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Effects of Dietary Inclusion of Black Cumin Seeds, Green Tea and Propolis Extraction on Growth Parameters, Body Composition and Economic Efficiency of Nile Tilapia, *Oreochromis niloticus*

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Abstract: In this study, we evaluated the effects of dietary Black Cumin Seeds (BS), green tea extract (GTE) and Propolis extract (ethanol extract of Propolis, EEP) on growth performance, body composition and economic efficiency of Nile Tilapia, Oreochromis niloticus. A total number of 240 Nile tilapia fingerlings averaging 23.34±0.13 g were divided into four groups, group 1 fed diet contained no additives (control) and the other three groups fed on basal diet which contained 0.75g GTE, 3g BS and 5g EEP for 12 weeks. The final body weight (g), weight gain (g) and FCR significantly increased in fish groups fed on BS followed by EEP and finally GTE supplemented diet. Groups fed diets supplemented by EEP and group fed on diet supplemented by GTE showed the lowest value for feed intake while there was no significance difference in feed intake between other Fish groups and control. The highest values of SGR were recorded in groups supplemented by BS then GTE and EEP. The protein efficiency ratio showed significance increase (p<0.5) in groups fed on diet supplemented by BS and EEP followed by group fed on GTE supplemented diet when compared with control group. On other hand, the addition of BS or GTE and EEP to diet improved the fish condition factor. The highest values for DM% was recorded in fish group fed on BS supplemented diet, the groups supplemented by BS, GTE and EEP showed a significance increase in values in CP content when compared with control. On other hand the lipid content of fish were decreased significantly in groups supplemented by BS, GTE and EEP in compared to control. Finally, addition of GTE, BS and EEP in fish diets revealed a more economically efficient production than in fish fed the Control diet.

Key words: Feed Additives • Growth • Nile Tilapia • Healthy Condition • Economic Efficiency

INTRODUCTION

Attempts to use the natural products of both plant and animal origin widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance. The feed additives improve the immunity, productivity and economic efficiency of fish via its improvement body weight, weight gain and feed conversion ratio [1].

Black cumin seed, *Nigella sativa*, an annual herb that belongs to the botanical family of *Ranuculaceae*, showed antibacterial fungicidal effects [2]. Some researchers have used black cumin seeds as enhancer for growth

performance and immune system of some fish species [3]. Green tea (GT; *Camellia sinensis* L.) is commonly known as green tea in the India and has been shown to contain various types of catechins, including more than 70% polyphenols and vitamins, nitrogenous compounds, caffeine, inorganic elements, lipids and carbohydrates [4].

The nonoxidized and unfermented leaves contain many components that have antitumorigenic, antiinflammatory, antioxidative, antiproliferative, antibacterial, antiviral and antiparasitic properties [5-7], that makes GT a possible candidate to be used as a feed additive to enhance fish growth and control the bacterial disease. Propolis, a green-brown resinous substance collected by

bees from various plant sources and mixed with secreted beeswax, is a multifunctional material used by bees in the construction, maintenance and protection of their hives. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various organic and mineral compounds [8]. The major active components of Propolis extracts are flavonoids and phenolic acids, comprising 25-30%, with many biological and pharmacological activities including antibacterial, antiviral, anti-inflammatory, anti-allergic and vasodilator actions, immunopotentiation and antitumor effects [9].

This study aimed to study the effect of some natural feed additives (BS, GT and Propolis extract) by such doses complete the work of other researches on growth, body composition and economic efficiency of Nile tilapia fingerlings.

MATERIALS AND METHODS

Experimental Fish and Culture: A total of 240 Nile tilapia fingerlings with initial body weight of 23.34±0.13 g (mean±SD) were divided into equal four triplicate groups (each replicate contained 20 fish) and the replicate of each fish group was stocked in its corresponding glass aquaria for two weeks to be acclimatized before the start of the experiment. The fish were located in 12 rectangular glass aquaria (30 x 40 x 150 cm) filled with dechlorinated tap water which continuously aerated by a small air compressor. Mean water temperature was 27±0.5°C and dissolved oxygen was 6.1±0.02 mg/L, while pH was 7.3±0.05.

