dried, broken up, sieved into pellets, and stored in airtight plastic bags until use.

After a week, when no clinical signs of illness were observed, 300 fish were randomly distributed into five triplicated experimental groups (20 fish / replicate), in a 0.18 m<sup>3</sup> tank for each group, supplied with 150L of dechlorinated freshwater) following a completely randomized design (CRD) (Festing and Altman 2002). During experimental feeding, fish were fed three times a day (09:00; 13:00; 17:00), six days a week until apparent satiation for 60 d. Continuous aeration, a natural photoperiod (12-hrs light/12-hrs dark cycle), and biweekly water exchange (60%) was maintained during the feeding trial. Dissolved oxygen (DO) concentration ( $5.65 \pm 0.96$  mg/l) and water temperature ( $27.19 \pm 1.26^{\circ}\text{C}$ ) was monitored once daily. Meanwhile, pH ( $6.7 \pm 0.08$ ), and ammonia (lower than 0.05 mg /L) were monitored on a weekly basis. The experiment was conducted according to the scientific research protocols of the University of Namibia, and had complied with all relevant local and international animal welfare laws, guidelines and policies (see Appendix D).

**Table 4.1** Formulation and composition of the experimental diets (%/100 g dry matter).

| Ingredients                      | Dietary groups |               |                |                |               |
|----------------------------------|----------------|---------------|----------------|----------------|---------------|
|                                  | 1              | 2             | 3              | 4              | 5             |
| Fish meal (60% CP)               | 28.50          | 28.50         | 28.50          | 28.50          | 28.50         |
| Cow peas (25% CP)                | 22.50          | 22.50         | 22.50          | 22.50          | 22.50         |
| Corn grain (10.2% CP)            | 8.40           | 8.40          | 8.40           | 8.40           | 8.40          |
| Wheat flour (11.7% CP)           | 13.90          | 13.90         | 13.90          | 13.90          | 13.90         |
| Pearl millet (12.5% CP)          | 22.70          | 22.70         | 22.70          | 22.70          | 22.70         |
| Vegetable oil                    | 3.00           | 3.00          | 3.00           | 3.00           | 3.00          |
| Vitamin premix <sup>a</sup>      | 0.50           | 0.50          | 0.50           | 0.50           | 0.50          |
| Mineral premix <sup>b</sup>      | 0.50           | 0.50          | 0.50           | 0.50           | 0.50          |
| <b>Total</b>                     | <b>100 .00</b> | <b>100.00</b> | <b>100 .00</b> | <b>100 .00</b> | <b>100.00</b> |
| GPE                              | 0.00           | 0.50          | 1.0 0          | 2.00           | 4.00          |
| <u>Proximate composition (%)</u> |                |               |                |                |               |
| Dry matter                       | 92.73          | 91.69         | 91.69          | 91.71          | 91.73         |
| Crude protein                    | 31.50          | 31.21         | 31. 45         | 31.28          | 31.42         |
| Crude lipid                      | 4.91           | 4.83          | 4.77           | 4.82           | 4.78          |
| Ash                              | 4.37           | 4.29          | 4.32           | 4.39           | 4.38          |
| Gross energy (KJ/g diet)         | 16.12          | 16.18         | 16.11          | 16.38          | 16.40         |

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

<sup>b</sup>Mineral premix (g kg<sup>-1</sup>): KH<sub>2</sub>PO<sub>4</sub>, 502; MgSO<sub>4</sub>. 7H<sub>2</sub>O, 162; NaCl, 49.8; CaCO<sub>3</sub>, 336; Fe (II) gluconate, 10.9; MnSO<sub>4</sub>.H<sub>2</sub>O, 3.12; ZnSO<sub>4</sub>. 7H<sub>2</sub>O, 4.67; CuSO<sub>4</sub>. 5H<sub>2</sub>O, 0.62; KI, 0.16; CoCl<sub>2</sub>. 6H<sub>2</sub>O, 0.08; ammonium molybdate, 0.06; NaSeO<sub>3</sub>, 0.02.

#### 4.2.3 Growth and feed utilization parameters

All fish were weighed (together) at the start and the end of the experiment (after 60 days), to calculate weight gain (WG), absolute growth rate (AGR), and specific growth rate (SGR). To calculate the organo-somatic indices body length, body weight, liver and gutted weights of three fish from each replicate were recorded. Before these fish were sacrificed, they were anaesthetized with 100mg MS-222, tricane methane sulfonate (Biodynamic Pty, Ltd, Namibia). The amount of feed consumed and mortality in each replicate was recorded throughout the experimental period to account for feed intake (FI) and survival rate, respectively. Growth performance and feed utilization were assessed in terms of WG, AGR, SGR, feed intake (FI), feed conversion ratio (FCR), and feed efficiency ratio (FER). Calculations were conducted as indicated in Chapter 3, Section 3.2.3, Equations 1 to 9.

#### 4.2.4 Haematological parameters

Blood samples were collected and haematological parameters were analyzed as described in Chapter 3, Section 3.2.4.

#### 4.2.5 Proximate body composition analysis

Fish whole body proximate composition analyses were carried out as laid out in Chapter 3, Section 3.2.5.

#### 4.2.6 Low pH stress challenge experiment

After the initial sampling, stocking density in each dietary group was adjusted to 10 fish / tank. They were then exposed to low pH (pH 5.2 -5.5) for three days (72 h). The water

pH was adjusted by adding 4N HCl and 4N NaOH, and was renewed daily, as demonstrated by Lin and Chen (2008). During this experiment pH, temperature ( $29 \pm 1.2^{\circ}\text{C}$ ), DO ( $> 4 \text{ mg/L}$ ), and  $\text{NH}_3\text{-N}$  concentration ( $< 0.08 \text{ mg/L}$ ) were monitored daily. Fish mortality was recorded at three 24-h intervals to determine the survival:

$$(12) \text{ Survival probability} = (\text{Number of fish survived})/(\text{Initial number of fish})$$

#### 4.2.7 Statistical analyses

Data were statistically analyzed using descriptive statistics in SPSS® (version 21, IMB Corp, Armonk, NY, USA). Normality and homogeneity of variance were confirmed using Kolmogorov-Smirnov and Levene's test, respectively. Treatment effects were analyzed using one-way analysis of variance (ANOVA). Significant differences between the group means were further compared using Duncan's Multiple Range Test (DMRT).  $P < 0.05$  was considered statistically significant. The second order polynomial regression model ( $y = b_0 + b_1x + b_2x^2$ , where,  $y$  = maximum WG or FER,  $x$  = optimum inclusion level) (Zeitoun et al. 1976) was used to estimate the optimum dietary GPE requirement for *C. gariepinus* juveniles to produce maximum growth and maximum feed efficiency ratio. The survival (probability) of fish at low pH treatment group was estimated using Kaplan–Meier analysis (Jelkić et al. 2014). Breslow (generalized Wilcoxon), Tarone-ware, and log-rank (Mantel-cox) tests were used to determine the significant difference ( $P < 0.05$ ) between groups at each sampling interval of the pH challenge.

### 4.3 Results

#### 4.3.1 Fish growth and feed utilization

Throughout the experimental period, the fish appeared healthy and no mortality was recorded. After 60 d of feeding, fish in all dietary groups increased in wet weight (Figure 4.1). Fish fed GPE supplemented diet at 1.0% ( $124.05 \pm 7.06$  g) and 2.0% ( $126.16 \pm 5.21$  g) presented significantly higher FW than those fed a control and those fed 4.0% ( $P < 0.05$ ). As a result, the WG, SGR, and AGR of fish fed 1.0% (WG =  $111.39 \pm 8.36$  g; SGR =  $3.83 \pm 0.18$  g; AGR =  $1.86 \pm 0.08$  g) and 2.0% GPE supplemented diet (WG =  $113.51 \pm 8.36$  g; SGR =  $3.89 \pm 0.07\%$ ; AGR =  $1.89 \pm 0.08$ ) were significantly higher than control and 4.0% ( $P < 0.05$ ), with fish fed 2.0% GPE presenting the highest growth, followed by those fed 1.0%, 0.5%, while those fed 4.0% (WG =  $54.77 \pm 1.73$ ; SGR =  $2.91 \pm 0.05$ ). Fish fed GPE had the lowest growth among dietary groups ( $P < 0.05$ ). Furthermore, there were no significant differences observed between groups in the organo-somatic indices (VSI and HSI) and CF ( $P > 0.05$ ) (Table 4.2). Generally, the same response observed in growth parameters was reflected in the feed utilization parameter (FI) (Figure 4.2) ( $P < 0.05$ ). Based on the second polynomial regression on WG against dietary garlic inclusion level ( $y = -11.809x^2 + 41.688x + 167$ ,  $R^2 = 0.765$   $P = 0.001$ ), or FER against dietary garlic inclusion level ( $y = -0.056x^2 + 0.189x + 0.807$ ,  $R^2 = 0.522$ ,  $P = 0.031$ ), the optimum dietary GPE inclusion level (%) was estimated to be 1.77% ( $Y = -11.809x^2 + 41.688x + 167$ ,  $R^2 = 0.765$   $P = 0.001$ ) and 1.69% GPE ( $y = -0.056x^2 + 0.189x + 0.807$ ,  $R^2 = 0.522$ ,  $P = 0.031$ ) for growth and feed utilization, respectively (Figure 4.1, 4.2).

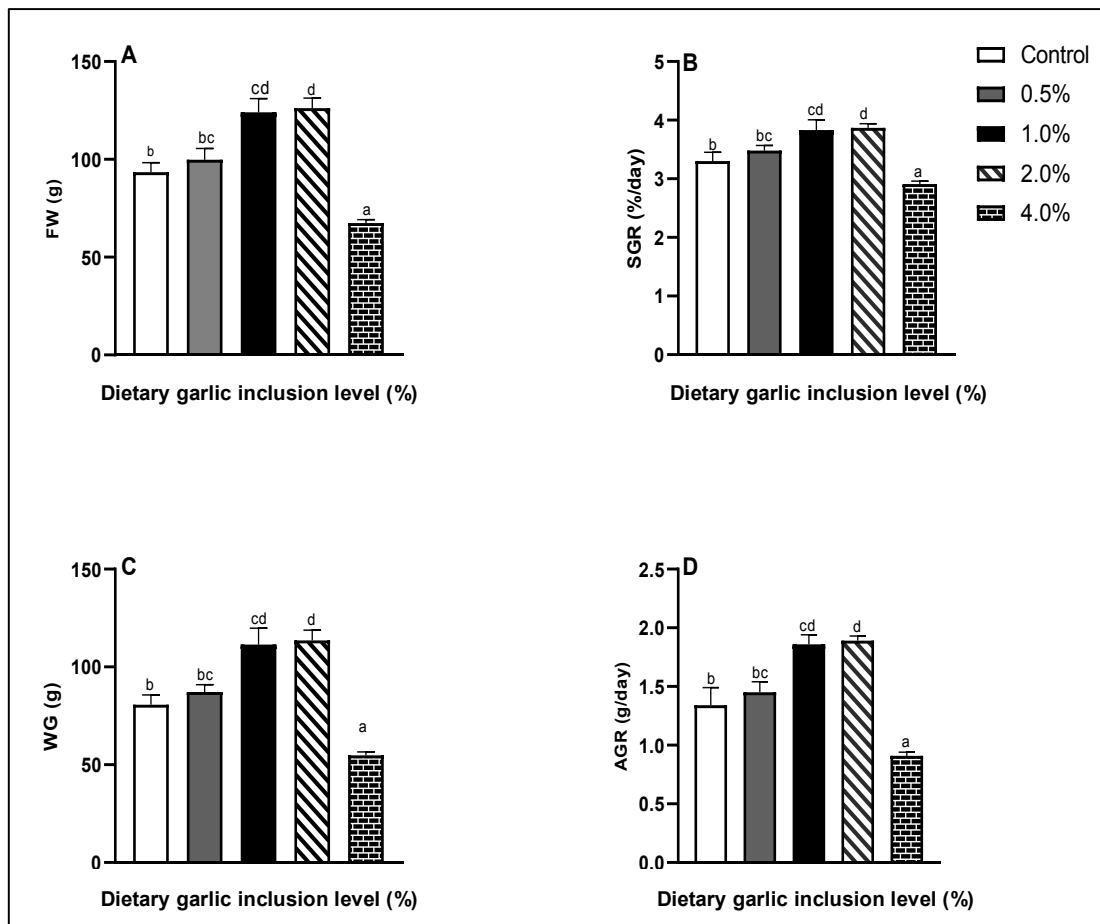


**Figure 4.1** Final weight (FW) (A), specific growth rate (SGR) (B), weight gain (WG) (C), and absolute growth rate (AGR) (D), of African catfish, *C. gariepinus* juveniles fed four garlic (*Allium sativum*) polysaccharide extracts (GPE) supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ). Values were expressed as mean  $\pm$  standard error; WG:  $y = -11.809x^2 + 41.688x + 167$ ,  $R^2 = 0.765$ ,  $P = 0.001$  (second order polynomial regression model).

**Table 4.2** Organo-somatic indices, condition factor, and survival (%) of the African catfish, *C. gariepinus* fingerlings fed four garlic (*Allium sativum*) crude polysaccharide extracts supplemented diets and a control diet for 60 d.

| Parameters | Dietary <i>A. vera</i> inclusion level (%) |                          |                          |                          |                          |
|------------|--------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|            | Control                                    | 0.5                      | 1.0                      | 2.0                      | 4.0                      |
| VSI        | 7.60 ± 0.32 <sup>a</sup>                   | 8.08 ± 0.51 <sup>a</sup> | 7.81 ± 0.30 <sup>a</sup> | 7.54 ± 0.16 <sup>a</sup> | 7.58 ± 0.13 <sup>a</sup> |
| HSI        | 3.04 ± 0.16 <sup>a</sup>                   | 3.11 ± 0.13 <sup>a</sup> | 3.22 ± 0.16 <sup>a</sup> | 3.38 ± 0.28 <sup>a</sup> | 2.96 ± 0.09 <sup>a</sup> |
| CF         | 0.54 ± 0.01 <sup>a</sup>                   | 0.56 ± 0.20 <sup>a</sup> | 0.59 ± 0.13 <sup>a</sup> | 0.58 ± 0.01 <sup>a</sup> | 0.60 ± 0.05 <sup>a</sup> |
| Survival   | 100 ± 0.00 <sup>a</sup>                    | 100 ± 0.00 <sup>a</sup>  | 100 ± 0.00 <sup>a</sup>  | 100 ± 0.00 <sup>a</sup>  | 100 ± 0.00 <sup>a</sup>  |

Data are expressed as mean ± standard error (M ± SE). Values with different superscript letters in the same row are not significantly different ( $P > 0.05$ ) from the control. Where VSI = viscerosomatic index, HSI = hepatosomatic index, and CF = condition factor.

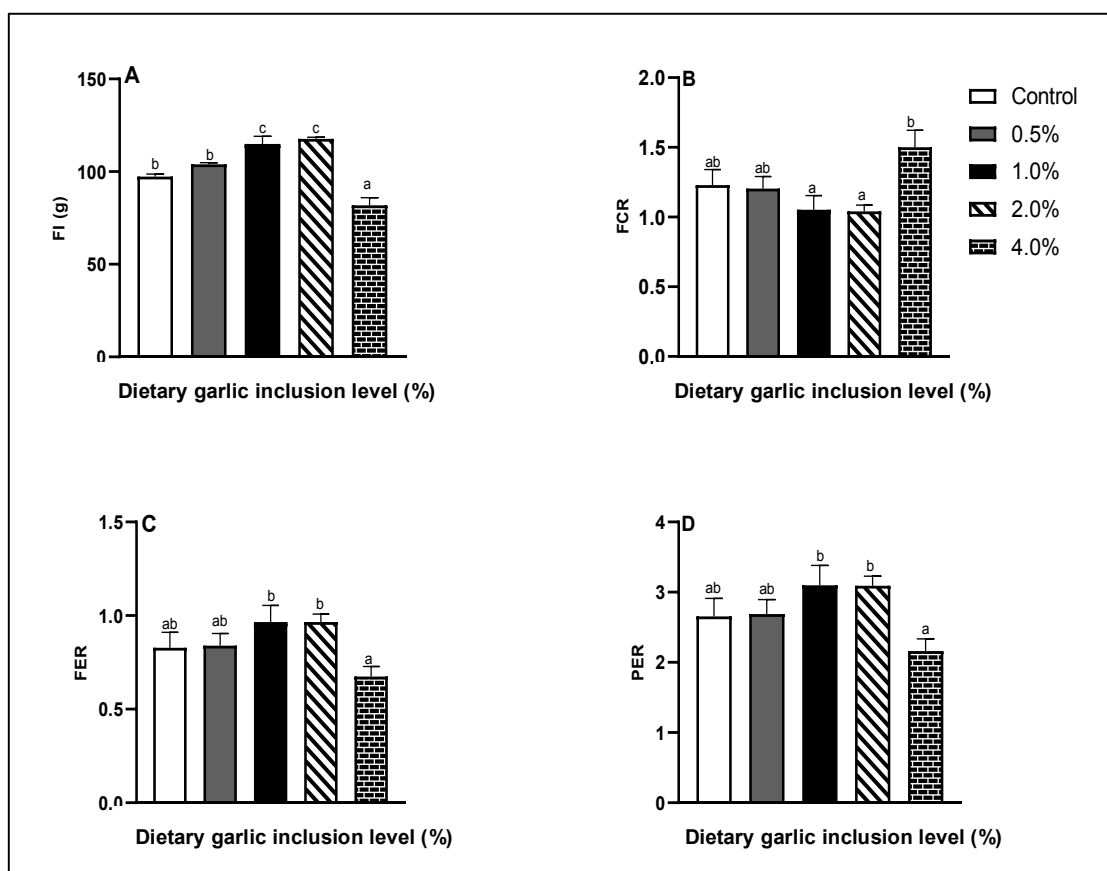


**Figure 4.2** Feed intake (FI) (A), feed conversion ratio (FCR) (B), feed efficiency ratio (FER) (C), and protein efficiency ratio (PER) (D), of the African catfish, *C. gariepinus* juveniles fed four garlic (*Allium sativum*) polysaccharides extracts (GPE) supplemented diets and an un-supplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error; FER:  $y = -0.056x^2 + 0.189x + 0.807$ ,  $R^2 = 0.522$ ,  $P = 0.031$  (second order polynomial regression model).

#### 4.3.2 Haematological indices

There were no significant differences in most of the haematological indices (i.e. WBC, Hg, Hct, MON, LYM, GRAN, MCV, RDW<sub>a</sub>, MCH, and PLT) between dietary groups (Figure 4.3, 4.4, 4.5) ( $P > 0.05$ ). On the other hand, the significant differences were observed in RBC counts (Figure 4.3A) and MCHC (Figure 4.4C) between dietary groups ( $P < 0.05$ ). The RBC counts were significantly higher in fish supplemented with 0.5% GPE ( $2.01 \pm 0.07$ ), followed by those fed 1.0% ( $1.96 \pm 0.22$ ), and 2.0% GPE ( $1.88 \pm 0.12$ ) when compared to the unsupplemented ones ( $1.36 \pm 0.11$ ) and the 4.0% GPE ( $1.29 \pm 0.22$ ) ( $P > 0.05$ ). MCHC levels were significantly higher in fish fed 1.0% ( $554.83 \pm 7.82$ ), and 0.5% ( $553.82 \pm 6.21$ ) GPE when compared to all other groups ( $P < 0.05$ ).

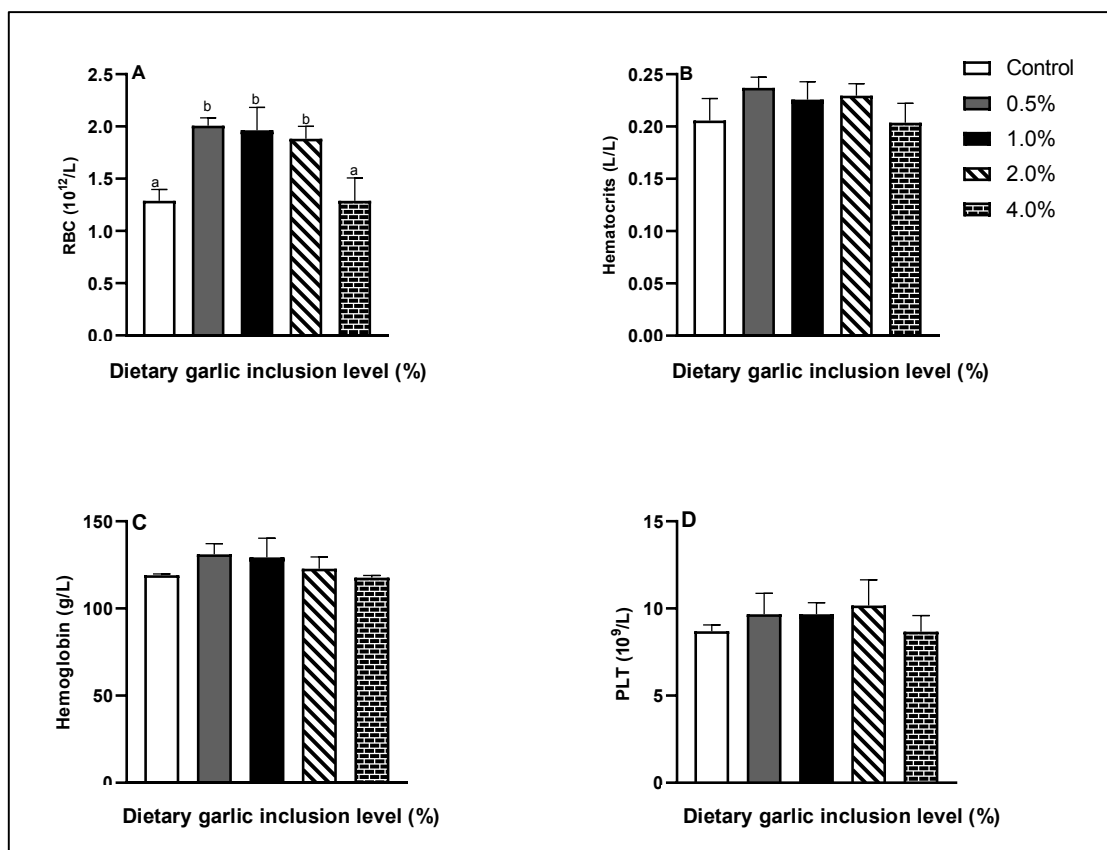


**Figure 4.3** Red blood cell counts (RBC) (A), haematocrit levels (B), haemoglobin concentration (C), and platelet counts (PLT) (D) of African catfish, *C. gariepinus* fingerlings fed four garlic (*Allium sativum*) polysaccharides extracts (GPE) supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

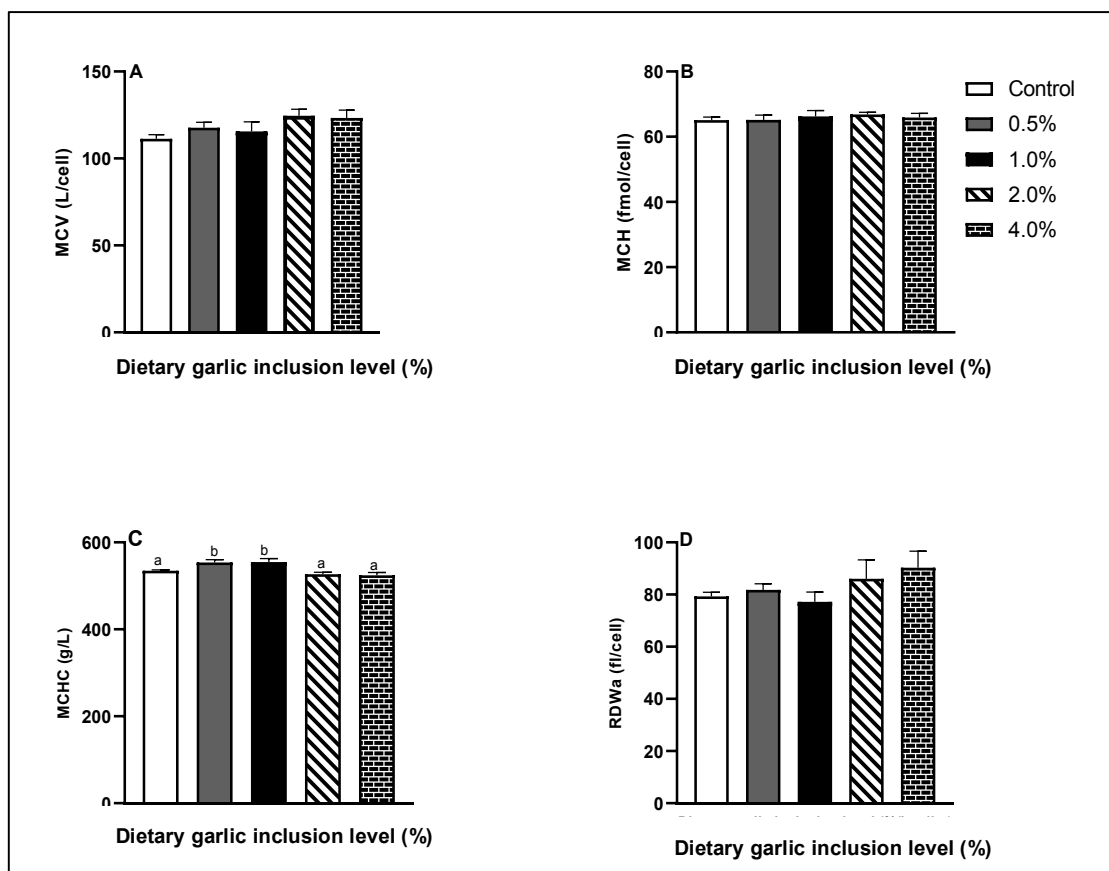


**Figure 4.4** Mean corpuscular volume (MCV) (A), mean corpuscular haemoglobin level (MCH) (B), mean corpuscular haemoglobin concentration (MCHC) (C), and Red blood cell distribution width (RDWc) (D) of African catfish, *C. gariepinus* juveniles fed four garlic (*Allium sativum*) polysaccharides extracts supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

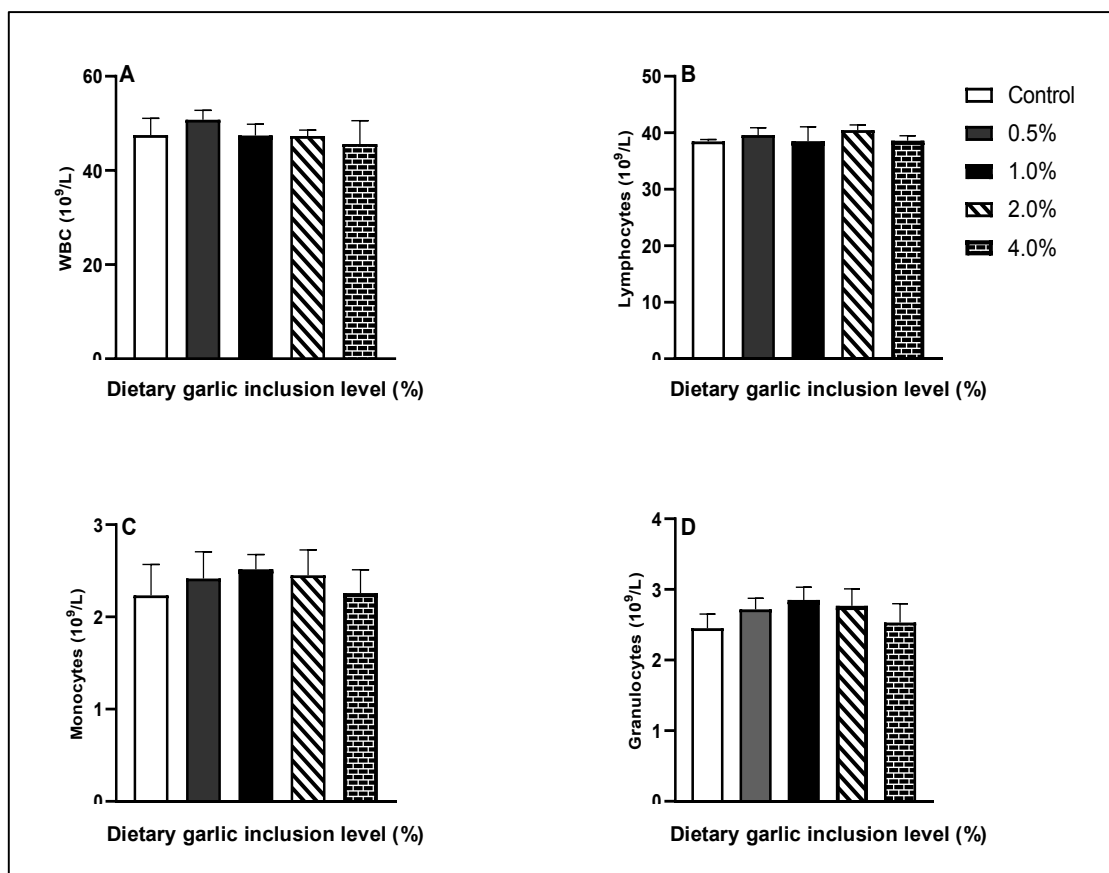


**Figure 4.5** White blood cell counts (WBC) (A), lymphocyte counts (B), monocyte counts (C), and granulocytes (D) of African catfish, *C. gariepinus* juveniles fed four garlic (*Allium sativum*) polysaccharides extracts (GPE) supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

#### 4.3.3 Proximate body composition

There were no significant differences in the whole body composition indices (protein, moisture, lipid, and ash composition) of fish between dietary groups ( $P > 0.05$ ) (Table 4.3).

**Table 4.3** Selected whole body composition parameters of African catfish, *C. gariepinus* juveniles fed four garlic (*Allium sativum*) polysaccharides extracts (GPE) supplemented diets and un-supplemented diet for 60 d.

| Parameters   | Control                   | Dietary garlic inclusion level (%) |                           |                           |                           |
|--------------|---------------------------|------------------------------------|---------------------------|---------------------------|---------------------------|
|              |                           | 0.5                                | 1.0                       | 2.0                       | 4.0                       |
| Moisture (%) | 72.30 ± 0.06 <sup>a</sup> | 72.79 ± 0.40 <sup>a</sup>          | 72.04 ± 0.34 <sup>a</sup> | 72.04 ± 0.63 <sup>a</sup> | 72.77 ± 0.87 <sup>a</sup> |
| Protein (%)  | 69.94 ± 1.19 <sup>a</sup> | 70.72 ± 0.70 <sup>a</sup>          | 71.88 ± 1.21 <sup>a</sup> | 72.22 ± 0.36 <sup>a</sup> | 70.35 ± 0.84 <sup>a</sup> |
| Lipid (%)    | 8.89 ± 0.31 <sup>a</sup>  | 8.99 ± 1.91 <sup>a</sup>           | 8.57 ± 0.99 <sup>a</sup>  | 7.83 ± 0.22 <sup>a</sup>  | 77 ± 0.50 <sup>a</sup>    |
| Ash (%)      | 6.41 ± 1.43 <sup>a</sup>  | 6.87 ± 0.51 <sup>a</sup>           | 6.73 ± 0.32 <sup>a</sup>  | 6.99 ± 0.06 <sup>a</sup>  | 7.25 ± 0.31 <sup>a</sup>  |

Values (Mean ± Standard Error, M±SE) within the same row with the same superscripts letters are not significantly different ( $P > 0.05$ ).

#### 4.3.4 Low pH challenge

Based on the Breslow (generalized Wilcoxon,  $P = 0.25$ ), Tarone-ware ( $P = 0.16$ ), and log rank (Mantel-Cox,  $P = 0.09$ ) tests, garlic supplement had no significant effect on the survival probability of fish after low water pH exposure ( $P > 0.05$ ) (Figure 4.6).

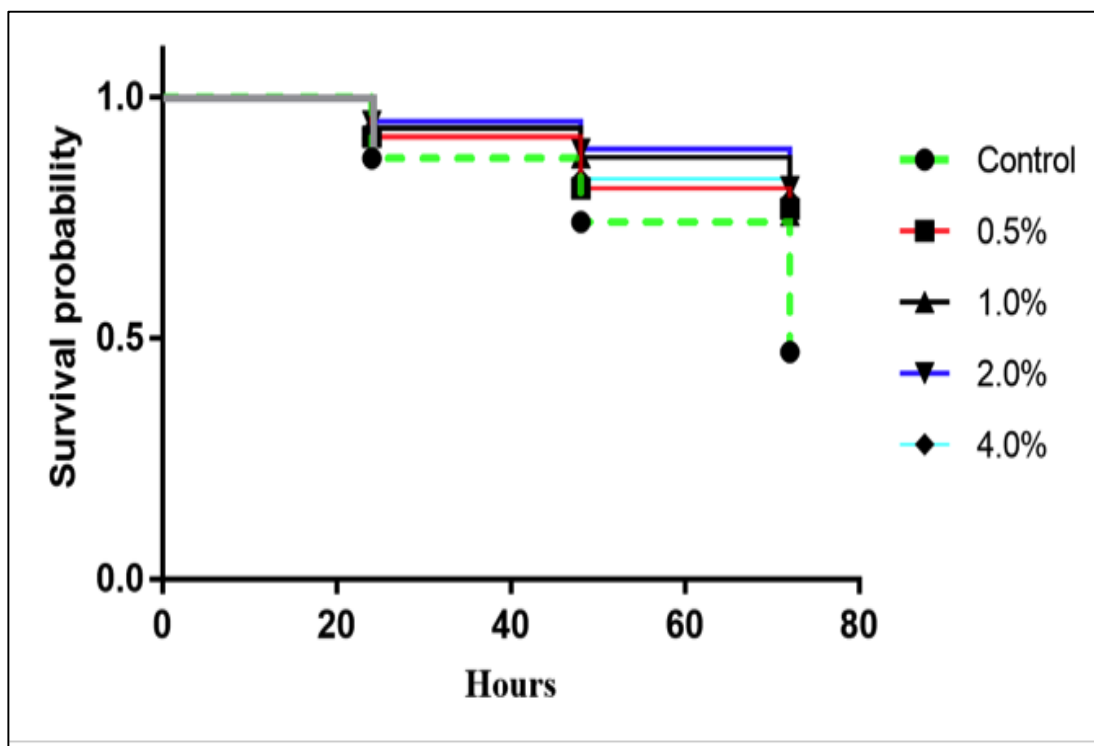


**Figure 4.6** Kaplan-Meier: low pH challenge survival probability of African catfish, *C. gariepinus* juveniles fed four garlic (*Allium sativum*) polysaccharides extracts (GPE) supplemented diets and an unsupplemented diet (control) for 60 d.

