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EVALUATION NON-CONVENTIONAL DIETS (ROSEMARY LEAVES (*Rosmarinus officinalis*) AND GINGER RHIZOME (*Zingiber officinale*) ON GROWTH PERFORMANCE, FEED UTILIZATION AND IMMUNE RESPONSE OF RED TILAPIA FINGERLINGS (*Oreochromis* sp.)

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ABSTRACT

The present study conducted at Mari culture Research Center, Arish University, North Sinai, Egypt. This study was carried out to investigate the effects of dietary inclusion of different levels of Ginger rhizome and Rosemary leaves on growth rates, feed utilization and immune response of red tilapia. 140 fish were distributed in 14 glass aquaria for all experiments ten fingerlings per aquarium with an average initial weight 6.1±0.2 g and an average initial length 6.4 ±0.5 cm. The experiment includes seven treatments (two replicates). T1 group was used as control. T2, T3 and T4 groups were fed the experimental diets adding Rosemary leaves (0.5%, 1% and 1.5% respectively) and T5, T6 and T7 groups were fed the experimental diets adding ginger rhizome (0.5%, 1% and 1.5% respectively). Fish were fed diets containing 30 % crude protein twice a day with an adjustment of the feeding rate by 3% day of the live weight of the fish. This experiment lasted for 14 weeks. At the end of experiment, body weight, length and chemical analysis of whole body of red Tilapia were measured to determine productive performance, feed utilization and blood parameters. The results of growth parameters indicated that T4 recorded high values for most growth parameters and feed utilization. T1 recorded the highest condition factor. Values of composition of blood parameters showed significant variations among all fish groups. In conclusion, the Rosemary leaves could be added to red Tilapia diets to improve growth rates, feed utilization and immune response.

INTRODUCTION

Medicinal plants and herbs have beneficial effects throughout its biological properties such as antimicrobial **(Sagdıc and Ozcan, 2003)** anticoccidial **(Giannenas *et al.*, 2003)**, antifungal **(Soliman and Badeaa, 2002)**, antispazmolytic **(Meister *et al.*, 1999)** and antioxidant **(Lee and Shibamoto, 2002)** impacts. Moreover, herbs frequently have digestion stimulating, immune boosting and antiseptic effects **(Cabuk *et al.*, 2003)** Furthermore, medicinal plants and herbs can also increase resistance to disease *via* optimizing the function of the immune system **(Al-Beitawi *et al.*, 2010)**.

In aquaculture, some medicinal plants and their bioactive components displayed several beneficial properties as antibacterial, antioxidant, immunostimulant, growth promotion and anti-stress like rosemary (*Rosmarinus officinale*) **(Naiel *et al.*, 2019)**, thyme (*Thymus vulgaries*) **(Abd El-Naby *et al.*, 2020)** and *Moringa oleifera* **(Ibrahim**

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***et al.*, 2019)**. Blood parameters is important pointers of fish health status in aquaculture systems **(Fazio *et al.*, 2018)**. A strong link was found between nutritional supplementation with medicinal herbs and improved blood parameters, oxidative status, and immunological response in aquaculture **(Acar *et al.*, 2018; Zargar *et al.*, 2019; Parrino *et al.*, 2020)**.

Rosemary (*Rosmarinus officinalis*) is a tiny evergreen medicinal herb **(Peterson and Talcott, 2013)**. Rosemary leaf has also been widely utilized as a natural medical plant due to its immunostimulatory, anti-inflammatory, antioxidant, antibacterial qualities and antibacterial **(Charles, 2013)**. **Hassan *et al.* (2018)** reported that fish fed a diet supplemented with rosemary displayed high significant in growth performance and feed utilization tilapia. Furthermore, adding rosemary leaves powder (RLP) with 0.5 percent in diets improved significantly antioxidant status and immunity of tilapia **(Naiel *et al.*, 2019)**. Ginger is a famous medicine plants having polyphenols, flavonoids, carotenoids, tannin, saponin, alkaloids, vitamins, steroids and minerals **(Talpur *et al.*, 2013)**. Besides, it is rich in natural antioxidants such as, zingerone, shogaol and gingerols **(Hori *et al.*, 2003)** .

