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Enhancing Growth Performance and Omega-3 Fatty Acid Composition of the Nile Tilapia through Dietary Supplementation of *Andrographis paniculata* Leaves Extract

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ABSTRACT

The Nile tilapia (*Oreochromis niloticus*) is a globally important aquaculture species valued for its rapid growth and high consumer demand. However, improving its nutritional quality particularly the content of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) remains a major challenge in sustainable fish farming. Natural phytogenic additives have emerged as promising alternatives to synthetic growth promoters, since they enhance nutrient absorption, immunity, and feed efficiency while minimizing environmental impact.. Among them, *Andrographis paniculata* (sambiloto) is known to contain andrographolide and flavonoid compounds with bioactive potential for improving fish metabolism and health. This study aimed to evaluate the effects of dietary supplementation of *A. paniculata* leaves extract at different inclusion levels on the growth performance, feed utilization, and lipid composition of the Nile tilapia. The experiment was conducted for 60 days using a completely randomized design with four treatments (0, 2, 4, and 6 mg/kg feed) and three replicates each. Juvenile tilapia (10 ± 0.3 g) were stocked at a density of 10 fish per 20 L aquarium and fed to apparent satiation three times daily. The results showed that *A. paniculata* supplementation significantly improved final body weight, feed conversion ratio, protein content, and tissue EPA and DHA levels, with the best outcomes at 6 mg/kg feed. These findings confirm the potential of *A. paniculata* as a natural, multifunctional additive for enhancing tilapia productivity and product quality in sustainable aquaculture systems.

INTRODUCTION

The Nile tilapia, *Oreochromis niloticus* has expanded rapidly and become one of the most important fish aquaculture industries worldwide due to its wide environmental tolerance, fast growth, and high market demand **(Lusiana *et al.*, 2021; Siddique *et al.*,**

2024; Chen *et al.*, 2025; Kwikiriza *et al.*, 2025). However, maintaining its competitive value in the global market requires improving the nutritional quality of fish while sustaining production efficiency **(Prakash *et al.*, 2025)**. Consumers increasingly value fish rich in high-quality protein and long-chain polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for their well-established roles in human cardiovascular and neurological health **(Rodrigues *et al.*, 2024)**. Intensified aquaculture systems, on the other hand, often result in stress, suboptimal feed conversion, and vulnerability to disease outbreaks **(Gadhiya *et al.*, 2025)**. Thus, the development of innovative feeding strategies and sustainable dietary additives is essential to enhance both growth performance and product quality in tilapia culture **(Alemayehu *et al.*, 2018)**.

Improving the nutritional profile of cultured fish, especially by enriching omega-3 fatty acids such as EPA (C20:5n3) and DHA (C22:6n3), has become a central focus of functional aquafeed development. Several studies have demonstrated that replacing traditional fish oil with alternative lipid sources, including microalgal oils rich in DHA, can effectively increase DHA deposition in tilapia fillets and improve feed conversion efficiency **(Sarker *et al.*, 2020)**. For example, complete replacement of fish oil with *Schizochytrium* sp. in tilapia diets resulted in significantly higher DHA content and improved growth performance **(Trevi *et al.*, 2024)**. These findings highlight that dietary manipulation through targeted lipid sources not only affects growth but also enhances the nutritional value of aquaculture products, meeting the growing demand for health-oriented fish consumption.

Beyond lipid sources, phytogenic feed additives bioactive compounds derived from plants have attracted increasing attention as natural growth promoters and immunostimulants in aquaculture. Numerous studies have shown that phytogenic supplements can improve digestive enzyme activity, nutrient absorption, feed utilization, immune responses, and resistance to bacterial infections in fish **(Mohammady *et al.*, 2022; Poolsawat *et al.*, 2022)**. The use of phytogenics is particularly important under current restrictions on antibiotic application in aquaculture and the global shift toward residue-free, eco-friendly production systems **(Gruber *et al.*, 2025)**. Hence, incorporating plant-based bioactive ingredients into aquafeeds represents a promising strategy for sustainable intensification of tilapia culture.

Among potential phytogenic sources, *Andrographis paniculata* (commonly known as sambiloto) has gained interest for its broad pharmacological properties, primarily attributed to andrographolide compounds **(Jayakumar *et al.*, 2013; Lichwiarska *et al.*, 2025)**. Experimental trials have reported that supplementation of *A. paniculata* extracts in the Nile tilapia diets reduced mortality following *Streptococcus agalactiae* infection and improved immune response **(Rattanachaikunsopon & Phumkhachorn, 2009)**. More recently, andrographolide was shown to enhance growth, feed efficiency, and disease resistance in the Nile tilapia **(Kengkittipat *et al.*, 2025)**. Similar immunostimulatory and antioxidant benefits have been documented in *Labeo rohita* **(Basha *et al.*, 2013)**, suggesting that *A. paniculata* possesses strong potential as a functional feed additive in aquaculture.

