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Assessment of the nutritional value and histopathological effects of adding propolis to the diet of Nile tilapia (*Oreochromis niloticus)*.

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Abstract

The experiment was conducted using 12 ponds to evaluate the effect of dietary honey bee propolis supplementation on Nile tilapia. Four levels of honey bee propolis were tested (C=0, T_1=4, T_2=8, T_3=16 g/kg diet), with each treatment replicated in three hapa. Nile tilapia fingerlings (2.1 g) were stocked at a rate of 60 fingerlings per hapa and fed twice daily, with feed amounts adjusted biweekly based on weight changes. Intestinal samples were preserved in 10% neutral-buffered formalin for histological analysis. The T_3 group exhibited the highest ($P<0.05$) final body weight (FBW), weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV), followed by T_2. No significant differences were observed in body crude protein, moisture, fat, or ash content among the groups. Histological examination revealed no pathological changes in the intestines of fish fed the control diet. Fish in T_1 showed elongated intestinal villi and a reduced number of goblet cells. The T_2 group demonstrated significantly enhanced villus length and goblet cell proliferation, indicating improved nutrient absorption. However, T_3 fish exhibited shorter villi and fewer goblet cells. Overall, the results suggest that propolis supplementation, particularly at 8 g/kg (T_2), improves growth performance, nutrient absorption, and gut health in Nile tilapia, making it a promising strategy for enhancing aquaculture production

Keywords: Propolis; Histological; Nile tilapia; Villi; Feed conversion ratio.

Introduction

The rising demand for aquaculture products and the global need for high-quality protein render aquaculture a profitable business; however, its development has relied mainly on the escalating use of chemical compounds, primarily to manage infective organisms, as the countryside of the employed aquaculture system becomes more intensive. These chemicals enhance output by enlarging larval vital and feed efficiency, alleviating transport stress, managing infections, and combating organisms that contribute to water condition deterioration **[1].**

The synthetic chemical agents employed in those agricultural methods adversely affect the environment and the health of animals and humans due to their environmental toxicity **[2, 3].**

Thus, there is a heightened imperative to substitute chemical complexes with natural yields in aquaculture to alleviate the hazards and negative influences linked to synthetic medications **[4].**

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Aquaculture employs natural substances like insecticides, antibiotics, herbicides, insecticides, and disinfectants **[5].** Propolis is recognized for its antibacterial, antioxidant, antiseptic, bacteriostatic attributes, and anti-inflammatory, as well as its provision of nutritional supplements including fatty acids, carotenoids, immunostimulants, vitamins, hormones, and adjuvants **[6].**

Nile tilapia (*O. niloticus*) was the second largely supplied fish species, following carp (including silver carp, grass carp, and common carp), with a whole productivity of 3.7 million tons **[7].**

Propolis is viscous, waxen rosin synthesized by bees from a mixture of plant exudations, wax, pollen, and an enzyme portrayed in bee spittle, exhibiting sterile, antibacterial, anti-parasitic, bacteriostatic, anti-inflammatory, and antioxidant characteristics. Natural goods like Propolis may serve several purposes in aquaculture, mitigating the risks and side effects associated with artful chemicals, including detrimental environmental impacts and harmful influences on animal and human health owing to ecotoxicity.

Regrettably, the advancement of aquaculture relies on the application of pesticides to manage several diseases impacting the business **[8].**

The positive role of honey bee propolis in resisting oxidative stress and preventing damage to the brain tissue of rainbow trout was also reported **[9].**

This work proposes to study the effect of appending honey bee propolis to the feed on the growth performance, vitality, body composition, and survival rate of Nile tilapia fingerlings (Oreochromis niloticus), using different doses of propolis to reach the best dose with high significance.

MATERIALS AND METHODS

Experimental fish and diet preparation

Fingerlings of Nile tilapia were obtained from General Authority for Fish Resources Development (GAFRD), Sahari region, Aswan city

Four isonitrogenous diets were developed using market components. The constitution and chemical analysis of the trial diets are presented in Table 1. The dry materials were sifted using a sieve with a 0.3 mm aperture prior to incorporation into the diet. Emulsified oil was combined with an equivalent volume of water containing 0.7% phosphatidylcholine (lecithin), according to **[10]**, in the experimental meals. The hot water was amalgamated with the mixtures at a ratio of 50% for pelleting.