#### 4.4 Discussion

In the current study, the significant increase in growth parameters (WG, FW, SGR, and AGR), and in feed intake observed in fish fed GPE enriched diets compared to other groups disagree with findings of different dietary garlic extracts supplementation in African catfish, *C. gariepinus*. For instance, no significant growth was observed in African catfish, *C. gariepinus* fingerlings when fed diets supplemented with garlic peels (Thanikachalam et al. 2010; Eirna et al. 2016), and garlic clove crude extracts (Eirna et al. 2016; Onomu 2019). However, dietary garlic extracts supplementation was reported to significantly improve feed utilization as well as growth in various aquaculture species,

as demonstrated in the present study. Abu-Elala et al. (2016) reported a significant increase in FW, WG, and significantly lower FCR in Nile tilapia, *O. niloticus* after being fed a diet supplemented with garlic crude powder compared to a control. Similarly, significant improvement in growth and feed utilization indices were reported in Caspian roach, *Rutilus rutilus* (Ghehdarijani et al. 2016), orange-spotted grouper, *Epinephelus coioides* (Guo et al. 2012), Asian sea bass, *Lates calcarifer* (Talpur and Ikhwanuddin 2012), and sterlet sturgeon, *Acipenser rutheni* (Lee et al. 2014) after being fed garlic crude powder. Studies that had garlic supplemented at varying inclusion levels indicated that growth and feed utilization response was dose-dependent. In most cases, the poorest growth was observed at highest inclusion levels (Guo et al. 2012; Talpur and Ikhwanuddin 2012; Ghehdarijani et al. 2016), as also demonstrated in the current study. This could be a result of the pungent smell possessed by garlic, which might act as feed deterrents, hence lower feed palatability and poor growth in fish (Lee et al. 2014).

It is now common to assess the herbal extract health effects in animals including fish using haematological indices such as WBC, RBC, Hct, Hb, erythrocyte indices (i.e. MCV, MCH, and MCHC), and differential leukocyte counts (i.e. lymphocytes, neutrophils, basophils, eosinophils, and monocytes). The present study observed an improvement in haematological parameters, and high resistance to low pH stress in fish fed garlic-supplemented diets. These findings agree with those of Thanikachalam et al. (2010) who reported that dietary garlic peels significantly enhanced RBC, WBC, and increased resistance of *C. gariepinus* against *Aeromonas hydrophila*. Similarly, increased haematological indices as well as increased resistance against stressors were reported in *O. mykiss* (Nya and Austin 2011), *Lates calcarifer* (Talpur and Ikhwanuddin

2012), *Huso huso* (Kanani et al. 2014), and in *Dicentrarcus labrax* (Saleh et al. 2015) following dietary garlic supplementation. This is indeed an indication that garlic has the ability to enhance the oxygen carrying capacity, non-specific or innate immunity (Fazlolah-zadeh et al. 2011) in fish, and as a result, producing healthy animals that can withstand physiological stress under culture conditions (Houston 1997).

Improved growth, feed utilization, health parameters, as well as increased stress resistance in fish fed diets supplemented with garlic crude polysaccharide extracts in the present study, could be attributed to many factors. Previous studies have attributed these effects to allicin (Khalil et al. 2001; Lee and Gao 2012), which is the most bioactive compound found in garlic (Rahman and Lowe 2006; Yoo et al. 2010). Its mode of action seems to be well understood. It improves the gastrointestinal motility, and modulates the secretion of various digestive enzymes to enhance digestion and nutrient absorption (Diab et al. 2010). It also promotes the performance of intestinal flora, inhibits deleterious bacteria while intensifying beneficial bacteria such as *Lactobacillus* and *Bifidus*, thus improving the utilization of energy and growth (Diab et al. 2010; Büyükdeveci et al. 2018). However, allicin at higher dosages is known to be harmful to fish, resulting in reduced growth and sometimes even death in fish, because of its ability to interfere with the normal metabolism and mitosis (Yang et al. 2010). Thus optimization of garlic extracts in aquaculture feed is important.

In addition, herbal polysaccharides (sometimes referred to as prebiotics) are other ingredients that have been reported to improve growth of fish through nutrient utilization and health improvement (Merrifield and Ringo 2014; Mohan et al. 2019). Interestingly,

there seem to be similarities in the mode of action between prebiotics and allicin in promoting growth in fish. Like allicin, prebiotics enhance growth in fish by improving feed digestibility, and availability of nutrients from feedstuffs, and shorten the feed transit time, which enhances digestive enzymes (Patel and Srinivasan 2004), and reduce the amount of feed substrate available in the gut for proliferation of pathogenic bacteria (Citarasu 2010). Prebiotics do this through their ability to modulate the interaction between gut autochthonous morphology and gut microbiota, as highlighted by Denev et al. (2009) and Carbone and Faggio (2016).

Ji et al. (2009) has explained that, growth in fish fed dietary herbal extracts could also be enhanced as a result of their ability to promote lipid metabolism, which spare protein for growth, and lead to the repression of lipid accumulation. As a result, fish muscle quality (high protein, and low lipid content) would be improved (Lee and Gao 2012), as demonstrated in the current study.

In conclusion, garlic crude polysaccharide extracts improved growth, feed utilization, health, meat quality as well as resistance against low water pH in African catfish, *C. gariepinus* juveniles. Based on the second order polynomial regression analysis, dietary inclusion level between 1.69% and 1.77% of garlic crude polysaccharide extracts /kg of basal diet was estimated optimal to support growth and feed utilization in African catfish juvenile culture, respectively. Future studies should focus on using purified garlic extracts to understand their effects on digestive enzymes as well as intestinal bacterial community in addition to biometric parameters and immunological indices.

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**CHAPTER FIVE: THE EFFECTS OF DIETARY GARLIC (*ALLIUM SATIVUM*) AND *ALOE VERA* POLYSACCHARIDES (1:1 MIXTURES) SUPPLEMENTATION ON GROWTH, HAEMATOLOGICAL PARAMETERS, WHOLE BODY COMPOSITION, AND SURVIVAL AT LOW PH IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*) JUVENILES**

**Abstract**

This experiment evaluated the effects of dietary *Allium sativum* and *Aloe vera* polysaccharides 1:1 mixture on growth performance, feed utilization, haematological parameters, resistance against low water pH, and whole body composition of African catfish (*Clarias gariepinus*) juveniles. Fish (initial weight,  $12.28 \pm 1.26$  g) were divided into five triplicate groups before being fed diets supplemented with 0% (control), 0.5%, 1.0%, 2.0% and 4.0% *A. vera* and *A. sativum* polysaccharides mixture (1:1 ratio). Fish fed 1.0% *A. vera-A. sativum* mixture supplemented diet had a significant increase in growth (FW =  $90.60 \pm 3.98$  g, WG =  $78.32 \pm 3.98$  g, SGR =  $3.33 \pm 0.07$  g, and AGR =  $1.24 \pm 0.08$ ), compared to those fed a control ( $P < 0.05$ ). Similarly, feed utilization indices were significantly improved in fish fed the 1.0% *A. vera-A. sativum* mixture supplemented diet when compared to all other dietary groups ( $P < 0.05$ ). The optimum dietary *A. vera-A. sativum* mixture inclusion level was estimated to be 0.70%, and 0.66% for growth and feed utilization respectively. Overall, the *A. vera-A. sativum* mixture improved haematological indices when compared to unsupplemented fish. Fish fed 1.0% of *A. vera-A. sativum* mixture had the highest survival probability (90%, 80%, 70% post 24h, 48h, and 72h, respectively) throughout the low water pH (5.2-5.5) challenge period. Moreover, significantly lower lipid contents (%) were reported in fish fed diets supplemented with the 2.0% ( $6.69 \pm 0.36$ ), 4.0% ( $7.18 \pm 0.24$ ), and 1.0% ( $7.44 \pm 0.29$ ) *A. vera-A. sativum* mixture than those fed a control diet ( $9.31 \pm 0.71$ ) ( $P < 0.05$ ).

In conclusion, *A. vera*-*A. sativum* polysaccharides mixture (1:1) is recommended as feed supplements in *C. gariepinus* juvenile culture.

**Keywords:** Aquaculture, *Clarias gariepinus*, Herbal mixtures, Immunostimulants, Stress resistance.

## 5.1 Introduction

In aquaculture, medicinal herbal extracts could either be studied alone (individual herb incorporated in the basal fish diet) or in combination (as mixture) with other medicinal herbs. Although positive benefits for individual herbs have been widely reported in farmed fish, synergistic/additive benefits were also unveiled when herbs were studied in combination. For instance, Ji et al. (2007a) reported the highest growth, immune response, and resistance of *Pagrus major* against *Vibrio anguillarum* after being fed a diet supplemented with a mixture of *Mussa medicate*, *Crataegi fructus*, *Artemisia capillaries*, and *Cnidium officinale* herbs (combination ratio 2:2:1:1) compared to those fed a mono-herbal supplemented diet, and control. The same was reported in *Paralichthy olivaceus* juveniles (Ji et al. 2007b) supplemented with the same herbs as demonstrated in (Ji et al. 2007a). Furthermore, a dietary mixture of *Astragalus membranaceus* and *Lonicera japonica* (combined in equal proportions, 1:1 ratio) in *Oreochromis niloticus* culture (Ardo et al. 2008), and a dietary mixture of *A. radix* and *Ganoderma lucidum* (1:1) in *Cyprinus carpio* (Yin et al. 2009) reported to evoke a significantly higher immune response activity and better protection against *Aeromonas hydrophila* pathogenic bacterium than the control. In the same line, a dietary mixture of the Chinese herbs supplemented at different levels (4, 8, 12, 16, and 20 g /kg diet) significantly improved growth performance, increased digestive enzyme activity, and enhanced immune response in *Lateolabrax japonicus* juveniles compared to the control, with the optimum dosage estimated between 8 and 12g /kg diet (Wang et al. 2018). The use of medicinal herbal mixtures seems to be another approach that can maximize the benefits associated with herbs in aquaculture.

Currently, no study has attempted to investigate the potential effects of garlic (*A. sativum*) and *Aloe vera* extract mixtures in aquaculture. However, reports on each herb's individual mixture with other herbs or natural products exist, and support the observation that the benefits of herbal extracts in fish could be amplified when administered as mixtures. A dietary mixture of garlic, ginger, and thyme (in a 1:1:1 ratio, at 1.0%/kg diet) was reported to significantly improve growth, overall health, and resistance of *Sparidentex hasta* fry against *Photobacterium damsela* (Jahanjoo et al. 2018). Positive synergistic effects of different garlic extracts mixed with other herbal extracts were also reported in *O. niloticus* (Abu-Elala et al. 2016; Hassan and Soltan 2016), *Dicentrarchus labrax* (Yilmaz et al. 2012; Yilmaz and Ergün 2012), and *Litopenaeus vannamei* (Huang et al. 2018). Similarly, dietary *A. vera* combined with *Strobilanthes crispus*, and *Vitex trifolia* extracts in a ratio of 1:1:1, at a dosage of 3.5 g/kg diet was reported to significantly enhance growth, health and tolerance against *Streptococcus agalactiae* of red hybrid tilapia (*Oreochromis* sp.) (Manaf et al. 2016). In the same vein, a mixture of *A. vera* and a natural product (propolis) was reported to improve health parameters in *O. niloticus* (Dotta et al. 2014, 2018). Hence, this present experiment was designed to evaluate the combined effects of dietary *A. vera* and *A. sativum* crude polysaccharides supplementation (at a 1:1 ratio) on growth, feed utilization, haematological indices, whole body composition, and survival at low water pH in African catfish, *C. gariepinus* juveniles.

## 5.2 Materials and methods

### 5.2.1 Preparation of experimental diets

A basal diet containing 31.20% crude protein, 17.82 KJ/g gross energy, and 5.12% lipid (Table 5.1) was used as a control diet. Four basal diets were supplemented with a mixture containing equal amounts (1:1) of *A. vera* and *A. sativum* polysaccharides crude extracts (*A. vera*-*A. sativum* mixture) at different inclusion levels (0.5, 1.0, 2.0, and 4.0%). The *A. vera* polysaccharides crude extract (30%) was a solvent-extracted and lyophilized commercial product (powder) purchased from Ningxia SangNutrition Biotech Inc., China, which consisted of acemannan, glucomannan, saponin, glycosides, galactan, mannose, aloin, and emodin. Garlic was also a solvent-extracted and lyophilized product (powder), which consisted of galactose, rhamnose, glucuronic acid, galacturonic acid, allicin, and alliin (commercial product from Shaanxi Fuheng Biotechnology, Co. Ltd, China). The procedures for manufacturing the experimental diets were similar to those explained in chapter 3 (section 3.2.1) and in chapter 4 (section 4.2.1).

**Table 5.1** Formulation and composition of the experimental diets (%/100 g dry matter).

| Ingredients                              | Dietary groups |               |                |                |               |
|------------------------------------------|----------------|---------------|----------------|----------------|---------------|
|                                          | 1              | 2             | 3              | 4              | 5             |
| Fish meal (60% CP)                       | 28.50          | 28.50         | 28.50          | 28.50          | 28.50         |
| Cow peas (25% CP)                        | 22.50          | 22.50         | 22.50          | 22.50          | 22.50         |
| Corn grain (10.2% CP)                    | 8.40           | 8.40          | 8.40           | 8.40           | 8.40          |
| Wheat flour (11.7% CP)                   | 13.90          | 13.90         | 13.90          | 13.90          | 13.90         |
| Pearl millet (12.5% CP)                  | 22.70          | 22.70         | 22.70          | 22.70          | 22.70         |
| Vegetable oil                            | 3.00           | 3.00          | 3.00           | 3.00           | 3.00          |
| Vitamin premix <sup>a</sup>              | 0.50           | 0.50          | 0.50           | 0.50           | 0.50          |
| Mineral premix <sup>b</sup>              | 0.50           | 0.50          | 0.50           | 0.50           | 0.50          |
| <b>Total</b>                             | <b>100 .00</b> | <b>100.00</b> | <b>100 .00</b> | <b>100 .00</b> | <b>100.00</b> |
| <i>A. vera</i> - <i>A. sativum</i> (1:1) | 0.00           | 0.50          | 1.0 0          | 2.00           | 4.00          |
| <u>Proximate composition (%)</u>         |                |               |                |                |               |
| Dry matter                               | 92.77          | 91.79         | 91.68          | 91.72          | 91.79         |
| Crude protein                            | 31.20          | 31.23         | 31. 26         | 31.22          | 31.21         |
| Crude lipid                              | 5.12           | 5.10          | 5.14           | 5.22           | 5.15          |
| Ash                                      | 4.75           | 4.69          | 4.72           | 4.79           | 4.81          |
| Gross energy (KJ/g diet)                 | 17.82          | 17.84         | 17.83          | 17.81          | 17.83         |

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

<sup>b</sup>Mineral premix (g kg<sup>-1</sup>): KH<sub>2</sub>PO<sub>4</sub>, 502; MgSO<sub>4</sub> · 7H<sub>2</sub>O, 162; NaCl, 49.8; CaCO<sub>3</sub>, 336; Fe (II) gluconate, 10.9; MnSO<sub>4</sub>·H<sub>2</sub>O, 3.12; ZnSO<sub>4</sub> · 7H<sub>2</sub>O, 4.67; CuSO<sub>4</sub> · 5H<sub>2</sub>O, 0.62; KI, 0.16; CoCl<sub>2</sub> · 6H<sub>2</sub>O, 0.08; ammonium molybdate, 0.06; NaSeO<sub>3</sub>, 0.02.

### 5.2.2 Fish and experimental design

African catfish, *C. gariepinus* (body weight  $12.28 \pm 1.26$  g, the same group of fish used in Chapter 4) were sourced from Onavivi Aquaculture Center (OAC) (government fish farm, Namibia). The fish were managed in the same way as described in chapter 3, section 3.2.1. After seven days, fish were randomly distributed into five groups that were replicated three times at a stocking density of 20 fish per replicate, in a  $0.18\text{m}^3$  tank each, supplied with 150L of dechlorinated freshwater. Each triplicate group represented a feeding group including a control (no herbal mixture), and *A. vera* and garlic extracts mixture-supplemented groups (0.5, 1.0, 2.0, and 4.0%). These diets were administered three times a day (09:00; 13: 00; 17:00), 6 days a week until apparent satiation for 60 days. Continuous aeration, a natural photoperiod (12-h light/12-h dark cycles), and biweekly water exchange (2/3) was maintained during the experimental period. DO ( $4.90 \pm 0.31$  mg/L), and water temperature ( $27.29 \pm 0.97^\circ\text{C}$ ) was monitored once daily, whiel pH ( $6.90 \pm 0.07$ ), and ammonia concentration were monitored on a weekly basis; the later was undetectable. This experiment was conducted according to the scientific research protocols of the University of Namibia, and had complied with all relevant local and international animal welfare laws, guidelines and policies (Appendix D).

### 5.2.3 Growth and feed utilization parameters

The growth and feed utilization parameters assessed in this experiment were the same as those studied and explained in Chapter 3, Section 3.2.3 in this thesis.

#### 5.2.4 Haematological parameters

The haematological parameters were evaluated as described in Chapter 3, Section 3.2.4 in this thesis.

#### 5.2.5 Proximate composition analysis

Proximate composition analysis was carried out as indicated in chapter 3, Section 3.3.4 in this thesis.

#### 5.2.6 Low pH stress challenge experiment

The pH stress challenge was carried out as described in chapter 3, Section 3.2.6, and Chapter 4, Section 4.2.6. During this experiment pH (5.2 - 5.5), water temperature ( $29 \pm 1.5^\circ\text{C}$ ), DO ( $> 4.60 \text{ mg/L}$ ), and  $\text{NH}_3\text{-N}$  concentration ( $< 0.05 \text{ mg/L}$ ) were monitored daily.

#### 5.2.7 Statistical analysis

Data were statistically analyzed using descriptive statistics in SPSS ® (version 21, IMB Corp, Armonk, NY, USA). Normality and homogeneity of variance were confirmed using Kolmogorov-Smirnov and Levene's test, respectively. One-way analysis of variance (ANOVA) was used 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. The dietary *A. vera-A. sativum* mixture optimum requirement for *C. gariepinus* juveniles was estimated by the broken-line regression analysis model ( $y_1 = b_0 + bx$ , if  $x \leq \text{requirement}$ ;  $y_2 = b_0 + bx$ , if  $x \geq \text{requirement}$ ) (Robbins et al. 1979). The survival (%) of fish in each treatment group

for low pH challenge 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 between groups at each sampling interval of the pH challenge ( $P < 0.05$ ).

### 5.3 Results

#### 5.3.1 Growth and feed utilization parameters

The dietary *A. vera*-*A. sativum* mixture had a significant effect on the growth and feed utilization parameters of *C. gariepinus* juveniles post 60 d administration ( $P < 0.05$ ). FW ( $90.60 \pm 3.98$  g), WG ( $78.32 \pm 3.98$  g), SGR ( $3.33 \pm 0.07$  g), and AGR ( $1.24 \pm 0.08$  g) were significantly higher in fish fed diets supplemented with 1.0% *A. vera*-*A. sativum* mixture when compared to those fed a control ( $P < 0.05$ ) (Figure 5.1). Fish fed 0.5% ( $2.29 \pm 0.17\%$ ) and 1.0% ( $2.04 \pm 0.05\%$ ) *A. vera*-*A. sativum* mixture supplemented diet registered a significantly higher HSI compared to all other groups ( $P < 0.05$ ) (Table 5.2). Viscerosomatic index was significantly higher in fish fed 4.0% ( $8.71 \pm 0.92\%$ ) (followed by those fed) 0.5% ( $7.45 \pm 0.42\%$ ), and 2.0% ( $7.36 \pm 0.38\%$ ), when compared to those fed a control diet ( $P < 0.05$ ) (Table 5.2). Dietary *A. vera*-*A. sativum* mixture had no significant effect on CF and survival of *C. gariepinus* juveniles during the feeding trial ( $P > 0.05$ ) (Table 5.2).

Furthermore, the dietary *A. vera*-*A. sativum* mixture significantly improved feed utilization indices such as FCR ( $1.26 \pm 0.07$ ), FER ( $0.80 \pm 0.04$ ), and PER ( $1.92 \pm 0.01$ ) at 1.0% inclusion level compared to all other groups ( $P < 0.05$ ) (Figure 5.2A). The

optimum dietary *A. vera*-*A. sativum* mixture inclusion level was estimated to be 0.70% and 0.66% /kg diet for growth (WG:  $y_1 = 6.72x + 56.27$ ,  $R^2 = 0.36$ ;  $y_2 = -1.28x + 61.88$ ,  $R^2 = 0.12$ ), and feed utilization (FER:  $y_1 = 0.07x + 0.57$ ,  $R^2 = 0.33$ ;  $y_2 = -0.0047x + 0.62$ ,  $R^2 = 0.03$ ), respectively (Figure 5.1, 5.2).

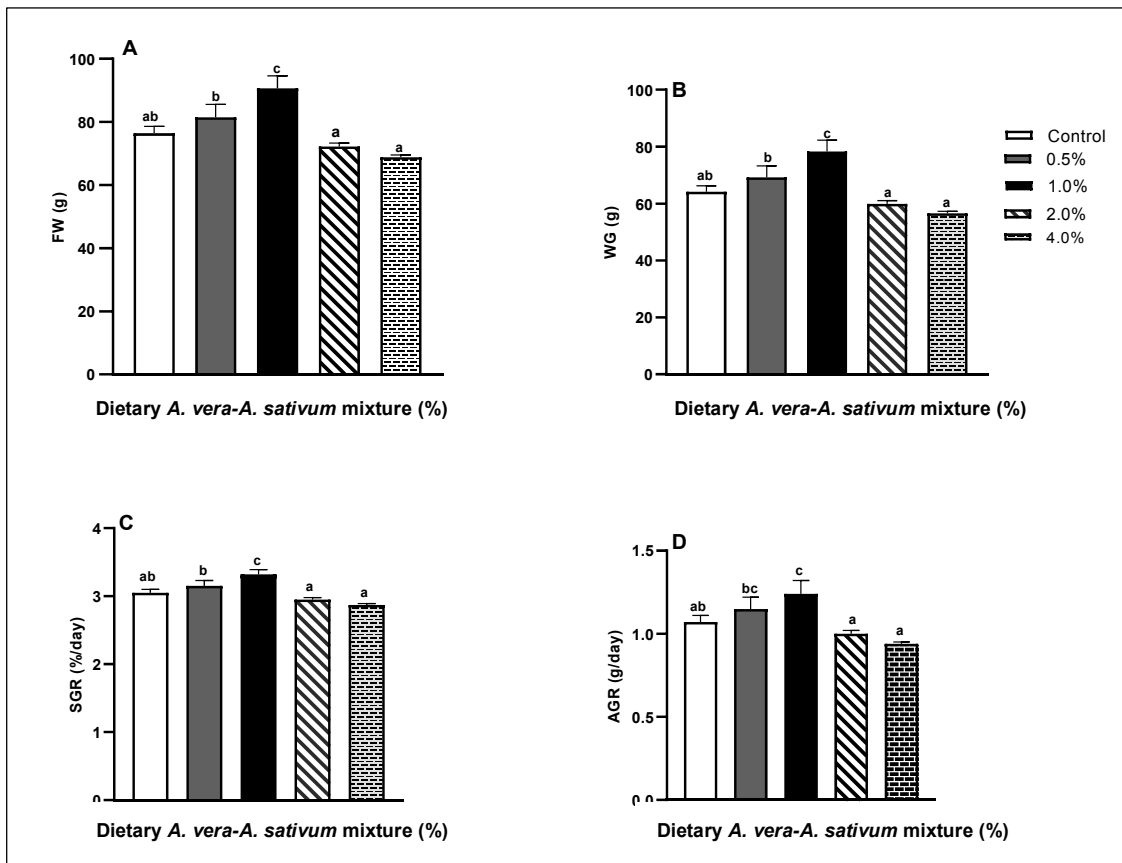


**Figure 5.1** Final weight (FW) (A), weight gain (WG) (B), Specific growth rate (SGR) (C), and absolute growth rate (AGR) (D) of African catfish, *C. gariepinus* juveniles fed four *A. vera*-*A. sativum* polysaccharide mixture (1:1) supplemented diets and an

unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference between dietary groups ( $P < 0.05$ ). Values were expressed as mean  $\pm$  standard error. WG:  $y_1 = 6.72x + 56.27$ ,  $R^2 = 0.36$ ;  $y_2 = -1.28x + 61.88$ ,  $R^2 = 0.12$  (broken-line regression model).

**Table 5.2** Organo-somatic indices, condition factor, and survival (%) of the African catfish, *C. gariepinus* fingerlings fed four *A. vera*-*A. sativum* polysaccharide mixture (1:1) supplemented diets and a control for 60 d.

| <u>Parameters</u> | <u>Dietary <i>A. vera</i> inclusion level (%)</u> |                           |                           |                           |                           |
|-------------------|---------------------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                   | Control                                           | 0.5                       | 1.0                       | 2.0                       | 4.0                       |
| VSI               | 5.38 ± 0.45 <sup>a</sup>                          | 7.45 ± 0.42 <sup>bc</sup> | 6.01 ± 0.30 <sup>ab</sup> | 7.36 ± 0.37 <sup>bc</sup> | 8.71 ± 0.92 <sup>c</sup>  |
| HSI               | 1.66 ± 0.11 <sup>a</sup>                          | 2.59 ± 0.17 <sup>bc</sup> | 2.59 ± 0.11 <sup>c</sup>  | 2.04 ± 0.04 <sup>ab</sup> | 2.04 ± 0.09 <sup>a</sup>  |
| CF                | 0.57 ± 0.02 <sup>ab</sup>                         | 0.52 ± 0.01 <sup>a</sup>  | 0.65 ± 0.03 <sup>b</sup>  | 0.65 ± 0.02 <sup>ab</sup> | 0.59 ± 0.05 <sup>ab</sup> |
| Survival          | 100 ± 0.00 <sup>a</sup>                           | 100 ± 0.00 <sup>a</sup>   | 100 ± 0.00 <sup>a</sup>   | 100 ± 0.00 <sup>a</sup>   | 100 ± 0.00 <sup>a</sup>   |

Data are expressed as mean ± standard error (M ± SE). Values with different superscript letters in the same row are not significantly different ( $P > 0.05$ ) from the control. Where VSI = viscerosomatic index, HSI = hepatosomatic index, and CF = condition factor.

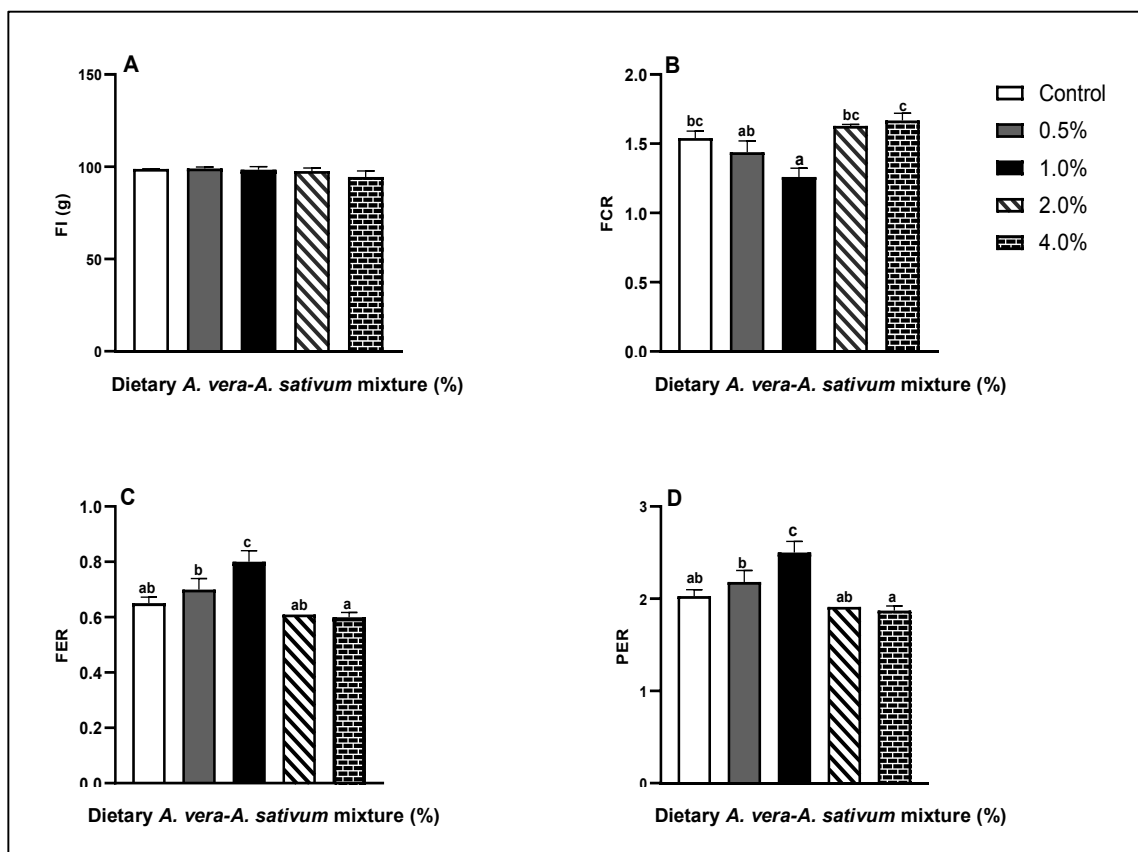


**Figure 5.3** Feed intake (FI) (A), feed conversion ratio (FCR) (B), feed efficiency ratio (FER) (C), and protein efficiency ratio (PER) (D) of the African catfish, *C. gariepinus* juveniles fed four *A. vera-A. sativum* polysaccharide mixture (1:1) supplemented diets and an unsupplemented diet (control) for 60 d. Values were expressed as mean  $\pm$  standard error. Different lower case letters denote a significant difference between dietary groups ( $P < 0.05$ ). FER:  $y_1 = 0.07x + 0.57$ ,  $R^2 = 0.33$ ;  $y_2 = -0.0047x + 0.62$ ,  $R_2 = 0.03$  (broken-line regression model).

### 5.3.2 Haematological parameters

In all haematological parameters (RBC, hematocrits, hemoglobin, PLT, MCV, MCH, MCHC, RDW<sub>a</sub>, WBC, lymphocytes, monocytes, and granulocytes) (Figures 5.3, 5.4, 5.5), a significant increase was only presented in RBC and PLT (Figure 5.3) ( $P < 0.05$ ).

Fish fed the 1.0% *A. vera*-*A. sativum* mixture supplemented diet presented a significantly higher RBC ( $1.92 \pm 0.06$ ) when compared to unsupplemented ones ( $1.40 \pm 0.15$ ) ( $P < 0.05$ ). Platelets significantly increased in fish fed the 2.0% *A. vera*-*A. sativum* mixture supplemented diet ( $38.17 \pm 4.13$ ) when compared to those fed a control diet ( $20.67 \pm 3.76$ ) ( $P < 0.05$ ).

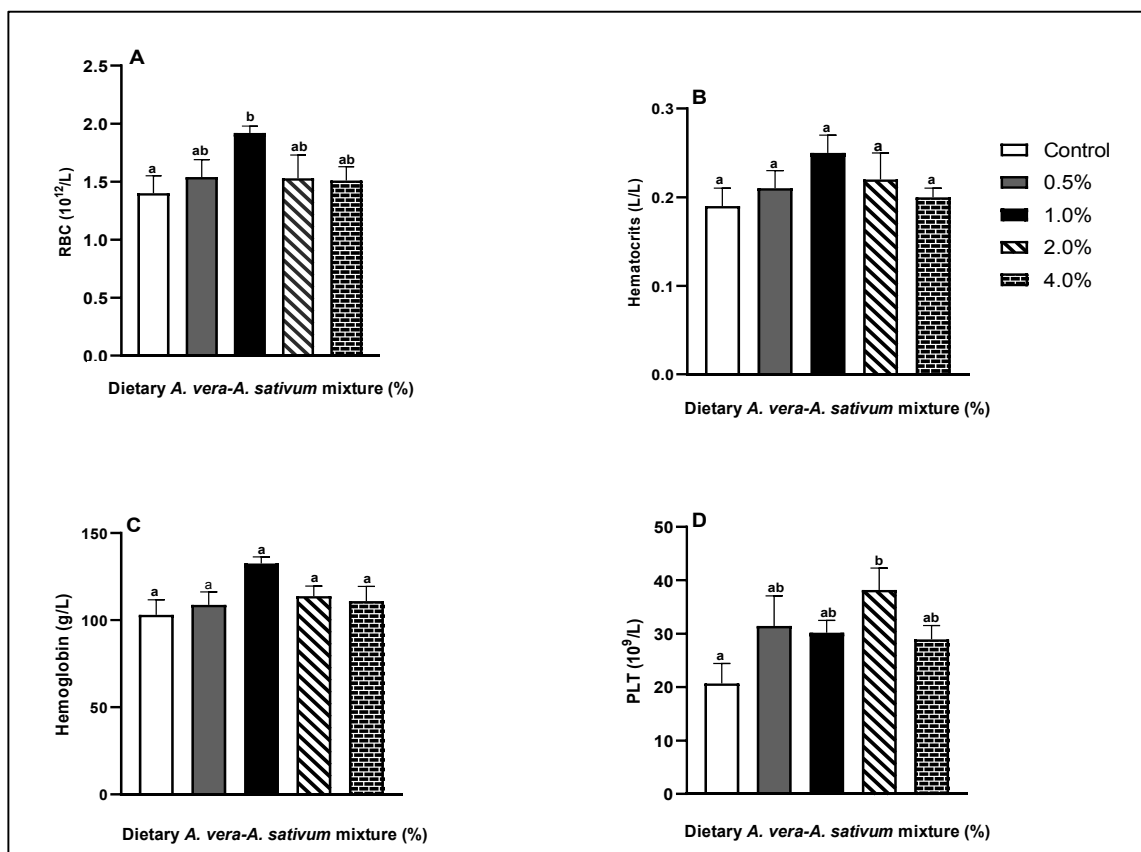


**Figure 5.3** Red blood cell counts (RBC) (A), hematocrits volume (B), hemoglobin concentration (C), and platelet counts (PLT) (D) of African catfish, *C. gariepinus* juveniles fed four *A. vera*-*A. sativum* polysaccharide mixture (1:1) supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference between dietary groups ( $P < 0.05$ ); Values were expressed as mean  $\pm$  standard error.