Despite growing evidence of the immunological and health-promoting effects of *A. paniculata*, little is known about its influence on lipid metabolism and the accumulation of long-chain omega-3 fatty acids such as EPA and DHA in the Nile tilapia. Most available studies have focused on immune modulation and pathogen resistance rather than nutritional enrichment of fish tissues. In contrast, research involving plant-derived oils has extensively examined DHA deposition and fatty acid remodeling in fish fillets **(Trevi *et al.*, 2024)**.

Although the possible role of *A. paniculata* in modulating lipid biosynthesis and improving the EPA and DHA content of the Nile tilapia fillets remains largely unexplored and warrants detailed investigation. Therefore, this study aimed to evaluate the effects of dietary supplementation of *A. paniculata* leaves extract at different inclusion levels on the growth performance and body composition particularly the protein, EPA and DHA contents of the Nile tilapia. Furthermore, the study sought to assess the potential of *A. paniculata* as a natural functional feed additive to enhance the nutritional quality of the farmed Nile tilapia while supporting sustainable and eco-friendly aquaculture practices.

MATERIALS AND METHODS

1. Preparation of Containers and Experimental Fish

The experimental containers used in this study were glass aquaria with a working water volume of 20 L each. Every aquarium was equipped with continuous aeration to maintain dissolved oxygen levels above 5 mg L^{-1} and prevent stratification. The test animals used were juvenile Nile tilapia with an average initial weight of 10 ± 0.3 g and total length of 5 ± 0.4 cm. Fish were acclimated for ten days in laboratory conditions and fed a commercial diet (30% crude protein) without any supplementation during this period. The stocking density was 10 fish per aquarium.

2. Preparation of *Andrographis paniculata* Leaves Extract

The extraction procedure followed the method of **Fardiyah *et al.* (2020)**. Fresh *A. paniculata* leaves were washed with distilled water, air-dried indoors at 30 °C to a constant weight, and ground into a fine powder. Approximately 100 g of dried powder was extracted with 96% ethanol as the solvent at a ratio of 1:10. The extraction process was carried out using the maceration method for 72 hours. The macerate was then filtered using Whatman No. 41 filter paper. The filtrate obtained was concentrated using a vacuum rotary evaporator at 50 °C until a thick extract was obtained. The concentrated extract was stored until use in feed formulation.

3. Experimental Design

A completely randomized design was employed in this study, consisting of four dietary treatments with three replicates each, as shown in Table (1). The treatments were formulated as follows: a control diet (0SE) without *A. paniculata* leaves extract; a diet supplemented with 2 mg/kg feed 2SE); a diet supplemented with 4 mg/kg feed (4SE); and a diet supplemented with 6 mg/kg feed (6SE). Fish were fed to apparent satiation three times daily at 08:00, 12:00, and 16:00 for a period of 30 days.

Table 1. Experimental design and dietary treatments of the Nile tilapia

Treatment	Description
0SE	Control diet without A. paniculata extract
2SE	Diet supplemented with A. paniculata extract 2 mg/kg feed
4SE	Diet supplemented with A. paniculata extract 4 mg/kg feed
6SE	Diet supplemented with A. paniculata extract 6 mg/kg feed

4. Observed Parameters

At the end of the experimental period, several biological parameters were evaluated to determine the effects of dietary *A. paniculata* supplementation on the performance and nutritional quality of the Nile tilapia. Growth performance was assessed by measuring the final body weight and total length of fish from each replicate. The parameters observed included weight gain (WG), length gain (LG), feed conversion ratio (FCR), feed efficiency (FE), and survival rate (SR), calculated according to the standard formulas described by **Tamrin *et al.* (2025)**. The whole-body protein content of the fish was determined through proximate based on **AOAC (1988)**. Meanwhile, the EPA and DHA contents in whole-body were quantified according to the method described by **Strobel *et al.* (2012)**.

5. Statistical Analysis

All data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to detect significant differences among treatments. When significant effects were found (P< 0.05), mean separation was performed using Duncan's Multiple Range Test (DMRT). Statistical analyses were performed using SPSS. Data normality and variance homogeneity were verified prior to analysis.

RESULTS

1. Growth performance

Dietary supplementation with *A. paniculata* leaves extract significantly affected (*P* < 0.05) weight gain, length gain, FCR, and feed efficiency compared to the control group (Table 2). The highest weight growth (25.84 ± 0.60 g) and length growth (5.77 ± 0.45 cm) were observed in fish fed with 6SE, followed by the 4SE group (21.70 ± 0.44 g; 3.88 ± 0.22 cm). Both were significantly higher than those of the 2SE and control treatments (*P* < 0.05). FCR decreased markedly with increasing extract dosage, with the lowest value recorded in the 6SE group (1.13 ± 0.04), indicating superior feed efficiency. Correspondingly, feed efficiency (FE) was highest in the 6SE treatment (88.41 ± 2.66 %) and lowest in the control (38.23 ± 1.13 %). The survival rate (SR) ranged from 86.67 % to 90.67 %, showing no significant difference among treatments (p > 0.05). These results suggest that the addition of *A. paniculata* extract at 4–6 mg/kg feed improved growth performance in the Nile tilapia.