Through studies, **Mahmoud *et al.* (2019)** found negative effects in growth performance and feed utilization when used Ginger (*Zingiber officinale*) rhizome in diets. However **Johnson-Ashun (2018)** reported that utilization ginger and garlic as feed supplements in Tilapia diets, the fish fed at levels (0.5, 1, 1.5 and 2%) as feed additives in diets. had no effect on the fish's growth, feed efficiency, blood parameters and lysozyme activity. Accordingly, the present work was carried out to study the effect of adding the medicinal plants Rosemary Leaves Powder (*Rosmarinus officinalis*) and Ginger (*Zingiber officinale*) rhizome with different concentrations (0.5%, 1% and 1.5%) as non-conventional diets to red Tilapia (*Oreochromis* sp.) diets on feed utilization, growth rates and immune response.

MATERIALS AND METHODS

The current work was carried out in Mari Culture Research Center (MRC), Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt. This experiment lasted for 14 weeks during July, August, September and October 2019 to evaluate the effect of using three levels of Rosemary (*Rosmarinus officinalis*) leaves and Ginger (*Zingiber officinale*) rhizome (0.5%, 1% and 1.5%, respectively) on red Tilapia fingerlings diets.

Experimental Fish and Facilities

Two hundred of red Tilapia fingerlings were obtained from El-kilo 21 Marine Fish Hatchery belonging to General Authority for Fish Resources Development (GAFRD), Egypt. The fish were transferred to Mari culture Research Center (MRC). A total of one hundred and forty fish were equally distributed in fourteen glass aquaria (60 X 40 X 50 cm) with total capacity of 120 liters. Ten fingerlings per aquarium were stocked with an average initial weight 6.1 ± 0.2 g and an average initial length 6.40 ± 0.5 cm. Fish were acclimatized for two weeks and fed commercial diet containing 30 % crude protein before start experiment. In the present work, all two replicate groups of fish hybrid red Tilapia (*Oreochromis* sp) for each treatment were fed the tested diets containing ~30 % crude protein isonitrogenous and (~456 Kcal/ 100 g gross energy, GE) isocaloric and fed twice a day at 8 AM and 4 PM with an adjustment of the feeding rate by 3%/day of the live weight of the fish and adjust the feeding rate every two weeks. Every two weeks, the total weight of the fish in each aquarium was measured to assess their growth.

The aquarium was daily cleaned and excreta were siphoned. The siphoned water was replaced with clean water of similar temperature before the first feeding in the morning. Each aquarium was supplied with compressed air. Salinity, water temperature, and pH were measured once every week. Water salinity and temperature were recorded using conductivity-temperature meter (SET). pH was measured using a pH-meter. The water quality were, temperature averaged 25.6 ± 1.52 °C, salinity averaged 28 ± 0.20 ppt, and the pH averaged 7.45 ± 0.02. The fish divided randomly into seven groups (two replicates per group). Control group was fed diet (T1) free from Rosemary leaves and Ginger rhizome, from (T2) to (T4) groups were fed the control diet supplied by Rosemary dry leaves powder (0.5 %, 1% and 1.5%, respectively) While, from (T5) to (T7) groups were fed the control diet supplied by Ginger dry rhizome powder (0.5%, 1% and 1.5%, respectively).

Experimental Diets

Ingredients were purchased from market at reasonable price. Solid ingredients were crushed into powder using a Lab-Mill and sieved before mixing. Diets were stored in the fridge at 4^oC until used. The diets formulation and chemical analysis are displayed in Table 1. Chemical analysis of the experimental diets were according to **(AOAC, 2000).**

Growth Performance and Feed Utilization

Growth performance and feed utilization were measured using the following equations: Weight gain (WG) = final weight (g) – initial weight (g); Gain % = (WG/W1) x 100; Condition factor (K) = (W/L^3) x 100, where, W is weight of fish in grams and L is total length of fish in cm; specific growth rate (SGR) = (LnW2 – LnW1)/ t X 100, Where, Ln is the natural log; W1 is initial body weight and W2 is the final body weight in grams and "t" is the experimental period in days; feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g); Feed efficiency (FE%) = gain in weight (g) / feed intake (g); protein efficiency ratio (PER) = weight gain (g)/protein ingested (g); protein productive value (PPV%) = (retained protein / protein intake) X 100 and EPV% = energy retained / energy intake . Retention of nutrients = (Final body weight x final nutrient concentration) - (Initial body weight x initial nutrient concentration).

