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Bromelain improves the growth, biochemical, and hematological profiles of the fingerlings of Nile Tilapia, *Oreochromis niloticus*

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ABSTRACT

A 6-week-long feeding trial experiment was conducted to study the efficacy of Bromelain, a blend of proteolytic enzymes present in pineapple wastes on growth performance, biochemical, and hematological profiles of the fingerlings of Nile tilapia, *Oreochromis niloticus.* For this, 240 Nile tilapia fingerlings (9 ± 0.11 cm) were fed a commercial diet, supplemented with different levels of pineapple peel extract (PPE) at 1:0, 1:1, 1:2, and 1:3 ratios. After 45 days of the feeding trial, growth parameters, biochemical constituents, and the level of blood cells were assessed. It was found that the growth parameters such as weight gain, feed efficiency ratio, and specific growth rate were increased ($p < 0.05$) along with the total protein and amino acid content and few hematological parameters; whereas the feed conversion ratio was found to be reduced significantly ($p > 0.05$) without changing the white blood cell count with PPE supplementation. Thus, the PPE can be a potential feed supplement in Nile tilapia aquaculture.

1. INTRODUCTION

Aquaculture is one of the fast-growing, food-producing sectors in the world, which helps to meet the increasing global protein demand. The success of aquaculture relies on the quality of feed, understanding of fish physiology, and the environmental management of the production system [1,2]. Feed is one of the utmost importance among the various factors that affect the aquaculture production system, especially the intensive system. It represents approximately 80% of the total operational cost [3]. Currently, the use of bioactive materials in fish feed as growth promoters is increasing, due to the excess use of antibiotics and other veterinary drugs that cause several side effects for the health of aquatic organisms and their environment [4], decreasing their therapeutic potential [5]. To avoid such environmental pollution, the by-products of the agro-industry can be used as a potential ingredient for the preparation of high-quality aquafeed and the waste generated can be utilized in a much effective manner [6].

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Among such several agro-industrial by-products thrown to the environment, the peel of pineapple, *Ananas comosus*, used in the present study, is a practicable option due to its availability and various other medicinal properties.

Bromelain, present in the aqueous extract of *A. comosus* [7], is a costly blend of proteolytic enzymes [8–11] and has considerable commercial importance as dietary supplements, cosmetics, food industry, etc. [12]. In aquaculture, the supplementation of bromelain in the basal diet can improve the digestibility, nutrient utilization, and assimilation in fishes [13,14]. Therefore, it is appropriate to consider pineapple peel as a functional supplement in aquafeed.

Nile tilapia, *Oreochromis niloticus*, is a widely cultivated freshwater fish of high economic importance and can tolerate a wide range of environmental and climatic conditions [15–18]. It is frequently referred to as the aquatic chicken [19]. But the contribution of India is very little with respect to the global production of tilapia. It is suggested that the proper distribution of improved stocks, better formulated and floating feeds, tightened biosecurity standards, etc. are essential to improve the production of tilapia [19].

An understanding of hematology provides information about the health status and physiological, as well as pathological, state of fishes [20]. However, to date, very little information is available on the impact of the incorporation of pineapple peel extract (PPE) in aquafeed, especially in Nile tilapia culture. Thus, the current study aims to examine the impact of PPE, supplemented with the basal diet on the growth, biochemical, and hematological parameters of the fingerlings of *O. niloticus.*

2. MATERIALS AND METHODS

2.1. PPE Preparation

Fresh and ripe pineapple (*A. comosus*) fruit was obtained from the local market, Bengaluru, Karnataka, India. The pineapple peel was peeled off using a knife, after cleaning the fruit with fresh water. PPE, used in the present study, was prepared according to Sharma *et al.* [21].

2.2. Preparation of the Feed

The commercially available feed (TAIYO Aini fish food) with minimum 35% crude protein was used as a basal diet for feeding trial experiments and mixed with varying amount PPE. Accordingly, three experimental diets (pellets) were prepared by supplementing the PPE with the ground basal diet in the ratios (basal diet:PPE) of 1:1, 1:2, and 1:3 and were designated as G_1, G_2, and G_3 respectively. The basal diet without any supplementation of PPE (1:0) was treated as the control group (Table 1).