Twelve ponds were used in this experiment to study the effect of appending Propolis in the diet of Nile tilapia fingerlings using four levels (0, 4, 8, and 16 g/Kg diet). Each one was replicated in three habas. Nile tilapia fingerlings (2.1g) were transferred to the experimental ponds at a rate of 60 fingerlings / haba. Each pond was supported by continuous artificial ventilation. The replacement ratios in the experimental diets were as follows:

C: zero Propolis (control diet).
T_1: 4 g Propolis.
T_2: 8 g Propolis.
T_3: 16 g Propolis.

Table 1. Composition and chemical analysis of the diets with the four levels of honey bee propolis s meal used to feed Nile tilapia fingerlings.

Items (g/kg)	C	T_1	T_2	T_3
Wheat flour	100	100	100	100
Propolis meal	0	4	8	16
Fishmeal	120	120	120	120
Wheat bran	150	146	142	134
Soybean meal	346	346	346	346
Corn meal	200	200	200	200
Corn oil	30	30	30	30
Sun flour oil	20	20	20	20
Vit.&min.mix*	30	30	30	30
Ascorbic acid	4	4	4	4
Proximate analysis on dry matter basis				
Dry mater	90.22	90.11	90.03	90.02
Moisture	9.78	9.89	9.97	9.98
Crude protein (N × 6.25)	33.22	32.94	31.92	31.46
Crude fat	7.24	7.45	7.40	7.20
Crude fiber	6.36	6.29	6.48	6.13
Ash	6.10	6.53	6.88	6.94
Carbohydrate (NFE)[u8]	47.08	46.79	47.32	48.27
Gross energy (GE) kcal/100g3 **	408.14	405.15	401.12	400.48

** Gross Energy (kcal/100 g DM) = Crude Protein x 5.64 + Ether Extract x 9.44 + Nitrogen-Free Extract x 4.11, as computed by **[11].**

* Each 100 grams contains the following vitamins and minerals: Minerals: Zn, 2.50 mg; Mn, 16.00 mg; Fe, 31.50 mg; Cu, 5.50 mg; I, 0.55 mg; Ca, 1.15 g; P, 450 mg. Vitamins: A, 7,500,000 IU; B1, 100 mg; B3, 500 mg; B6, 150 mg; B12, 2.5 mg; K, 100 mg; E, 100 mg; Folic acid, 100 mg; Pantothenic acid, 275 mg; D3, 7,500 IU.

Growth performance and feed utilization:

Fingerlings were fed with treatments diets twice daily and feed amounts are adjusted every two weeks based on weight change. At the ending of the experimental period, the fish in every pond were totaled and the weight of the group was taken to evaluate the absolute and relative average weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR), protein productive value (PPV), and protein efficiency ratio (PER) using the following formulae:

WG = final average weight (g) - beginning average weight (g).

SGR (% d–1) = 100 × (ln Wt − ln W0)/t, where Wt and W0 denote the end and initial body weights of fish, respectively, and t signifies the period of the feeding session.

FCR = dry weight of feed (g) / wet weight increase of fish (g); PER = wet weight gain of fish (g) / protein intake (g).

Protein intake (g) equals protein (%) in feed × total weight (g) of diet consumed / 100.

PPV % = 100 * (Pt - P0) / protein intake (g).

Where:

P0: Protein content in Nile tilapia fingerlings carcass at the start.

Pt: Protein content in Nile tilapia fingerlings carcass at the end.

Water temperature, pH, and dissolved oxygen levels were systematically observed in every pond. At the commencement and conclusion of the trial, examples of Nile tilapia fingerlings were examined for their biochemical makeup.