Blood Analysis

Caudal vessels were used to collect blood samples from six fish from each group at the end of each trial, and the blood samples were put in dry, clean centrifuge tubes. Serum was separated using a digital centrifuge at 3000 rpm for 15 minutes, then stored in plastic vials and kept at -20°C until biochemical analysis. The serum total protein (g/dl) was determined by the method of **(Doumas, 1975)**, while Serum albumin was determined according to the method of **(Doumas *et al.*, 1971)**. The globulin and albumin-globulin ratio were determined according to the method of **(Coles, 1986)**. The serum enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were assayed by the method to **(Reitman and Frankel, 1957)**. Serum lysozyme activity (µg/ml) was determined by the method of **(Ellis, 1990b)**.

Proximate Analysis

Proximate composition analyses of diets and whole body conducted according to **(AOAC, 2000)** methodology on dry matter basis.

Statistical Analyses

Mean values and standard error (mean ± SE) for each parameter of all groups were first calculated. Data were tested using the analysis of variance one way (ANOVA) using SAS **(SAS, 2004).** Where a significant difference was observed for a measured value, mean separated using Duncan's multiple range test **(Duncan, 1955),** at the 5% level.

Table 1. Proximate analysis of the experimental diets on dry matter basis

Diet	T1	T2	T3	T4	T5	T6	T7
Fish meal	20	20	20	20	20	20	20
Soybean meal	12	12	12	12	12	12	12
Corn gluten	10	10	10	10	10	10	10
Yellow corn	35	34.5	34	33.5	34.5	34	33.5
Wheat bran	15	15	15	15	15	15	15
Medicinal plants	0	0.5	1	1.5	0.5	1	1.5
Linseed oil	2	2	2	2	2	2	2
Fish oil	2	2	2	2	2	2	2
Vitamins and Minerals premix[1]	4	4	4	4	4	4	4
Proximate analysis							
DM	91.30	91.80	92.10	91.90	92.20	90.95	91.10
Crud protein	30.05	30.56	30.17	30.15	30.14	30.19	30.2
Ether extract	8.87	9.010	8.95	8.97	8.99	8.93	8.94
Ash	6.41	6.54	6.48	6.50	6.51	6.48	6.49
CF	3.86	4.05	3.98	4.07	4.15	3.93	3.96
NFE	50.81	49.84	50.42	50.31	50.21	50.47	50.41
GE[2]	456.4	456.7	456.3	455.9	455.6	456.4	456.3
DE[3]	407.9	408.3	407.9	407.6	407.3	408.0	407.9
ME[4]	269.5	271.0	269.9	269.8	269.8	269.9	270.0
P/E[5]	111.5	112.8	111.8	111.7	111.7	111.8	111.9

[1]One kilogram of minerals and vitamins premix contain: 65mg manganese sulfate ($MnSO_4$, 36 % Mn), 26mg pyridoxine HCl, 7.2mg thiamin HCl ,3077mg ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 1.2mg sodium chloride (NaCl, 39% Na and 61% Cl), 6mg riboflavin, 150mg copper sulfate ($CuSO_4.5H_2O$, 25 % Cu) and89 mg zinc sulfate ($ZnSO_4.7H_2O$, 40 % Zn), 28mg potassium iodide (KI, 24 % K and 76 % I) , 4800 IU Vitam A, 2400 IU cholecalciferol (Vitam D), 4g Vitam B2, 6g Vitam,B6 Vitam E, 4g Vitam B12, 8g Vitam K,6g Vitam B6, 4g pantothenic acid, 8g nicotinic acid, 400mg folic acid, 4g copper, 0.4g Iodine, 22g manganese, 22g zinc20mg biotin, 200mg choline, 12g Iron, , 0.04g selenium folic acid, 1.2mg niacin, 12mg d-calcium pantothenate,

[2]Gross energy (Kcal/100g) = 5.65 (CP %) + 9.45 (EE %) + 4.0 (NFE %) according **(NRC, 1993)**. [3]Digestable energy (Kcal/100g) = 5 (CP %) + 9 (EE %) + 3.5 (NFE %) according to **(NRC, 1993).** [4]Metabolizable Energy (Kcal/100g) = 3.9 (CP %) + 8 (EE %) + 1.6 NFE %) according to **(NRC, 1993).** [5]P/E (mg/ Kcal) = (mg Protein/Metabolizable energy Kcal) according to **(NRC, 1993).**