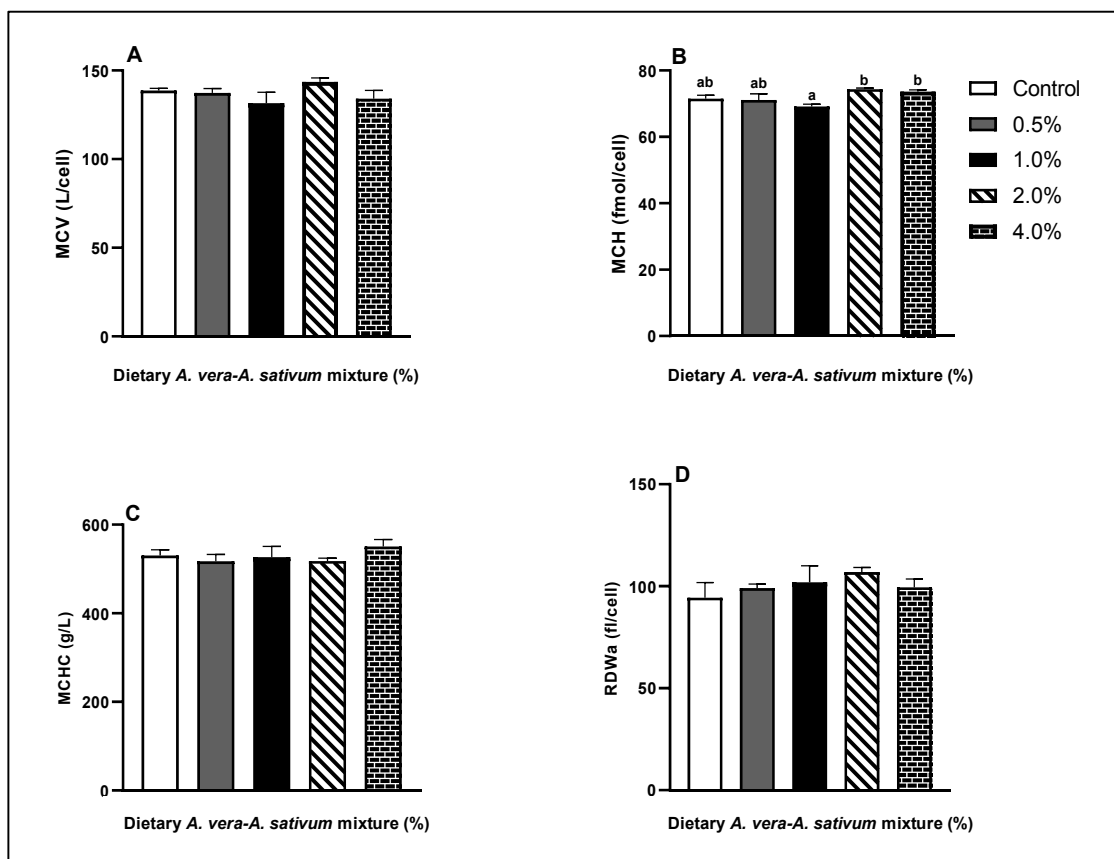


**Figure 5.4** Mean corpuscular volume (MCV) (A), mean corpuscular hemoglobin level (MCH) (B), mean corpuscular hemoglobin concentration (MCHC) (C), and red blood cell distribution width (RDWa) (D) of African catfish, *C. gariepinus* fingerlings fed four *A. vera*-*A. sativum* polysaccharide mixture (1:1) supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference between dietary groups ( $P < 0.05$ ). Values were expressed as mean  $\pm$  standard error.

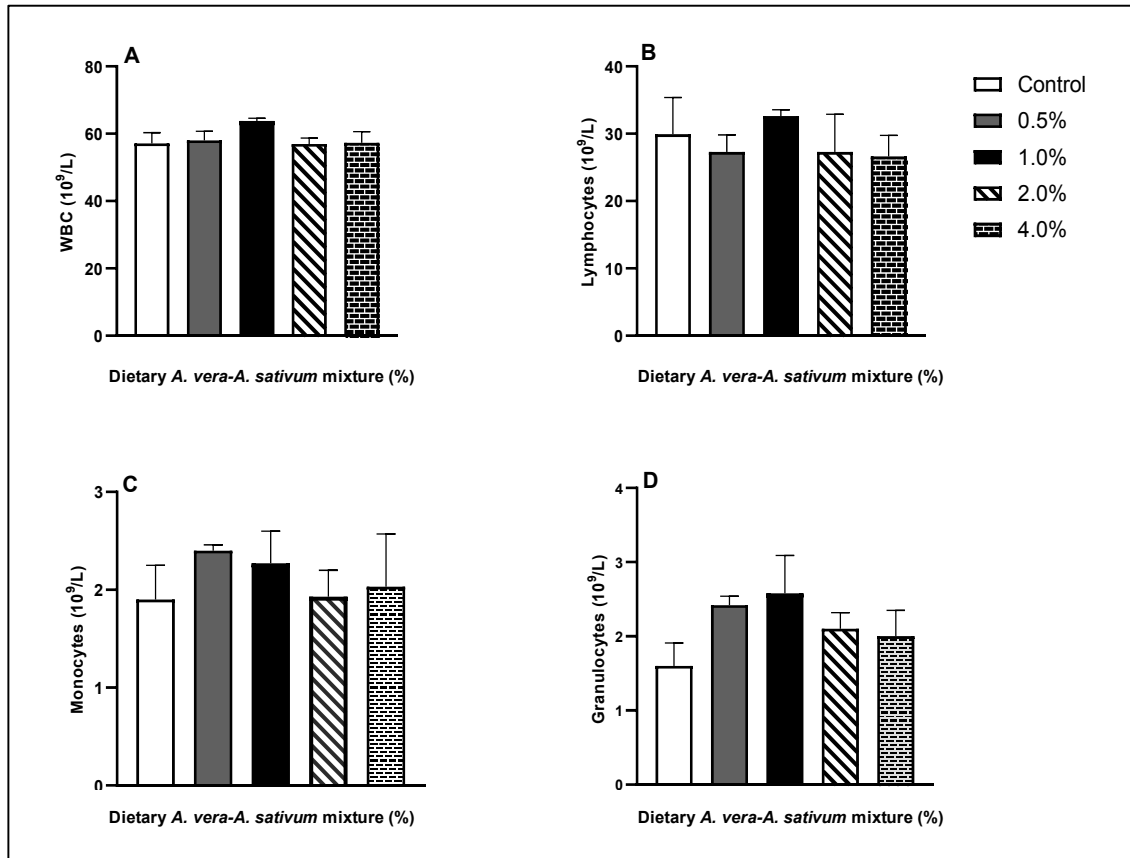


**Figure 5.5** White blood cell (WBC) (A), lymphocyte (B), monocyte (C), and granulocyte (D) counts of African catfish, *C. gariepinus* fed four *A. vera-A. sativum* polysaccharide mixture (1:1) supplemented diets and an unsupplemented diet (control) for 60 d. Different lower case letters denote a significant difference among dietary groups ( $P < 0.05$ ). Values were expressed as mean  $\pm$  standard error.

### 5.3.3 Low pH stress challenge experiment

Fish survival was significantly affected by dietary groups ( $P < 0.05$ ) at 24h, 48h, and 72h post low pH challenge, based on the Breslow (generalized Wilcoxon,  $P = 0.035$ ), Tarone-ware ( $P = 0.007$ ), and log-rank (Mantel-cox,  $P = 0.007$ ) tests (Figure. 5.6). Fish fed 1.0% *A. vera-A. sativum* mixture supplemented diet had the highest survival probability (90%, 80%, 70%, post 24h, 48h, and 72h, respectively) throughout the

challenge period. The lowest survival probabilities were presented in fish fed 4.0% (40% post 48h, and 15% post 72h) followed by those fed 2.0% (50% post 48h, and 28% post 72h). Survival probability was intermediate in fish fed 0.5% (72% post 48h, and 58% post 72h) and a control diet (62% post 48h, and 40% post 72h) throughout the challenge experiment.

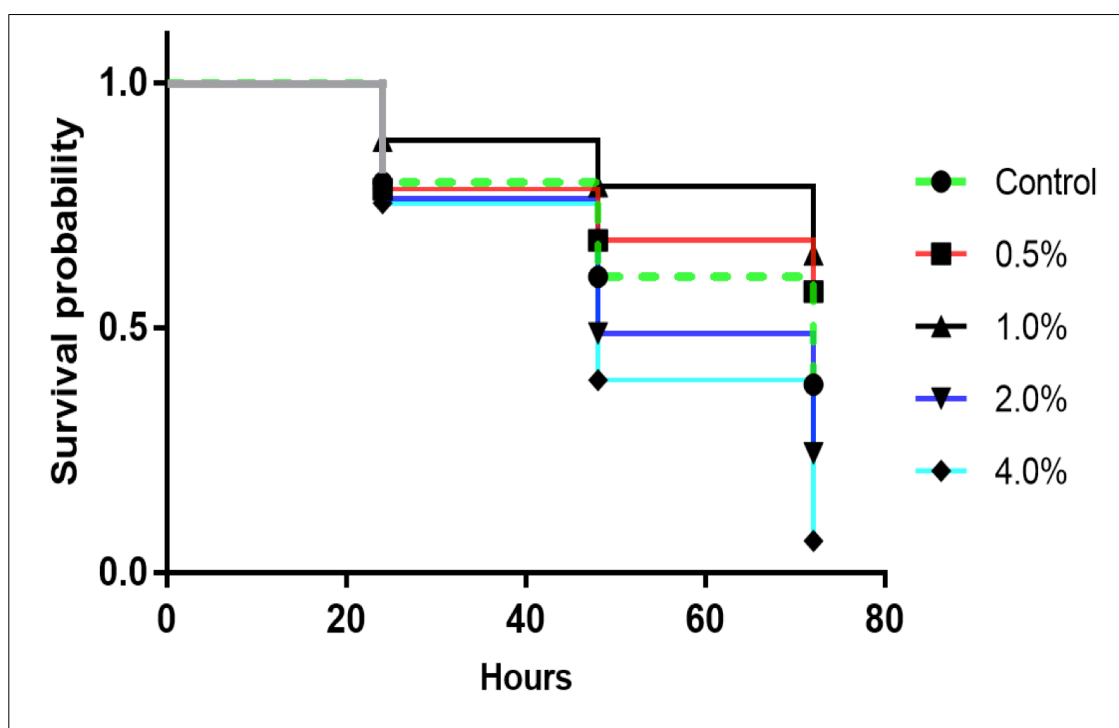


**Figure 5.6** Kaplan-Meier: low pH challenge survival probability of African catfish, *C. gariepinus* fingerlings fed four *A. vera*-*A. sativum* polysaccharide mixture (1:1) supplemented diets and an unsupplemented diet (control) for 60 d.

#### 5.3.4 Proximate body composition

The dietary *A. vera*-*A. sativum* mixture had no significant ( $P > 0.05$ ) effect on moisture, protein, and ash % of *C. gariepinus* juveniles (Table 5.3). A significantly lower lipid

content was observed in fish fed 2.0% ( $6.69 \pm 0.36$ ), 4.0% ( $7.18 \pm 0.24$ ) and 1.0% ( $7.44 \pm 0.29$ ) of the mixture when compared to the control ( $9.31 \pm 0.71$ ) ( $P < 0.05$ ).

**Table 5.3** Selected whole body composition parameters of African catfish, *C. gariepinus* juveniles fed four *A. vera*-*A. sativum* polysaccharide mixture (1:1) and an un-supplemented diet (control) for 60 d.

| <u>Parameters</u> | <u>Dietary <i>A. vera</i>-<i>A. sativum</i> mixture inclusion level (%)</u> |                      |                      |                    |                    |
|-------------------|-----------------------------------------------------------------------------|----------------------|----------------------|--------------------|--------------------|
|                   | Control                                                                     | 0.5                  | 1.0                  | 2.0                | 4.0                |
| Moisture (%)      | $72.30 \pm 0.06^a$                                                          | $72.79 \pm 0.40^a$   | $72.04 \pm 0.34^a$   | $72.06 \pm 0.63^a$ | $72.77 \pm 0.87^a$ |
| Protein (%)       | $70.75 \pm 0.99^a$                                                          | $73.70 \pm 1.92^a$   | $72.88 \pm 0.60^a$   | $76.50 \pm 3.11^a$ | $75.70 \pm 2.06^a$ |
| Lipid (%)         | $9.31 \pm 0.71^c$                                                           | $8.27 \pm 0.33^{bc}$ | $7.44 \pm 0.29^{ab}$ | $6.69 \pm 0.36^a$  | $7.18 \pm 0.24^a$  |
| Ash (%)           | $5.68 \pm 0.60^a$                                                           | $6.99 \pm 0.55^a$    | $6.33 \pm 0.97^a$    | $6.98 \pm 0.90^a$  | $7.29 \pm 0.20^a$  |

Values (Mean  $\pm$  Standard Error, M $\pm$ SE) within the same row with the same superscripts letters are not significantly different ( $P < 0.05$ ).

## 5.4 Discussion

In the current study, some growth and feed utilization parameters improved in fish fed *A. vera*-*A. sativum* polysaccharide mixture supplemented diets compared to those fed a control, and the optimum inclusion level ranged between 0.5% and 1.0%, but was mostly 1.0%. This is the first study reporting *A. vera* and *A. sativum* herbal mixture as a dietary supplement in fish. The findings are in accordance with previous studies, which reported *A. vera* or *A. sativum* as a single extract or a mixture with other herbs. *A. vera* as a single dietary supplement was reported to have increased growth in *C. gariepinus*

(Ibidunni et al. 2018), *C. carpio* (Mahdavi et al. 2013), *Oncorhynchus mykiss* (Heidarieh et al. 2013), and GIFT-*O. niloticus* (Gabriel et al. 2015). Increased growth was also reported in *O. niloticus* juveniles after being fed a diet supplemented with a herbal mixture comprised of *A. vera*, *S. crispus*, and *V. trifolia* powder, combined in equal proportions (1:1:1 ratio) (Manaf et al. 2016). Furthermore, *A. sativum* as an individual dietary growth promoter supplement has been widely reported in fish (Thanikachalam et al. 2010; Talpur and Ikhwanuddin 2012; Mehrim et al. 2014; Hassaan and Soltan 2016; Büyükdeveci et al. 2018). In the same vein, a dietary mixture of *A. sativum* and *Spirulina platensis* increased weight gain and specific growth rate, and improved the feed conversion ratio and the protein efficiency ratio in *O. niloticus* compared to a control (Abu-Elala et al. 2016). Moreover, higher growth and better feed utilization parameters were reported in *S. hasta* fry after being fed diets supplemented with herbal mixtures of *A. sativum*, *Zingiber officinale*, and *Thymus vulgaris* for eight weeks compared to a control (Jahanjoo et al. 2018).

*Allium sativum* and *A. vera* have been reported to have had no effect on the growth of fish after a short feeding duration (Labrador et al. 2016; Huang et al. 2018) or at high dietary inclusion levels (Mehrim et al. 2014; Gabriel et al. 2015). Accordingly, Liu et al. (2010) reported poor growth in *Macrobrachium rosenbergii* after being fed diets supplemented with anthraquinones extracts for four weeks, but significant growth improvement was only presented after six weeks when compared to a control.

Gabriel et al. (2015) also reported that 4.0% *A. vera* supplemented diets had no significant effect on the growth of GIFT-*O. niloticus* juveniles. The same inclusion level

(highest dosage, 4.0%) had no significant effect on the growth performance of *C. gariepinus* juveniles compared to the control, in the present study.

The improvement of some haematological parameters in fish fed the *A. vera*-*A. sativum* mixture supplemented diets observed in the present study is supported by several studies, which have reported them as single supplements or individually mixed with other herbs. Significantly higher haematological parameters were reported in *C. gariepinus* fingerlings after being fed a diet supplemented with *A. vera* leaves paste for 12 wks (Ibidunni et al. 2018) compared to a control. Improved haematological parameters were also reported in GIFT-*O. niloticus* fed diets supplemented with 100% *A. vera* powder (Gabriel et al. 2015). A mixture of *A. vera*, *V. trifolia*, and *S. crispus* has also been reported to significantly increase haematological parameters of red hybrid tilapia (*Oreochromis* sp.) post 60 days administration compared to a control (Manaf et al. 2016). Furthermore, haematological parameters of *O. mykiss* (Nya and Austin 2011; Esmaeili et al. 2017), *O. niloticus* (Shalaby et al. 2006; Aly and Mohamed 2010) *Lates calcarifer* (Talpur and Ikhwanuddin 2012), and *Labeo rohita* (Sahu et al. 2007) improved after being fed diets supplemented with *A. sativum* alone compared to a control.

Combining *A. sativum* with other herbs has also been reported to greatly improve haematological parameters in fish compared to a control. For instance, a dietary mixture of *A. sativum*, *Z. officinale*, and *T. vulgaris* was reported to significantly increase RBC and WBC in *S. hasta* fry when compared to a control (Jahanjoo et al. 2018). Yilmaz and Ergün (2012) observed that *D. labrax* juveniles supplemented with a dietary mixture of

garlic and ginger oil had the highest RBC and Hct, Hb, MCV, MCH, and MCHC when compared to those fed a control and diets separately supplemented with garlic or ginger. This is an indication that there are benefits in combining herbal extracts in fish feed as also shown in the present study.

Currently, there are limited studies that have demonstrated the mechanisms of action of herbal extracts as supplements in animals. Previous studies attributed the improved growth parameters, health parameters, and increased resistance against stress in fish following herbal extracts supplementation to their nutritional compositions as well as their non-nutritional factors (Lee and Gao 2012; Tremaroli and Backhed 2012; Zahran et al. 2014). Ji et al. (2007a) indicated that mixed herbs happen to evoke better beneficial synergistic effects in animals as demonstrated in the current study, because they may complement one another in terms of nutrients and other medicinal properties. Moreover, polysaccharides (prebiotics) present both in *A. vera* (Hamman 2008; Gupta and Malhotra 2012), and *A. sativum* (Kallel et al. 2015; Li et al. 2017) are some of the non-nutritional factors that have been reported to possess growth and health promoting properties in animals (Song et al. 2014; Mohan et al. 2019). Combining polysaccharides of different nature (acemannan, glucomannan, galactan, and mannose from *A. vera*, and galactose, rhamnose, glucuronic acid, and galacturonic acid from *A. sativum*) in fish diet as demonstrated in this study, might additively improve gut microbial community as well as the host's health (Patel and Srinivasan 2004; Citarasu 2010; Tremaroli and Backhed 2012) using mechanisms explained by Chen et al. (2003) and Yu et al. (2018).

In addition to polysaccharides, the improved growth and health of *C. gariepinus* juveniles in the present study, could be a result of additive effects of allicin from *A. sativum* and emodin from *A. vera*. Allicin, as indicated in Chapter 4, has the ability to improve feed utilization in fish by enhancing gastrointestinal motility, and modulating secretion of various digestive enzymes (Lee and Gao 2012). It also promotes the performance of intestinal flora, inhibits deleterious bacteria while intensifying beneficial bacteria, hence improving energy utilization, growth and health of the host (Diab et al. 2008). Similarly, emodin has been reported to enhance innate immune response, antioxidation, and increase disease resistance in fish (Devi et al. 2019). Moreover, growth and health of fish fed diets supplemented with herbs could also be attributed to their ability to promote lipid metabolism via increased bile production (Yilmaz et al. 2012), which spare protein for growth, and lead to the repression of lipid accumulation (Ji et al. 2009). Thus, fish muscle quality (i.e. low lipid) would be improved, as demonstrated in the current study.

On the contrary, medicinal herbs including *A. vera* (Taiwo et al. 2005; Gabriel et al. 2015) and *A. sativum* (Yang et al. 2010) may be harmful to fish and even deadly, at high dosages (Palanisamy et al. 2011). The premise was true when *C. gariepinus* juveniles were fed a diet supplemented with the highest inclusion level of *A. sativum* polysaccharides (4.0% /kg diet) (as demonstrated in Chapter 4 of this thesis). In agreement, the present study observed that the highest dosage (4% *A. vera*-*A. sativum* mixture) had negative effects on the resistance of *C. gariepinus* juveniles against low water pH. Administration of herbal extracts at higher dosage and over a long period of time may lead to a number of issues such as immune suppression (Sakai 1999), reduced

effectiveness (Harikrishnan et al. 2011), and overstimulation of the immune systems which affects the normal metabolic activities (Talpur and Ikhwanuddin 2013). Thus, resulting in fish that are unable to cope with physiological stress as demonstrated in the current study.

In conclusion, this study demonstrated that *A. vera* and *A. sativum* polysaccharides can be used in mixture for synergistic beneficial growth, feed utilization, health, and meat quality effects in African catfish, *C. gariepinus* juveniles. The dietary *A. vera*-*A. sativum* mixture inclusion level between 0.70% and 0.66% was estimated optimal to support growth and feed utilization in *C. gariepinus* juveniles.

Further studies should focus on using purified *A. vera* and *A. sativum* extracts. Extracts should be combined in different ratios and compared directly with using the extracts individually. The effects of the extracts on digestive enzymes as well as intestinal the fish bacterial community in addition to biometric and immunological parameters should also be tested. The relationship between time of administration and the effectiveness of the herbal extracts in fish also needs to be established.

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## CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

The poor fish health management in the Namibian aquaculture and elsewhere in the world is something that can be mitigated. Although not much has been done locally to improve this situation, previous studies have shown that the use of medicinal herbs in aquaculture as immuno-stimulants, growth promoters, feed utilization enhancers, appetizers, and anti-stress enhancers could be a sustainable solution. Hence, the aim of this study was to develop a phytogenic diet for the Namibian aquaculture sector from easily grown herbs and indigenous ingredients with the ability to improve fish growth, feed utilization, general health, and resistance of fish against culture conditions. For this purpose, the current study was designed to investigate the effects of dietary aloe vera (*Aloe vera*), and garlic (*Allium sativum*) crude polysaccharide extracts on growth performance, feed utilization, haematological parameters, whole body composition, and resistance against low water pH in African catfish, *C. gariepinus* under culture conditions.

The first part of this study investigated the potential effects of dietary *A. vera* polysaccharides (0.5%, 1.0%, 2.0%, and 4.0%) on growth performance, feed utilization, haemato-biochemical parameters, body composition, and resistance against low water pH (5.2 – 5.5) in *C. gariepinus* fingerlings, post 60 d feeding. This experiment showed the ability of *A. vera* polysaccharides (0.5% - 2.0%) to promote growth (final weight, weight gain, absolute growth rate, and specific growth rate) improve feed utilization

(feed conversion ratio, and protein efficiency ratio) health parameters (white blood cells, red blood cells, alanine aminotransferase, and aspartate aminotransferase enzyme), and increase resistance of *C. gariepinus* fingerlings against low water pH after 60 d of administration. The optimum dietary *A. vera* inclusion levels suitable for growth and feed utilization were estimated to be 1.79% and 1.77%, respectively, based on the second order polynomial regression model. Dietary *A. vera* had no effects on the proximate body composition of *C. gariepinus* fingerlings. Furthermore, the study has demonstrated poor growth performance, feed utilization, health parameters, and low resistance of fish against low water pH at the highest dietary *A. vera* inclusion level (4.0%). Overall, from this experiment, it can thus be concluded that *A. vera* polysaccharide extracts have the potential to be used as feed supplements to improve growth, health, and reduce stress in *C. gariepinus* fingerlings during culture.

The second part of the study investigated the effects of dietary garlic (*Allium sativum*) polysaccharide extracts (GPE) included at 0.5%, 1.0%, 2.0%, and 4.0% on growth performance, haematological parameters, whole body composition, and resistance against low water pH in *C. gariepinus* juveniles. From this study, GPE demonstrated better growth performance (final weight, weight gain, absolute growth rate, and specific growth rate), improved feed utilization (feed intake, feed conversion ratio, feed efficiency ratio, and protein efficiency ratio), and increased haematological parameters (red blood cells, and mean corpuscular haemoglobin concentration) in *C. gariepinus* juveniles, in a dose dependent fashion. As demonstrated in the first experiment, the highest dietary GPE level (4.0%) had no effects on fish growth performance, feed

utilization and health parameters. Moreover, GPE had no effects on proximate body composition, neither on the resistance of fish against low water pH. The optimum inclusion level for growth and feed utilization was estimated to be 1.69% and 1.77% *A. sativum*, respectively. In summary, this experiment presented that *A. sativum* polysaccharide extracts qualify to be used as phytogenics to promote growth and general health in *C. gariepinus* juveniles, especially in intensive aquaculture systems.

The last component of this study was designed to investigate the hypothesis that feeding *C. gariepinus* juveniles with a diet supplemented with *A. vera* and *A. sativum* polysaccharides mixture (1:1) (0.5%, 1.0%, 2.0% and 4.0%) would have effects on their growth performance, feed utilization, haematological indices, whole body composition, and survival at low water pH. This experiment has proven that dietary *A. vera*-*A. sativum* mixture (1:1) had the capacity to increase growth (final weight, weight gain, absolute growth rate, and specific growth rate) and improve feed utilization parameters (feed conversion ratio, feed efficiency ratio, and protein efficiency ratio) in *C. gariepinus* juveniles. The optimum dietary *A. vera*-*A. sativum* mixture inclusion levels were estimated to be 0.70% and 0.66% diet for growth and feed utilization respectively. Generally, haematological indices were increased in fish supplemented with *A. vera*-*A. sativum* mixture when compared to the control; no negative effects were presented in supplemented groups when compared to the unsupplemented ones. In terms of proximate body composition, fish that were supplemented with inclusion levels between 1.0%, and 4.0% *A. vera*-*A. sativum* mixture presented lower lipid content compared to the supplemented ones. Furthermore, fish that were supplemented with *A. vera*-*A.*

*sativum* mixture showed a stronger resistance against low water pH when compared to the unsupplemented ones. In conclusion, *A. vera* and *A. sativum* polysaccharides mixture (1:1) could be recommended as feed additives in aquaculture to promote growth, feed utilization, health, and increase fish resistance against stress during culture period.

In summary, *A. vera*, and *A. sativum* polysaccharide extracts (separately or mixture) could be recommended as possible alternative remedies to promote growth, feed utilization, health, and resistance of African catfish against acidic water in aquaculture systems, especially at dietary inclusion levels not higher than 2.0%. This study was the first to introduce *A. vera*, and *A. sativum* polysaccharide extracts and their mixtures as potential aquaculture feed additives, and the findings could still be of greater significance to the development of aquaculture in Namibia, and other sub-Saharan African countries experiencing the same plight of poor fish health management, and insignificant production return from aquaculture. This is encouraging to the fish farmers, and aquaculture scientists who could now choose to use cost effective aquaculture natural remedies, which are easily grown, safe to consumers, and their environment. Since the adoption of herbal extracts in aquaculture feed industry is lagging behind, this study also serves to market and promote the implementation of herbal extracts as feed supplements in the aquaculture feed industry. Furthermore, the study significantly contributes to the expansion of previous related work and knowledge on *A. vera*, and *A. sativum* and other medicinal herbs tested in aquaculture. Essentially, this study could be used as a review for understanding underlying theoretical principles of dietary herbal extract effects in

fish, which would provide scientists a basis for better future work in the field of aquaculture nutrition, especially in Namibia.

## **6.2 Recommendations**

Before *A. vera* and *A. sativum* polysaccharide extracts and their mixtures are adopted in *C. gariepinus* culture as feed additives more studies are still required, and the following recommendations should be considered:

(1) In this study, experimental facilities were a limitation, thus the study could not carry out parallel experiments to be able to compare the growth and physiological effects between individual herbs (*A. vera* and *A. sativum*) and their mixtures in *C. gariepinus*, thus this needs to be further tested. (2) Future studies should consider investigating these extract mixtures at different ratios to better understand their synergistic effects in fish and compare them directly with effects of each herb individually. (3) Additional experiments need to be carried out to determine to what extent the effects of these herbs (*A. vera* and *A. sativum* and their mixture) are influenced by the dosages (from 0.5% to 2%), and duration of exposure (i.e short 30 d and long-term 90 d). (4) Environmental factors play an important role in fish physiology including metabolism. Future studies should determine whether the observed *A. vera*, *A. sativum*, and their mixture effects in *C. gariepinus* are influenced by environmental factors such as temperature and light. (5) Herbal extracts have been acclaimed to improve growth and health status of fish through their ability to improve the intestinal microbiota. To confirm this mechanism, future studies should incorporate evaluating the composition and diversity of bacterial communities within the fish intestinal ecosystem following herbal extract administration. (6) In addition, since antimicrobial activity has been reported in *A. vera*

and *A. sativum*, future studies should investigate their preventative and curative effects in fish against pathogenic bacteria that are a problem in aquaculture. (7) Garlic and aloe vera are known to possess pungent smell and bitter tastes respectively, hence future studies should include sensory evaluation to determine the eating quality of fish products from dietary garlic and/or aloe vera supplemented fish. (8) Future studies should include a cost-benefit analysis of using herbal extracts in aquaculture. (9) Since the herbal extracts used in this study were commercial products, effective extraction and screening methods of the main constituents need to be explored locally, to better understand their functions. (10) Lastly, to ensure a sustainable aquaculture, mass production of these herbs in Namibia needs to be explored as their use in aquaculture may double the pressure already exerted by agricultural sectors and humans.