Preparation of Ethanol Extracts of Propolis (EEP): EEP was prepared following the method described by Cuesta et al. [10]. Briefly, crude Propolis sample was collected from a bee farm at Dakahlia province. EEP was prepared by adding 30 ml of absolute ethanol to 3g minced Propolis in air-sealed bottles which were continuously shaken in darkness for 24 hrs at room temperature. The extract was then filtered twice, dried under vacuum and stored in air-sealed bottles at 4°C until use.

Experimental Diets and Feeding Trial: Group 1 (control), groups 2,3 and 4 were supplemented with 0.75 g green tea, 3 g of black seed (BS) and 5 g Propolis extract (EEP), respectively. Green tea was suspended in 100 ml per one kg and blended with the other ingredients. Table (1) shows the formulation and composition of experimental diet, all fish groups were fed on the diets according to

Table 1: Dietary formulation and proximate composition of the basal diet for Nile tilapia, (*Oreochromis niloticus*)

Ingredients	%
Yellow corn	35.00
Wheat flour	10.00
Soybean meal	18.00
Fish meal	16.00
Poultry by-product meal	14.00
Vegetable oil	5.50
Vitamins and Minerals mixture*	1.50
Calculated composition	
DM,%	89.60
CP,%	30.80
EE,%	10.26
CF,%	2.42
NFE,%	39.00
Ash,%	7.12
DE, Kcal/ kg diet**	2940

body weight at the rate of 3% during the 12 weeks of the experiment. The fish were weighed at start and every three weeks throughout the experiment. Ten fish were taken at the beginning and end of the experiment from each group to measure their body length to determine the condition factor.

- Vitamin mixture provides (g, mg or I.U/kg diet):- Vit. A 5000 I.U, D3 200 I.U, E. 100 mg, K 10 mg, C 1000 mg, B1 10 mg, B2 15 mg, B6 7.5 mg, B12 0.1 mg, Biotin 0.2 mg, Folic acid 0.4 mg, Choline HCL 1 g, Inositol 3000 mg, Pantothenic acid 50 mg, Nicotine 100 mg, P-aminobenzonic acid 50 mg.
- Mineral mixture: each 1000 gm contains: potassium chloride 16 gm, sodium chloride 250 gm, phosphorous 25 gm, iron 10 gm, manganese 5 gm, magnesium 40 gm, zinc 5 gm, copper 5 gm, iodine 150 gm, nickel 90 gm, molybdenum 100 gm, cobalt 10 gm, selenium 10 gm and calcium carbonate till 1000 gm.
- Digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE, 2.5 kcal/gm [11].

Chemical Analysis: Proximate chemical analyses were made for diet ingredients and fish according to standard methods [12].

Calculation of Growth Indices: The growth rate and feed conversion ratio as well as protein utilization were calculated according to Castell and Tiews [13] while the condition factor was calculated according to Gjedrem and Gunnes [14] and Specific growth rate (SGR) and Survival rate (%) were calculated according [15].

Economic Efficiency Calculation: Economic efficiency was calculated according to the following equation: Y= [(A-B)/B X 100] [16] where, A is the selling cost of obtained gain and B is the feeding cost of obtained gain.

Statistical Analyses: The obtained data in this study were subjected to one-way ANOVA analysis of variance [17, 18]. Differences among treatment means were determined by Duncan's multiple range tests at a (P<0.05) level of significance [19].

RESULTS AND DISCUSSION

Survival and Growth Parameters: Survival rate (%) of fish ranged from 83.33% to 94.00% and was not significantly different among the dietary treatments (Table 2). These results agreed with [20] who reported that survival of juvenile Nile tilapia was not significantly affected by dietary inclusion of green tea leaves and dietary EEP supplementation of rainbow trout juveniles [21]. Moreover dietary supplementation of two levels (5 and 10g/kg diet) of Black cumin seeds (*Nigella sativa*) to fingerlings of Asian sea bass not affected the mortality rate [22].

The body weight development throughout the experimental period is present in Fig (1). The final results for growth performance parameters are present Table 2. The fish group fed on diet contained 3gm of black seed, recorded the best final body weight and weight gain (54.45 and 31.03 g, respectively) followed by the fish group fed on diet contained 5gm Propolis extract, (50.15 g and 26.74, respectively) then the fish group fed on diet contained 0.75 g green tea, (47.28 g and 24.03, respectively) in comparison with control group (43.39 g and 19.94, respectively).