RESULTS

Growth Performance and Feed Utilization

Results of initial and final body weight, weight gain, specific growth rate and average daily gain are presented in Table 2 as well as Figs. 1 and 2. The analysis of variance of these data indicated that there were significantly differences (P≤0.05) among treatments in specific growth rate, relative growth rate, gain in weight and average daily gain. The highest values recorder for T4; 1.67 ± 0.0468,412 ± 23.5, 24.2 ± 0.15 and 0.247 ± 0.00153, respectively. Final body length and Condition factor values show significantly differences (P≤0.05) among treatments. The highest value of final body length was recorded for T4 and the lowest value of final body length was recorded forT7.The highest condition factor was recorded for T1 (2.18 ± 0.034) but the lowest value was recorded for T4 (1.69 ± 0.137). There were no significant differences in survival rates among the groups.

Feed, protein, fat and energy intake of the experimental diets are displayed in Table 3 and Fig. 3. The analysis of variance revealed that all treatments had significant (P≤0.05) changes. T3 and T4 groups recorded the highest feed, protein, fat and energy intake. The lowest values of feed, protein, fat and energy intake were recorded for the T7 group.

There were significant differences (P ≤ 0.05) among treatments regarding FCR and FE. The highest FCR and FE were recoded for T7, T4 groups, respectively.

Protein Efficiency Ratio (PER) of tested diets was significantly different (P≤0.05) in the T4 group when compared with the control group. Table 3 shows the percentage of protein productive value (PPV) and energy productive value (EPV) of diets. The highest value was recorded for the T4 group and the lowest one was recorded for T7.

Body Composition and Energy Content of Whole-Body Fish

Analysis of whole-body composition on dry matters basis (DM), crude protein (CP), either extract (EE), ash and gross energy content for red tilapia are presented in Table 4.

There were significant (P≤0.05) among treatments in dry matter. The highest value of DM was recorded for T1 group and the lowest one was recorded for T7. There were high significant (P≤0.05) difference among groups in crude protein (CP). The highest value of CP was recorded for T4 group and the lowest value was recorded for T7 group. The highest values in either extract was recorded for T1 group and the lowest one was recorded for initial sample. Ash content also differed significantly (P≤0.05) among treatments. The highest value of ash content was recorded for T7 and the lowest value was recorded for T4 group.

Results in Table 5 show protein, fat, ash and energy retained. The results showed that no significant (P>0.05) in ash retained among all groups. But there were significantly different (P≤0.05) of protein, fat and energy retained among treatments. The highest value for each of protein, fat and energy retained was recorded for the T4 group. The lowest protein, energy and fat retained values were recorded for the T7 group.

Blood parameters

Serum biochemical parameters could be used as pointers of the nutritional and physiological status of red Tilapia fish (*Oreochromis* sp.); Results are presented in Table 6 and Figs. 4 and 5. It is obvious that dietary with all treatment significantly affect total protein, globulin, albumin, A/G ratio, lysozyme, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Red Tilapia fed T4 had high (P≤0.05) total protein, globulin, lysozyme (4.2 ± 0.08, 2.32±0.01 g/dl and 3.62 ± 0.09 µg/ml, respectively) as compared to the control group.

Table 2. Growth performance of red tilapia as affected by addition of Rosemary leaves and Ginger rhizome with different concentrations in fish diets