## APPENDICES

### Appendix A

**Table 1** Descriptive statistics of growth performance indices of African catfish (*Clarias gariepinus*) fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

|     |               | N  | Mean    | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Minimum | Maximum |
|-----|---------------|----|---------|----------------|------------|----------------------------------|-------------|---------|---------|
|     |               |    |         |                |            | Lower Bound                      | Upper Bound |         |         |
| FW  | Control       | 3  | 28.5658 | 5.34849        | 3.08795    | 15.2794                          | 41.8521     | 24.84   | 34.69   |
|     | Aloe 0.5%     | 3  | 40.6750 | 2.92487        | 1.68867    | 33.4092                          | 47.9408     | 37.41   | 43.06   |
|     | Aloe 1.0%     | 3  | 42.4873 | 11.21416       | 6.47450    | 14.6298                          | 70.3448     | 34.34   | 55.28   |
|     | Aloe 2%       | 3  | 37.1262 | 2.32638        | 1.34314    | 31.3471                          | 42.9052     | 34.50   | 38.93   |
|     | Aloe 3%       | 3  | 28.4480 | 7.30829        | 4.21944    | 10.2932                          | 46.6027     | 23.71   | 36.86   |
|     | Total         | 15 | 35.4604 | 8.33087        | 2.15102    | 30.8470                          | 40.0739     | 23.71   | 55.28   |
| WG  | Control       | 3  | 25.5158 | 5.34849        | 3.08795    | 12.2294                          | 38.8021     | 21.79   | 31.64   |
|     | Aloe 0.5%     | 3  | 37.6250 | 2.92487        | 1.68867    | 30.3592                          | 44.8908     | 34.36   | 40.01   |
|     | Aloe 1.0%     | 3  | 39.4373 | 11.21416       | 6.47450    | 11.5798                          | 67.2948     | 31.29   | 52.23   |
|     | Aloe 2%       | 3  | 34.0762 | 2.32638        | 1.34314    | 28.2971                          | 39.8552     | 31.45   | 35.88   |
|     | Aloe 3%       | 3  | 25.3980 | 7.30829        | 4.21944    | 7.2432                           | 43.5527     | 20.66   | 33.81   |
|     | Total         | 15 | 32.4104 | 8.33087        | 2.15102    | 27.7970                          | 37.0239     | 20.66   | 52.23   |
| AGR | Control       | 3  | 0.4253  | 0.08914        | 0.05147    | 0.2038                           | 0.6467      | 0.36    | 0.53    |
|     | 0.5% Aloe     | 3  | 0.6271  | 0.04875        | 0.02814    | 0.506                            | 0.7482      | 0.57    | 0.67    |
|     | 1.0% Aloe     | 3  | 0.6573  | 0.1869         | 0.10791    | 0.193                            | 1.1216      | 0.52    | 0.87    |
|     | 2.0% Aloe     | 3  | 0.5679  | 0.03877        | 0.02239    | 0.4716                           | 0.6643      | 0.52    | 0.6     |
|     | 4.0% Aloe     | 3  | 0.4233  | 0.1218         | 0.07032    | 0.1207                           | 0.7259      | 0.34    | 0.56    |
|     | Total         | 15 | 0.5402  | 0.13885        | 0.03585    | 0.4633                           | 0.6171      | 0.34    | 0.87    |
| SGR | Control       | 3  | 3.7100  | .29969         | .17303     | 2.9655                           | 4.4544      | 3.50    | 4.05    |
|     | Aloe 0.5%     | 3  | 4.3145  | .12184         | .07034     | 4.0119                           | 4.6172      | 4.18    | 4.41    |
|     | Aloe 1.0%     | 3  | 4.3537  | .41921         | .24203     | 3.3124                           | 5.3951      | 4.04    | 4.83    |
|     | Aloe 2%       | 3  | 4.1631  | .10610         | .06126     | 3.8995                           | 4.4267      | 4.04    | 4.24    |
|     | Aloe 3%       | 3  | 3.6874  | .40532         | .23401     | 2.6805                           | 4.6943      | 3.42    | 4.15    |
|     | Total         | 15 | 4.0457  | .39442         | .10184     | 3.8273                           | 4.2642      | 3.42    | 4.83    |
| HSI | Control       | 3  | 1.7093  | .16916         | .09766     | 1.2891                           | 2.1295      | 1.51    | 1.81    |
|     | Aloe 0.5%     | 3  | 1.5020  | .08861         | .05116     | 1.2819                           | 1.7221      | 1.45    | 1.60    |
|     | Aloe 1.0%     | 3  | 1.5913  | .33665         | .19437     | .7550                            | 2.4276      | 1.22    | 1.87    |
|     | Aloe 2%       | 3  | 1.4524  | .28232         | .16300     | .7511                            | 2.1538      | 1.22    | 1.77    |
|     | Aloe 3% Total | 3  | 1.5507  | .28419         | .16408     | .8447                            | 2.2567      | 1.35    | 1.88    |
|     | Total         | 15 | 1.5611  | .22919         | .05918     | 1.4342                           | 1.6881      | 1.22    | 1.88    |
| VSI | Control       | 3  | 7.4550  | 1.28961        | .74456     | 4.2514                           | 10.6585     | 6.30    | 8.84    |
|     | Aloe 0.5%     | 3  | 8.6792  | 4.95929        | 2.86325    | -3.6404                          | 20.9987     | 5.55    | 14.40   |
|     | Aloe 1.0%     | 3  | 6.5742  | .93910         | .54219     | 4.2413                           | 8.9070      | 5.55    | 7.39    |
|     | Aloe 2%       | 3  | 5.6448  | .38014         | .21947     | 4.7005                           | 6.5891      | 5.32    | 6.06    |
|     | Aloe 3%       | 3  | 8.3386  | 4.41271        | 2.54768    | -2.6232                          | 19.3003     | 5.70    | 13.43   |
|     | Total         | 15 | 7.3383  | 2.83226        | .73129     | 5.7699                           | 8.9068      | 5.32    | 14.40   |
| CFI | control       | 3  | .6801   | .00573         | .00331     | .6659                            | .6944       | .68     | .69     |
|     | Aloe 0.5%     | 3  | .6886   | .05755         | .03323     | .5457                            | .8316       | .62     | .72     |
|     | Aloe 1.0%     | 3  | .7289   | .03029         | .01749     | .6536                            | .8041       | .71     | .76     |
|     | Aloe 2%       | 3  | .6954   | .01270         | .00733     | .6638                            | .7269       | .69     | .71     |
|     | Aloe 3%       | 3  | .6981   | .05633         | .03252     | .5582                            | .8380       | .63     | .74     |
|     | Total         | 15 | .6982   | .03713         | .00959     | .6777                            | .7188       | .62     | .76     |

**Table 2** Descriptive statistics of feed utilization indices and survival rate of African catfish (*Clarias gariepinus*) fingerlings fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

| Parameters | N  | Mean    | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Minimum | Maximum |
|------------|----|---------|----------------|------------|----------------------------------|-------------|---------|---------|
|            |    |         |                |            | Lower Bound                      | Upper Bound |         |         |
| <b>FCR</b> |    |         |                |            |                                  |             |         |         |
| Control    | 3  | 1.9441  | .32460         | .18741     | 1.1377                           | 2.7504      | 1.59    | 2.22    |
| Aloe0.5%   | 3  | 1.4141  | .12804         | .07393     | 1.0961                           | 1.7322      | 1.27    | 1.50    |
| Aloe1.0%   | 3  | 1.3410  | .38020         | .21951     | .3966                            | 2.2855      | .93     | 1.68    |
| Aloe2%     | 3  | 1.5847  | .12915         | .07456     | 1.2639                           | 1.9055      | 1.44    | 1.67    |
| Aloe3%     | 3  | 1.9862  | .48121         | .27783     | .7908                            | 3.1816      | 1.44    | 2.33    |
| Total      | 15 | 1.6540  | .38669         | .09984     | 1.4399                           | 1.8682      | .93     | 2.33    |
| <b>FI</b>  |    |         |                |            |                                  |             |         |         |
| Control    | 3  | 48.5467 | 3.79108        | 2.18878    | 39.1291                          | 57.9642     | 44.22   | 51.26   |
| Aloe0.5%   | 3  | 53.0100 | 3.20823        | 1.85227    | 45.0403                          | 60.9797     | 50.71   | 56.68   |
| Aloe1.0%   | 3  | 50.1000 | 2.13721        | 1.23392    | 44.7909                          | 55.4091     | 48.54   | 52.54   |
| Aloe2%     | 3  | 53.8533 | 3.20988        | 1.85323    | 45.8795                          | 61.8271     | 51.53   | 57.52   |
| Aloe3%     | 3  | 48.1067 | .49715         | .28703     | 46.8717                          | 49.3417     | 47.59   | 48.58   |
| Total      | 15 | 50.7233 | 3.38657        | .87441     | 48.8479                          | 52.5988     | 44.22   | 57.52   |
| <b>FER</b> |    |         |                |            |                                  |             |         |         |
| Control    | 3  | .5248   | .09411         | .05433     | .2911                            | .7586       | .45     | .63     |
| Aloe0.5%   | 3  | .7112   | .06774         | .03911     | .5430                            | .8795       | .67     | .79     |
| Aloe1.0%   | 3  | .7928   | .25152         | .14522     | .1680                            | 1.4176      | .60     | 1.08    |
| Aloe2%     | 3  | .6340   | .05414         | .03126     | .4995                            | .7685       | .60     | .70     |
| Aloe3%     | 3  | .5272   | .14696         | .08485     | .1621                            | .8922       | .43     | .70     |
| Total      | 15 | .6380   | .16164         | .04173     | .5485                            | .7275       | .43     | 1.08    |
| <b>PER</b> |    |         |                |            |                                  |             |         |         |
| Control    | 3  | .8505   | .17828         | .10293     | .4076                            | 1.2934      | .73     | 1.05    |
| Aloe0.5%   | 3  | 1.2542  | .09750         | .05629     | 1.0120                           | 1.4964      | 1.15    | 1.33    |
| Aloe1.0%   | 3  | 1.3146  | .37381         | .21582     | .3860                            | 2.2432      | 1.04    | 1.74    |
| Aloe2%     | 3  | 1.1359  | .07755         | .04477     | .9432                            | 1.3285      | 1.05    | 1.20    |
| Aloe3%     | 3  | .8466   | .24361         | .14065     | .2414                            | 1.4518      | .69     | 1.13    |
| Total      | 15 | 1.0803  | .27770         | .07170     | .9266                            | 1.2341      | .69     | 1.74    |
| <b>SUR</b> |    |         |                |            |                                  |             |         |         |
| Control    | 3  | 88.3333 | 7.63763        | 4.40959    | 69.3604                          | 107.3062    | 80.00   | 95.00   |
| Aloe0.5%   | 3  | 90.0000 | 5.00000        | 2.88675    | 77.5793                          | 102.4207    | 85.00   | 95.00   |
| Aloe1.0%   | 3  | 90.0000 | 5.00000        | 2.88675    | 77.5793                          | 102.4207    | 85.00   | 95.00   |
| Aloe2%     | 3  | 93.3333 | 2.88675        | 1.66667    | 86.1622                          | 100.5044    | 90.00   | 95.00   |
| Aloe3%     | 3  | 85.0000 | 5.00000        | 2.88675    | 72.5793                          | 97.4207     | 80.00   | 90.00   |
| Total      | 15 | 89.3333 | 5.30049        | 1.36858    | 86.3980                          | 92.2686     | 80.00   | 95.00   |

**Table 3** Descriptive statistics of haemato-biochemical indices of African catfish (*Clarias gariepinus*) fingerlings fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

|      |           | N  | Mean     | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Minimum | Maximum |
|------|-----------|----|----------|----------------|------------|----------------------------------|-------------|---------|---------|
|      |           |    |          |                |            | Lower Bound                      | Upper Bound |         |         |
| WBC  | control   | 3  | 36.6833  | 1.62583        | .93868     | 32.6445                          | 40.7221     | 35.25   | 38.45   |
|      | Aloe 0.5% | 3  | 45.6333  | 6.87138        | 3.96719    | 28.5639                          | 62.7028     | 39.90   | 53.25   |
|      | Aloe 1.0% | 3  | 43.1833  | 3.13860        | 1.81207    | 35.3866                          | 50.9801     | 39.80   | 46.00   |
|      | Aloe 2%   | 3  | 35.6333  | 3.44867        | 1.99109    | 27.0664                          | 44.2003     | 31.90   | 38.70   |
|      | Aloe 4%   | 3  | 28.2000  | 6.62891        | 3.82721    | 11.7329                          | 44.6671     | 24.15   | 35.85   |
|      | Total     | 15 | 37.8667  | 7.54204        | 1.94735    | 33.6900                          | 42.0433     | 24.15   | 53.25   |
| Lym  | control   | 3  | 32.3333  | 2.97588        | 1.71812    | 24.9408                          | 39.7258     | 29.40   | 35.35   |
|      | Aloe 0.5% | 3  | 41.4000  | 5.16890        | 2.98426    | 28.5597                          | 54.2403     | 37.45   | 47.25   |
|      | Aloe 1.0% | 3  | 38.6333  | 2.90359        | 1.67639    | 31.4204                          | 45.8463     | 35.65   | 41.45   |
|      | Aloe 2%   | 3  | 32.3667  | 3.82143        | 2.20630    | 22.8737                          | 41.8596     | 28.80   | 36.40   |
|      | Aloe 4%   | 3  | 26.3000  | 7.23809        | 4.17892    | 8.3196                           | 44.2804     | 21.30   | 34.60   |
|      | Total     | 15 | 34.2067  | 6.78362        | 1.75152    | 30.4500                          | 37.9633     | 21.30   | 47.25   |
| MON  | control   | 3  | 1.8667   | .58381         | .33706     | .4164                            | 3.3169      | 1.35    | 2.50    |
|      | Aloe 0.5% | 3  | 2.2000   | .86747         | .50083     | .0451                            | 4.3549      | 1.45    | 3.15    |
|      | Aloe 1.0% | 3  | 2.3500   | .18028         | .10408     | 1.9022                           | 2.7978      | 2.15    | 2.50    |
|      | Aloe 2%   | 3  | 1.4333   | .23629         | .13642     | .8464                            | 2.0203      | 1.25    | 1.70    |
|      | Aloe 4%   | 3  | .9667    | .20817         | .12019     | .4496                            | 1.4838      | .80     | 1.20    |
|      | Total     | 15 | 1.7633   | .67174         | .17344     | 1.3913                           | 2.1353      | .80     | 3.15    |
| GRAN | control   | 3  | 2.4833   | .80829         | .46667     | .4754                            | 4.4912      | 1.75    | 3.35    |
|      | Aloe 0.5% | 3  | 2.0333   | .94384         | .54493     | -.3113                           | 4.3780      | 1.00    | 2.85    |
|      | Aloe 1.0% | 3  | 2.2000   | .22913         | .13229     | 1.6308                           | 2.7692      | 2.00    | 2.45    |
|      | Aloe 2%   | 3  | 1.8333   | .82815         | .47813     | -.2239                           | 3.8906      | 1.05    | 2.70    |
|      | Aloe 4%   | 3  | .9333    | .63311         | .36553     | -.6394                           | 2.5061      | .45     | 1.65    |
|      | Total     | 15 | 1.8967   | .82494         | .21300     | 1.4398                           | 2.3535      | .45     | 3.35    |
| HCT  | control   | 3  | .2342    | .03522         | .02033     | .1467                            | .3217       | .19     | .25     |
|      | Aloe 0.5% | 3  | .2797    | .01522         | .00879     | .2419                            | .3175       | .27     | .30     |
|      | Aloe 1.0% | 3  | .2695    | .05110         | .02950     | .1426                            | .3964       | .22     | .32     |
|      | Aloe 2%   | 3  | .2352    | .03720         | .02148     | .1428                            | .3276       | .21     | .28     |
|      | Aloe 4%   | 3  | .1868    | .04734         | .02733     | .0692                            | .3044       | .16     | .24     |
|      | Total     | 15 | .2411    | .04735         | .01222     | .2148                            | .2673       | .16     | .32     |
| MCV  | control   | 3  | 130.9000 | 1.98053        | 1.14346    | 125.9801                         | 135.8199    | 129.15  | 133.05  |

|      |           |    |          |          |          |          |          |        |        |
|------|-----------|----|----------|----------|----------|----------|----------|--------|--------|
|      | Aloe 0.5% | 3  | 131.5500 | 1.98053  | 1.14346  | 126.6301 | 136.4699 | 129.80 | 133.70 |
|      | Aloe 1.0% | 3  | 131.0833 | 6.53153  | 3.77098  | 114.8581 | 147.3085 | 126.00 | 138.45 |
|      | Aloe 2%   | 3  | 126.4000 | 7.83374  | 4.52281  | 106.9399 | 145.8601 | 120.55 | 135.30 |
|      | Aloe 4%   | 3  | 121.5000 | 5.99020  | 3.45844  | 106.6195 | 136.3805 | 115.15 | 127.05 |
|      | Total     | 15 | 128.2867 | 6.09606  | 1.57400  | 124.9108 | 131.6626 | 115.15 | 138.45 |
| RDWa | control   | 3  | 97.9167  | 7.68315  | 4.43587  | 78.8307  | 117.0027 | 89.60  | 104.75 |
|      | Aloe 0.5% | 3  | 90.1333  | 1.00540  | .58047   | 87.6358  | 92.6309  | 89.30  | 91.25  |
|      | Aloe 1.0% | 3  | 91.6500  | 7.52446  | 4.34425  | 72.9582  | 110.3418 | 84.50  | 99.50  |
|      | Aloe 2%   | 3  | 85.2000  | 6.90489  | 3.98654  | 68.0473  | 102.3527 | 80.70  | 93.15  |
|      | Aloe 4%   | 3  | 84.4000  | 3.01164  | 1.73877  | 76.9187  | 91.8813  | 81.10  | 87.00  |
|      | Total     | 15 | 89.8600  | 7.10111  | 1.83350  | 85.9275  | 93.7925  | 80.70  | 104.75 |
| HGB  | control   | 3  | 121.1667 | 17.14886 | 9.90090  | 78.5665  | 163.7668 | 101.50 | 133.00 |
|      | Aloe 0.5% | 3  | 144.1667 | 5.34634  | 3.08671  | 130.8856 | 157.4477 | 139.50 | 150.00 |
|      | Aloe 1.0% | 3  | 140.8333 | 23.67664 | 13.66972 | 82.0173  | 199.6494 | 119.00 | 166.00 |
|      | Aloe 2%   | 3  | 128.3333 | 13.41951 | 7.74776  | 94.9974  | 161.6693 | 118.00 | 143.50 |
|      | Aloe 4%   | 3  | 103.1667 | 23.67664 | 13.66972 | 44.3506  | 161.9827 | 89.00  | 130.50 |
|      | Total     | 15 | 127.5333 | 21.56921 | 5.56915  | 115.5887 | 139.4780 | 89.00  | 166.00 |
| MCHC | control   | 3  | 522.0000 | 12.01041 | 6.93421  | 492.1645 | 551.8355 | 508.50 | 531.50 |
|      | Aloe 0.5% | 3  | 516.0000 | 9.36750  | 5.40833  | 492.7298 | 539.2702 | 505.50 | 523.50 |
|      | Aloe 1.0% | 3  | 524.5000 | 11.82159 | 6.82520  | 495.1335 | 553.8665 | 511.00 | 533.00 |
|      | Aloe 2%   | 3  | 551.8333 | 29.47174 | 17.01552 | 478.6215 | 625.0452 | 519.00 | 576.00 |
|      | Aloe 4%   | 3  | 559.6667 | 16.26602 | 9.39119  | 519.2596 | 600.0737 | 543.00 | 575.50 |
|      | Total     | 15 | 534.8000 | 23.30757 | 6.01799  | 521.8927 | 547.7073 | 505.50 | 576.00 |
| MCH  | control   | 3  | 68.3500  | 2.54313  | 1.46828  | 62.0325  | 74.6675  | 65.65  | 70.70  |
|      | Aloe 0.5% | 3  | 67.9500  | 1.90000  | 1.09697  | 63.2301  | 72.6699  | 66.35  | 70.05  |
|      | Aloe 1.0% | 3  | 68.6833  | 1.87239  | 1.08102  | 64.0321  | 73.3346  | 67.10  | 70.75  |
|      | Aloe 2%   | 3  | 69.6667  | .53463   | .30867   | 68.3386  | 70.9948  | 69.20  | 70.25  |
|      | Aloe 4%   | 3  | 67.9333  | 1.51438  | .87433   | 64.1714  | 71.6953  | 66.20  | 69.00  |
|      | Total     | 15 | 68.5167  | 1.65709  | .42786   | 67.5990  | 69.4343  | 65.65  | 70.75  |
| RBC  | control   | 3  | 1.7917   | .29616   | .17099   | 1.0560   | 2.5274   | 1.45   | 1.98   |
|      | Aloe 0.5% | 3  | 2.1233   | .12332   | .07120   | 1.8170   | 2.4297   | 2.04   | 2.27   |
|      | Aloe 1.0% | 3  | 2.0417   | .28593   | .16508   | 1.3314   | 2.7520   | 1.77   | 2.34   |
|      | Aloe 2%   | 3  | 1.8595   | .17564   | .10141   | 1.4232   | 2.2958   | 1.72   | 2.06   |
|      | Aloe 4%   | 3  | 1.5217   | .32521   | .18776   | .7138    | 2.3295   | 1.30   | 1.90   |
|      | Total     | 15 | 1.8676   | .30545   | .07887   | 1.6984   | 2.0367   | 1.30   | 2.34   |
| PLT  | control   | 3  | 22.8333  | 7.00595  | 4.04489  | 5.4296   | 40.2371  | 16.00  | 30.00  |
|      | Aloe 0.5% | 3  | 11.1667  | 1.60728  | .92796   | 7.1740   | 15.1594  | 10.00  | 13.00  |
|      | Aloe 1.0% | 3  | 14.3333  | 6.21155  | 3.58624  | -1.0970  | 29.7637  | 10.50  | 21.50  |
|      | Aloe 2%   | 3  | 19.1667  | 7.63763  | 4.40959  | .1938    | 38.1396  | 12.50  | 27.50  |
|      | Aloe 4%   | 3  | 12.1667  | 3.61709  | 2.08833  | 3.1813   | 21.1520  | 8.00   | 14.50  |

|      |           |    |          |           |          |          |          |        |        |
|------|-----------|----|----------|-----------|----------|----------|----------|--------|--------|
|      | Total     | 15 | 15.9333  | 6.63289   | 1.71260  | 12.2602  | 19.6065  | 8.00   | 30.00  |
| ALT  | control   | 3  | 90.0000  | 35.36948  | 20.42058 | 2.1373   | 177.8627 | 60.00  | 129.00 |
|      | Aloe 0.5% | 3  | 44.8333  | 9.56992   | 5.52519  | 21.0603  | 68.6063  | 37.00  | 55.50  |
|      | Aloe 1.0% | 3  | 51.3333  | 14.35560  | 8.28821  | 15.6720  | 86.9946  | 35.50  | 63.50  |
|      | Aloe 2%   | 3  | 72.5000  | 19.83053  | 11.44916 | 23.2382  | 121.7618 | 56.00  | 94.50  |
|      | Aloe 4%   | 3  | 110.6667 | 28.08173  | 16.21299 | 40.9078  | 180.4255 | 79.50  | 134.00 |
|      | Total     | 15 | 73.8667  | 32.02926  | 8.26992  | 56.1295  | 91.6039  | 35.50  | 134.00 |
| AST  | control   | 3  | 483.8333 | 187.99224 | 108.5377 | 16.8347  | 950.8320 | 338.00 | 696.00 |
|      | Aloe 0.5% | 3  | 140.1667 | 7.07696   | 4.08588  | 122.5865 | 157.7468 | 132.00 | 144.50 |
|      | Aloe 1.0% | 3  | 176.8333 | 41.46183  | 23.93800 | 73.8364  | 279.8302 | 140.50 | 222.00 |
|      | Aloe 2%   | 3  | 328.1667 | 76.83803  | 44.36246 | 137.2904 | 519.0429 | 242.50 | 391.00 |
|      | Aloe 4%   | 3  | 268.5000 | 143.36405 | 82.77127 | -87.6360 | 624.6360 | 106.50 | 379.00 |
|      | Total     | 15 | 279.5000 | 158.05119 | 40.80864 | 191.9742 | 367.0258 | 106.50 | 696.00 |
| Glu  | control   | 3  | 3.9667   | .35119    | .20276   | 3.0943   | 4.8391   | 3.60   | 4.30   |
|      | Aloe 0.5% | 3  | 2.8000   | 1.03320   | .59652   | .2334    | 5.3666   | 1.70   | 3.75   |
|      | Aloe 1.0% | 3  | 3.0833   | .25166    | .14530   | 2.4582   | 3.7085   | 2.85   | 3.35   |
|      | Aloe 2%   | 3  | 5.5500   | 3.97524   | 2.29510  | -4.3250  | 15.4250  | 2.75   | 10.10  |
|      | Aloe 4%   | 3  | 2.9833   | .67515    | .38980   | 1.3062   | 4.6605   | 2.30   | 3.65   |
|      | Total     | 15 | 3.6767   | 1.90130   | .49091   | 2.6238   | 4.7296   | 1.70   | 10.10  |
| CHOL | control   | 3  | 3.78     | 0.621     | 0.359    | 2.23     | 5.32     | 3      | 4      |
|      | Aloe 0.5% | 3  | 3.66     | 0.545     | 0.315    | 2.31     | 5.02     | 3      | 4      |
|      | Aloe 1.0% | 3  | 3.86     | 0.253     | 0.146    | 3.23     | 4.49     | 4      | 4      |
|      | Aloe 2.0% | 3  | 3.69     | 0.527     | 0.304    | 2.38     | 5        | 3      | 4      |
|      | Aloe 4.0% | 3  | 3.41     | 0.758     | 0.438    | 1.52     | 5.29     | 3      | 4      |
|      | Total     | 15 | 3.68     | 0.503     | 0.13     | 3.4      | 3.96     | 3      | 4      |
| TG   | control   | 3  | 2.6      | 0.105     | 0.061    | 2.33     | 2.86     | 2      | 3      |
|      | Aloe 0.5% | 3  | 2.27     | 0.193     | 0.111    | 1.79     | 2.75     | 2      | 2      |
|      | Aloe 1.0% | 3  | 2.2      | 0.321     | 0.186    | 1.4      | 3        | 2      | 3      |
|      | Aloe 2.0% | 3  | 2.3      | 0.672     | 0.388    | 0.63     | 3.97     | 2      | 3      |
|      | Aloe 4.0% | 3  | 2.23     | 0.186     | 0.108    | 1.77     | 2.69     | 2      | 2      |
|      | Total     | 15 | 2.32     | 0.336     | 0.087    | 2.13     | 2.5      | 2      | 3      |

**Table 4** Test of homogeneity of variance in growth, feed utilization, survival, and haemato-biochemical indices of African catfish (*Clarias gariepinus*) fingerlings fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

|      | <b>Levene<br/>Statistic</b> | <b>df1</b> | <b>df2</b> | <b>Sig.</b> |
|------|-----------------------------|------------|------------|-------------|
| FW   | 3.990                       | 4          | 10         | .535        |
| WG   | 3.990                       | 4          | 10         | .635        |
| FCR  | 2.386                       | 4          | 10         | .121        |
| FI   | 2.886                       | 4          | 10         | .079        |
| FER  | 3.700                       | 4          | 10         | .425        |
| PER  | 3.990                       | 4          | 10         | .055        |
| HSI  | 1.771                       | 4          | 10         | .211        |
| VSI  | 7.376                       | 4          | 10         | .055        |
| SUr  | .585                        | 4          | 10         | .681        |
| SGR  | 3.272                       | 4          | 10         | .058        |
| CF   | 5.613                       | 4          | 10         | .052        |
| WBC  | 2.511                       | 4          | 10         | .108        |
| Lym  | 1.753                       | 4          | 10         | .215        |
| MONO | 2.894                       | 4          | 10         | .079        |
| GRAN | 1.020                       | 4          | 10         | .443        |
| HCT  | 1.336                       | 4          | 10         | .322        |
| MCV  | 2.471                       | 4          | 10         | .112        |
| RDWa | 2.005                       | 4          | 10         | .170        |
| HGB  | 1.825                       | 4          | 10         | .201        |
| MCHC | 1.855                       | 4          | 10         | .195        |
| MCH  | 1.242                       | 4          | 10         | .354        |
| RBC  | 1.230                       | 4          | 10         | .358        |
| PLT  | 1.438                       | 4          | 10         | .291        |
| ALT  | 1.737                       | 4          | 10         | .218        |
| AST  | 4.785                       | 4          | 10         | .060        |
| Glu  | 8.740                       | 4          | 10         | .053        |
| CHOL | 1.34                        | 4          | 10         | 0.321       |
| TG   | 2.293                       | 4          | 10         | 0.131       |
| AGR  | 3.99                        | 4          | 10         | 0.065       |

**Table 5** Analysis of variances (ANOVA) of growth and feed utilization indices of African catfish (*Clarias gariepinus*) fingerlings fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

|     |                | Sum of Squares | df | Mean Square | F     | Sig.  |
|-----|----------------|----------------|----|-------------|-------|-------|
| FW  | Between Groups | 528.163        | 4  | 132.041     | 2.977 | .074  |
|     | Within Groups  | 443.483        | 10 | 44.348      |       |       |
|     | Total          | 971.647        | 14 |             |       |       |
| WG  | Between Groups | 528.163        | 4  | 132.041     | 2.977 | .074  |
|     | Within Groups  | 443.483        | 10 | 44.348      |       |       |
|     | Total          | 971.647        | 14 |             |       |       |
| FCR | Between Groups | 1.064          | 4  | .266        | 2.585 | .102  |
|     | Within Groups  | 1.029          | 10 | .103        |       |       |
|     | Total          | 2.093          | 14 |             |       |       |
| FI  | Between Groups | 80.997         | 4  | 20.249      | 2.545 | .105  |
|     | Within Groups  | 79.566         | 10 | 7.957       |       |       |
|     | Total          | 160.564        | 14 |             |       |       |
| FER | Between Groups | .163           | 4  | .041        | 2.016 | .168  |
|     | Within Groups  | .202           | 10 | .020        |       |       |
|     | Total          | .366           | 14 |             |       |       |
| PER | Between Groups | .587           | 4  | .147        | 2.977 | .074  |
|     | Within Groups  | .493           | 10 | .049        |       |       |
|     | Total          | 1.080          | 14 |             |       |       |
| HSI | Between Groups | .115           | 4  | .029        | .463  | .762  |
|     | Within Groups  | .621           | 10 | .062        |       |       |
|     | Total          | .735           | 14 |             |       |       |
| VSI | Between Groups | 18.792         | 4  | 4.698       | .502  | .735  |
|     | Within Groups  | 93.512         | 10 | 9.351       |       |       |
|     | Total          | 112.304        | 14 |             |       |       |
| SUr | Between Groups | 110.000        | 4  | 27.500      | .971  | .465  |
|     | Within Groups  | 283.333        | 10 | 28.333      |       |       |
|     | Total          | 393.333        | 14 |             |       |       |
| SGR | Between Groups | 1.266          | 4  | .317        | 3.471 | .050  |
|     | Within Groups  | .912           | 10 | .091        |       |       |
|     | Total          | 2.178          | 14 |             |       |       |
| CF  | Between Groups | .004           | 4  | .001        | .676  | .624  |
|     | Within Groups  | .015           | 10 | .002        |       |       |
|     | Total          | .019           | 14 |             |       |       |
| AGR | Between Group  | 0.147          | 4  | 0.037       | 2.977 | 0.074 |
|     | Within Groups  | 0.123          | 10 | 0.012       |       |       |
|     | Total          | 0.27           | 14 |             |       |       |

**Table 6** Quadratic regression model output on weight gain and feed efficiency ratio against dietary *A. vera* crude polysaccharide extracts inclusion level in African catfish (*Clarias gariepinus*) fingerlings' culture.

**WG**

|                                     |                             |                   |                            |        |       |
|-------------------------------------|-----------------------------|-------------------|----------------------------|--------|-------|
| Model Summary                       |                             |                   |                            |        |       |
| R                                   | R Square                    | Adjusted R Square | Std. Error of the Estimate |        |       |
| 0.609                               | 0.37                        | 0.265             | 7.141                      |        |       |
| The Independent Variable is Groups. |                             |                   |                            |        |       |
| ANOVA                               |                             |                   |                            |        |       |
|                                     | Sum of Squares              | df                | Mean Square                | F      | Sig.  |
| Regression                          | 359.797                     | 2                 | 179.899                    | 3.528  | 0.042 |
| Residual                            | 611.85                      | 12                | 50.987                     |        |       |
| Total                               | 971.647                     | 14                |                            |        |       |
| The Independent Variable is Groups. |                             |                   |                            |        |       |
| Coefficients                        |                             |                   |                            |        |       |
|                                     | Unstandardized Coefficients | Standardized t    |                            | Sig.   |       |
|                                     | B                           | Std. Error        | Beta                       |        |       |
| Groups                              | 9.95                        | 4.966             | 1.748                      | 2.004  | 0.036 |
| Groups <sup>2</sup>                 | -2.778                      | 1.162             | -2.087                     | -2.391 | 0.034 |
| (Constant)                          | 29.291                      | 3.506             |                            | 8.355  | 0     |
| FER                                 |                             |                   |                            |        |       |
| Model Summary                       |                             |                   |                            |        |       |
| R                                   | R Square                    | Adjusted R Square | Std. Error of the Estimate |        |       |
| 0.498                               | 0.248                       | 0.123             | 0.151                      |        |       |
| The Independent Variable is Groups. |                             |                   |                            |        |       |
| ANOVA                               |                             |                   |                            |        |       |
|                                     | Sum of Squares              | df                | Mean Square                | F      | Sig.  |
| Regression                          | 0.091                       | 2                 | 0.045                      | 1.98   | 0.045 |
| Residual                            | 0.275                       | 12                | 0.023                      |        |       |
| Total                               | 0.366                       | 14                |                            |        |       |
| The Independent Variable is Groups. |                             |                   |                            |        |       |
| Coefficients                        |                             |                   |                            |        |       |
|                                     | Unstandardized Coefficients | Standardized t    |                            | Sig.   |       |
|                                     | B                           | Std. Error        | Beta                       |        |       |
| Groups                              | 0.152                       | 0.105             | 1.378                      | 1.445  | 0.048 |
| Groups <sup>2</sup>                 | -0.043                      | 0.025             | -1.672                     | -1.754 | 0.105 |
| (Constant)                          | 0.593                       | 0.074             |                            | 7.983  | 0     |

**Table 7** Analysis of variances (ANOVA) of haemato-biochemical indices of African catfish (*Clarias gariepinus*) fingerlings fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

|      |                | Sum of Squares | df | Mean Square | F     | Sig. |
|------|----------------|----------------|----|-------------|-------|------|
| WBC  | Between Groups | 565.262        | 4  | 141.315     | 6.115 | .009 |
|      | Within Groups  | 231.092        | 10 | 23.109      |       |      |
|      | Total          | 796.353        | 14 |             |       |      |
| Lym  | Between Groups | 422.249        | 4  | 105.562     | 4.755 | .021 |
|      | Within Groups  | 221.995        | 10 | 22.200      |       |      |
|      | Total          | 644.244        | 14 |             |       |      |
| MONO | Between Groups | 3.867          | 4  | .967        | 3.946 | .036 |
|      | Within Groups  | 2.450          | 10 | .245        |       |      |
|      | Total          | 6.317          | 14 |             |       |      |
| GRAN | Between Groups | 4.161          | 4  | 1.040       | 1.938 | .181 |
|      | Within Groups  | 5.367          | 10 | .537        |       |      |
|      | Total          | 9.527          | 14 |             |       |      |
| HCT  | Between Groups | .016           | 4  | .004        | 2.589 | .101 |
|      | Within Groups  | .015           | 10 | .002        |       |      |
|      | Total          | .031           | 14 |             |       |      |
| MCV  | Between Groups | 224.756        | 4  | 56.189      | 1.901 | .187 |
|      | Within Groups  | 295.512        | 10 | 29.551      |       |      |
|      | Total          | 520.267        | 14 |             |       |      |
| RDWa | Between Groups | 359.148        | 4  | 89.787      | 2.589 | .101 |
|      | Within Groups  | 346.813        | 10 | 34.681      |       |      |
|      | Total          | 705.961        | 14 |             |       |      |
| HGB  | Between Groups | 3265.400       | 4  | 816.350     | 2.514 | .108 |
|      | Within Groups  | 3247.833       | 10 | 324.783     |       |      |
|      | Total          | 6513.233       | 14 |             |       |      |
| MCHC | Between Groups | 4595.567       | 4  | 1148.892    | 3.817 | .039 |
|      | Within Groups  | 3009.833       | 10 | 300.983     |       |      |
|      | Total          | 7605.400       | 14 |             |       |      |
| MCH  | Between Groups | 6.118          | 4  | 1.530       | .473  | .755 |
|      | Within Groups  | 32.325         | 10 | 3.232       |       |      |
|      | Total          | 38.443         | 14 |             |       |      |

|      |                |                |    |           |       |       |
|------|----------------|----------------|----|-----------|-------|-------|
| RBC  | Between Groups | .664           | 4  | .166      | 2.582 | .102  |
|      | Within Groups  | .643           | 10 | .064      |       |       |
|      | Total          | 1.306          | 14 |           |       |       |
| PLT  | Between Groups | 292.600        | 4  | 73.150    | 2.262 | .135  |
|      | Within Groups  | 323.333        | 10 | 32.333    |       |       |
|      | Total          | 615.933        | 14 |           |       |       |
| ALT  | Between Groups | 8901.233       | 4  | 2225.308  | 4.075 | .033  |
|      | Within Groups  | 5461.000       | 10 | 546.100   |       |       |
|      | Total          | 14362.233      | 14 |           |       |       |
| AST  | Between Groups | 222587.33<br>3 | 4  | 55646.833 | 4.377 | .027  |
|      | Within Groups  | 127135.16      | 10 | 12713.517 |       |       |
|      | Total          | 349722.50      | 14 |           |       |       |
| Glu  | Between Groups | 15.584         | 4  | 3.896     | 1.112 | .403  |
|      | Within Groups  | 35.025         | 10 | 3.503     |       |       |
|      | Total          | 50.609         | 14 |           |       |       |
| CHOL | Between Groups | 0.35           | 4  | 0.087     | 0.273 | 0.889 |
|      | Within Groups  | 3.197          | 10 | 0.32      |       |       |
|      | Total          | 3.547          | 14 |           |       |       |
| TG   | Between Groups | 0.306          | 4  | 0.077     | 0.6   | 0.671 |
|      | Within Groups  | 1.276          | 10 | 0.128     |       |       |
|      | Total          | 1.583          | 14 |           |       |       |