Fish and feed analyses:

Feed samples were kept for their proximate composition analysis, which includes moisture, protein, fat, and ash content, following the guidelines set by **[12]** NFE was determined using the following equation: NFE = 100 - [Moisture % + Ash % + Lipid % + Protein % + Fiber %]. The gross energy content of the diets was calculated based on the following values: 5.64, 9.44, and 4.11 kcal/100g for protein, fat, and nitrogen-free extract, respectively **[11].**

Histopathological examination

Three Nile tilapia fingerlings from each group were taken for embedding paraffin sections. The Nile tilapia fingerlings were immediately washed with distilled water, dissected, eviscerated, and collected the intestine which was immediately stabled in 10% neutral buffer formalin for histological examination, according to **[13]** and **[14],** three parts were excised from intestine and hepatopancreas that subjected to climbing grades of alcohol (70%, 80%, 90%, 100%) for dehydration, after that cleaning by methyl benzoate, then fixed in paraffin wax. Pieces were cut using semi-automated sliding microtome at 3 to 5 µm stiffness. After that, the paraffin sections mounted on glass slides, dried, deparaffinized in xylol, rehydrated in a graded alcohol chain (100%, 90%, 80% and 70%), and stained with Harris's Haematoxylin and Eosin (H&E).

The tarnished sections were watched and analyzed using a DMLS light microscope (Leica, Germany) equipped with an MC120 HD camera (Leica).

Statistical analysis:

Analysis of variance (ANOVA) was conducted to evaluate significant differences ($p < .05$) among the treatment means related to growth, feed usage, and body composition. All statistical analyses were operated with IBM-SPSS version 28 software.

RESULTS AND DISCUSSION

Growth performance

Fig (1) illustrates the survival rate, final body weight (FBW), weight gain (WG), and specific growth rate (SGR) of Nile tilapia fingerlings subjected to four distinct amounts of Propolis supplementation.

The group receiving T_3 exhibited the most significant ($P<0.05$) final body weight (FBW) and weight gain (WG), followed by the group receiving T_2, while the control diet (C) and T_1 groups recorded the lowly ($P<0.05$) FBW and WG. **El-Hais *et al*. [15]**. The current investigation concurs that fish given diets enriched with propolis extract (3g and 6g) exhibited significantly higher ultimate body weight contrasted to the control diet ($P\geq0.05$).

A study by **[16]** corroborated these findings, revealing that the enclosure of 1 g/kg alcoholic extract of propolis in the diet of post-larvae and fingerling Nile tilapia improved condition factor and augmented body protein accretion. The improvement in the condition factor component

suggests that the propolis extract stimulates better nutritional status in Nile tilapias during the early phases, possibly leading to greater growth rates, productivity, survival, and reproduction in succeeding phases.

Deng *et al*. [17] identified a notable discrepancy in the impact of propolis insertion between the current study and prior research, while an enhancement in fish body composition was observed in all instances. In adolescent rainbow trout (Oncorhynchus mykiss), supplementation with alcoholic extract of propolis up to 4 g/kg did not affect body composition.

Ebtehal and El-Sayed [18] observed a distinct trend indicating that ultimate body weight reduced as the proportion of propolis in the diets raised, with significant variation across all treatments. The group fed with T_3 and T_2 exhibited the most significant specific growth rate (SGR), followed by the T_1 group, whilst the group fed on the control diet (C) of Nile tilapia (O. niloticus) demonstrated the lowest significant SGR.

Evidence from numerous studies robustly indicates that including propolis extract into Nile tilapia diets significantly enhances the specific growth rate (SGR) and final weight. The research by **[15]** highlights that fish administered propolis extract at doses of 3g and 6g had a markedly superior specific growth rate (SGR) in contrast to the control diet. Furthermore, **[19]** corroborated this tendency, indicating that the combination of propolis into the diet of Nile tilapia fingerlings considerably enhanced their growth performance, leading to higher final weight and specific growth rate (SGR). Furthermore, **[20]** corroborated these findings, determining that the maximum growth rate, average daily gain, and specific growth rate were attained by the utilization of crude propolis.

Fig (1): Effect of four levels of Propolis (0, 4, 8 and 16g) on a: Survival b: Final Body weight (FBW) c: Weight gain (WG) and d: specific growth rate (SGR) of *Nile tilapia* fingerlings (*Oreochromis niloticus*), 2.1g initial BW.