Table 2. Weight growth, length growth, FCR, feed efficiency, and survival rate of Nile tilapia fed diets containing different levels of *A. paniculata* leaves extract in 30 days

Parameter	Treatment				*P* value
	0SE	2SE	4SE	6SE	
Weight Growth (g)	13.45 ± 2.09^{a}	15.71 ± 1.28^{a}	21.7 ± 0.44^{b}	25.84 ± 0.60^{c}	*
Length Growth (cm)	1.91 ± 0.29^{a}	2.30 ± 0.28^{a}	3.88 ± 0.22^{b}	5.77 ± 0.45^{c}	*
FCR	2.37 ± 0.28^{a}	2.27 ± 0.23a^{b}	1.85 ± 0.30^{b}	1.13 ± 0.04^{c}	*
Feed efficiency (%)	38.23 ± 1.13^{a}	59.28 ± 10.30^{b}	82.27 ± 1.71^{c}	88.41 ± 2.66^{c}	*
Survival rate (%)	89.33 ± 15.14^{a}	86.67 ± 16.65^{a}	90.67 ± 12.86^{a}	88.00 ± 20.78^{a}	NS

Values are presented as mean ± standard deviation (SD). Different superscript letters in the same row indicate significant differences among treatments according to Duncan’s Multiple Range Test (p < 0.05). Treatments: 0SE = control (no supplementation A. paniculata leaves extract); 2SE = supplementation A. paniculata leaves extract 2 mg/kg feed; 4SE = supplementation A. paniculata leaves extract 4 mg/kg feed; 6SE = supplementation *A. paniculata* leaves extract 6 mg/kg feed; * = significant; NS = no significant.

2. Whole-body Protein, EPA, and DHA Content

The dietary inclusion of *A. paniculata* extract significantly influenced (*P* < 0.05) the whole-body protein content of the Nile tilapia (Table 3). The highest crude protein value was recorded in fish fed the 6SE diet (52.70 ± 0.42%), followed by 2SE (45.15 ± 0.10%) and 4SE (42.34 ± 0.21%). The lowest protein content was found in the control group (41.53 ± 0.52%). The EPA and DHA levels in whole-body Nile tilapia exhibited significant improvements (*P* < 0.05) with the increasing inclusion of *A. paniculata* extract. Fish fed with 6SE showed the highest EPA content (1910.32 ± 0.64 %) and DHA content (720.51 ± 1.02 %), followed by the 4SE and 2SE groups. The lowest values were observed in the control (EPA: 1888.84 ± 1.02 %; DHA: 695.09 ± 0.60 %).

Table 3. Protein, EPA, and DHA Content of whole-body Nile tilapia fed diets containing different levels of A. paniculata leaves extract in 30 days.

Parameter	Treatment				*P* value
	0SE	**2SE**	**4SE**	**6SE**	
Body Protein (%)	41.53 ± 0.52^a	45.15 ± 0.10^c	42.34 ± 0.21^b	52.70 ± 0.42^d	*
DHA (%)	695.09 ± 0.60^a	716.22 ± 1.07^c	711.68 ± 0.99^b	720.51 ± 1.02^d	*
EPA (%)	1888.84 ± 1.02^a	1895.60 ± 1.08^b	1903.26 ± 1.23^c	1910.32 ± 0.64^d	*

Values are presented as mean ± standard deviation (SD). Different superscript letters in the same row indicate significant differences among treatments according to Duncan's Multiple Range Test ($P < 0.05$). Treatments: 0SE = control (no supplementation A. paniculata leaves extract); 2SE = supplementation A. paniculata leaves extract 2 mg/kg feed; 4SE = supplementation A. paniculata leaves extract 4 mg/kg feed; 6SE = supplementation *A. paniculata* leaves extract 6 mg/kg feed; * = significant.

DISCUSSION

This study demonstrated that dietary supplementation with *A. paniculata* leaves extract enhanced growth performance, body protein content, and fatty acid composition of the Nile tilapia. These improvements support the hypothesis that phytogenic feed additives can stimulate physiological and metabolic processes associated with growth and nutrient utilization in aquaculture species **(Kesselring *et al.*, 2021; Wang *et al.*, 2024; Hassan *et al.*, 2025; Pudota *et al.*, 2025)**. The positive responses observed in this study suggest that *A. paniculata* acts as a functional bioactive ingredient capable of improving productivity and nutritional quality in aquaculture species.