**Table 8** Post hoc test (Duncan multiple range test) of growth, survival and feed utilization indices of African catfish (*Clarias gariepinus*) fingerlings fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

| Homogeneous Subsets Post Hoc Tests                     |   |                         |         |                                                        |   |                         |         |                                                        |   |                         |  |
|--------------------------------------------------------|---|-------------------------|---------|--------------------------------------------------------|---|-------------------------|---------|--------------------------------------------------------|---|-------------------------|--|
| Final Weight                                           |   |                         |         | Weight Gain                                            |   |                         |         | Hepatosomatic index                                    |   |                         |  |
| Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |  |
| groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |  |
|                                                        |   | 1                       | 2       |                                                        |   | 1                       | 2       |                                                        |   | 1                       |  |
| Aloe 4%                                                | 3 | 28.4480                 |         | Aloe 4%                                                | 3 | 25.3980                 |         | Aloe 2%                                                | 3 | 1.4524                  |  |
| control                                                | 3 | 28.5658                 |         | control                                                | 3 | 25.5158                 |         | Aloe 0.5%                                              | 3 | 1.5020                  |  |
| Aloe 2%                                                | 3 | 37.1262                 | 37.1262 | Aloe 2%                                                | 3 | 34.0762                 | 34.0762 | Aloe 4%                                                | 3 | 1.5507                  |  |
| Aloe 0.5%                                              | 3 | 40.6750                 | 40.6750 | Aloe 0.5%                                              | 3 | 37.6250                 | 37.6250 | Aloe 1.0%                                              | 3 | 1.5913                  |  |
| Aloe 1.0%                                              | 3 |                         | 42.4873 | Aloe 1.0%                                              | 3 |                         | 39.4373 | control                                                | 3 | 1.7093                  |  |
| Sig.                                                   |   | .062                    | .369    | Sig.                                                   |   | .062                    | .369    | Sig.                                                   |   | .271                    |  |
| Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |  |
| a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |  |
| Specific Growth Rate                                   |   |                         |         | Absolute Growth Rate                                   |   |                         |         | Viserosomatic index                                    |   |                         |  |
| Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |  |
| groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |  |
|                                                        |   | 1                       | 2       |                                                        |   | 1                       | 2       |                                                        |   | 1                       |  |
| Aloe 4%                                                | 3 | 3.6874                  |         | 4.0% aloe                                              | 3 | 0.4233                  |         | Aloe 2%                                                | 3 | 5.6448                  |  |
| control                                                | 3 | 3.7100                  |         | control                                                | 3 | 0.4253                  |         | Aloe 1.0%                                              | 3 | 6.5742                  |  |
| Aloe 2%                                                | 3 | 4.1631                  | 4.1631  | 2.0% aloe                                              | 3 | 0.5679                  | 0.5679  | control                                                | 3 | 7.4550                  |  |
| Aloe 0.5%                                              | 3 |                         | 4.3145  | 0.5% aloe                                              | 3 | 0.6271                  | 0.6271  | Aloe 4%                                                | 3 | 8.3386                  |  |
| Aloe 1.0%                                              | 3 |                         | 4.3537  | 1.0% aloe                                              | 3 |                         | 0.6573  | Aloe 0.5%                                              | 3 | 8.6792                  |  |
| Sig.                                                   |   | .095                    | .477    | Sig.                                                   |   | 0.062                   | 0.369   | Sig.                                                   |   | .288                    |  |
| Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |  |
| a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |  |
| Feed Conversion Ratio                                  |   |                         |         | Feed Intake                                            |   |                         |         | Survival                                               |   |                         |  |
| Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |  |
| groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |  |
|                                                        |   | 1                       | 2       |                                                        |   | 1                       | 2       |                                                        |   | 1                       |  |
| Aloe 1.0%                                              | 3 | 1.3410                  |         | Aloe 4%                                                | 3 | 48.1067                 |         | Aloe 4%                                                | 3 | 85.0000                 |  |
| Aloe 0.5%                                              | 3 | 1.4141                  | 1.4141  | control                                                | 3 | 48.5467                 | 48.5467 | control                                                | 3 | 88.3333                 |  |
| Aloe 2%                                                | 3 | 1.5847                  | 1.5847  | Aloe 1.0%                                              | 3 | 50.1000                 | 50.1000 | Aloe 0.5%                                              | 3 | 90.0000                 |  |
| control                                                | 3 | 1.9441                  | 1.9441  | Aloe 0.5%                                              | 3 | 53.0100                 | 53.0100 | Aloe 1.0%                                              | 3 | 90.0000                 |  |
| Aloe 4%                                                | 3 |                         | 1.9862  | Aloe 2%                                                | 3 |                         | 53.8533 | Aloe 2%                                                | 3 | 93.3333                 |  |
| Sig.                                                   |   | .057                    | .069    | Sig.                                                   |   | .075                    | .057    | Sig.                                                   |   | .108                    |  |
| Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |  |
| a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |  |
| Feed Efficiency Ratio                                  |   |                         |         | Protein Efficiency Ratio                               |   |                         |         | Condition Factor                                       |   |                         |  |
| Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |         | Duncan                                                 |   |                         |  |
| groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |         | groups                                                 | N | Subset for alpha = 0.05 |  |
|                                                        |   | 1                       |         |                                                        |   | 1                       | 2       |                                                        |   | 1                       |  |
| control                                                | 3 | .5248                   |         | Aloe 4%                                                | 3 | .8466                   |         | control                                                | 3 | .6801                   |  |
| Aloe 4%                                                | 3 | .5272                   |         | control                                                | 3 | .8505                   |         | Aloe 0.5%                                              | 3 | .6886                   |  |
| Aloe 2%                                                | 3 | .6340                   |         | Aloe 2%                                                | 3 | 1.1359                  | 1.1359  | Aloe 2%                                                | 3 | .6954                   |  |
| Aloe 0.5%                                              | 3 | .7112                   |         | Aloe 0.5%                                              | 3 | 1.2542                  | 1.2542  | Aloe 4%                                                | 3 | .6981                   |  |
| Aloe 1.0%                                              | 3 | .7928                   |         | Aloe 1.0%                                              | 3 |                         | 1.3146  | Aloe 1.0%                                              | 3 | .7289                   |  |
| Sig.                                                   |   | .060                    |         | Sig.                                                   |   | .062                    | .369    | Sig.                                                   |   | .189                    |  |
| Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |  |
| a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |         | a. Uses Harmonic Mean Sample Size = 3.000.             |   |                         |  |

**Table 9** Post hoc test (Duncan multiple range test) of haemato-biochemical indices of African catfish (*Clarias gariepinus*) fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

| WBC                                                   |   |                         |          | Lym                                                   |   |                         |          | MONO                                                  |   |                         |          |                                                       |   |                         |         |
|-------------------------------------------------------|---|-------------------------|----------|-------------------------------------------------------|---|-------------------------|----------|-------------------------------------------------------|---|-------------------------|----------|-------------------------------------------------------|---|-------------------------|---------|
| Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |          |                                                       |   |                         |         |
| groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          |                                                       |   |                         |         |
| Aloe 4%                                               | 3 | 28.2000                 |          | Aloe 4%                                               | 3 | 28.3000                 |          | Aloe 4%                                               | 3 | .9667                   |          |                                                       |   |                         |         |
| Aloe 2%                                               | 3 | 35.6333                 | 35.6333  | control                                               | 3 | 32.3333                 | 32.3333  | Aloe 2%                                               | 3 | 1.4333                  | 1.4333   |                                                       |   |                         |         |
| control                                               | 3 | 36.6833                 | 36.6833  | Aloe 2%                                               | 3 | 32.3667                 | 32.3667  | control                                               | 3 | 1.8667                  | 1.8667   |                                                       |   |                         |         |
| Aloe                                                  | 3 |                         | 43.1833  | Aloe                                                  | 3 |                         | 38.6333  | Aloe                                                  | 3 |                         | 2.2000   |                                                       |   |                         |         |
| 1.0%                                                  |   |                         |          | 1.0%                                                  |   |                         |          | 0.5%                                                  |   |                         |          |                                                       |   |                         |         |
| Aloe                                                  | 3 |                         |          | Aloe                                                  | 3 |                         | 41.4000  | Aloe                                                  | 3 |                         | 2.3500   |                                                       |   |                         |         |
| 0.5%                                                  |   |                         |          | 0.5%                                                  |   |                         |          | 1.0%                                                  |   |                         |          |                                                       |   |                         |         |
| Sig.                                                  |   | .066                    | .096     | Sig.                                                  |   | .163                    | .052     | Sig.                                                  |   | .059                    | .060     |                                                       |   |                         |         |
| Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          |                                                       |   |                         |         |
| a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          |                                                       |   |                         |         |
| GRAN                                                  |   |                         |          | HCT                                                   |   |                         |          | MCV                                                   |   |                         |          | RDW                                                   |   |                         |         |
| Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |         |
| groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |         |
| Aloe 4%                                               | 3 | .9333                   |          | Aloe 4%                                               | 3 | .1868                   |          | Aloe 4%                                               | 3 | 121.5000                |          | Aloe 4%                                               | 3 | 84.4000                 |         |
| Aloe 2%                                               | 3 | 1.8333                  | 1.8333   | control                                               | 3 | .2342                   | .2342    | Aloe 2%                                               | 3 | 126.4000                |          | Aloe 2%                                               | 3 | 85.2000                 |         |
| Aloe                                                  | 3 | 2.0333                  | 2.0333   | Aloe 2%                                               | 3 | .2352                   | .2352    | control                                               | 3 | 130.9000                |          | Aloe                                                  | 3 | 90.1333                 | 90.1333 |
| 0.5%                                                  |   |                         |          | Aloe                                                  | 3 |                         | .2695    | Aloe                                                  | 3 | 131.0833                |          | Aloe                                                  | 3 | 91.6500                 | 91.6500 |
| 1.0%                                                  |   |                         |          | Aloe                                                  | 3 |                         | .2797    | Aloe                                                  | 3 | 131.5500                |          | Aloe                                                  | 3 |                         | 97.9167 |
| control                                               | 3 |                         | 2.4833   | Aloe                                                  | 3 |                         |          | Aloe                                                  | 3 |                         |          | control                                               | 3 |                         |         |
| Sig.                                                  |   | .076                    | .334     | Sig.                                                  |   | .180                    | .215     | Sig.                                                  |   | .064                    | .153     | Sig.                                                  |   | .190                    | .153    |
| Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |         |
| a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |         |
| HGB                                                   |   |                         |          | MCHC                                                  |   |                         |          | MCH                                                   |   |                         |          | RBC                                                   |   |                         |         |
| Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |         |
| groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |         |
| Aloe 4%                                               | 3 | 103.1667                |          | Aloe                                                  | 3 | 516.0000                |          | Aloe 4%                                               | 3 | 67.9333                 |          | Aloe 4%                                               | 3 | 1.5217                  |         |
| control                                               | 3 | 121.1667                | 121.1667 | Aloe 0.5%                                             | 3 | 522.0000                | 522.0000 | Aloe                                                  | 3 | 67.9500                 |          | control                                               | 3 | 1.7917                  | 1.7917  |
| Aloe 2%                                               | 3 | 128.3333                | 128.3333 | Aloe                                                  | 3 | 524.5000                | 524.5000 | Aloe 0.5%                                             | 3 | 68.3500                 |          | Aloe 2%                                               | 3 | 1.8595                  | 1.8595  |
| Aloe                                                  | 3 |                         | 140.8333 | Aloe 1.0%                                             | 3 |                         | 551.8333 | Aloe                                                  | 3 | 68.6833                 |          | Aloe                                                  | 3 |                         | 2.0417  |
| 1.0%                                                  |   |                         |          | Aloe 2%                                               | 3 |                         | 559.6667 | Aloe 1.0%                                             | 3 | 69.6667                 |          | Aloe                                                  | 3 |                         | 2.1233  |
| Aloe                                                  | 3 |                         | 144.1667 | Aloe 4%                                               | 3 |                         |          | Aloe 2%                                               | 3 | 69.6667                 |          | Aloe                                                  | 3 |                         |         |
| 0.5%                                                  |   |                         |          | Sig.                                                  |   | .133                    | .176     | Sig.                                                  |   | .301                    | .150     | Sig.                                                  |   | .150                    | .166    |
| Sig.                                                  |   |                         |          | Sig.                                                  |   | .580                    | .072     | Sig.                                                  |   | .592                    |          | Sig.                                                  |   | .150                    | .166    |
| Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |         |
| a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |         |
| PLT                                                   |   |                         |          | ALT                                                   |   |                         |          | ALT                                                   |   |                         |          | Glu                                                   |   |                         |         |
| Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |          | Duncan                                                |   |                         |         |
| groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |          | groups                                                | N | Subset for alpha = 0.05 |         |
| Aloe                                                  | 3 | 11.1667                 |          | Aloe                                                  | 3 | 44.8333                 |          | Aloe                                                  | 3 | 140.1667                |          | Aloe                                                  | 3 | 2.8000                  |         |
| 0.5%                                                  |   |                         |          | Aloe 0.5%                                             | 3 | 51.3333                 |          | Aloe 0.5%                                             | 3 | 176.8333                |          | Aloe 0.5%                                             | 3 | 2.9833                  |         |
| Aloe 4%                                               | 3 | 12.1667                 | 12.1667  | Aloe 1.0%                                             | 3 | 72.5000                 | 72.5000  | Aloe 1.0%                                             | 3 | 268.5000                |          | Aloe 4%                                               | 3 | 3.0833                  |         |
| Aloe                                                  | 3 | 14.3333                 | 14.3333  | Aloe 2%                                               | 3 | 90.0000                 | 90.0000  | Aloe 2%                                               | 3 | 328.1667                |          | Aloe                                                  | 3 |                         |         |
| 1.0%                                                  |   |                         |          | control                                               | 3 | 110.6667                | 110.6667 | Aloe 4%                                               | 3 | 328.1667                | 328.1667 | control                                               | 3 | 3.9667                  |         |
| Aloe 2%                                               | 3 | 19.1667                 | 19.1667  | Aloe 4%                                               | 3 |                         |          | control                                               | 3 | 483.8333                | 483.8333 | Aloe 2%                                               | 3 | 5.5500                  |         |
| control                                               | 3 |                         | 22.8333  | Sig.                                                  |   | .139                    | .058     | Sig.                                                  |   | .086                    | .122     | Sig.                                                  |   | .129                    |         |
| Sig.                                                  |   |                         |          | Sig.                                                  |   | .052                    | .085     | Sig.                                                  |   | .086                    | .122     | Sig.                                                  |   | .129                    |         |
| Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |         |
| a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |         |
| CHOL                                                  |   |                         |          | TG                                                    |   |                         |          |                                                       |   |                         |          |                                                       |   |                         |         |
| Duncan                                                |   |                         |          | Duncan                                                |   |                         |          |                                                       |   |                         |          |                                                       |   |                         |         |
| Groups                                                | N | Subset for alpha = 0.05 |          | Groups                                                | N | Subset for alpha = 0.05 |          |                                                       |   |                         |          |                                                       |   |                         |         |
| 4.0%Aloe                                              | 3 | 3.41                    |          | 1.0%Aloe                                              | 3 | 2.2                     |          |                                                       |   |                         |          |                                                       |   |                         |         |
| 0.5%Aloe                                              | 3 | 3.66                    |          | 4.0%Aloe                                              | 3 | 2.23                    |          |                                                       |   |                         |          |                                                       |   |                         |         |
| 2.0%Aloe                                              | 3 | 3.69                    |          | 0.5%Aloe                                              | 3 | 2.27                    |          |                                                       |   |                         |          |                                                       |   |                         |         |
| control                                               | 3 | 3.78                    |          | 2.0%Aloe                                              | 3 | 2.3                     |          |                                                       |   |                         |          |                                                       |   |                         |         |
| 1.0%Aloe                                              | 3 | 3.86                    |          | control                                               | 3 | 2.6                     |          |                                                       |   |                         |          |                                                       |   |                         |         |
| Sig.                                                  |   | 0.387                   |          | Sig.                                                  |   | 0.24                    |          |                                                       |   |                         |          |                                                       |   |                         |         |
| Means for groups in homogeneous subsets are displayed |   |                         |          | Means for groups in homogeneous subsets are displayed |   |                         |          |                                                       |   |                         |          |                                                       |   |                         |         |
| a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          | a. Uses Harmonic Mean Sample Size = 3.000.            |   |                         |          |                                                       |   |                         |          |                                                       |   |                         |         |

**Table 10** Kaplan–Meier analysis (survival) output of African catfish (*Clarias gariepinus*) fingerlings subjected to low water pH after being fed 30% *Aloe vera* crude polysaccharide extracts supplemented diets for 60 d.

| Overall Comparisons                  |            |    |       |
|--------------------------------------|------------|----|-------|
|                                      | Chi-Square | df | Sig.  |
| Log Rank<br>(Mantel-Cox)             | 22.304     | 4  | 0     |
| Breslow<br>(Generalized<br>Wilcoxon) | 18.479     | 4  | 0.001 |
| Tarone-Ware                          | 20.407     | 4  | 0     |

Test of equality of survival distributions for the different levels of Groups.

## Appendix B

**Table 1** Descriptive statistics of growth performance indices of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

|     |             | N  | Mean     | Descriptives   |            |                                  |             |         |         |
|-----|-------------|----|----------|----------------|------------|----------------------------------|-------------|---------|---------|
|     |             |    |          | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Minimum | Maximum |
|     |             |    |          |                |            | Lower Bound                      | Upper Bound |         |         |
| FW  | Control     | 3  | 93.3833  | 15.45116       | 8.92073    | 55.0005                          | 131.7661    | 83.28   | 111.17  |
|     | 0.5% Garlic | 3  | 99.79    | 10.09144       | 5.8263     | 74.7215                          | 124.8585    | 91.93   | 111.17  |
|     | 1.0% Garlic | 3  | 124.0467 | 21.47994       | 12.40145   | 70.6875                          | 177.4058    | 99.67   | 140.2   |
|     | 2.0% Garlic | 3  | 126.16   | 9.14271        | 5.27854    | 103.4483                         | 148.8717    | 116.15  | 134.07  |
|     | 4.0% Garlic | 3  | 67.4233  | 3.00057        | 1.73238    | 59.9695                          | 74.8772     | 64.7    | 70.64   |
|     | Total       | 15 | 102.1607 | 25.1027        | 6.48149    | 88.2593                          | 116.0621    | 64.7    | 140.2   |
| WG  | Control     | 3  | 80.7333  | 15.45116       | 8.92073    | 42.3505                          | 119.1161    | 70.63   | 98.52   |
|     | 0.5% Garlic | 3  | 87.14    | 10.09144       | 5.8263     | 62.0715                          | 112.2085    | 79.28   | 98.52   |
|     | 1.0% Garlic | 3  | 111.3967 | 21.47994       | 12.40145   | 58.0375                          | 164.7558    | 87.02   | 127.55  |
|     | 2.0% Garlic | 3  | 113.5093 | 9.1438         | 5.27918    | 90.7949                          | 136.2238    | 103.5   | 121.42  |
|     | 4.0% Garlic | 3  | 54.7733  | 3.00057        | 1.73238    | 47.3195                          | 62.2272     | 52.05   | 57.99   |
|     | Total       | 15 | 89.5105  | 25.10262       | 6.48147    | 75.6092                          | 103.4119    | 52.05   | 127.55  |
| AGR | Control     | 3  | 1.3456   | 0.25752        | 0.14868    | 0.7058                           | 1.9853      | 1.18    | 1.64    |
|     | 0.5% Garlic | 3  | 1.4523   | 0.16819        | 0.0971     | 1.0345                           | 1.8701      | 1.32    | 1.64    |
|     | 1.0% Garlic | 3  | 1.8566   | 0.358          | 0.20669    | 0.9673                           | 2.7459      | 1.45    | 2.13    |
|     | 2.0% Garlic | 3  | 1.8918   | 0.1524         | 0.08799    | 1.5132                           | 2.2704      | 1.72    | 2.02    |
|     | 4.0% Garlic | 3  | 0.9129   | 0.05001        | 0.02887    | 0.7887                           | 1.0371      | 0.87    | 0.97    |
|     | Total       | 15 | 1.4918   | 0.41838        | 0.10802    | 1.2602                           | 1.7235      | 0.87    | 2.13    |
| SGR | Control     | 3  | 3.3601   | 0.26626        | 0.15373    | 2.6986                           | 4.0215      | 3.18    | 3.67    |
|     | 0.5% Garlic | 3  | 3.4801   | 0.1658         | 0.09573    | 3.0682                           | 3.8919      | 3.35    | 3.67    |
|     | 1.0% Garlic | 3  | 3.8315   | 0.30517        | 0.17619    | 3.0734                           | 4.5896      | 3.48    | 4.05    |
|     | 2.0% Garlic | 3  | 3.8747   | 0.12283        | 0.07091    | 3.5696                           | 4.1799      | 3.74    | 3.98    |
|     | 4.0% Garlic | 3  | 2.9084   | 0.09201        | 0.05312    | 2.6798                           | 3.1369      | 2.82    | 3       |
|     | Total       | 15 | 3.4909   | 0.40443        | 0.10442    | 3.267                            | 3.7149      | 2.82    | 4.05    |
| CF  | Control     | 3  | 0.5383   | 0.11939        | 0.06893    | 0.2417                           | 0.8348      | 0.41    | 0.64    |
|     | 0.5% Garlic | 3  | 0.5559   | 0.15847        | 0.0915     | 0.1623                           | 0.9496      | 0.43    | 0.74    |
|     | 1.0% Garlic | 3  | 0.4886   | 0.12481        | 0.07206    | 0.1785                           | 0.7986      | 0.4     | 0.63    |
|     | 2.0% Garlic | 3  | 0.4752   | 0.12187        | 0.07036    | 0.1725                           | 0.778       | 0.34    | 0.56    |
|     | 4.0% Garlic | 3  | 0.6955   | 0.07618        | 0.04398    | 0.5062                           | 0.8847      | 0.63    | 0.78    |
|     | Total       | 15 | 0.5507   | 0.13182        | 0.03404    | 0.4777                           | 0.6237      | 0.34    | 0.78    |
| HSI | Control     | 3  | 3.0371   | 0.27138        | 0.15668    | 2.363                            | 3.7113      | 2.72    | 3.21    |
|     | 0.5% Garlic | 3  | 3.1108   | 0.21973        | 0.12686    | 2.565                            | 3.6567      | 2.92    | 3.35    |
|     | 1.0% Garlic | 3  | 3.1309   | 1.34593        | 0.77707    | -0.2126                          | 6.4744      | 2.28    | 4.68    |
|     | 2.0% Garlic | 3  | 3.3756   | 0.47652        | 0.27512    | 2.1918                           | 4.5593      | 3.04    | 3.92    |
|     | 4.0% Garlic | 3  | 2.9651   | 0.15163        | 0.08754    | 2.5884                           | 3.3417      | 2.79    | 3.07    |
|     | Total       | 15 | 3.1239   | 0.57669        | 0.1489     | 2.8045                           | 3.4433      | 2.28    | 4.68    |
| VSI | Control     | 3  | 8.7699   | 0.56246        | 0.32474    | 7.3727                           | 10.1671     | 8.12    | 9.14    |
|     | 0.5% Garlic | 3  | 8.8767   | 1.87241        | 1.08104    | 4.2254                           | 13.528      | 6.78    | 10.38   |
|     | 1.0% Garlic | 3  | 7.1096   | 0.52361        | 0.30231    | 5.8089                           | 8.4103      | 6.55    | 7.58    |
|     | 2.0% Garlic | 3  | 7.5411   | 0.27391        | 0.15814    | 6.8607                           | 8.2215      | 7.34    | 7.85    |
|     | 4.0% Garlic | 3  | 7.5795   | 0.2216         | 0.12794    | 7.029                            | 8.1299      | 7.4     | 7.83    |
|     | Total       | 15 | 7.9754   | 1.07094        | 0.27651    | 7.3823                           | 8.5684      | 6.55    | 10.38   |

**Table 2** Descriptive statistics of feed utilization indices and survival rate of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| Descriptives |             |    |          |                |            |                                  |             |         |         |
|--------------|-------------|----|----------|----------------|------------|----------------------------------|-------------|---------|---------|
|              |             | N  | Mean     | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Minimum | Maximum |
|              |             |    |          |                |            | Lower Bound                      | Upper Bound |         |         |
| FI           | Control     | 3  | 97.2733  | 2.44549        | 1.41191    | 91.1984                          | 103.3483    | 94.8    | 99.69   |
|              | 0.5% Garlic | 3  | 103.8933 | 1.61745        | 0.93383    | 99.8754                          | 107.9113    | 102.04  | 105.02  |
|              | 1.0% Garlic | 3  | 114.8833 | 7.16894        | 4.13899    | 97.0747                          | 132.692     | 107.93  | 122.25  |
|              | 2.0% Garlic | 3  | 117.6267 | 1.67455        | 0.9668     | 113.4668                         | 121.7865    | 116.34  | 119.52  |
|              | 4.0% Garlic | 3  | 81.82    | 7.2303         | 4.17441    | 63.8589                          | 99.7811     | 75.96   | 89.9    |
|              | Total       | 15 | 103.0993 | 13.99492       | 3.61347    | 95.3492                          | 110.8495    | 75.96   | 122.25  |
| FCR          | Control     | 3  | 1.2292   | 0.19245        | 0.11111    | 0.7511                           | 1.7073      | 1.01    | 1.38    |
|              | 0.5% Garlic | 3  | 1.2038   | 0.15016        | 0.0867     | 0.8308                           | 1.5769      | 1.04    | 1.32    |
|              | 1.0% Garlic | 3  | 1.0532   | 0.17354        | 0.1002     | 0.6221                           | 1.4843      | 0.9     | 1.24    |
|              | 2.0% Garlic | 3  | 1.0405   | 0.08036        | 0.0464     | 0.8409                           | 1.2402      | 0.96    | 1.12    |
|              | 4.0% Garlic | 3  | 1.5012   | 0.21081        | 0.12171    | 0.9775                           | 2.0249      | 1.31    | 1.73    |
|              | Total       | 15 | 1.2056   | 0.22302        | 0.05758    | 1.0821                           | 1.3291      | 0.9     | 1.73    |
| FER          | Control     | 3  | 0.8282   | 0.14045        | 0.08109    | 0.4793                           | 1.1771      | 0.73    | 0.99    |
|              | 0.5% Garlic | 3  | 0.8399   | 0.11102        | 0.0641     | 0.5641                           | 1.1157      | 0.75    | 0.97    |
|              | 1.0% Garlic | 3  | 0.9663   | 0.15436        | 0.08912    | 0.5829                           | 1.3498      | 0.81    | 1.11    |
|              | 2.0% Garlic | 3  | 0.9648   | 0.07401        | 0.04273    | 0.781                            | 1.1487      | 0.89    | 1.04    |
|              | 4.0% Garlic | 3  | 0.6748   | 0.09243        | 0.05337    | 0.4452                           | 0.9044      | 0.58    | 0.76    |
|              | Total       | 15 | 0.8548   | 0.14964        | 0.03864    | 0.7719                           | 0.9377      | 0.58    | 1.11    |
| PER          | Control     | 3  | 2.6544   | 0.45016        | 0.2599     | 1.5361                           | 3.7727      | 2.33    | 3.17    |
|              | 0.5% Garlic | 3  | 2.692    | 0.35584        | 0.20544    | 1.808                            | 3.5759      | 2.42    | 3.09    |
|              | 1.0% Garlic | 3  | 3.0972   | 0.49474        | 0.28564    | 1.8682                           | 4.3263      | 2.58    | 3.57    |
|              | 2.0% Garlic | 3  | 3.0924   | 0.23722        | 0.13696    | 2.5031                           | 3.6817      | 2.85    | 3.33    |
|              | 4.0% Garlic | 3  | 2.1627   | 0.29626        | 0.17104    | 1.4268                           | 2.8987      | 1.86    | 2.45    |
|              | Total       | 15 | 2.7398   | 0.4796         | 0.12383    | 2.4742                           | 3.0053      | 1.86    | 3.57    |