Feed utilization

The feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV) of Nile tilapia fingerlings (*O. niloticus*), nourished with four varying levels of Propolis meal, are shown in Table 2. Fish fed on T_3 (16 g propolis) had the best significant feed conversion ratio (FCR), being 1.24 followed by T_2 (8 g propolis), being 1.28. There was no significant differences was detected between the group fed on T_1 (4 g propolis) and the control group. Fish fed on T_3 gave the best value of PER (2.60) followed by T_2 (2.49). No significant differences were detected between groups fed on T_3 and that fed on T_2 (2.60and 2.49). Otherwise, group fed control diet gave the lowest PER (1.77). The highest (P<.05) PPV value were obtained for Nile tilapia fingerlings fed T_3 (40.65) followed by T_2, T_1 and control being 39.0, 35.66, 29.93 respectively.

Table 2. Feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV) of Nile tilapia fingerlings (*O. niloticus*), fed at four levels of Propolis for 8 Wks.

u	C	T_1	T_2	T_3
FCR	1.87 ± 0.02^a	1.66 ± 0.01^b	1.28 ± 0.04^c	1.24 ± 0.02^c
PER	1.77 ± 0.02^c	2.15 ± 0.01^b	2.49 ± 0.08^a	2.60 ± 0.05^a
PPV	29.93 ± 0.29^c	35.66 ± 1.5^b	39.0 ± 1.8^a	40.65 ± 0.83^a

$^{a, b, c,}$ within the same row without a common superscript are significantly different (p <0.05).

Body Composition

The table (3) presents the body composition constituents of hydration, protein, fat, and ash. The experimental groups exhibited no significant variations (P>0.05) in body composition regarding crude protein, hydration, fat, and ash across varying levels of propolis during the study period.

Table 3. Chemical composition of Nile tilapia fingerlings (*O. niloticus*), fed at four levels of honey bee Propolis for 8 Wks.

Items	C	T_1	T_2	T_3
Moisture	72.80±0.47	73.25±0.43	72.97±0.25	72.50±0.31
Crude protein	64.58±0.33	65.05±0.23	64.85±0.24	65.34±0.10
Crude Fat	15.01±0.41	14.74±0.39	15.18±0.22	14.77±0.04
Ash	20.40±0.10	20.21±0.17	19.98±0.12	19.80±0.07

Despite the sole notable alteration in the body structure of Nile tilapia fingerlings administered alcoholic extract of propolis in the study by **[21]** being an increase in crude protein levels, this serves as a strong pointer of the productive growth of these fish. Propolis addition enhanced crude protein levels in Nile tilapia fingerlings receiving 5g/kg of propolis extract in their diet and in juvenile eels consuming 2.5 and 10g of dry propolis/kg in their diet **[22].**

However, these researchers observed an elevation in moisture, mineral content, and a decrease in lipids with escalating quantities of propolis (5g/kg to 40g/kg, respectively), a phenomenon not evidenced in the current study **[21 , 22].**

Intestinal Histology:

In several studies, the addition of honey bee propolis to fish food has been found to have a significant impact on intestinal histology. Intestinal histology refers to the microscopic structure

and organization of the intestinal tissue, which is important for nutrient absorption and overall gut health in fish. In the current study the intestines of Nile tilapia fingerlings that were fed on C diet without any supplements (Fig. 2A), no specific pathological changes were observed. However, the Nile tilapia fingerlings that were fed the T_1 diet showed long villi and a low numeral of goblet cells (Fig. 2B). Those fed the diet T_2 exhibited a significant rise in the number and length of villi that filled the intestinal lumen, as well as abundant goblet cells (Fig. 2B). Meanwhile, Nile tilapia fingerlings fed the diet T_3 showed short intestinal villi and a low numeral of goblet cells.