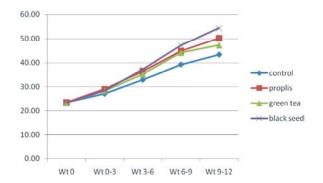


Fig. 1: Effect of dietary supplement on body weight development (g), throughout the experiment

The feed intake significantly decreased in fish groups fed on diet supplemented by GTE then EEP in comparison with the control group, while there was no significant difference between groups fed black cumin seed and control group.

On other hand, feed conversion ratio (FCR), revealed that fish groups fed on diet contained 3 gm of BS or 5 g EEP recorded the best values for FCR followed by fish groups fed on diet contained 0.75 gm GTE when compared with the control group. Moreover, the protein efficiency ratio (PER) values were significantly increased in fish groups supplemented with EEP and BS followed by GTE when compared with the control group. These results agree with Abdel-Tawwab and Medhat Seden [20] who mentioned that final fish weight, weight gain, FCR, PER and SGR% significantly (P<0.05) increased with the GT leaves extract up to 0.5g/kg diet, while there was no significance in final fish weight, weight gain, FCR, PER and SGR between the control group and inclusion of 1g/kg of green tea extract. Similarly [22] observed improvement growth performance of Mugil cephalus fed

Table 2: Effect of experimental diets on all over growth performance of Nile tilapia (means±SE)

•	Experimental groups					
Parameters	Control	Propolis extract (EEP)	GT leaves extract	BS		
Initial BW (g)	23.45±0.13	23.40±0.40	23.25±0.25	23.26±0.25		
Final BW (g)	43.39±1.63 ^D	50.15±1.72 b	47.28±1.00 °	54.45±2.34 a		
Body gain (g)	19.94±0.70 d	26.74±0.56 b	24.03±1.21 °	31.03±0.45 a		
Specific growth rate (%)	0.73±0.02 °	0.91±0.01 b	0.84±0.04 b	1.01±0.01 a		
Feed intake (g)	44.41 ± 0.63^{a}	37.43±0.50°	39.35 ± 0.49^{b}	44.47 ± 0.48^a		
Feed conversion ratio	2.23±0.05 a	1.40±0.05 °	1.67±0.11 ^b	1.43±0.03 °		
Condition factor	1.70±0.13 °	1.96±0.10 ^b	1.82±0.01 a	2.39±0.20 ab		
Protein efficiency ratio	1.28±0.03 °	2.03±0.07 a	1.72±0.11 b	2.00±0.05 a		
Survival (%)	83.33±0.67°	94.00±0.85a	92.67±0.33 ^a	89.67±0.33b		

Means with different superscript letters denote significant difference (P<0.05).

Table 3: Effect of experimental diets on body composition performance of Nile tilapia

		Groups			
Nutrient (%)	Initial	Propolis extract (EEP)	GT leaves extract	BS	
Dry matter	18.64±0.43 °	23.84±0.06 ab	24.59±0.20 ab	24.90±0.29 a	
Protein	56.12±0.22 °	62.68±0.37 a	62.49±0.35 a	64.27±0.51 a	
Fat	14.33±0.16 a	13.80±0.20 b	12.33±0.15 b	13.19±0.12 b	
Ash	15.54±0.22 °	17.00±0.50 b	16.80±0.15 b	18.87±0.50 a	

Means with different superscript letters denote significant difference (P<0.05).

on 5 g and 10g/kg diet of dietary supplementation of black cumin seeds or 6 weeks while there was no significance difference in feed intake. Increased specific growth rate and food conversion ratio in Labeo rohita fingerlings fed four different levels of black seed (0.1, 0.5, 1.0 and 5g /kg feed) for 60 days [23]. On other hand, Deng et al. [21] stated that dietary EEP supplementation 2 and 4 g/kg level significantly improved the final weight, weight gain, specific growth rate and PER of rainbow trout. Previous studies with Nile tilapia (Oreochromis niloticus) showed that the incorporation of 1.83-2.74 g kg⁻¹ brown Propolis extract, 10 g kg-1 EEP or crude Propolis increased the growth performance and decreased the feed conversion ratio [24]. In terrestrial animals, the antimicrobial and/or antioxidant activities of the components of EEP could result in better intestinal health and improved digestion and absorption and thereby improved the growth performance [25]. Abdel-Tawwab and Medhat Seden [20] stated that the inclusion of dietary GT in diet may enhance the nutrients digestibility leading to improving the nutrient utilization, which explain the better growth and feed utilization. Moreover, GT may play an inhibiting role with the potential pathogens in the digestive tract, enhance the population of beneficial microorganisms and enhance the microbial enzyme activity that consequently improves the feed digestibility and nutrient absorption. Lin et al. [26] reported that traditional CMH may influence digestive processes by enhancing enzyme activity and improving digestibility of nutrients and thus may increase the diet evacuation rate as herb levels increased.