**Table 3** Descriptive statistics of haematological indices and body proximate composition parameters of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

|      |             | N  | Mean     | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             |       |       |
|------|-------------|----|----------|----------------|------------|----------------------------------|-------------|-------|-------|
|      |             |    |          |                |            | Lower Bound                      | Upper Bound | Mini  | Max   |
| WBC  | Control     | 3  | 47.5     | 6.15386        | 3.55293    | 32.213                           | 62.787      | 42.1  | 54.2  |
|      | 0.5% Garlic | 3  | 50.7667  | 3.4858         | 2.01253    | 42.1074                          | 59.4259     | 46.75 | 53    |
|      | 1.0% Garlic | 3  | 41.4333  | 3.84231        | 2.21836    | 31.8885                          | 50.9782     | 37.2  | 44.7  |
|      | 2.0% Garlic | 3  | 47.3167  | 1.92635        | 1.11218    | 42.5313                          | 52.102      | 45.35 | 49.2  |
|      | 4.0% Garlic | 3  | 47.65    | 11.22731       | 6.48209    | 19.7598                          | 75.5402     | 40.65 | 60.6  |
|      | Total       | 15 | 46.9333  | 6.13464        | 1.58396    | 43.5361                          | 50.3306     | 37.2  | 60.6  |
| LYM  | Control     | 3  | 38.4833  | 0.57951        | 0.33458    | 37.0437                          | 39.9229     | 37.95 | 39.1  |
|      | 0.5% Garlic | 3  | 42.6167  | 2.22785        | 1.28625    | 37.0824                          | 48.151      | 40.05 | 44.05 |
|      | 1.0% Garlic | 3  | 38.5     | 4.45084        | 2.5697     | 27.4435                          | 49.5565     | 33.4  | 41.6  |
|      | 2.0% Garlic | 3  | 42.4667  | 1.594          | 0.9203     | 38.507                           | 46.4264     | 40.75 | 43.9  |
|      | 4.0% Garlic | 3  | 38.62    | 1.50622        | 0.86962    | 34.8783                          | 42.3617     | 37.15 | 40.16 |
|      | Total       | 15 | 40.1373  | 2.89966        | 0.74869    | 38.5316                          | 41.7431     | 33.4  | 44.05 |
| MON  | Control     | 3  | 2.2333   | 0.57951        | 0.33458    | 0.7937                           | 3.6729      | 1.7   | 2.85  |
|      | 0.5% Garlic | 3  | 3.4167   | 0.50083        | 0.28916    | 2.1725                           | 4.6608      | 2.85  | 3.8   |
|      | 1.0% Garlic | 3  | 1.4167   | 0.27538        | 0.15899    | 0.7326                           | 2.1007      | 1.1   | 1.6   |
|      | 2.0% Garlic | 3  | 2.45     | 0.47697        | 0.27538    | 1.2651                           | 3.6349      | 2.15  | 3     |
|      | 4.0% Garlic | 3  | 2.5      | 1.47309        | 0.85049    | -1.1594                          | 6.1594      | 1.6   | 4.2   |
|      | Total       | 15 | 2.4033   | 0.93512        | 0.24145    | 1.8855                           | 2.9212      | 1.1   | 4.2   |
| GRAN | Control     | 3  | 2.45     | 0.69462        | 0.40104    | 0.7245                           | 4.1755      | 2     | 3.25  |
|      | 0.5% Garlic | 3  | 4.7167   | 0.7911         | 0.45674    | 2.7515                           | 6.6819      | 3.85  | 5.4   |
|      | 1.0% Garlic | 3  | 2.85     | 0.83217        | 0.48045    | 0.7828                           | 4.9172      | 2.25  | 3.8   |
|      | 2.0% Garlic | 3  | 2.7667   | 0.59231        | 0.34197    | 1.2953                           | 4.2381      | 2.4   | 3.45  |
|      | 4.0% Garlic | 3  | 2.0333   | 0.63311        | 0.36553    | 0.4606                           | 3.6061      | 1.55  | 2.75  |
|      | Total       | 15 | 2.9633   | 1.12971        | 0.29169    | 2.3377                           | 3.5889      | 1.55  | 5.4   |
| HCT  | Control     | 3  | 0.2058   | 0.071          | 0.04099    | 0.0295                           | 0.3822      | 0.14  | 0.28  |
|      | 0.5% Garlic | 3  | 0.237    | 0.01754        | 0.01013    | 0.1934                           | 0.2806      | 0.22  | 0.26  |
|      | 1.0% Garlic | 3  | 0.2257   | 0.02954        | 0.01705    | 0.1523                           | 0.299       | 0.2   | 0.26  |
|      | 2.0% Garlic | 3  | 0.2295   | 0.01994        | 0.01151    | 0.18                             | 0.279       | 0.22  | 0.25  |
|      | 4.0% Garlic | 3  | 0.2036   | 0.03227        | 0.01863    | 0.1235                           | 0.2838      | 0.17  | 0.22  |
|      | Total       | 15 | 0.2203   | 0.03582        | 0.00925    | 0.2005                           | 0.2402      | 0.14  | 0.28  |
| MCV  | Control     | 3  | 111.35   | 4.13068        | 2.38485    | 101.0888                         | 121.6112    | 108.6 | 116.1 |
|      | 0.5% Garlic | 3  | 117.6833 | 5.39776        | 3.1164     | 104.2745                         | 131.0921    | 111.6 | 121.9 |
|      | 1.0% Garlic | 3  | 115.5333 | 9.63397        | 5.56217    | 91.6012                          | 139.4654    | 106.4 | 125.6 |

|          |             |    |          |          |          |          |          |        |        |
|----------|-------------|----|----------|----------|----------|----------|----------|--------|--------|
|          | 2.0% Garlic | 3  | 124.5167 | 6.63067  | 3.82822  | 108.0452 | 140.9882 | 118.05 | 131.3  |
|          | 4.0% Garlic | 3  | 123.2833 | 7.91033  | 4.56703  | 103.633  | 142.9337 | 114.15 | 127.95 |
|          | Total       | 15 | 118.4733 | 7.79242  | 2.01199  | 114.158  | 122.7886 | 106.4  | 131.3  |
| RDWa     | Control     | 3  | 79.2667  | 2.80238  | 1.61795  | 72.3052  | 86.2282  | 77     | 82.4   |
|          | 0.5% Garlic | 3  | 81.8     | 4.00905  | 2.31463  | 71.841   | 91.759   | 79.05  | 86.4   |
|          | 1.0% Garlic | 3  | 77.2167  | 6.48389  | 3.74348  | 61.1098  | 93.3235  | 72.45  | 84.6   |
|          | 2.0% Garlic | 3  | 86.0333  | 12.53239 | 7.23558  | 54.9011  | 117.1655 | 74.25  | 99.2   |
|          | 4.0% Garlic | 3  | 90.2667  | 10.96099 | 6.32833  | 63.0381  | 117.4953 | 79.6   | 101.5  |
|          | Total       | 15 | 82.9167  | 8.53032  | 2.20252  | 78.1927  | 87.6406  | 72.45  | 101.5  |
| HGB      | Control     | 3  | 119      | 1.5      | 0.86603  | 115.2738 | 122.7262 | 117.5  | 120.5  |
|          | 0.5% Garlic | 3  | 131      | 10.75872 | 6.21155  | 104.2739 | 157.7261 | 120.5  | 142    |
|          | 1.0% Garlic | 3  | 129.3333 | 18.92969 | 10.92906 | 82.3094  | 176.3573 | 116    | 151    |
|          | 2.0% Garlic | 3  | 122.8333 | 11.55783 | 6.67291  | 94.1221  | 151.5446 | 112    | 135    |
|          | 4.0% Garlic | 3  | 117.6667 | 2.25462  | 1.30171  | 112.0659 | 123.2675 | 115.5  | 120    |
|          | Total       | 15 | 123.9667 | 10.89473 | 2.81301  | 117.9334 | 130      | 112    | 151    |
| MCHC     | Control     | 3  | 534.6667 | 3.17543  | 1.83333  | 526.7785 | 542.5549 | 531    | 536.5  |
|          | 0.5% Garlic | 3  | 553.8333 | 10.75097 | 6.20707  | 527.1264 | 580.5402 | 542    | 563    |
|          | 1.0% Garlic | 3  | 554.8333 | 13.54929 | 7.82269  | 521.175  | 588.4916 | 542    | 569    |
|          | 2.0% Garlic | 3  | 526.8333 | 8.00521  | 4.62181  | 506.9473 | 546.7194 | 519    | 535    |
|          | 4.0% Garlic | 3  | 524.3333 | 10.86662 | 6.27384  | 497.3392 | 551.3275 | 514.5  | 536    |
|          | Total       | 15 | 538.9    | 15.90283 | 4.10609  | 530.0933 | 547.7067 | 514.5  | 569    |
| MCH      | Control     | 3  | 65.1     | 1.67108  | 0.9648   | 60.9488  | 69.2512  | 63.7   | 66.95  |
|          | 0.5% Garlic | 3  | 65.1833  | 2.48713  | 1.43595  | 59.005   | 71.3617  | 62.85  | 67.8   |
|          | 1.0% Garlic | 3  | 66.2333  | 3.06159  | 1.76761  | 58.6279  | 73.8387  | 62.7   | 68.1   |
|          | 2.0% Garlic | 3  | 66.8     | 1.2278   | 0.70887  | 63.75    | 69.85    | 65.75  | 68.15  |
|          | 4.0% Garlic | 3  | 65.8667  | 2.40849  | 1.39054  | 59.8836  | 71.8497  | 63.35  | 68.15  |
|          | Total       | 15 | 65.8367  | 2.02639  | 0.52321  | 64.7145  | 66.9588  | 62.7   | 68.15  |
| RBC      | Control     | 3  | 1.3517   | 0.18724  | 0.1081   | 0.8865   | 1.8168   | 1.15   | 1.52   |
|          | 0.5% Garlic | 3  | 2.0083   | 0.12868  | 0.07429  | 1.6887   | 2.328    | 1.86   | 2.09   |
|          | 1.0% Garlic | 3  | 1.9633   | 0.37978  | 0.21927  | 1.0199   | 2.9068   | 1.71   | 2.4    |
|          | 2.0% Garlic | 3  | 1.8833   | 0.20306  | 0.11724  | 1.3789   | 2.3878   | 1.65   | 2.02   |
|          | 4.0% Garlic | 3  | 1.2883   | 0.37869  | 0.21864  | 0.3476   | 2.2291   | 1.05   | 1.73   |
|          | Total       | 15 | 1.699    | 0.39888  | 0.10299  | 1.4781   | 1.9199   | 1.05   | 2.4    |
| PLT      | Control     | 3  | 8.7      | 0.60828  | 0.35119  | 7.189    | 10.211   | 8      | 9.1    |
|          | 0.5% Garlic | 3  | 9.6667   | 2.08167  | 1.20185  | 4.4955   | 14.8378  | 8      | 12     |
|          | 1.0% Garlic | 3  | 9.6667   | 1.1547   | 0.66667  | 6.7982   | 12.5351  | 9      | 11     |
|          | 2.0% Garlic | 3  | 11.1667  | 2.5658   | 1.48137  | 4.7929   | 17.5405  | 9      | 14     |
|          | 4.0% Garlic | 3  | 8.6667   | 1.60728  | 0.92796  | 4.674    | 12.6594  | 7.5    | 10.5   |
|          | Total       | 15 | 9.5733   | 1.74907  | 0.45161  | 8.6047   | 10.5419  | 7.5    | 14     |
| Moisture | Control     | 3  | 72.2957  | 0.10966  | 0.06331  | 72.0232  | 72.5681  | 72.23  | 72.42  |
|          | 0.5% Garlic | 3  | 72.7933  | 0.6877   | 0.39704  | 71.085   | 74.5017  | 72     | 73.22  |
|          | 1.0% Garlic | 3  | 72.043   | 0.5876   | 0.33925  | 70.5833  | 73.5027  | 71.4   | 72.55  |

|         |             |    |         |         |         |         |         |       |       |
|---------|-------------|----|---------|---------|---------|---------|---------|-------|-------|
|         | 2.0% Garlic | 3  | 72.063  | 1.09591 | 0.63272 | 69.3406 | 74.7854 | 70.82 | 72.89 |
|         | 4.0% Garlic | 3  | 72.7712 | 1.51152 | 0.87268 | 69.0164 | 76.526  | 71.39 | 74.39 |
|         | Total       | 15 | 72.3932 | 0.85626 | 0.22108 | 71.9191 | 72.8674 | 70.82 | 74.39 |
| Ash     | Control     | 3  | 6.4067  | 2.48528 | 1.43488 | 0.2329  | 12.5805 | 3.9   | 8.87  |
|         | 0.5% Garlic | 3  | 6.87    | 0.87504 | 0.50521 | 4.6963  | 9.0437  | 5.9   | 7.6   |
|         | 1.0% Garlic | 3  | 6.7333  | 0.55293 | 0.31924 | 5.3598  | 8.1069  | 6.1   | 7.12  |
|         | 2.0% Garlic | 3  | 6.99    | 0.11136 | 0.06429 | 6.7134  | 7.2666  | 6.87  | 7.09  |
|         | 4.0% Garlic | 3  | 7.248   | 0.53007 | 0.30603 | 5.9312  | 8.5648  | 6.93  | 7.86  |
|         | Total       | 15 | 6.8496  | 1.07732 | 0.27816 | 6.253   | 7.4462  | 3.9   | 8.87  |
| Lipid   | Control     | 3  | 8.8867  | 0.53613 | 0.30953 | 7.5549  | 10.2185 | 8.31  | 9.37  |
|         | 0.5% Garlic | 3  | 8.9933  | 3.32506 | 1.91973 | 0.7334  | 17.2532 | 5.68  | 12.33 |
|         | 1.0% Garlic | 3  | 8.5785  | 1.70714 | 0.98562 | 4.3377  | 12.8193 | 6.67  | 9.96  |
|         | 2.0% Garlic | 3  | 6.829   | 0.38882 | 0.22449 | 5.8631  | 7.7949  | 6.47  | 7.24  |
|         | 4.0% Garlic | 3  | 6.767   | 0.8713  | 0.50304 | 4.6026  | 8.9314  | 5.8   | 7.49  |
|         | Total       | 15 | 8.0109  | 1.79944 | 0.46461 | 7.0144  | 9.0074  | 5.68  | 12.33 |
| Protein | Control     | 3  | 69.9367 | 2.06776 | 1.19382 | 64.8001 | 75.0733 | 67.98 | 72.1  |
|         | 0.5% Garlic | 3  | 70.7233 | 1.205   | 0.69571 | 67.7299 | 73.7167 | 69.86 | 72.1  |
|         | 1.0% Garlic | 3  | 71.88   | 2.11026 | 1.21836 | 66.6378 | 77.1222 | 69.66 | 73.86 |
|         | 2.0% Garlic | 3  | 72.22   | 0.62602 | 0.36143 | 70.6649 | 73.7751 | 71.65 | 72.89 |
|         | 4.0% Garlic | 3  | 70.3533 | 1.46603 | 0.84641 | 66.7115 | 73.9951 | 68.97 | 71.89 |
|         | Total       | 15 | 71.0227 | 1.6279  | 0.42032 | 70.1212 | 71.9242 | 67.98 | 73.86 |

**Table 4** Test of homogeneity of variance in growth, feed utilization, haemato-biochemical indices, and body proximate composition parameters of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| Test of Homogeneity of Variances |                  |     |     |       |
|----------------------------------|------------------|-----|-----|-------|
|                                  | Levene Statistic | df1 | df2 | Sig.  |
| WG                               | 3.386            | 4   | 10  | 0.054 |
| AGR                              | 3.386            | 4   | 10  | 0.054 |
| SGR                              | 2.6              | 4   | 10  | 0.1   |
| CF                               | 0.697            | 4   | 10  | 0.611 |
| HSI                              | 8.46             | 4   | 10  | 0.052 |
| VSI                              | 5.595            | 4   | 10  | 0.063 |
| FI                               | 2.43             | 4   | 10  | 0.116 |
| FCR                              | 0.764            | 4   | 10  | 0.572 |
| FER                              | 0.629            | 4   | 10  | 0.653 |
| PER                              | 0.629            | 4   | 10  | 0.653 |
| WBC                              | 4.364            | 4   | 10  | 0.057 |
| LYM                              | 4.43             | 4   | 10  | 0.06  |
| MONO                             | 4.958            | 4   | 10  | 0.058 |
| GRAN                             | 0.214            | 4   | 10  | 0.925 |
| HCT                              | 1.333            | 4   | 10  | 0.323 |
| MCV                              | 0.617            | 4   | 10  | 0.66  |
| RDWa                             | 1.402            | 4   | 10  | 0.302 |
| HGB                              | 3.863            | 4   | 10  | 0.058 |
| MCHC                             | 0.929            | 4   | 10  | 0.485 |
| MCH                              | 0.906            | 4   | 10  | 0.496 |
| RBC                              | 2.563            | 4   | 10  | 0.104 |
| PLT                              | 1.877            | 4   | 10  | 0.191 |
| Moisture                         | 2.573            | 4   | 10  | 0.103 |
| Ash                              | 2.521            | 4   | 10  | 0.107 |
| Lipid                            | 2.234            | 4   | 10  | 0.138 |
| protein                          | 0.849            | 4   | 10  | 0.525 |
| FW                               | 3.387            | 4   | 10  | 0.054 |

**Table 5** Analysis of variances (ANOVA) of growth and feed utilization indices of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| ANOVA |                |                |    |             |        |       |
|-------|----------------|----------------|----|-------------|--------|-------|
|       |                | Sum of Squares | df | Mean Square | F      | Sig.  |
| FW    | Between Groups | 7032.927       | 4  | 1758.232    | 9.827  | 0.002 |
|       | Within Groups  | 1789.111       | 10 | 178.911     |        |       |
|       | Total          | 8822.038       | 14 |             |        |       |
| WG    | Between Groups | 7032.831       | 4  | 1758.208    | 9.827  | 0.002 |
|       | Within Groups  | 1789.151       | 10 | 178.915     |        |       |
|       | Total          | 8821.982       | 14 |             |        |       |
| AGR   | Between Groups | 1.954          | 4  | 0.488       | 9.827  | 0.002 |
|       | Within Groups  | 0.497          | 10 | 0.05        |        |       |
|       | Total          | 2.451          | 14 |             |        |       |
| SGR   | Between Groups | 1.86           | 4  | 0.465       | 10.809 | 0.001 |
|       | Within Groups  | 0.43           | 10 | 0.043       |        |       |
|       | Total          | 2.29           | 14 |             |        |       |
| CF    | Between Groups | 0.092          | 4  | 0.023       | 1.522  | 0.268 |
|       | Within Groups  | 0.151          | 10 | 0.015       |        |       |
|       | Total          | 0.243          | 14 |             |        |       |
| HSI   | Between Groups | 0.289          | 4  | 0.072       | 0.165  | 0.951 |
|       | Within Groups  | 4.367          | 10 | 0.437       |        |       |
|       | Total          | 4.656          | 14 |             |        |       |
| VSI   | Between Groups | 7.616          | 4  | 1.904       | 2.255  | 0.135 |
|       | Within Groups  | 8.441          | 10 | 0.844       |        |       |
|       | Total          | 16.057         | 14 |             |        |       |
| FI    | Between Groups | 324.632        | 4  | 81.158      | 15.711 | 0     |
|       | Within Groups  | 51.657         | 10 | 5.166       |        |       |
|       | Total          | 376.289        | 14 |             |        |       |
| FCR   | Between Groups | 0.46           | 4  | 0.115       | 8.441  | 0.003 |
|       | Within Groups  | 0.136          | 10 | 0.014       |        |       |
|       | Total          | 0.596          | 14 |             |        |       |
| FER   | Between Groups | 1.247          | 4  | 0.312       | 7.329  | 0.005 |
|       | Within Groups  | 0.425          | 10 | 0.043       |        |       |
|       | Total          | 1.672          | 14 |             |        |       |
| PER   | Between Groups | 7.814          | 4  | 1.954       | 9.827  | 0.002 |
|       | Within Groups  | 1.988          | 10 | 0.199       |        |       |

**Table 6** Analysis of variances (ANOVA) of haematological and body proximate composition indices of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| ANOVA |                |                |    |             |       |       |
|-------|----------------|----------------|----|-------------|-------|-------|
|       |                | Sum of Squares | df | Mean Square | F     | Sig.  |
| WBC   | Between Groups | 137.778        | 4  | 34.445      | 0.885 | 0.507 |
|       | Within Groups  | 389.095        | 10 | 38.91       |       |       |
|       | Total          | 526.873        | 14 |             |       |       |
| LYM   | Between Groups | 57.875         | 4  | 14.469      | 2.418 | 0.117 |
|       | Within Groups  | 59.837         | 10 | 5.984       |       |       |
|       | Total          | 117.713        | 14 |             |       |       |
| MON   | Between Groups | 6.122          | 4  | 1.531       | 2.501 | 0.109 |
|       | Within Groups  | 6.12           | 10 | 0.612       |       |       |
|       | Total          | 12.242         | 14 |             |       |       |
| GRAN  | Between Groups | 12.762         | 4  | 3.191       | 6.25  | 0.009 |
|       | Within Groups  | 5.105          | 10 | 0.511       |       |       |
|       | Total          | 17.867         | 14 |             |       |       |
| HCT   | Between Groups | 0.003          | 4  | 0.001       | 0.43  | 0.784 |
|       | Within Groups  | 0.015          | 10 | 0.002       |       |       |
|       | Total          | 0.018          | 14 |             |       |       |
| MCV   | Between Groups | 359.003        | 4  | 89.751      | 1.828 | 0.2   |
|       | Within Groups  | 491.102        | 10 | 49.11       |       |       |
|       | Total          | 850.104        | 14 |             |       |       |
| RDWa  | Between Groups | 332.387        | 4  | 83.097      | 1.211 | 0.365 |
|       | Within Groups  | 686.342        | 10 | 68.634      |       |       |
|       | Total          | 1018.728       | 14 |             |       |       |
| HGB   | Between Groups | 431.733        | 4  | 107.933     | 0.878 | 0.511 |
|       | Within Groups  | 1230           | 10 | 123         |       |       |
|       | Total          | 1661.733       | 14 |             |       |       |
| MCHC  | Between Groups | 2557.767       | 4  | 639.442     | 6.506 | 0.008 |
|       | Within Groups  | 982.833        | 10 | 98.283      |       |       |
|       | Total          | 3540.6         | 14 |             |       |       |
| MCH   | Between Groups | 6.167          | 4  | 1.542       | 0.3   | 0.871 |
|       | Within Groups  | 51.32          | 10 | 5.132       |       |       |
|       | Total          | 57.487         | 14 |             |       |       |
| RBC   | Between Groups | 1.466          | 4  | 0.367       | 4.818 | 0.02  |
|       | Within Groups  | 0.761          | 10 | 0.076       |       |       |
|       | Total          | 2.227          | 14 |             |       |       |
| PLT   | Between Groups | 12.423         | 4  | 3.106       | 1.021 | 0.442 |

|          |                |        |    |       |       |       |
|----------|----------------|--------|----|-------|-------|-------|
| Moisture | Within Groups  | 30.407 | 10 | 3.041 |       |       |
|          | Total          | 42.829 | 14 |       |       |       |
|          | Between Groups | 1.633  | 4  | 0.408 | 0.473 | 0.755 |
| Ash      | Within Groups  | 8.632  | 10 | 0.863 |       |       |
|          | Total          | 10.264 | 14 |       |       |       |
|          | Between Groups | 1.166  | 4  | 0.291 | 0.193 | 0.936 |
| Lipid    | Within Groups  | 15.083 | 10 | 1.508 |       |       |
|          | Total          | 16.249 | 14 |       |       |       |
|          | Between Groups | 14.995 | 4  | 3.749 | 1.236 | 0.356 |
| protein  | Within Groups  | 30.336 | 10 | 3.034 |       |       |
|          | Total          | 45.332 | 14 |       |       |       |
|          | Between Groups | 11.657 | 4  | 2.914 | 1.145 | 0.39  |
|          | Within Groups  | 25.444 | 10 | 2.544 |       |       |

**Table 7** Post hoc test (Duncan multiple range test) of growth parameters of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| Weight Gain                                            |   |                                           |         |             | Final Weight                                           |             |                                           |             |         |         |        |
|--------------------------------------------------------|---|-------------------------------------------|---------|-------------|--------------------------------------------------------|-------------|-------------------------------------------|-------------|---------|---------|--------|
| Duncan a                                               |   |                                           |         |             | Duncan a                                               |             |                                           |             |         |         |        |
| Groups                                                 | N | Subset for alpha = 0.05                   |         |             | Groups                                                 | N           | Subset for alpha = 0.05                   |             |         |         |        |
|                                                        |   | 1                                         | 2       | 3           | 4                                                      |             | 1                                         | 2           | 3       | 4       |        |
| 4.0% Garlic                                            | 3 | 54.7733                                   |         |             |                                                        | 4.0% Garlic | 3                                         | 67.4233     |         |         |        |
| Control                                                | 3 |                                           | 80.7333 |             |                                                        | Control     | 3                                         |             | 93.3833 |         |        |
| 0.5% Garlic                                            | 3 |                                           | 87.14   | 87.14       |                                                        | 0.5% Garlic | 3                                         |             | 99.79   |         |        |
| 1.0% Garlic                                            | 3 |                                           |         | 111.397     | 111.4                                                  | 1.0% Garlic | 3                                         |             | 124.047 | 124.047 |        |
| 2.0% Garlic                                            | 3 |                                           |         |             | 113.51                                                 | 2.0% Garlic | 3                                         |             |         | 126.16  |        |
| Sig.                                                   |   | 1                                         | 0.57    | 0.051       | 0.85                                                   | Sig.        |                                           | 1           | 0.57    | 0.051   | 0.85   |
| Means for groups in homogeneous subsets are displayed. |   |                                           |         |             | Means for groups in homogeneous subsets are displayed. |             |                                           |             |         |         |        |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                                           |         |             | a Uses Harmonic Mean Sample Size = 3.000.              |             |                                           |             |         |         |        |
| Specific Growth Rate                                   |   |                                           |         |             | Absolute Growth Rate                                   |             |                                           |             |         |         |        |
| Duncan a                                               |   |                                           |         |             | Duncan a                                               |             |                                           |             |         |         |        |
| Groups                                                 | N | Subset for alpha = 0.05                   |         |             | Groups                                                 | N           | Subset for alpha = 0.05                   |             |         |         |        |
|                                                        |   | 1                                         | 2       | 3           | 4                                                      |             | 1                                         | 2           | 3       | 4       |        |
| 4.0% Garlic                                            | 3 | 2.9084                                    |         |             |                                                        | 4.0% Garlic | 3                                         | 0.9129      |         |         |        |
| Control                                                | 3 |                                           | 3.3601  |             |                                                        | Control     | 3                                         |             | 1.3456  |         |        |
| 0.5% Garlic                                            | 3 |                                           | 3.4801  | 3.4801      |                                                        | 0.5% Garlic | 3                                         |             | 1.4523  | 1.4523  |        |
| 1.0% Garlic                                            | 3 |                                           |         | 3.8315      | 3.8315                                                 | 1.0% Garlic | 3                                         |             |         | 1.8566  | 1.8566 |
| 2.0% Garlic                                            | 3 |                                           |         |             | 3.8747                                                 | 2.0% Garlic | 3                                         |             |         |         | 1.8918 |
| Sig.                                                   |   | 1                                         | 0.495   | 0.065       | 0.804                                                  | Sig.        |                                           | 1           | 0.57    | 0.051   | 0.85   |
| Means for groups in homogeneous subsets are displayed. |   |                                           |         |             | Means for groups in homogeneous subsets are displayed. |             |                                           |             |         |         |        |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                                           |         |             | a Uses Harmonic Mean Sample Size = 3.000.              |             |                                           |             |         |         |        |
| Condition Factor                                       |   | Hepatosomatic Index                       |         |             | Viscerosomatic Index                                   |             |                                           |             |         |         |        |
| Duncan a                                               |   |                                           |         | Duncan a    |                                                        |             | Duncan a                                  |             |         |         |        |
| Groups                                                 | N | Subset for alpha = 0.05                   |         |             | Groups                                                 | N           | Subset for alpha = 0.05                   |             |         |         |        |
|                                                        |   | 1                                         |         | Groups      | N                                                      | 1           |                                           | Groups      | N       | 1       |        |
| 2.0% Garlic                                            | 3 | 0.4752                                    |         | 4.0% Garlic | 3                                                      | 2.9651      |                                           | 1.0% Garlic | 3       | 7.1096  |        |
| 1.0% Garlic                                            | 3 | 0.4886                                    |         | Control     | 3                                                      | 3.0371      |                                           | 2.0% Garlic | 3       | 7.5411  |        |
| Control                                                | 3 | 0.5383                                    |         | 0.5% Garlic | 3                                                      | 3.1108      |                                           | 4.0% Garlic | 3       | 7.5795  |        |
| 0.5% Garlic                                            | 3 | 0.5559                                    |         | 1.0% Garlic | 3                                                      | 3.1309      |                                           | Control     | 3       | 8.7699  |        |
| 4.0% Garlic                                            | 3 | 0.6955                                    |         | 2.0% Garlic | 3                                                      | 3.3756      |                                           | 0.5% Garlic | 3       | 8.8767  |        |
| Sig.                                                   |   | 0.072                                     |         | Sig.        |                                                        | 0.497       |                                           | Sig.        |         | 0.056   |        |
| Means for groups in homogeneous subsets                |   | Means for groups in homogeneous subsets   |         |             | Means for groups in homogeneous subsets                |             | Means for groups in homogeneous subsets   |             |         |         |        |
| a Uses Harmonic Mean Sample Size = 3.000.              |   | a Uses Harmonic Mean Sample Size = 3.000. |         |             | a Uses Harmonic Mean Sample Size = 3.000.              |             | a Uses Harmonic Mean Sample Size = 3.000. |             |         |         |        |

**Table 8** Post hoc test (Duncan multiple range test) of feed utilization indices of African catfish (*Clarias gariepinus*) juveniles fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| <b>FI</b>                                              |   |                         |         | <b>FCR</b>                                             |   |                         |        |
|--------------------------------------------------------|---|-------------------------|---------|--------------------------------------------------------|---|-------------------------|--------|
| Duncan a group                                         | N | Subset for alpha = 0.05 |         | Duncan a group                                         | N | Subset for alpha = 0.05 |        |
|                                                        |   | 1                       | 2       |                                                        |   | 1                       | 2      |
| 4% garlic                                              | 3 | 81.82                   |         | 2% garlic                                              | 3 | 1.0405                  |        |
| control                                                | 3 |                         | 97.2733 | 1% garlic                                              | 3 | 1.0532                  |        |
| 0.5% garlic                                            | 3 |                         | 103.893 | 0.5% garlic                                            | 3 | 1.2038                  | 1.2038 |
| 1% garlic                                              | 3 |                         |         | control                                                | 3 | 1.2292                  | 1.2292 |
| 2% garlic                                              | 3 |                         |         | 4% garlic                                              | 3 |                         | 1.5012 |
| <b>Sig.</b>                                            |   | 1                       | 0.122   | <b>Sig.</b>                                            |   | 0.228                   | 0.064  |
| Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |        |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |         | a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |        |
| <b>FER</b>                                             |   |                         |         | <b>PER</b>                                             |   |                         |        |
| Duncan a group                                         | N | Subset for alpha = 0.05 |         | Duncan a group                                         | N | Subset for alpha = 0.05 |        |
|                                                        |   | 1                       | 2       |                                                        |   | 1                       | 2      |
| 4% garlic                                              | 3 | 0.6748                  |         | 4% garlic                                              | 3 | 2.163                   |        |
| control                                                | 3 | 0.8282                  | 0.8282  | control                                                | 3 | 2.654                   | 2.6544 |
| 0.5% garlic                                            | 3 | 0.8399                  | 0.8399  | 0.5% garlic                                            | 3 | 2.692                   | 2.692  |
| 2% garlic                                              | 3 |                         | 0.9648  | 2% garlic                                              | 3 |                         | 3.0924 |
| 1% garlic                                              | 3 |                         | 0.9663  | 1% garlic                                              | 3 |                         | 3.0972 |
| <b>Sig.</b>                                            |   | 0.133                   | 0.211   | <b>Sig.</b>                                            |   | 0.133                   | 0.211  |
| Means for groups in homogeneous subsets are displayed. |   |                         |         | Means for groups in homogeneous subsets are displayed. |   |                         |        |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |         | a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |        |

**Table 9** Quadratic regression model output on weight gain and feed efficiency ratio against dietary garlic crude polysaccharide inclusion level in African catfish (*Clarias gariepinus*) juveniles' culture.

|                                        |                             |                   |                            |        |       |
|----------------------------------------|-----------------------------|-------------------|----------------------------|--------|-------|
| WG                                     |                             |                   |                            |        |       |
| Model Summary                          |                             |                   |                            |        |       |
| R                                      | R Square                    | Adjusted R Square | Std. Error of the Estimate |        |       |
| 0.875                                  | 0.765                       | 0.726             | 13.142                     |        |       |
| The Independent Variable is Groups.    |                             |                   |                            |        |       |
|                                        |                             |                   |                            |        |       |
| ANOVA                                  |                             |                   |                            |        |       |
|                                        | Sum of Squares              | df                | Mean Square                | F      | Sig.  |
| Regression                             | 6749.62                     | 2                 | 3374.81                    | 19.541 | 0     |
| Residual                               | 2072.418                    | 12                | 172.701                    |        |       |
| Total                                  | 8822.038                    | 14                |                            |        |       |
| The Independent Variable is Groups.    |                             |                   |                            |        |       |
|                                        |                             |                   |                            |        |       |
| Coefficients                           |                             |                   |                            |        |       |
|                                        | Unstandardized Coefficients |                   | Standardized Coefficients  |        | Sig.  |
|                                        | B                           | Std. Error        | Beta                       |        |       |
| Groups                                 | 41.689                      | 9.14              | 2.431                      | 4.561  | 0.001 |
| Groups <sup>2</sup> * 10 <sup>-2</sup> | -11.809                     | 2.138             | -2.944                     | -5.524 | 0     |
| (Constant)                             | 77.167                      | 6.452             |                            | 11.961 | 0     |
|                                        |                             |                   |                            |        |       |
| FER                                    |                             |                   |                            |        |       |
| Model Summary                          |                             |                   |                            |        |       |
| R                                      | R Square                    | Adjusted R Square | Std. Error of the Estimate |        |       |
| 0.722                                  | 0.522                       | 0.442             | 0.112                      |        |       |
| The Independent Variable is Groups.    |                             |                   |                            |        |       |
|                                        |                             |                   |                            |        |       |
| ANOVA                                  |                             |                   |                            |        |       |
|                                        | Sum of Squares              | df                | Mean Square                | F      | Sig.  |
| Regression                             | 0.163                       | 2                 | 0.082                      | 6.54   | 0.012 |
| Residual                               | 0.15                        | 12                | 0.012                      |        |       |
| Total                                  | 0.313                       | 14                |                            |        |       |
| The Independent Variable is Groups.    |                             |                   |                            |        |       |
|                                        |                             |                   |                            |        |       |
| Coefficients                           |                             |                   |                            |        |       |
|                                        | Unstandardized Coefficients |                   | Standardized Coefficients  |        | Sig.  |
|                                        | B                           | Std. Error        | Beta                       |        |       |
| Groups                                 | 0.189                       | 0.078             | 1.853                      | 2.436  | 0.031 |
| Groups <sup>2</sup> * 10 <sup>-2</sup> | -0.056                      | 0.018             | -2.322                     | -3.052 | 0.01  |
| (Constant)                             | 0.807                       | 0.055             |                            | 14.696 | 0     |
|                                        |                             |                   |                            |        |       |