The enhanced growth performance observed in the Nile tilapia fed diets supplemented with *A. paniculata* extract may be attributed to improved feed palatability, stimulation of digestive enzymes, and better nutrient absorption. The main bioactive compound of *A. paniculata* is andrographolide **(Villedieu-Percheron *et al.*, 2019; Mussard *et al.*, 2020; Wang *et al.*, 2025)**. Previous studies have reported positive effects of andrographolide on fish growth and immunity. For instance, andrographolide significantly improved the growth performance and immune responses of *Labeo rohita* **(Basha *et al.*, 2013)** and showed effectiveness against *Aeromonas hydrophila* infection **(Palanikani *et al.*, 2020)**. Similarly, supplementation enhanced growth performance, antioxidant capacity, and intestinal integrity in *Monopterus albus* **(Shi *et al.*, 2020)**, improved growth and nutrient digestibility in *Cyprinus carpio* **(Mishra *et al.*, 2023)**, and enhanced growth, immunity, and resistance against *A. hydrophila* in *Pangasianodon hypophthalmus* **(Maiti *et al.*, 2023)**. Moreover, it promoted growth and immunity in *Penaeus vannamei*, providing protection against *Vibrio alginolyticus* **(Yin *et al.*, 2023)**, and improved protein utilization, hematological indices, and feed efficiency in hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) **(Ear *et al.*, 2024)**. In the Nile tilapia, andrographolide-based phytogenic supplementation significantly improved growth, feed utilization, liver health, and resistance to bacterial infection **(Kengkittipat *et al.*, 2025)**. Therefore, the growth-promoting effects of *A. paniculata* observed in the present study are consistent with previous findings linking its bioactive components to enhanced growth performance.

The increase in whole-body protein content observed in fish fed *A. paniculata* extract may be associated with andrographolide's stimulatory effect on protein synthesis and amino acid metabolism. **Shi *et al.* (2020)** reported that andrographolide enhanced digestive enzyme activities such as lipase, protease, trypsin, and amylase in *M. albus*. Similarly, **Mishra *et al.* (2023)** found improved digestibility coefficients of dietary nutrients particularly protein, fiber, carbohydrates, and energy in *C. carpio*. Enhanced protease activity consequently contributes to greater protein deposition in fish muscle, as also reported in *P. vannamei* and *L. rohita* **(Yao *et al.*, 2019; Maryam *et al.*, 2022)**. Thus, the increased whole-body protein content observed in this study reflects improved nutrient absorption and an anabolic response stimulated by the bioactive components of *A. paniculata*.

Supplementation with *A. paniculata* also elevated whole-body levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in Nile tilapia. EPA and DHA are essential omega-3 fatty acids vital for fish health and nutritional value. Previous studies have demonstrated that dietary inclusion of plant-derived bioactives can enhance the fatty acid profile of fish **(Esmaeili *et al.*, 2017; Ramezanzadeh *et al.*, 2021)**. The proposed mechanism involves the antioxidant activity of phytochemicals, which may prevent oxidation of polyunsaturated fatty acids particularly n-3 fatty acids thereby maintaining or increasing their levels in fish tissue **(Ramezanzadeh *et al.*, 2021)**. *A. paniculata* is also known for its strong antioxidant properties **(Baru-Venkata *et al.*, 2023; Hamid *et al.*, 2023)**. Therefore, the observed increases in whole-body EPA and DHA in this study can be attributed to the antioxidant potential of *A. paniculata*.

To the best of our knowledge, this is the first study demonstrating a significant positive effect of A. paniculata supplementation on EPA and DHA levels in fish. Previous reports in goats indicated that diets containing *A. paniculata* could elevate EPA and DHA, though the effects were not significant **(Karami *et al.*, 2011; Yusuf *et al.*, 2018)**. Overall, these findings suggest that dietary supplementation with *A. paniculata* enhances growth performance, whole-body protein content, and omega-3 fatty acid composition (EPA and DHA) in the Nile tilapia, highlighting its potential as a promising phytogenic additive in aquaculture nutrition.

CONCLUSION

The present study demonstrated that dietary supplementation with *A. paniculata* leaves extract effectively enhanced growth performance, whole-body protein content, and the levels of EPA and DHA in Nile tilapia, with the optimal effects observed at a dosage of 6 mg/kg feed. Therefore, these results confirm the potential of A. paniculata as a natural phytogenic additive capable of improving nutrient assimilation and lipid metabolism while promoting sustainable aquaculture practices. Incorporating *A. paniculata* into tilapia diets may serve as a viable and eco-friendly alternative to synthetic growth promoters, contributing to enhanced fish quality, health, and environmentally responsible aquaculture production.

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