2.3. Experimental Setup

Nile tilapia, *O. niloticus*, fingerlings were collected from the Fisheries Research and Information Centre, Hebbal, Bengaluru, Karnataka, and were acclimatized to the laboratory conditions for at least 2 weeks, in a 500 l cement tank to minimize stress and were fed a basal diet. Two hundred and forty fingerlings (length = 9 ± 0.11 cm and weight = 16.18 ± 1.13 g) were randomly distributed into different tanks with three replicates each at a stocking density of 20 fingerlings per tank on the 16th day. Both control and experimental fish were fed twice a day with their respective diets *ad libitum*, at 10.00 and 18.00 hours for 45 days. Water quality parameters such as temperature (25.23°C ± 0.8°C), pH (7.18 ± 0.14), and dissolved oxygen (5 ± 0.56 mg l^{-1}) were maintained.

$$WG(\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$LG(\%) = \frac{\text{Final length}(\text{cm}) - \text{Initial length}(\text{cm})}{\text{Initial length}(\text{cm})} \times 100$$

$$FCR = \frac{\text{Feed intake}}{\text{Weight gain}}$$

$$FER = \frac{1}{FCR}$$

$$SGR(\%\text{ per day}) = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Number of days}} \times 100$$

2.4. Growth Parameters

The parameters of growth, such as weight gain (WG), length gain (LG), feed conversion ratio (FCR), feed efficiency ratio (FER), and specific growth rate (SGR) were determined by using the following formulae, every 15 days.

2.5. Biochemical Composition

Once in 15 days, the fingerlings were randomly selected from each of the tanks. After being anesthetized with clove oil (at a concentration of 500 µl l^{-1}), muscle tissue was dissected out and used for the estimation of total protein [22], total carbohydrate [23], total lipid [24], and free amino acid content [25], according to the standard protocol.

2.6. Hematological Analysis

For hematological studies, the blood samples of three fish per tank were collected either by puncturing the heart and/or caudal vein using a 1 mL insulin syringe, rinsed with 2.7% ethylenediamine tetraacetic acid solution, once in 15 days. Hematological parameters such as red blood cell and white blood cell counts (by hemocytometry according to Rao and Chakrabarti [26]), hemoglobin concentration (spectrophotometrically at 540 nm with cyanmethemoglobin method of Citarasu *et al.* [27]), and hematocrit (microcentrifuge method) were estimated by following the standard methods.

2.7. Statistical Analysis

The obtained data were subjected to the suitable statistical tool (two-way analysis of variance) using the GraphPad Prism 9.1.0 software, by keeping the significance level ($p < 0.05$). The results are presented as mean ± SD.

3. RESULTS AND DISCUSSION

Proper utilization of wastes of agricultural by-products as fish feed ingredients reduces the environmental contaminants and increases the farmers' income [28]. Therefore, an experiment was

Table 1: The proximate compositions of the feeds (as per the manufacturers).

Constituents	Experimental groups (%)			
	Control (1:0)	Group 1 (1:1)	Group 2 (1:2)	Group 3 (1:3)
Crude protein	35	35	35	35
Crude fiber	8	8	8	8
Crude fat	2	2	2	2
Moisture	10	10	10	10
PPE (feed:PPE ratio)	0	1:1	1:2	1:3

Table 2: Growth performances of *O. niloticus* fingerlings fed with PPE supplemented diet on the 45th day.

Parameters	Control (1:0)	Group 1 (1:1)	Group 2 (1:2)	Group 3 (1:3)
WG (%)	37.15 ± 0.30[a]	41.90 ± 0.32[b]	44.61 ± 0.21[c]	47.66 ± 0.42[d]
LG (%)	17.65 ± 0.11[a]	17.25 ± 0.20[a]	17.37 ± 0.32[a]	17.44 ± 0.39[a]
Feed conversion ratio	2.03 ± 0.05[a]	1.85 ± 0.12[b]	1.55 ± 0.11[c]	1.32 ± 0.08[d]
Feed efficiency ratio	1.55 ± 0.11[a]	1.43 ± 0.07[a]	1.00 ± 0.05[b]	0.77 ± 0.05[c]
SGR	2.56 ± 0.04[a]	2.78 ± 0.05[b]	2.92 ± 0.05[c]	3.01 ± 0.02[d]

Means within a row with different superscripts differ significantly at $p < 0.05$.

Table 3: Biochemical profiles of *O. niloticus* fingerlings fed with PPE-supplemented diet on the 45th day (mg/g of body weight).

Profiles	Control (1:0)	Group 1 (1:1)	Group 2 (1:2)	Group 3 (1:3)
Total protein	40.20 ± 0.20[a]	41.50± 0.20[b]	43.90 ± 0.20[c]	46.00 ± 1.73[d]
Total carbohydrate	20.10 ± 0.89[a]	19.80 ± 0.39[a]	20.00 ± 0.87[a]	20.50 ± 0.30[a]
Total lipids	4.66 ± 0.47[a]	5.67 ± 0.57[a]	5.07 ± 0.57[a]	5.33 ± 0.57[a]
Total free amino acids	12.60 ± 0.12[a]	14.00 ± 0.35[b]	15.27 ± 0.23[c]	16.47 ± 0.23[d]

Means within a row with different superscripts differ significantly at $p < 0.05$.