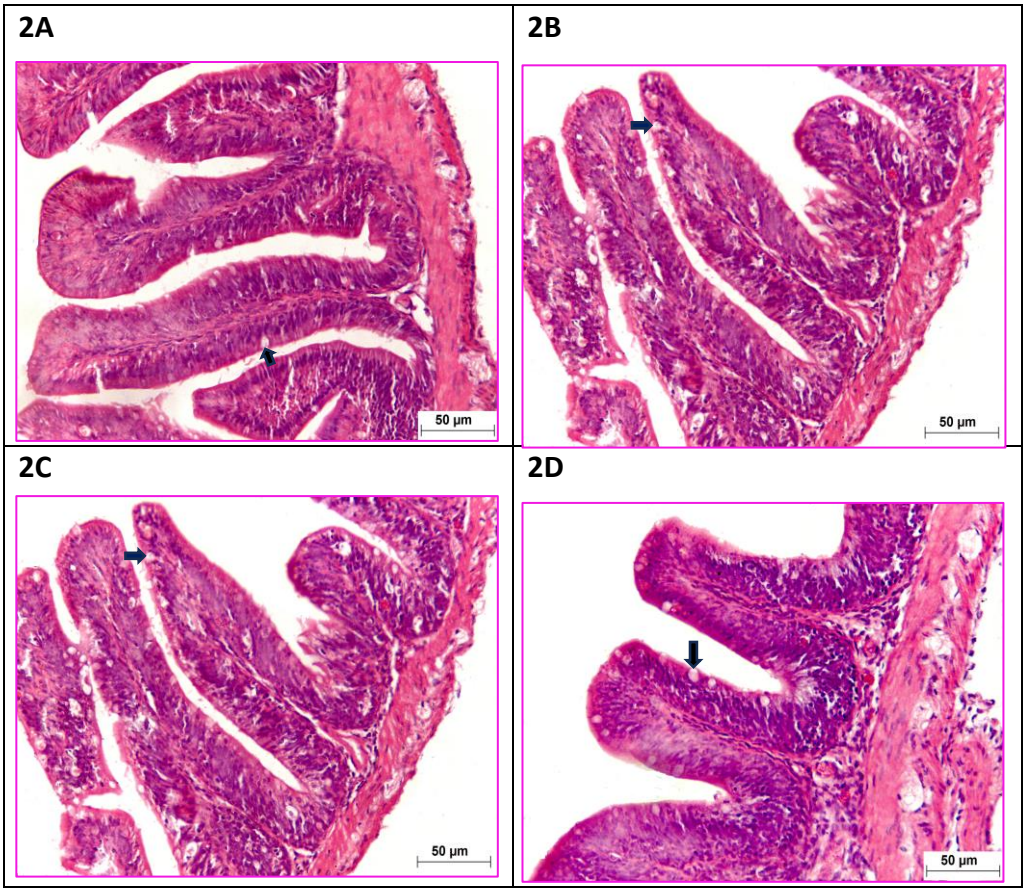

Figure 2. Intestinal sections stained with H&E stain from the experimental groups showed: control treatment (2A): normal intestinal villi with a normal numeral of goblet cells (black arrow). T_1 group (2B): showing: tall villi and low number of goblet cells (black arrow). T_2 group (2C): showing: a remarkable increase in the number and length of the villi occluded the intestinal lumen and abundant goblet cells (black arrow). T_3 group (2D): showing: short intestinal villi and low numeral of goblet cells (black arrow).

Fazio *et al*. [23] Investigated the effects of food supplementation with propolis extract on the intestinal histology of European sea bass (*Dicentrarchus labrax*). The findings shown that fish administered propolis extract displayed enhanced intestinal morphology, characterized by greater villus height and crypt depth, relative to those on a control diet. The findings indicate that propolis supplementation may improve nutrient absorption and enhance overall gut health in fish.

A study by **[24]** revealed that including propolis into the diet of African catfish resulted in enhanced intestinal villi height and width, along with an increase in goblet cell density. This indicates improved nutrition assimilation and mucosal protection. A study by **[25]** revealed that Nile tilapia consuming a propolis-enriched diet showed elevated villi height and crypt depth, signifying improved nutrient absorption and general gastrointestinal health.

These findings align with the results described by **[26],** who investigated the effects of propolis on the histological structure of rainbow trout intestines. The researchers observed a significant increase in both villi height and surface area when propolis was added to the fish food, indicating improved absorptive capacity and digestive function.

CONCLUSION

The findings of the current study recommend that adding honey bee propolis to the diet of Nile tilapia fingerlings can significantly improve their growth performance, nutritional efficiency, and body composition. When honey bee propolis was added to fish meal at a level of up to 4 g/kg diet , the honey bee propolis showed better growth and had more villi and goblet cells in their intestines, indicating enhanced nutrient absorption and overall gut health. This suggests that honey bee propolis supplementation could be a promising strategy for improving farmed Nile tilapia fingerlings production.

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