**Table 10** Post hoc test (Duncan multiple range test) of haematological indices, and body proximate composition parameters of African catfish (*Clarias gariepinus*) fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| WBC                                                    |   |          |                         | LYM                                                    |   |         |                         | HGB                                                    |   |          |                         | PLT                                                    |   |         |                         |
|--------------------------------------------------------|---|----------|-------------------------|--------------------------------------------------------|---|---------|-------------------------|--------------------------------------------------------|---|----------|-------------------------|--------------------------------------------------------|---|---------|-------------------------|
| Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         | Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         |
| Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 | Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 |
|                                                        |   |          | 1                       |                                                        |   |         | 1                       |                                                        |   |          | 1                       |                                                        |   |         | 1                       |
| 1.0% Garlic                                            | 3 | 41.4333  |                         | Control                                                | 3 | 38.4833 |                         | 4.0% Garlic                                            | 3 | 117.6667 |                         | 4.0% Garlic                                            | 3 | 8.6667  |                         |
| 2.0% Garlic                                            | 3 | 47.3167  |                         | 1.0% Garlic                                            | 3 | 38.5    |                         | Control                                                | 3 | 119      |                         | Control                                                | 3 | 8.7     |                         |
| Control                                                | 3 | 47.5     |                         | 4.0% Garlic                                            | 3 | 38.62   |                         | 2.0% Garlic                                            | 3 | 122.8333 |                         | 0.5% Garlic                                            | 3 | 9.6667  |                         |
| 4.0% Garlic                                            | 3 | 47.65    |                         | 2.0% Garlic                                            | 3 | 42.4667 |                         | 1.0% Garlic                                            | 3 | 129.3333 |                         | 1.0% Garlic                                            | 3 | 9.6667  |                         |
| 0.5% Garlic                                            | 3 | 50.7667  |                         | 0.5% Garlic                                            | 3 | 42.6167 |                         | 0.5% Garlic                                            | 3 | 131      |                         | 2.0% Garlic                                            | 3 | 11.1667 |                         |
| Sig.                                                   |   | 0.123    |                         | Sig.                                                   |   | 0.086   |                         | Sig.                                                   |   | 0.205    |                         | Sig.                                                   |   | 0.137   |                         |
| Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         | Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         |
| MONO                                                   |   |          |                         | GRAN                                                   |   |         |                         | MCHC                                                   |   |          |                         | Moisture                                               |   |         |                         |
| Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         | Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         |
| Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 | Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 |
|                                                        |   |          | 1                       |                                                        |   |         | 1                       |                                                        |   |          | 1                       |                                                        |   |         | 1                       |
| 1.0% Garlic                                            | 3 | 1.4167   |                         | 4.0% Garlic                                            | 3 | 2.0333  |                         | 4.0% Garlic                                            | 3 | 524.3333 |                         | 1.0% Garlic                                            | 3 | 72.043  |                         |
| Control                                                | 3 | 2.2333   | 2.2333                  | Control                                                | 3 | 2.45    |                         | 2.0% Garlic                                            | 3 | 526.8333 |                         | 2.0% Garlic                                            | 3 | 72.063  |                         |
| 2.0% Garlic                                            | 3 | 2.45     | 2.45                    | 2.0% Garlic                                            | 3 | 2.7667  |                         | Control                                                | 3 | 534.6667 |                         | Control                                                | 3 | 72.2957 |                         |
| 4.0% Garlic                                            | 3 | 2.5      |                         | 1.0% Garlic                                            | 3 | 2.85    |                         | 0.5% Garlic                                            | 3 | 553.8333 |                         | 4.0% Garlic                                            | 3 | 72.7712 |                         |
| 0.5% Garlic                                            | 3 |          | 3.4167                  | 0.5% Garlic                                            | 3 |         | 4.7167                  | 1.0% Garlic                                            | 3 | 554.8333 |                         | 0.5% Garlic                                            | 3 | 72.7933 |                         |
| Sig.                                                   |   | 0.145    | 0.115                   | Sig.                                                   |   | 0.221   | 1                       | Sig.                                                   |   | 0.251    | 0.904                   | Sig.                                                   |   | 0.382   |                         |
| Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         | Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         |
| GRAN                                                   |   |          |                         | HCT                                                    |   |         |                         | MCH                                                    |   |          |                         | Ash                                                    |   |         |                         |
| Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         | Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         |
| Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 | Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 |
|                                                        |   |          | 1                       |                                                        |   |         | 1                       |                                                        |   |          | 1                       |                                                        |   |         | 1                       |
| 4.0% Garlic                                            | 3 | 2.0333   |                         | 4.0% Garlic                                            | 3 | 0.2036  |                         | Control                                                | 3 | 65.1     |                         | Control                                                | 3 | 6.4067  |                         |
| Control                                                | 3 | 2.45     |                         | Control                                                | 3 | 0.2058  |                         | 0.5% Garlic                                            | 3 | 65.1833  |                         | 1.0% Garlic                                            | 3 | 6.7333  |                         |
| 2.0% Garlic                                            | 3 | 2.7667   |                         | 1.0% Garlic                                            | 3 | 0.2257  |                         | 4.0% Garlic                                            | 3 | 65.8667  |                         | 0.5% Garlic                                            | 3 | 6.87    |                         |
| 1.0% Garlic                                            | 3 | 2.85     |                         | 2.0% Garlic                                            | 3 | 0.2295  |                         | 1.0% Garlic                                            | 3 | 66.2333  |                         | 2.0% Garlic                                            | 3 | 6.99    |                         |
| 0.5% Garlic                                            | 3 |          | 4.7167                  | 0.5% Garlic                                            | 3 | 0.237   |                         | 2.0% Garlic                                            | 3 | 66.8     |                         | 4.0% Garlic                                            | 3 | 7.248   |                         |
| Sig.                                                   |   | 0.221    | 1                       | Sig.                                                   |   | 0.358   |                         | Sig.                                                   |   | 0.415    |                         | Sig.                                                   |   | 0.456   |                         |
| Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         | Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         |
| MCV                                                    |   |          |                         | RDW                                                    |   |         |                         | RBC                                                    |   |          |                         | Lipid                                                  |   |         |                         |
| Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         | Duncan a                                               |   |          |                         | Duncan a                                               |   |         |                         |
| Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 | Groups                                                 |   | N        | Subset for alpha = 0.05 | Groups                                                 |   | N       | Subset for alpha = 0.05 |
|                                                        |   |          | 1                       |                                                        |   |         | 1                       |                                                        |   |          | 1                       |                                                        |   |         | 1                       |
| Control                                                | 3 | 111.35   |                         | 1.0% Garlic                                            | 3 | 77.2167 |                         | 4.0% Garlic                                            | 3 | 1.2883   |                         | 4.0% Garlic                                            | 3 | 6.767   |                         |
| 1.0% Garlic                                            | 3 | 115.5333 |                         | Control                                                | 3 | 79.2667 |                         | Control                                                | 3 | 1.3517   |                         | 2.0% Garlic                                            | 3 | 6.829   |                         |
| 0.5% Garlic                                            | 3 | 117.6833 |                         | 0.5% Garlic                                            | 3 | 81.8    |                         | 2.0% Garlic                                            | 3 |          | 1.8833                  | 1.0% Garlic                                            | 3 | 8.5785  |                         |
| 4.0% Garlic                                            | 3 | 123.2833 |                         | 2.0% Garlic                                            | 3 | 86.0333 |                         | 1.0% Garlic                                            | 3 |          | 1.9633                  | Control                                                | 3 | 8.8867  |                         |
| 2.0% Garlic                                            | 3 | 124.5167 |                         | 4.0% Garlic                                            | 3 | 90.2667 |                         | 0.5% Garlic                                            | 3 |          | 2.0083                  | 0.5% Garlic                                            | 3 | 8.9933  |                         |
| Sig.                                                   |   | 0.061    |                         | Sig.                                                   |   | 0.106   |                         | Sig.                                                   |   | 0.784    | 0.608                   | Sig.                                                   |   | 0.18    |                         |
| Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         | Means for groups in homogeneous subsets are displayed. |   |          |                         | Means for groups in homogeneous subsets are displayed. |   |         |                         |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         | a Uses Harmonic Mean Sample Size = 3.000.              |   |         |                         |
| Protein                                                |   |          |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| Duncan a                                               |   |          |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| Groups                                                 |   | N        | Subset for alpha = 0.05 |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
|                                                        |   |          | 1                       |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| Control                                                | 3 | 69.9367  |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| 4.0% Garlic                                            | 3 | 70.3533  |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| 0.5% Garlic                                            | 3 | 70.7233  |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| 1.0% Garlic                                            | 3 | 71.88    |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| 2.0% Garlic                                            | 3 | 72.22    |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| Sig.                                                   |   | 0.138    |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| Means for groups in homogeneous subsets are displayed. |   |          |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |          |                         |                                                        |   |         |                         |                                                        |   |          |                         |                                                        |   |         |                         |

**Table 11** Kaplan–Meier analysis (survival) output of African catfish (*Clarias gariepinus*) juveniles subjected to low water pH after being fed 30% *Allium sativum* crude polysaccharide extracts supplemented diets for 60 d.

| Overall Comparisons                                                           | Chi-Square | df | Sig.  |
|-------------------------------------------------------------------------------|------------|----|-------|
| Log-Rank (Mantel-Cox)                                                         | 8.153      | 4  | 0.086 |
| Breslow-Generalized Wilcoxon                                                  | 5.413      | 4  | 0.248 |
| Tarone-Ware                                                                   | 6.582      | 4  | 0.16  |
| Test of equality of survival distributions for the different levels of Group. |            |    |       |

## Appendix C

**Table 1** Descriptive statistics of growth performance indices of African catfish (*Clarias gariepinus*) fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

|     |         | N  | Mean    | Std. Deviation | Std. Error | 95% Confidence Interval |             | Minimum | Maximum |
|-----|---------|----|---------|----------------|------------|-------------------------|-------------|---------|---------|
|     |         |    |         |                |            | Lower Bound             | Upper Bound |         |         |
| FW  | Control | 3  | 76.4267 | 3.68609        | 2.12816    | 67.2699                 | 85.5834     | 72.23   | 79.14   |
|     | 0.50%   | 3  | 81.5    | 7.01986        | 4.05292    | 64.0617                 | 98.9383     | 75.5    | 89.22   |
|     | 1.00%   | 3  | 90.6    | 6.8942         | 3.98037    | 73.4739                 | 107.7261    | 84.2    | 97.9    |
|     | 2.00%   | 3  | 72.18   | 1.98071        | 1.14356    | 67.2597                 | 77.1003     | 69.94   | 73.7    |
|     | 4.00%   | 3  | 68.8067 | 1.28535        | 0.7421     | 65.6137                 | 71.9997     | 67.4    | 69.92   |
|     | Total   | 15 | 77.9027 | 8.89392        | 2.2964     | 72.9774                 | 82.828      | 67.4    | 97.9    |
| WG  | Control | 3  | 64.1467 | 3.68609        | 2.12816    | 54.9899                 | 73.3034     | 59.95   | 66.86   |
|     | 0.50%   | 3  | 69.22   | 7.01986        | 4.05292    | 51.7817                 | 86.6583     | 63.22   | 76.94   |
|     | 1.00%   | 3  | 78.32   | 6.8942         | 3.98037    | 61.1939                 | 95.4461     | 71.92   | 85.62   |
|     | 2.00%   | 3  | 59.9    | 1.98071        | 1.14356    | 54.9797                 | 64.8203     | 57.66   | 61.42   |
|     | 4.00%   | 3  | 56.5267 | 1.28535        | 0.7421     | 53.3337                 | 59.7197     | 55.12   | 57.64   |
|     | Total   | 15 | 65.6227 | 8.89392        | 2.2964     | 60.6974                 | 70.548      | 55.12   | 85.62   |
| AGR | Control | 3  | 1.0691  | 0.06143        | 0.03547    | 0.9165                  | 1.2217      | 1       | 1.11    |
|     | 0.50%   | 3  | 1.1537  | 0.117          | 0.06755    | 0.863                   | 1.4443      | 1.05    | 1.28    |
|     | 1.00%   | 3  | 1.2442  | 0.14478        | 0.08359    | 0.8846                  | 1.6039      | 1.08    | 1.36    |
|     | 2.00%   | 3  | 0.9983  | 0.03301        | 0.01906    | 0.9163                  | 1.0803      | 0.96    | 1.02    |
|     | 4.00%   | 3  | 0.9421  | 0.02142        | 0.01237    | 0.8889                  | 0.9953      | 0.92    | 0.96    |
|     | Total   | 15 | 1.0815  | 0.13483        | 0.03481    | 1.0068                  | 1.1562      | 0.92    | 1.36    |
| SGR | Control | 3  | 3.046   | 0.08143        | 0.04701    | 2.8437                  | 3.2482      | 2.95    | 3.11    |
|     | 0.50%   | 3  | 3.1503  | 0.14179        | 0.08186    | 2.7981                  | 3.5026      | 3.03    | 3.31    |
|     | 1.00%   | 3  | 3.3276  | 0.12616        | 0.07284    | 3.0142                  | 3.641       | 3.21    | 3.46    |
|     | 2.00%   | 3  | 2.9516  | 0.04604        | 0.02658    | 2.8372                  | 3.0659      | 2.9     | 2.99    |
|     | 4.00%   | 3  | 2.872   | 0.03123        | 0.01803    | 2.7944                  | 2.9496      | 2.84    | 2.9     |
|     | Total   | 15 | 3.0695  | 0.18349        | 0.04738    | 2.9679                  | 3.1711      | 2.84    | 3.46    |
| CF  | Control | 3  | 0.5732  | 0.04538        | 0.0262     | 0.4604                  | 0.6859      | 0.52    | 0.61    |
|     | 0.50%   | 3  | 0.521   | 0.11966        | 0.06908    | 0.2237                  | 0.8182      | 0.43    | 0.66    |
|     | 1.00%   | 3  | 0.6598  | 0.04546        | 0.02624    | 0.5469                  | 0.7727      | 0.61    | 0.7     |
|     | 2.00%   | 3  | 0.6507  | 0.03689        | 0.0213     | 0.559                   | 0.7424      | 0.62    | 0.69    |
|     | 4.00%   | 3  | 0.5862  | 0.04948        | 0.02857    | 0.4633                  | 0.7091      | 0.53    | 0.63    |
|     | Total   | 15 | 0.5982  | 0.07763        | 0.02004    | 0.5552                  | 0.6412      | 0.43    | 0.7     |
| HSI | Control | 3  | 1.6639  | 0.20712        | 0.11958    | 1.1494                  | 2.1784      | 1.52    | 1.9     |
|     | 0.50%   | 3  | 2.2914  | 0.3016         | 0.17413    | 1.5422                  | 3.0406      | 2.01    | 2.61    |
|     | 1.00%   | 3  | 2.5857  | 0.19175        | 0.1107     | 2.1094                  | 3.062       | 2.38    | 2.76    |
|     | 2.00%   | 3  | 2.0434  | 0.08202        | 0.04735    | 1.8397                  | 2.2472      | 1.97    | 2.13    |
|     | 4.00%   | 3  | 2.0386  | 0.16135        | 0.09316    | 1.6377                  | 2.4394      | 1.86    | 2.18    |
|     | Total   | 15 | 2.1246  | 0.35924        | 0.09275    | 1.9257                  | 2.3235      | 1.52    | 2.76    |
| VSI | Control | 3  | 5.3794  | 0.77144        | 0.44539    | 3.463                   | 7.2958      | 4.82    | 6.26    |
|     | 0.50%   | 3  | 7.4522  | 0.7268         | 0.41962    | 5.6467                  | 9.2577      | 6.96    | 8.29    |
|     | 1.00%   | 3  | 6.0683  | 0.46262        | 0.26709    | 4.9191                  | 7.2175      | 5.63    | 6.55    |
|     | 2.00%   | 3  | 7.3617  | 0.64865        | 0.3745     | 5.7503                  | 8.973       | 6.61    | 7.74    |
|     | 4.00%   | 3  | 8.7078  | 1.59186        | 0.91906    | 4.7534                  | 12.6622     | 7.31    | 10.44   |
|     | Total   | 15 | 6.9939  | 1.43483        | 0.37047    | 6.1993                  | 7.7885      | 4.82    | 10.44   |

**Table 2** Descriptive statistics of feed utilization indices of African catfish (*Clarias gariepinus*) fed *Aloe vera*-*Allium sativum* polysaccharide mixture supplemented diets for 60 d.

|     |         | N  | Mean    | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Minimum | Maximum |
|-----|---------|----|---------|----------------|------------|----------------------------------|-------------|---------|---------|
|     |         |    |         |                |            | Lower Bound                      | Upper Bound |         |         |
| FI  | Control | 3  | 98.7567 | 0.35445        | 0.20464    | 97.8762                          | 99.6372     | 98.35   | 99      |
|     | 0.50%   | 3  | 99.0683 | 1.31302        | 0.75808    | 95.8066                          | 102.3301    | 97.56   | 99.91   |
|     | 1.00%   | 3  | 98.3897 | 2.95949        | 1.70866    | 91.0379                          | 105.7415    | 94.99   | 100.39  |
|     | 2.00%   | 3  | 97.6533 | 2.86572        | 1.65452    | 90.5345                          | 104.7722    | 94.48   | 100.04  |
|     | 4.00%   | 3  | 94.4433 | 5.72532        | 3.30552    | 80.2208                          | 108.6658    | 88.98   | 100.4   |
|     | Total   | 15 | 97.6623 | 3.22265        | 0.83208    | 95.8776                          | 99.4469     | 88.98   | 100.4   |
| FCR | Control | 3  | 1.5433  | 0.09452        | 0.05457    | 1.3085                           | 1.7781      | 1.47    | 1.65    |
|     | 0.50%   | 3  | 1.4433  | 0.14012        | 0.0809     | 1.0953                           | 1.7914      | 1.3     | 1.58    |
|     | 1.00%   | 3  | 1.2633  | 0.11372        | 0.06566    | 0.9808                           | 1.5458      | 1.17    | 1.39    |
|     | 2.00%   | 3  | 1.63    | 0.01           | 0.00577    | 1.6052                           | 1.6548      | 1.62    | 1.64    |
|     | 4.00%   | 3  | 1.67    | 0.08718        | 0.05033    | 1.4534                           | 1.8866      | 1.61    | 1.77    |
|     | Total   | 15 | 1.51    | 0.17271        | 0.04459    | 1.4144                           | 1.6056      | 1.17    | 1.77    |
| FER | Control | 3  | 0.6496  | 0.03865        | 0.02231    | 0.5536                           | 0.7456      | 0.61    | 0.68    |
|     | 0.50%   | 3  | 0.6987  | 0.06953        | 0.04014    | 0.526                            | 0.8714      | 0.63    | 0.77    |
|     | 1.00%   | 3  | 0.7962  | 0.06806        | 0.03929    | 0.6271                           | 0.9653      | 0.72    | 0.85    |
|     | 2.00%   | 3  | 0.6133  | 0.00278        | 0.0016     | 0.6065                           | 0.6202      | 0.61    | 0.62    |
|     | 4.00%   | 3  | 0.5996  | 0.02932        | 0.01693    | 0.5268                           | 0.6725      | 0.57    | 0.62    |
|     | Total   | 15 | 0.6715  | 0.08435        | 0.02178    | 0.6248                           | 0.7182      | 0.57    | 0.85    |
| PER | Control | 3  | 2.03    | 0.12078        | 0.06973    | 1.7299                           | 2.33        | 1.89    | 2.12    |
|     | 0.50%   | 3  | 2.1834  | 0.21728        | 0.12545    | 1.6437                           | 2.7232      | 1.98    | 2.41    |
|     | 1.00%   | 3  | 2.4882  | 0.21268        | 0.12279    | 1.9598                           | 3.0165      | 2.25    | 2.67    |
|     | 2.00%   | 3  | 1.9167  | 0.00868        | 0.00501    | 1.8952                           | 1.9383      | 1.91    | 1.92    |
|     | 4.00%   | 3  | 1.8739  | 0.09162        | 0.0529     | 1.6463                           | 2.1015      | 1.77    | 1.94    |
|     | Total   | 15 | 2.0984  | 0.26359        | 0.06806    | 1.9525                           | 2.2444      | 1.77    | 2.67    |

**Table 3** Descriptive statistics of haematological indices and body composition proximate parameters of African catfish (*Clarias gariepinus*) fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

|      |         | N  | Mean    | Std. Deviation | Std. Error | 95% Confidence Interval for Mean |             | Min  | Max  |
|------|---------|----|---------|----------------|------------|----------------------------------|-------------|------|------|
|      |         |    |         |                |            | Lower Bound                      | Upper Bound |      |      |
| WBC  | Control | 3  | 57.0667 | 5.62257        | 3.24619    | 43.0994                          | 71.0339     | 50.6 | 60.8 |
|      | 0.50%   | 3  | 58.0333 | 4.67047        | 2.6965     | 46.4312                          | 69.6354     | 53.9 | 63.1 |
|      | 1.00%   | 3  | 63.7833 | 1.38774        | 0.80121    | 60.336                           | 67.2307     | 62.3 | 65.0 |
|      | 2.00%   | 3  | 56.9833 | 2.93783        | 1.69616    | 49.6854                          | 64.2813     | 53.9 | 59.7 |
|      | 4.00%   | 3  | 57.3333 | 5.68888        | 3.28448    | 43.2014                          | 71.4653     | 50.9 | 61.7 |
|      | Total   | 15 | 58.64   | 4.58212        | 1.1831     | 56.1025                          | 61.1775     | 50.6 | 65.0 |
| Lym  | Control | 3  | 29.9    | 9.50631        | 5.48847    | 6.285                            | 53.515      | 20.2 | 39.2 |
|      | 0.50%   | 3  | 27.2667 | 4.43772        | 2.56212    | 16.2428                          | 38.2906     | 23.2 | 32   |
|      | 1.00%   | 3  | 32.6333 | 1.5987         | 0.92301    | 28.6619                          | 36.6047     | 30.9 | 34.0 |
|      | 2.00%   | 3  | 27.2833 | 9.71807        | 5.61073    | 3.1423                           | 51.4244     | 20.4 | 5    |
|      | 4.00%   | 3  | 26.6333 | 5.40494        | 3.12054    | 13.2067                          | 40.0599     | 20.5 | 38.4 |
|      | Total   | 15 | 28.7433 | 6.25785        | 1.61577    | 25.2779                          | 32.2088     | 20.2 | 30.7 |
| Mon  | Control | 3  | 1.9     | 0.60828        | 0.35119    | 0.389                            | 3.411       | 1.2  | 2.3  |
|      | 0.50%   | 3  | 2.4     | 0.1            | 0.05774    | 2.1516                           | 2.6484      | 2.3  | 2.5  |
|      | 1.00%   | 3  | 2.2667  | 0.57951        | 0.33458    | 0.8271                           | 3.7063      | 1.6  | 2.65 |
|      | 2.00%   | 3  | 1.9333  | 0.46188        | 0.26667    | 0.786                            | 3.0807      | 1.4  | 2.2  |
|      | 4.00%   | 3  | 2.0333  | 0.92916        | 0.53645    | -0.2748                          | 4.3415      | 1.4  | 3.1  |
|      | Total   | 15 | 2.1067  | 0.54474        | 0.14065    | 1.805                            | 2.4083      | 1.2  | 3.1  |
| Gran | Control | 3  | 1.6     | 0.52915        | 0.30551    | 0.2855                           | 2.9145      | 1.2  | 2.2  |
|      | 0.50%   | 3  | 2.4167  | 0.20207        | 0.11667    | 1.9147                           | 2.9186      | 2.2  | 2.6  |
|      | 1.00%   | 3  | 2.5833  | 0.88929        | 0.51343    | 0.3742                           | 4.7924      | 1.8  | 3.55 |
|      | 2.00%   | 3  | 2.1     | 0.37749        | 0.21794    | 1.1623                           | 3.0377      | 1.7  | 2.45 |
|      | 4.00%   | 3  | 2       | 0.60828        | 0.35119    | 0.489                            | 3.511       | 1.3  | 2.4  |
|      | Total   | 15 | 2.14    | 0.59797        | 0.1544     | 1.8089                           | 2.4711      | 1.2  | 3.55 |
| HCT  | Control | 3  | 0.1947  | 0.03675        | 0.02122    | 0.1034                           | 0.286       | 0.17 | 0.24 |
|      | 0.50%   | 3  | 0.2117  | 0.03523        | 0.02034    | 0.1241                           | 0.2992      | 0.18 | 0.25 |
|      | 1.00%   | 3  | 0.253   | 0.03005        | 0.01735    | 0.1783                           | 0.3277      | 0.22 | 0.27 |
|      | 2.00%   | 3  | 0.2202  | 0.05144        | 0.0297     | 0.0924                           | 0.348       | 0.18 | 0.28 |
|      | 4.00%   | 3  | 0.2013  | 0.0168         | 0.0097     | 0.1596                           | 0.2431      | 0.18 | 0.22 |
|      | Total   | 15 | 0.2162  | 0.03691        | 0.00953    | 0.1957                           | 0.2366      | 0.17 | 0.28 |

|      |         |   |                    |          |                    |          |                    |        |             |
|------|---------|---|--------------------|----------|--------------------|----------|--------------------|--------|-------------|
| MCV  | Control | 3 | 138.733<br>3       | 2.01329  | 1.16237            | 133.732  | 143.734<br>6       | 136.6  | 140.<br>6   |
|      | 0.50%   | 3 | 137.316<br>7       | 4.28554  | 2.47426            | 126.6708 | 147.962<br>5       | 133.7  | 142.<br>05  |
|      | 1.00%   | 3 | 131.616<br>7       | 10.53664 | 6.08333            | 105.4422 | 157.791<br>1       | 119.7  | 139.<br>7   |
|      | 2.00%   | 3 | 143.583<br>3       | 3.90587  | 2.25506            | 133.8806 | 153.286<br>1       | 139.1  | 146.<br>25  |
|      | 4.00%   | 3 | 133.966<br>7       | 8.33447  | 4.81191            | 113.2627 | 154.670<br>6       | 127    | 143.<br>2   |
|      |         | 1 | 137.043            |          |                    |          | 140.931            |        | 146.        |
|      | Total   | 5 | 3                  | 7.02124  | 1.81288            | 133.1551 | 6                  | 119.7  | 25          |
| RDWa | Control | 3 | 94.4               | 12.79414 | 7.3867             | 62.6176  | 126.182<br>4       | 79.9   | 104.<br>1   |
|      | 0.50%   | 3 | 99.0167<br>101.866 | 3.49154  | 2.01584            | 90.3432  | 107.690<br>1       | 95.2   | 102.<br>05  |
|      | 1.00%   | 3 | 7                  | 14.13164 | 8.15891            | 66.7617  | 136.971<br>6       | 90     | 117.<br>5   |
|      | 2.00%   | 3 | 106.983<br>3       | 3.83873  | 2.21629            | 97.4474  | 116.519<br>3       | 104.45 | 111.<br>4   |
|      | 4.00%   | 3 | 99.5333            | 6.90386  | 3.98595            | 82.3832  | 116.683<br>5       | 92.5   | 106.<br>3   |
|      |         | 1 |                    |          |                    |          | 105.331            |        | 117.        |
|      | Total   | 5 | 100.36             | 8.97779  | 2.31806            | 95.3883  | 7                  | 79.9   | 5           |
| HGB  | Control | 3 | 103<br>108.833     | 14.93318 | 8.62168            | 65.9039  | 140.096<br>1       | 92     | 120         |
|      | 0.50%   | 3 | 3                  | 12.829   | 7.40683            | 76.9643  | 140.702<br>3       | 98     | 123<br>137. |
|      | 1.00%   | 3 | 132.5<br>113.833   | 6.61438  | 3.81881<br>15.7330 | 116.069  | 148.931            | 125    | 5           |
|      | 2.00%   | 3 | 3                  | 27.25038 | 2                  | 46.1396  | 181.527<br>146.826 | 91     | 144         |
|      | 4.00%   | 3 | 111<br>113.833     | 14.42221 | 8.32666            | 75.1733  | 7                  | 95     | 123         |
|      |         | 1 |                    |          |                    |          | 123.493            |        |             |
|      | Total   | 5 | 3                  | 17.44447 | 4.50414            | 104.1729 | 8                  | 91     | 144         |
| MCHC | Control | 3 | 530.333<br>3       | 22.50185 | 12.9914<br>5       | 474.4356 | 586.231<br>582.932 | 505    | 548         |
|      | 0.50%   | 3 | 517.333<br>3       | 26.40707 | 15.2461<br>3       | 451.7345 | 1                  | 494    | 546         |
|      | 1.00%   | 3 | 526.833<br>3       | 41.28054 | 23.8333<br>3       | 424.2868 | 629.379<br>9       | 503    | 574.<br>5   |
|      | 2.00%   | 3 | 517.833<br>3       | 11.36148 | 6.55956<br>15.6027 | 489.6098 | 546.056<br>8       | 507.5  | 530         |
|      | 4.00%   | 3 | 550.666<br>7       | 27.02468 | 1                  | 483.5336 | 617.799<br>7       | 520    | 571         |
|      |         | 1 |                    |          |                    |          | 543.208            |        | 574.        |
|      | Total   | 5 | 528.6              | 26.37924 | 6.81109            | 513.9917 | 3                  | 494    | 5           |
| MCH  | Control | 3 | 71.5               | 1.83303  | 1.0583             | 66.9465  | 76.0535            | 69.9   | 73.5        |
|      | 0.50%   | 3 | 71.0667            | 3.26548  | 1.88532            | 62.9548  | 79.1786            | 67.3   | 73.1        |
|      | 1.00%   | 3 | 69.15              | 1.13027  | 0.65256            | 66.3423  | 71.9577            | 68.2   | 70.4        |
|      | 2.00%   | 3 | 74.35              | 0.6265   | 0.36171            | 72.7937  | 75.9063            | 73.75  | 75          |
|      | 4.00%   | 3 | 73.6333            | 0.9609   | 0.55478            | 71.2463  | 76.0203            | 72.6   | 74.5        |
|      |         | 1 |                    |          |                    |          |                    |        |             |
|      | Total   | 5 | 71.94              | 2.47099  | 0.63801            | 70.5716  | 73.3084            | 67.3   | 75          |
| RBC  | Control | 3 | 1.4                | 0.25981  | 0.15               | 0.7546   | 2.0454             | 1.25   | 1.7         |
|      | 0.50%   | 3 | 1.5417             | 0.25624  | 0.14794            | 0.9051   | 2.1782             | 1.34   | 1.83        |
|      | 1.00%   | 3 | 1.92               | 0.09539  | 0.05508            | 1.683    | 2.157              | 1.83   | 2.02        |

|          |         |    |         |         |         |         |         |       |       |
|----------|---------|----|---------|---------|---------|---------|---------|-------|-------|
|          | 2.00%   | 3  | 1.5317  | 0.34808 | 0.20096 | 0.667   | 2.3963  | 1.23  | 1.91  |
|          | 4.00%   | 3  | 1.5133  | 0.21385 | 0.12347 | 0.9821  | 2.0446  | 1.28  | 1.7   |
|          | Total   | 15 | 1.5813  | 0.27865 | 0.07195 | 1.427   | 1.7356  | 1.23  | 2.02  |
| PLT      | Control | 3  | 20.6667 | 6.50641 | 3.75648 | 4.5039  | 36.8295 | 14    | 27    |
|          | 0.50%   | 3  | 31.4333 | 9.81139 | 5.66461 | 7.0605  | 55.8062 | 23.8  | 42.5  |
|          | 1.00%   | 3  | 30.1667 | 4.0104  | 2.31541 | 20.2043 | 40.1291 | 26    | 34    |
|          | 2.00%   | 3  | 38.1667 | 7.14726 | 4.12647 | 20.4119 | 55.9214 | 32    | 46    |
|          | 4.00%   | 3  | 28.9333 | 4.56216 | 2.63397 | 17.6003 | 40.2664 | 24    | 33    |
|          | Total   | 15 | 29.8733 | 8.12399 | 2.09761 | 25.3744 | 34.3722 | 14    | 46    |
| Moisture | Control | 3  | 72.2957 | 0.10966 | 0.06331 | 72.0232 | 72.5681 | 72.23 | 72.42 |
|          | 0.50%   | 3  | 72.7933 | 0.6877  | 0.39704 | 71.085  | 74.5017 | 72    | 73.22 |
|          | 1.00%   | 3  | 72.043  | 0.5876  | 0.33925 | 70.5833 | 73.5027 | 71.4  | 72.55 |
|          | 2.00%   | 3  | 72.063  | 1.09591 | 0.63272 | 69.3406 | 74.7854 | 70.82 | 72.89 |
|          | 4.00%   | 3  | 72.7757 | 1.51075 | 0.87223 | 69.0228 | 76.5286 | 71.39 | 74.39 |
|          | Total   | 15 | 72.3941 | 0.85648 | 0.22114 | 71.9198 | 72.8684 | 70.82 | 74.39 |
| Ash      | Control | 3  | 5.6833  | 1.04505 | 0.60336 | 3.0873  | 8.2794  | 5.07  | 6.89  |
|          | 0.50%   | 3  | 6.9867  | 0.94522 | 0.54572 | 4.6386  | 9.3347  | 6.03  | 7.92  |
|          | 1.00%   | 3  | 6.3333  | 1.67739 | 0.96844 | 2.1665  | 10.5002 | 4.98  | 8.21  |
|          | 2.00%   | 3  | 6.98    | 1.55393 | 0.89716 | 3.1198  | 10.8402 | 5.21  | 8.12  |
|          | 4.00%   | 3  | 7.29    | 0.33779 | 0.19502 | 6.4509  | 8.1291  | 6.98  | 7.65  |
|          | Total   | 15 | 6.6547  | 1.18489 | 0.30594 | 5.9985  | 7.3108  | 4.98  | 8.21  |
| Lipid    | Control | 3  | 9.3133  | 1.23087 | 0.71064 | 6.2557  | 12.371  | 7.91  | 10.21 |
|          | 0.50%   | 3  | 8.2667  | 0.56801 | 0.32794 | 6.8557  | 9.6777  | 7.89  | 8.92  |
|          | 1.00%   | 3  | 7.44    | 0.50478 | 0.29143 | 6.1861  | 8.6939  | 6.98  | 7.98  |
|          | 2.00%   | 3  | 6.6933  | 0.6309  | 0.36425 | 5.1261  | 8.2606  | 5.97  | 7.13  |
|          | 4.00%   | 3  | 7.18    | 0.41073 | 0.23714 | 6.1597  | 8.2003  | 6.89  | 7.65  |
|          | Total   | 15 | 7.7787  | 1.13552 | 0.29319 | 7.1498  | 8.4075  | 5.97  | 10.21 |
| Protein  | Control | 3  | 70.7467 | 1.7065  | 0.98525 | 66.5075 | 74.9858 | 68.92 | 72.3  |
|          | 0.50%   | 3  | 73.7    | 3.3208  | 1.91726 | 65.4507 | 81.9493 | 69.89 | 75.98 |
|          | 1.00%   | 3  | 72.7    | 1.03068 | 0.59506 | 70.1397 | 75.2603 | 72.09 | 73.89 |
|          | 2.00%   | 3  | 76.5033 | 5.38749 | 3.11047 | 63.1201 | 89.8866 | 70.3  | 80.01 |
|          | 4.00%   | 3  | 75.6967 | 3.57033 | 2.06133 | 66.8275 | 84.5659 | 71.98 | 79.1  |
|          | Total   | 15 | 73.8693 | 3.56392 | 0.9202  | 71.8957 | 75.843  | 68.92 | 80.01 |