Evaluation of the Health Condition: Concerning the evaluation of the health condition of the experimental fish, there were found that no changes in the normal behavior of the fish because the tested fish were able to respond positively to all tested reflexes especially the escape reflex, in which the fish during and at the end of the experiment were able to exhibit normal responses in fish pond. Lucky [27] reported that sick fish do not react to external agitation and were not able to exhibit normal

behavioral responses (lost of reflexes) and therefore can be easily caught by dip net or by hand be course sick fish are not energetic. There were no marked difference in the behavioral responses between the tested fish groups, which received diets supplemented with different feed additives and fish of control group.

The fish reacted rapidly to external agitation so they quickly submerge to greater depth, swim away from the water Surface and in general their escape movements were energetic and their general healthy condition were good without any changes of the color of the tested fish.

Whole Body Composition: The results of whole body composition are present in Table 3. The results showed that the highest values of DM content were recorded in group fed on diet supplemented with black cumin seed, while the CP content were significantly (P<0.05) increased in groups fed green tea, black seed and Propolis extract while the EE content of the same groups significantly (P>0.05) decreased when compared with control group. These results agree with Abdel-Tawwab and Medhat Seden [20] who confirmed that inclusion of GT extract up to 0.5g and 1g/kg diet increased protein content, while 0.5g /kg diet significantly increased total lipids 1g/kg diet significantly decreased total lipids content.

Whole-body protein and lipid content of juvenile eel, *Anguilla japonica* increased up to peak values at the Propolis level supplementation of 0.5%, this may explained as it is possible that flavonoids in Propolis enhance food ingestion and absorption, nutrient metabolism, as well as specific and nonspecific immune responses in eel. On other hand, there were no significant differences in the whole body compositions of trout juveniles fed on 1, 2 and 4 g kg⁻¹ diet propolisfor 10 weeks [28].

Abdel wahab and El-Bahr [22] observed that DM, CP, EE and crude ash contents of Asian seabass not significantly affected by addition of 5 and 10 gm black cumin seed to the diet.

Table 4: Effect of experimental diets on Economic efficiency

		Groups		
Parameters (%)	Control	Propolis extract (EEP)	GT leaves extract	BS
Diet cost (L.E * /kg diet)	4.69	5.94	4.74	4.48
Feeding cost of obtained gain (L.E *)	0.20	0.22	0.14	0.15
Fish selling cost of obtained gain (L.E */kg live weight)	12.00	12.00	12.00	12.00
selling cost of obtained gain (L.E *)	0.24	0.32	0.34	0.34
Economic efficiency (%)	17.58	44.32	136.16	131.04

L.E * Egyptian Pound.

Condition Factor (K): The results of condition factor (k) at the end of the experiment are present in (Table 2). Fish groups fed on diets supplemented with green tea, black seed (BS) and Propolis extract (EEP) had higher body condition factor in comparison with the control group. These results are supported by Gjedrem and Gunnes [14] who reported that the high condition factor is an indicator to the good nutritional (healthy) state of fish.

Economical Efficiency: The effect of experimental diets on the economic efficiency in different fish groups are presented in (Table 4). The highest economic efficiency was recorded with fish groups fed diet contained green tea leaves extract (136.16%), followed by fish group fed on diet contained black cumin seed (131.04%) then Propolis extract supplemented diet (44.32%) in comparison with control group (17.58%). Addition of feed additives to fish diets can improve the immunity, productivity and economic efficiency of fish via its improvement body weight, weight gain and feed conversion ratio [1].

CONCLUSION

The results of the present study indicated that dietary inclusion of black cumin seeds, green tea and Propolis extract exhibited good growth performance, health condition of Nile tilapia fingerlings.

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