Table 4: Hematological parameters *O. niloticus* fingerlings fed with PPE-supplemented diet on the 45th day.

	Control (1:0)	Group 1 (1:1)	Group 2 (1:2)	Group 3 (1:3)
Hematocrit (%)	21.07 ± 0.18[a]	23.80 ± 0.20[b]	24.20± 0.20[b]	24.89 ± 0.05[c]
Hemoglobin	20.10 ± 0.89[a]	19.80 ± 0.39[a]	20.00 ± 0.87[a]	20.50 ± 0.30[a]
RBC (10^6 μl^{-1})	4.66 ± 0.47[a]	4.87 ± 0.37[a]	5.57 ± 0.29[b]	5.83 ± 0.25[b]
WBC ($10^3 \mu l^{-1}$)	12.60 ± 1.12[a]	12.00 ± 1.35[a]	13.27 ± 1.23[a]	12.47 ± 1.23[a]

Means within a row with different superscripts differ significantly at $p < 0.05$.

conducted to study the impact of PPE on the growth performance, biochemical composition, and hematology of the fingerlings of Nile tilapia.

3.1. Growth Parameters

The results of growth parameters revealed that the fingerlings of Nile tilapia fed a PPE-supplemented basal diet for 45 days resulted in an enhancement of growth parameters, except LG, with an increasing concentration of PPE (Table 2), most notably in G_3. These results are similar for fish like *Ctenopharyngodon idella*, *Cyprinus carpio*, *Clarias* sp., *Acipenser ruthenus*, etc. [28–30]. It is interesting to mention that apart from fishes, a significant increase in growth performance was also noticed in chickens [31].

The PPE contains high amounts of phenolic compounds, like gallic acid and ferulic acid, which can improve the growth performances in fish and other organisms [31–33]. Bromelain present in the PPE significantly boosts the enzymatic activity of pepsin and enhances nutrient digestion and absorption by influencing the gut tissue and intestinal absorptive cells [34,35] and also the acceptable flavor and palatability of pineapple. However, significantly low growth performances were also registered in fish along with other livestock, such as rabbits and pigs, with a high dose of pineapple peel and its by-products [36,5]. This can be attributed to the fact that the presence of high-fiber contents can reduce the digestion of other dietary components, thereby decreasing digestibility and absorption of nutrients and energy [37,38].

3.2. Biochemical Profile

According to Ribeiro *et al.* [39], the digestive enzymes are used to evaluate the digestive capacity of the animal as they are the most important factors influencing nutrient utilization in the gastrointestinal tract. In the present study, dietary supplementation of PPE increased total protein and total free amino acid level significantly ($p \leq 0.05$) with the increase in PPE extract in *O. niloticus* fingerlings (Table 3). On the other hand, the level of carbohydrate and lipid content was found insignificant ($p > 0.05$) among the experimental groups, on the 45th day, compared to the control. With respect to carbohydrates and lipid, similar results were observed in *Litopenaeus vannamei, A. ruthenus, Anguilla Anguilla,* and *Cyprinus carpio* [21,30,40]. This result may be attributed to the bromelain supplementation which could improve the digestibility of fish diets [41]. However, Wiszniewski *et al.* [30] observed that the crude protein tended to decrease with the increased bromelain concentration and insignificant differences were noted in the proximate composition of Nile tilapia [41].

3.3. Hematological Parameters

Improving the immune system is one of the target goals for feed supplementation in aquaculture and is also essential for monitoring fish general health and physiological status [42]. In the present investigation, it is found that dietary inclusion of PPE significantly enhanced the hematocrit and red blood cell count ($p < 0.05$) in *O. niloticus* with an increasing in PPE, whereas hemoglobin and leucocyte count were unaffected after 45 days of the dietary treatments (Table 4). The increased erythrocyte count is a positive response to an improved immune system [43]. To establish the mode of action between bromelain and hematological parameters, further studies are highly warranted since there is no clear understanding of these parameters in fishes.

4. CONCLUSION

The data of the present study demonstrate that the *PPE*-supplemented diet significantly improve the growth performance, biochemical profiles and immunity status of Nile tilapia, *O. niloticus*, fingerlings. However, further studies are warranted to understand the mode of action between bromelain and hematological parameters.

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6. AUTHORS' CONTRIBUTION

KV and CL: Conceived the idea and designed the experiments.

JGR, JBSR, and KV: Carried out the experiments and wrote the manuscript.

KV, CL, JGR, and JBSR: Corrected, edited, and finalized the manuscript.

All the authors contributed equally to this manuscript and agreed to submit it for publication.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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