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

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ABSTRACT

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1. Experimental fish and management

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

2.2. Preparation of diets and experimental design

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

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