**Table 4** Test of homogeneity of variance in growth, feed utilization, haematological, and body proximate composition indices of African catfish (*Clarias gariepinus*) juveniles fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

| Test of Homogeneity of Variances |                  |     |     |       |
|----------------------------------|------------------|-----|-----|-------|
|                                  | Levene Statistic | df1 | df2 | Sig.  |
| FW                               | 2.259            | 4   | 10  | 0.135 |
| WG                               | 1.988            | 4   | 10  | 0.172 |
| AGR                              | 3.451            | 4   | 10  | 0.051 |
| SGR                              | 1.797            | 4   | 10  | 0.206 |
| CF                               | 2.932            | 4   | 10  | 0.076 |
| HSI                              | 1.043            | 4   | 10  | 0.432 |
| VSI                              | 1.897            | 4   | 10  | 0.188 |
| FI                               | 2.488            | 4   | 10  | 0.11  |
| FCR                              | 1.7              | 4   | 10  | 0.226 |
| FER                              | 2.424            | 4   | 10  | 0.117 |
| PER                              | 2.408            | 4   | 10  | 0.118 |
| WBC                              | 1.896            | 4   | 10  | 0.188 |
| Lym                              | 1.945            | 4   | 10  | 0.179 |
| Mono                             | 3.771            | 4   | 10  | 0.054 |
| Gran                             | 1.847            | 4   | 10  | 0.197 |
| HCT                              | 1.285            | 4   | 10  | 0.339 |
| MCV                              | 2.979            | 4   | 10  | 0.074 |
| RDWa                             | 2.651            | 4   | 10  | 0.096 |
| HGB                              | 1.889            | 4   | 10  | 0.189 |
| MCHC                             | 1.96             | 4   | 10  | 0.177 |
| MCH                              | 4.141            | 4   | 10  | 0.051 |
| RBC                              | 1.287            | 4   | 10  | 0.339 |
| PLT                              | 1.06             | 4   | 10  | 0.425 |
| moisture                         | 2.538            | 4   | 10  | 0.106 |
| Ash                              | 2.099            | 4   | 10  | 0.156 |
| Lipid                            | 2.51             | 4   | 10  | 0.108 |
| Protein                          | 2.845            | 4   | 10  | 0.082 |

**Table 5** Analysis of variances (ANOVA) of growth and feed utilization indices of African catfish (*Clarias gariepinus*) juveniles fed *Aloe vera*-*Allium sativum* polysaccharide mixture supplemented diets for 60 d.

| ANOVA |                |                |    |             |        |       |
|-------|----------------|----------------|----|-------------|--------|-------|
|       |                | Sum of Squares | df | Mean Square | F      | Sig.  |
| FW    | Between Groups | 875.483        | 4  | 218.871     | 9.436  | 0.002 |
|       | Within Groups  | 231.942        | 10 | 23.194      |        |       |
|       | Total          | 1107.425       | 14 |             |        |       |
| WG    | Between Groups | 875.483        | 4  | 218.871     | 9.436  | 0.002 |
|       | Within Groups  | 231.942        | 10 | 23.194      |        |       |
|       | Total          | 1107.425       | 14 |             |        |       |
| AGR   | Between Groups | 0.175          | 4  | 0.044       | 5.459  | 0.014 |
|       | Within Groups  | 0.08           | 10 | 0.008       |        |       |
|       | Total          | 0.255          | 14 |             |        |       |
| SGR   | Between Groups | 0.38           | 4  | 0.095       | 10.379 | 0.001 |
|       | Within Groups  | 0.091          | 10 | 0.009       |        |       |
|       | Total          | 0.471          | 14 |             |        |       |
| CF    | Between Groups | 0.04           | 4  | 0.01        | 2.239  | 0.137 |
|       | Within Groups  | 0.045          | 10 | 0.004       |        |       |
|       | Total          | 0.084          | 14 |             |        |       |
| HSI   | Between Groups | 1.4            | 4  | 0.35        | 8.604  | 0.003 |
|       | Within Groups  | 0.407          | 10 | 0.041       |        |       |
|       | Total          | 1.807          | 14 |             |        |       |
| VSI   | Between Groups | 20.238         | 4  | 5.059       | 5.894  | 0.011 |
|       | Within Groups  | 8.584          | 10 | 0.858       |        |       |
|       | Total          | 28.822         | 14 |             |        |       |
| FI    | Between Groups | 42.196         | 4  | 10.549      | 1.022  | 0.442 |
|       | Within Groups  | 103.2          | 10 | 10.32       |        |       |
|       | Total          | 145.396        | 14 |             |        |       |
| FCR   | Between Groups | 0.319          | 4  | 0.08        | 8.11   | 0.004 |
|       | Within Groups  | 0.098          | 10 | 0.01        |        |       |
|       | Total          | 0.418          | 14 |             |        |       |
| FER   | Between Groups | 0.076          | 4  | 0.019       | 8.027  | 0.004 |
|       | Within Groups  | 0.024          | 10 | 0.002       |        |       |
|       | Total          | 0.1            | 14 |             |        |       |
| PER   | Between Groups | 0.742          | 4  | 0.185       | 8.027  | 0.004 |
|       | Within Groups  | 0.231          | 10 | 0.023       |        |       |
|       | Total          | 0.973          | 14 |             |        |       |

**Table 6** Analysis of variances (ANOVA) of haematological and body proximate composition indices of African catfish (*Clarias gariepinus*) juveniles fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

| ANOVA    |                | Sum of Squares | df | Mean Square | F     | Sig.  |
|----------|----------------|----------------|----|-------------|-------|-------|
| WBC      | Between Groups | 101.248        | 4  | 25.312      | 1.314 | 0.33  |
|          | Within Groups  | 192.693        | 10 | 19.269      |       |       |
|          | Total          | 293.941        | 14 |             |       |       |
| Lym      | Between Groups | 75.703         | 4  | 18.926      | 0.401 | 0.804 |
|          | Within Groups  | 472.547        | 10 | 47.255      |       |       |
|          | Total          | 548.249        | 14 |             |       |       |
| Mono     | Between Groups | 0.569          | 4  | 0.142       | 0.397 | 0.806 |
|          | Within Groups  | 3.585          | 10 | 0.359       |       |       |
|          | Total          | 4.154          | 14 |             |       |       |
| Gran     | Between Groups | 1.758          | 4  | 0.439       | 1.353 | 0.317 |
|          | Within Groups  | 3.248          | 10 | 0.325       |       |       |
|          | Total          | 5.006          | 14 |             |       |       |
| HCT      | Between Groups | 0.006          | 4  | 0.002       | 1.211 | 0.365 |
|          | Within Groups  | 0.013          | 10 | 0.001       |       |       |
|          | Total          | 0.019          | 14 |             |       |       |
| MCV      | Between Groups | 253.851        | 4  | 63.463      | 1.455 | 0.287 |
|          | Within Groups  | 436.318        | 10 | 43.632      |       |       |
|          | Total          | 690.169        | 14 |             |       |       |
| RDWa     | Between Groups | 252.444        | 4  | 63.111      | 0.72  | 0.597 |
|          | Within Groups  | 875.967        | 10 | 87.597      |       |       |
|          | Total          | 1128.411       | 14 |             |       |       |
| HGB      | Between Groups | 1496.5         | 4  | 374.125     | 1.354 | 0.317 |
|          | Within Groups  | 2763.833       | 10 | 276.383     |       |       |
|          | Total          | 4260.333       | 14 |             |       |       |
| MCHC     | Between Groups | 2207.767       | 4  | 551.942     | 0.733 | 0.59  |
|          | Within Groups  | 7534.333       | 10 | 753.433     |       |       |
|          | Total          | 9742.1         | 14 |             |       |       |
| MCH      | Between Groups | 52.248         | 4  | 13.062      | 3.93  | 0.036 |
|          | Within Groups  | 33.233         | 10 | 3.323       |       |       |
|          | Total          | 85.481         | 14 |             |       |       |
| RBC      | Between Groups | 0.469          | 4  | 0.117       | 1.895 | 0.188 |
|          | Within Groups  | 0.618          | 10 | 0.062       |       |       |
|          | Total          | 1.087          | 14 |             |       |       |
| PLT      | Between Groups | 470.836        | 4  | 117.709     | 2.598 | 0.101 |
|          | Within Groups  | 453.153        | 10 | 45.315      |       |       |
|          | Total          | 923.989        | 14 |             |       |       |
| Moisture | Between Groups | 1.643          | 4  | 0.411       | 0.476 | 0.753 |

|         |                |         |    |        |       |       |
|---------|----------------|---------|----|--------|-------|-------|
|         | Within Groups  | 8.627   | 10 | 0.863  |       |       |
|         | Total          | 10.27   | 14 |        |       |       |
| Ash     | Between Groups | 4.999   | 4  | 1.25   | 0.853 | 0.524 |
|         | Within Groups  | 14.656  | 10 | 1.466  |       |       |
|         | Total          | 19.655  | 14 |        |       |       |
| Lipid   | Between Groups | 12.733  | 4  | 3.183  | 5.985 | 0.01  |
|         | Within Groups  | 5.318   | 10 | 0.532  |       |       |
|         | Total          | 18.052  | 14 |        |       |       |
| protein | Between Groups | 64.272  | 4  | 16.068 | 1.415 | 0.298 |
|         | Within Groups  | 113.549 | 10 | 11.355 |       |       |
|         | Total          | 177.821 | 14 |        |       |       |

**Table 7** Post hoc test (Duncan multiple range test) of growth, and feed utilization indices of African catfish (*Clarias gariepinus*) fingerlings fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

| Final Weight                                           |   |                         |     | Weight Gain                                            |   |                         |     | Absolute Growth Rate                                   |   |                         |       |
|--------------------------------------------------------|---|-------------------------|-----|--------------------------------------------------------|---|-------------------------|-----|--------------------------------------------------------|---|-------------------------|-------|
| Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |       |
| Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2     |
| 4.00%                                                  | 3 | 68.8                    |     | 4.00%                                                  | 3 | 56.53                   |     | 4.00%                                                  | 3 | 0.942                   |       |
| 2.00%                                                  | 3 | 72.2                    |     | 2.00%                                                  | 3 | 59.9                    |     | 2.00%                                                  | 3 | 0.998                   | 1     |
| Control                                                | 3 | 76.4                    | 76  | Control                                                | 3 | 64.15                   | 64  | Control                                                | 3 | 1.069                   | 1     |
| 0.50%                                                  | 3 |                         | 82  | 0.50%                                                  | 3 |                         | 69  | 0.50%                                                  | 3 |                         | 1.154 |
| 1.00%                                                  | 3 |                         |     | 1.00%                                                  | 3 |                         |     | 1.00%                                                  | 3 |                         | 1.244 |
| Sig.                                                   |   | 0.09                    | 0.2 | Sig.                                                   |   | 0.094                   | 0.2 | Sig.                                                   |   | 0.127                   | 0.243 |
| Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |       |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.                  |   |                         |       |
| Specific Growth Rate                                   |   |                         |     | Condition Factor                                       |   |                         |     | Hepatosomatic Index                                    |   |                         |       |
| Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |       |
| Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2     |
| 4.00%                                                  | 3 | 2.87                    |     | 0.50%                                                  | 3 | 0.521                   |     | Control                                                | 3 | 1.664                   |       |
| 2.00%                                                  | 3 | 2.95                    |     | Control                                                | 3 | 0.573                   | 0.6 | 4.00%                                                  | 3 | 2.039                   | 2     |
| Control                                                | 3 | 3.05                    | 3   | 4.00%                                                  | 3 | 0.586                   | 0.6 | 2.00%                                                  | 3 | 2.043                   | 2     |
| 0.50%                                                  | 3 |                         | 3.2 | 2.00%                                                  | 3 | 0.651                   | 0.7 | 0.50%                                                  | 3 |                         | 2.291 |
| 1.00%                                                  | 3 |                         |     | 1.00%                                                  | 3 |                         | 0.7 | 1.00%                                                  | 3 |                         | 2.586 |
| Sig.                                                   |   | 0.06                    | 0.2 | Sig.                                                   |   | 0.05                    | 0.2 | Sig.                                                   |   | 0.052                   | 0.104 |
| Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |       |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.                  |   |                         |       |
| Viscerosomatic Index                                   |   |                         |     | Feed Intake                                            |   |                         |     | Feed Conversion Ratio                                  |   |                         |       |
| Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |       |
| Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2     |
| Control                                                | 3 | 5.38                    |     | 4.00%                                                  | 3 | 94.44                   |     | 1.00%                                                  | 3 | 1.263                   |       |
| 1.00%                                                  | 3 | 6.07                    | 6.1 | 2.00%                                                  | 3 | 97.65                   |     | 0.50%                                                  | 3 | 1.443                   | 1     |
| 2.00%                                                  | 3 |                         | 7.4 | 1.00%                                                  | 3 | 98.39                   |     | Control                                                | 3 |                         | 1.543 |
| 0.50%                                                  | 3 |                         | 7.5 | Control                                                | 3 | 98.76                   |     | 2.00%                                                  | 3 |                         | 1.63  |
| 4.00%                                                  | 3 |                         |     | 0.50%                                                  | 3 | 99.07                   |     | 4.00%                                                  | 3 |                         | 1.67  |
| Sig.                                                   |   | 0.38                    | 0.1 | Sig.                                                   |   | 0.136                   |     | Sig.                                                   |   | 0.05                    | 0.166 |
| Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |       |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.                  |   |                         |       |
| Feed efficiency ratio                                  |   |                         |     | Protein Efficiency Ratio                               |   |                         |     |                                                        |   |                         |       |
| Duncan a                                               | N | Subset for alpha = 0.05 |     | Duncan a                                               | N | Subset for alpha = 0.05 |     |                                                        |   |                         |       |
| Groups                                                 |   | 1                       | 2   | Groups                                                 |   | 1                       | 2   |                                                        |   |                         |       |
| 4.00%                                                  | 3 | 0.6                     |     | 4.00%                                                  | 3 | 1.874                   |     |                                                        |   |                         |       |
| 2.00%                                                  | 3 | 0.61                    | 0.6 | 2.00%                                                  | 3 | 1.917                   | 1.9 |                                                        |   |                         |       |
| Control                                                | 3 | 0.65                    | 0.6 | Control                                                | 3 | 2.03                    | 2   |                                                        |   |                         |       |
| 0.50%                                                  | 3 |                         | 0.7 | 0.50%                                                  | 3 |                         | 2.2 |                                                        |   |                         |       |
| 1.00%                                                  | 3 |                         |     | 1.00%                                                  | 3 |                         |     |                                                        |   |                         |       |
| Sig.                                                   |   | 0.26                    | 0.1 | Sig.                                                   |   | 0.257                   | 0.1 |                                                        |   |                         |       |
| Means for groups in homogeneous subsets are displayed. |   |                         |     | Means for groups in homogeneous subsets are displayed. |   |                         |     |                                                        |   |                         |       |
| a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     | a Uses Harmonic Mean Sample Size = 3.000.              |   |                         |     |                                                        |   |                         |       |

**Table 8** Quadratic regression model output on weight gain and feed efficiency ratio against dietary *Aloe vera-Allium sativum* polysaccharide mixture in African catfish (*Clarias gariepinus*) juveniles' culture.

|                                   |                            |                          |                            |        |       |
|-----------------------------------|----------------------------|--------------------------|----------------------------|--------|-------|
| WG                                |                            |                          |                            |        |       |
| Model Summary                     |                            |                          |                            |        |       |
| R                                 | R Square                   | Adjusted R Square        | Std. Error of the Estimate |        |       |
| 0.239                             | 0.057                      | -0.1                     | 4.806                      |        |       |
| The Independent Variables Groups. |                            |                          |                            |        |       |
|                                   |                            |                          |                            |        |       |
| ANOVA                             |                            |                          |                            |        |       |
|                                   | Sum of Squares             | df                       | Mean Square                | F      | Sig.  |
| Regression                        | 16.729                     | 2                        | 8.365                      | 0.362  | 0.704 |
| Residual                          | 277.212                    | 12                       | 23.101                     |        |       |
| Total                             | 293.941                    | 14                       |                            |        |       |
| The Independent Variables Groups. |                            |                          |                            |        |       |
|                                   |                            |                          |                            |        |       |
| Coefficients                      |                            |                          |                            |        |       |
|                                   | Unstandardized Coefficient | Standardized Coefficient | t                          | Sig.   |       |
|                                   | B                          | Std. Error               | Beta                       |        |       |
| Groups                            | 2.014                      | 3.343                    | 0.643                      | 0.603  | 0.558 |
| Groups <sup>2</sup>               | -0.578                     | 0.782                    | -0.789                     | -0.739 | 0.474 |
| (Constant)                        | 58.075                     | 2.36                     |                            | 24.612 | 0     |
|                                   |                            |                          |                            |        |       |
| FER                               |                            |                          |                            |        |       |
| Model Summary                     |                            |                          |                            |        |       |
| R                                 | R Square                   | Adjusted R Square        | Std. Error of the Estimate |        |       |
| 0.193                             | 0.037                      | -0.123                   | 0.046                      |        |       |
| The Independent Variables Groups. |                            |                          |                            |        |       |
|                                   |                            |                          |                            |        |       |
| ANOVA                             |                            |                          |                            |        |       |
|                                   | Sum of Squares             | df                       | Mean Square                | F      | Sig.  |
| Regression                        | 0.001                      | 2                        | 0                          | 0.231  | 0.797 |
| Residual                          | 0.025                      | 12                       | 0.002                      |        |       |
| Total                             | 0.026                      | 14                       |                            |        |       |
| The Independent Variables Groups. |                            |                          |                            |        |       |
|                                   |                            |                          |                            |        |       |
| Coefficients                      |                            |                          |                            |        |       |
|                                   | Unstandardized Coefficient | Standardized Coefficient | t                          | Sig.   |       |
|                                   | B                          | Std. Error               | Beta                       |        |       |
| Groups                            | 0.021                      | 0.032                    | 0.704                      | 0.653  | 0.526 |
| Groups <sup>2</sup>               | -0.004                     | 0.007                    | -0.626                     | -0.58  | 0.573 |
| (Constant)                        | 0.588                      | 0.023                    |                            | 26.034 | 0     |

**Table 9** Post hoc test (Duncan multiple range test) of haemato-biochemical indices of African catfish (*Clarias gariepinus*) fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

|                    |                      |                         |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
|--------------------|----------------------|-------------------------|------|--|--------------------|-----------------|-------------------------|-----|--|------------------|-------------------|-------------------------|----|-------|
| Duncan a<br>Groups | <b>WBC</b><br>N      | Subset for alpha = 0.0  |      |  | Duncan a<br>Groups | <b>Lym</b><br>N | Subset for alpha = 0.05 |     |  | Duncan<br>Groups | <b>Mono</b><br>N  | Subset for alpha = 0.05 |    |       |
|                    |                      | 1                       |      |  |                    |                 | 1                       |     |  |                  |                   | 1                       |    |       |
| 2.00%              | 3                    | 57                      |      |  | 4.00%              | 3               | 26.63                   |     |  | Control          | 3                 | 1.9                     |    |       |
| Control            | 3                    | 57.1                    |      |  | 0.50%              | 3               | 27.27                   |     |  | 2.00%            | 3                 | 1.933                   |    |       |
| 4.00%              | 3                    | 57.3                    |      |  | 2.00%              | 3               | 27.28                   |     |  | 4.00%            | 3                 | 2.033                   |    |       |
| 0.50%              | 3                    | 58                      |      |  | Control            | 3               | 29.9                    |     |  | 1.00%            | 3                 | 2.267                   |    |       |
| 1.00%              | 3                    | 63.8                    |      |  | 1.00%              | 3               | 32.63                   |     |  | 0.50%            | 3                 | 2.4                     |    |       |
| Sig.               |                      | 0.11                    |      |  | Sig.               |                 | 0.347                   |     |  | Sig.             |                   | 0.367                   |    |       |
| Duncan a<br>Groups | <b>Granu</b><br>N    | Subset for alpha = 0.0  |      |  | Duncan a<br>Groups | <b>HCT</b><br>N | Subset for alpha = 0.05 |     |  | Duncan<br>Groups | <b>MCV</b><br>N   | Subset for alpha = 0.05 |    |       |
|                    |                      | 1                       |      |  |                    |                 | 1                       |     |  |                  |                   | 1                       |    |       |
| Control            | 3                    | 1.6                     |      |  | Control            | 3               | 0.195                   |     |  | 1.00%            | 3                 | 131.6                   |    |       |
| 4.00%              | 3                    | 2                       |      |  | 4.00%              | 3               | 0.201                   |     |  | 4.00%            | 3                 | 134                     |    |       |
| 2.00%              | 3                    | 2.1                     |      |  | 0.50%              | 3               | 0.212                   |     |  | 0.50%            | 3                 | 137.3                   |    |       |
| 0.50%              | 3                    | 2.42                    |      |  | 2.00%              | 3               | 0.22                    |     |  | Control          | 3                 | 138.7                   |    |       |
| 1.00%              | 3                    | 2.58                    |      |  | 1.00%              | 3               | 0.253                   |     |  | 2.00%            | 3                 | 143.6                   |    |       |
| Sig.               |                      | 0.08                    |      |  | Sig.               |                 | 0.097                   |     |  | Sig.             |                   | 0.069                   |    |       |
| Duncan a<br>Groups | <b>RDWa</b><br>N     | Subset for alpha = 0.0  |      |  | Duncan a<br>Groups | <b>HGB</b><br>N | Subset for alpha = 0.05 |     |  | Duncan<br>Groups | <b>MCHC</b><br>N  | Subset for alpha = 0.05 |    |       |
|                    |                      | 1                       |      |  |                    |                 | 1                       |     |  |                  |                   | 1                       |    |       |
| Control            | 3                    | 94.4                    |      |  | Control            | 3               | 103                     |     |  | 0.50%            | 3                 | 517.3                   |    |       |
| 0.50%              | 3                    | 99                      |      |  | 0.50%              | 3               | 108.8                   |     |  | 2.00%            | 3                 | 517.8                   |    |       |
| 4.00%              | 3                    | 99.5                    |      |  | 4.00%              | 3               | 111                     |     |  | 1.00%            | 3                 | 526.8                   |    |       |
| 1.00%              | 3                    | 102                     |      |  | 2.00%              | 3               | 113.8                   |     |  | Control          | 3                 | 530.3                   |    |       |
| 2.00%              | 3                    | 107                     |      |  | 1.00%              | 3               | 132.5                   |     |  | 4.00%            | 3                 | 550.7                   |    |       |
| Sig.               |                      | 0.16                    |      |  | Sig.               |                 | 0.074                   |     |  | Sig.             |                   | 0.201                   |    |       |
| Duncan a<br>Groups | <b>MCH</b><br>N      | Subset for alpha = 0.0  |      |  | Duncan a<br>Groups | <b>RBC</b><br>N | Subset for alpha = 0.05 |     |  | Duncan<br>Groups | <b>PLT</b><br>N   | Subset for alpha = 0.05 |    |       |
|                    |                      | 1                       | 2    |  |                    |                 | 1                       | 2   |  |                  |                   | 1                       | 2  |       |
| 1.00%              | 3                    | 69.2                    |      |  | Control            | 3               | 1.4                     |     |  | Control          | 3                 | 20.67                   |    |       |
| 0.50%              | 3                    | 71.1                    | 71.1 |  | 4.00%              | 3               | 1.513                   | 1.5 |  | 4.00%            | 3                 | 28.93                   | 29 |       |
| Control            | 3                    | 71.5                    | 71.5 |  | 2.00%              | 3               | 1.532                   | 1.5 |  | 1.00%            | 3                 | 30.17                   | 30 |       |
| 4.00%              | 3                    |                         | 73.6 |  | 0.50%              | 3               | 1.542                   | 1.5 |  | 0.50%            | 3                 | 31.43                   | 31 |       |
| 2.00%              | 3                    |                         | 74.4 |  | 1.00%              | 3               |                         | 1.9 |  | 2.00%            | 3                 |                         | 38 |       |
| Sig.               |                      | 0.16                    | 0.07 |  | Sig.               |                 | 0.529                   | 0.1 |  | Sig.             |                   | 0.098                   | 0  |       |
| Duncan a<br>Groups | <b>Moisture</b><br>N | Subset for alpha = 0.0  |      |  | Duncan a<br>Groups | <b>Ash</b><br>N | Subset for alpha = 0.05 |     |  | Duncan<br>Groups | <b>Lipid</b><br>N | Subset for alpha = 0.05 |    |       |
|                    |                      | 1                       |      |  |                    |                 | 1                       |     |  |                  |                   | 1                       | 2  | 3     |
| 1.00%              | 3                    | 72                      |      |  | Control            | 3               | 5.683                   |     |  | 2.00%            | 3                 | 6.693                   |    |       |
| 2.00%              | 3                    | 72.1                    |      |  | 1.00%              | 3               | 6.333                   |     |  | 4.00%            | 3                 | 7.18                    | 7  |       |
| Control            | 3                    | 72.3                    |      |  | 2.00%              | 3               | 6.98                    |     |  | 1.00%            | 3                 | 7.44                    | 7  |       |
| 4.00%              | 3                    | 72.8                    |      |  | 0.50%              | 3               | 6.987                   |     |  | 0.50%            | 3                 |                         | 8  | 8.267 |
| 0.50%              | 3                    | 72.8                    |      |  | 4.00%              | 3               | 7.29                    |     |  | Control          | 3                 |                         |    | 9.313 |
| Sig.               |                      | 0.38                    |      |  | Sig.               |                 | 0.165                   |     |  | Sig.             |                   | 0.259                   | 0  | 0.109 |
| Duncan a<br>Groups | <b>Protein</b><br>N  | Subset for alpha = 0.05 |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
|                    |                      | 1                       |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
| Control            | 3                    | 70.7                    |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
| 1.00%              | 3                    | 72.7                    |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
| 0.50%              | 3                    | 73.7                    |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
| 4.00%              | 3                    | 75.7                    |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
| 2.00%              | 3                    | 76.5                    |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |
| Sig.               |                      | 0.08                    |      |  |                    |                 |                         |     |  |                  |                   |                         |    |       |

**Table 10** Kaplan–Meier analysis (survival) output of African catfish (*Clarias gariepinus*) fingerlings subjected to low water pH after being fed *Aloe vera-Allium sativum* polysaccharide mixture supplemented diets for 60 d.

| Overall Comparisons                                                            |            |    |       |
|--------------------------------------------------------------------------------|------------|----|-------|
|                                                                                | Chi-Square | df | Sig.  |
| Log-Rank (Mantel-Cox)                                                          | 19.705     | 4  | 0.001 |
| Breslow-Generalized Wilcoxon)                                                  | 10.375     | 4  | 0.035 |
| Tarone-Ware                                                                    | 14.144     | 4  | 0.007 |
| Test of equality of survival distributions for the different levels of Groups. |            |    |       |

## Appendix D

### (1) Research permission letter from the director of postgraduate studies.

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>CENTRE FOR POSTGRADUATE STUDIES</b></p> <p>University of Namibia, Private Bag 13301, Windhoek, Namibia<br/>340 Mandume Ndemutayo Avenue, Pioneers Park<br/>☎ +264 61 206 3275/4662; Fax +264 61 206 3290; URL: <a href="http://www.unam.edu.na">http://www.unam.edu.na</a></p>                                                                                                                                                                                                         | <br><b>UNAM</b><br><small>UNIVERSITY OF NAMIBIA</small> |
| <b>RESEARCH PERMISSION LETTER</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                            |
| <p><b>Student Name:</b> Mr Ndakalimwe Naftal Gabriel</p> <p><b>Student number:</b> 200516566</p> <p><b>Programme :</b> <i>PhD (Fisheries &amp; Aquatic Sciences)</i></p> <p><b>Approved research title:</b> Dietary Aloe and Garlic Polysaccharides: Effects on Growth Performance, Body Composition and Haematological Parameters of <i>Clarias Gariepinus</i></p>                                                                                                                          |                                                                                                                                            |
| <b>TO WHOM IT MAY CONCERN</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                            |
| <p>I hereby confirm that the above mentioned student is registered at the University of Namibia for the programme indicated. The proposed study met all the requirements as stipulated in the University guidelines and has been approved by the relevant committees.</p> <p>The proposal adheres to ethical principles as per attached Ethical Clearance Certificate. Permission is hereby granted to carry out the research as described in the approved proposal.</p> <p>Best Regards</p> |                                                                                                                                            |
| <br>_____<br>Dr Marius Hedimbi<br><b>Director: Centre for Postgraduate Studies</b><br>Tel: +264 61 2063275<br>E-mail: <a href="mailto:directorpgs@unam.na">directorpgs@unam.na</a>                                                                                                                                                                                                                        | <p><i>24 May 2017</i><br/>_____<br/>Date</p>                                                                                               |

(2) Research ethical clearance from the postgraduate studies committee.



**ETHICAL CLEARANCE CERTIFICATE**

**Ethical Clearance Reference Number:** SNC/197/2017      **Date:** 24 April, 2017

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

**Title of Project:** Dietary Aloe And Garlic Polysaccharides: Effects On Growth, Performance, Body Composition And Haematological Parameters Of *Clarias Gariepinus*

**Nature/Level of Project:** Doctorate

**Researcher:** Ndakalimwe, Naftal Gabriel

**Student Number:** 200516566

**Faculty:** Sam Nujoma Campus

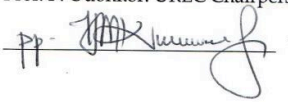
**Supervisors:** Prof. Professor Edosa Omoregie (Main) Pro. Percy Chimwamurombe (Co)

Take note of the following:

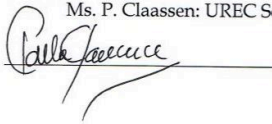
- (a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
- (b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
- (c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
- (d) The UREC retains the right to:
  - (i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
  - (ii) Request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. P. Odonkor: UREC Chairperson



Ms. P. Claassen: UREC Secretary



(3) Supervision agreement letter.

ANNEX 1

SUPERVISION AGREEMENT BETWEEN GRADUATE STUDENT AND SUPERVISOR

This supervision agreement between

Name of student: Ndakalimwe N. Gabriel.....  
Student number: 200516566.....  
Faculty: Agriculture and Natural Resources.....  
Department: Fisheries and Aquatic Sciences.....

and (on behalf of UNAM)

Name of supervisor: Professor Edosa Omoregie.....  
Department: Fisheries and Aquatic Sciences.....

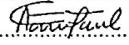
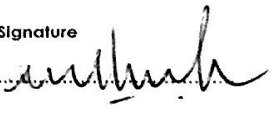
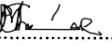
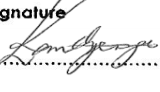
And (if applicable)

Name of co-supervisor: Professor Percy Chimwamurombe....  
Department: Biological Sciences.....

Regarding post-graduate research for the degree of: Fisheries & Aquatic Sciences

Research topic:  
Dietary aloe and garlic polysaccharides: Effects on growth performance,  
body composition and haematological parameters of *Clarias gariepinus*.....

By signing this document, both student and supervisor(s) acknowledge their understanding and obligations of the general expectations and responsibilities regarding the supervision of the thesis/dissertation as contained in the UNAM Postgraduate Studies regulations prospectus.

|                                          |                                                                                      |                |
|------------------------------------------|--------------------------------------------------------------------------------------|----------------|
| N.N. Gabriel.....                        |   | 3 April 2017   |
| Name of Student                          | Signature                                                                            | Date           |
| Prof. E. Omoregie.....                   |  | 04 April, 2017 |
| Name of Supervisor                       | Signature                                                                            | Date           |
| Prof. P. Chimwamurombe                   |  | 4th April 2017 |
| Name of co-supervisor (where applicable) | Signature                                                                            | Date           |
| Mr. L. Kandjengo.....                    |  | 5 April 2017   |
| Name of Department HoD                   | Signature                                                                            | Date           |
| Name of Faculty HoD: PGS                 | Signature                                                                            | Date           |

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(4) Supervision letter from the current main supervisor Dr. Margit Wilhelm (2018 - 2019), department of fisheries and aquatic sciences, University of Namibia.

**SAM NUJOMA CAMPUS**

Sam Nujoma Campus, P.O. Box 462 Omaruru Street,  
North Dune, Henties Bay, Namibia  
☎ +264 64 502 600; URL: <http://www.unam.edu.na>



**SUBMISSION**

To: Prof. H. Bello  
HOD: Postgraduate Studies  
Faculty of Agriculture  
University of Namibia

From: Dr. Margit Wilhelm  
Department of Fisheries and Aquatic Sciences  
Sam Nujoma Campus  
University of Namibia  
Henties Bay

Date: 13 June 2018

**Postgraduate student supervision**

This is to inform you that I agree to supervise the following student: Mr. Naftal Gabriel (200516566) for his PhD Degree studies in the Department of Fisheries and Aquatic Sciences. His project title is: "Dietary aloe and garlic polysaccharides: Effects on growth, performance, body composition and haematological parameters of *Clarias gariepinus*". I am replacing Prof. Edosa Omoregie, who is no longer employed at the University of Namibia.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'M. Wilhelm', is written over a horizontal line.

Margit Wilhelm, PhD  
Email: [mwilhelm@unam.na](mailto:mwilhelm@unam.na)

(5) Supervision letter from Dr. Michael Habte-Tsion (co-supervisor, 2018 -2019),  
department of aquaculture nutrition, Kentucky State University, USA.



COLLEGE OF AGRICULTURE, FOOD SCIENCE, AND SUSTAINABLE SYSTEMS  
DIVISION OF AQUACULTURE

Aquaculture Research Center, 103 Athletic Road, Frankfort, KY 40601  
[www.ksuaquaculture.org](http://www.ksuaquaculture.org)

Date: 30 January 2018

TO WHOM IT MAY CONCERN  
School of Postgraduate Studies  
University of Namibia, Namibia

Subject: **PhD Candidate Supervision**

Dear Sir/Madam,

This is kindly to inform you that I have agreed to be external supervisor of Mr. Ndakalimwe Naftal Gabriel (Student's Registration No.: 200516566) for his PhD research project entitled "Dietary *Aloe* and garlic polysaccharides: effects on the growth performance, body composition and hematological parameters of *Clarias Gariepinus*", based on his request.

If you need any further information on this, please let me know.

Thanks  
Best regards;

A handwritten signature in blue ink, appearing to read "Michael Habte-Tsion".

Michael

.....  
**H.-Michael Habte-Tsion, Ph.D.**  
Research Associate - Aquaculture Nutrition  
Kentucky State University | Division of Aquaculture  
103 Athletic Dr., Frankfort, KY 40601, USA  
E-mail: [hab.andemichael@kysu.edu](mailto:hab.andemichael@kysu.edu) or  
[mike2692011@gmail.com](mailto:mike2692011@gmail.com)