	Experimental Diet						
Diet	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Initial weight (g/fish)	6.2 ± 0.45	6 ± 0.4	6.4 ± 0.2	5.9 ± 0.3	6.1 ± 0.35	6.2 ± 0.65	6.1 ± 0.5
Final weight (g/fish)	24.9 ± 0.685^{cd}	25.4 ± 0.6^{cd}	27.7 ± 0.19^{b}	30.1 ± 0.15^{a}	25.8 ± 0.08^{c}	24.3 ± 0.255^{d}	22.6 ± 0.08^{e}
Gain in weight (g/fish)	18.7 ± 0.235^{c}	19.4 ± 1^{c}	21.3 ± 0.39^{b}	24.2 ± 0.15^{a}	19.7 ± 0.27^{bc}	18.1 ± 0.395^{cd}	16.5 ± 0.58^{d}
Average daily gain (g/fish/day)	0.191 ± 0.0024^{c}	0.198 ± 0.0102^{c}	0.218 ± 0.00398^{b}	0.247 ± 0.00153^{a}	0.201 ± 0.00276^{bc}	0.185 ± 0.00403^{cd}	0.169 ± 0.00592^{d}
Relative growth rate (%)	303 ± 18.2^{b}	326 ± 38.4^{ab}	334 ± 16.5^{ab}	412 ± 23.5^{a}	324 ± 23^{ab}	296 ± 37.4^{b}	273 ± 31.9^{b}
Specific growth rate (%)	1.42 ± 0.0461^{ab}	1.47 ± 0.0922^{ab}	1.5 ± 0.0389^{ab}	1.67 ± 0.0468^{a}	1.47 ± 0.0554^{ab}	1.4 ± 0.0967^{b}	1.34 ± 0.0874^{b}
Survival rate (%)	95 ± 5^{a}	80 ± 10^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	90 ± 10^{a}	100 ± 0^{a}
Initial length (cm)	6.4 ± 0.1	6.9 ± 0.1	6.4 ± 0.4	6.7 ± 0.5	6.35± 0.35	6.85 ± 0.35	6.95 ± 0.05
Finial length (cm)	10.4 ± 0.15^{d}	11.3 ± 0.2^{b}	11.4 ± 0.15^{b}	12.2 ± 0.35^{a}	11.1 ± 0.05^{bc}	10.6 ± 0.1^{cd}	10.3 ± 0.125^{d}
Gain length (cm)	4.05 ± 0.05^{cd}	4.4 ± 0.1^{bc}	5.05 ± 0.25^{ab}	5.45 ± 0.15^{a}	4.8 ± 0.3^{ab}	3.75 ± 0.25^{cd}	3.37 ± 0.175^{d}
Condition factor (K)	2.18 ± 0.034^{a}	1.77 ± 0.135^{bc}	1.85 ± 0.0853^{bc}	1.69 ± 0.137^{c}	1.86 ± 0.0192^{abc}	2.04 ± 0.0792^{ab}	2.06 ± 0.0819^{ab}

*Means followed by different letters in each row are significantly different (P<0.05)

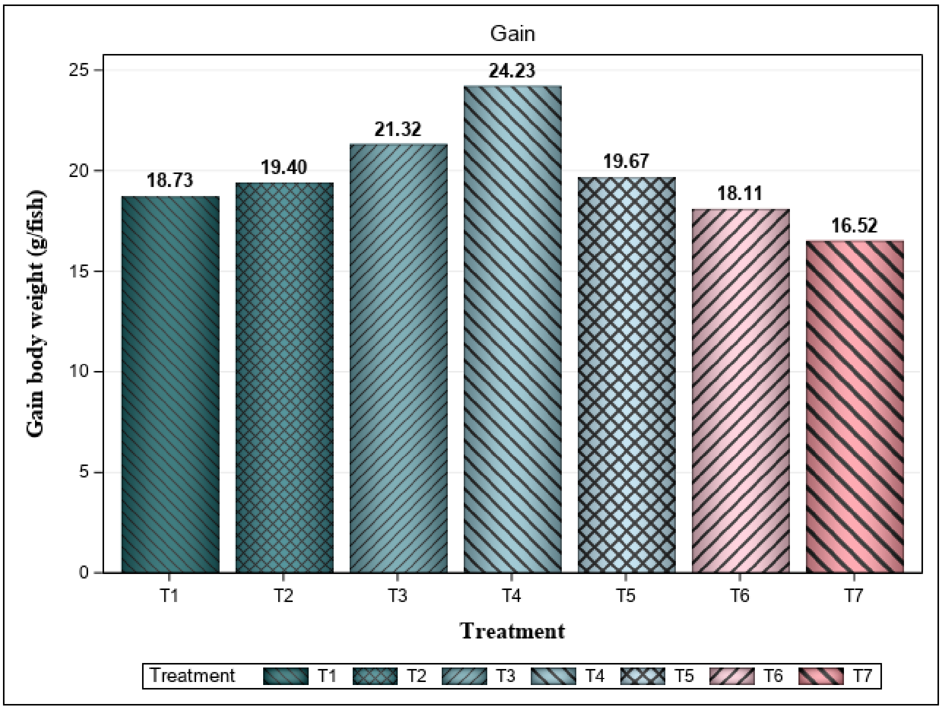


Fig. 1. Effect of adding medicinal plants to the diets for red Tilapia (*Oreochromis* sp.) on the gain weight

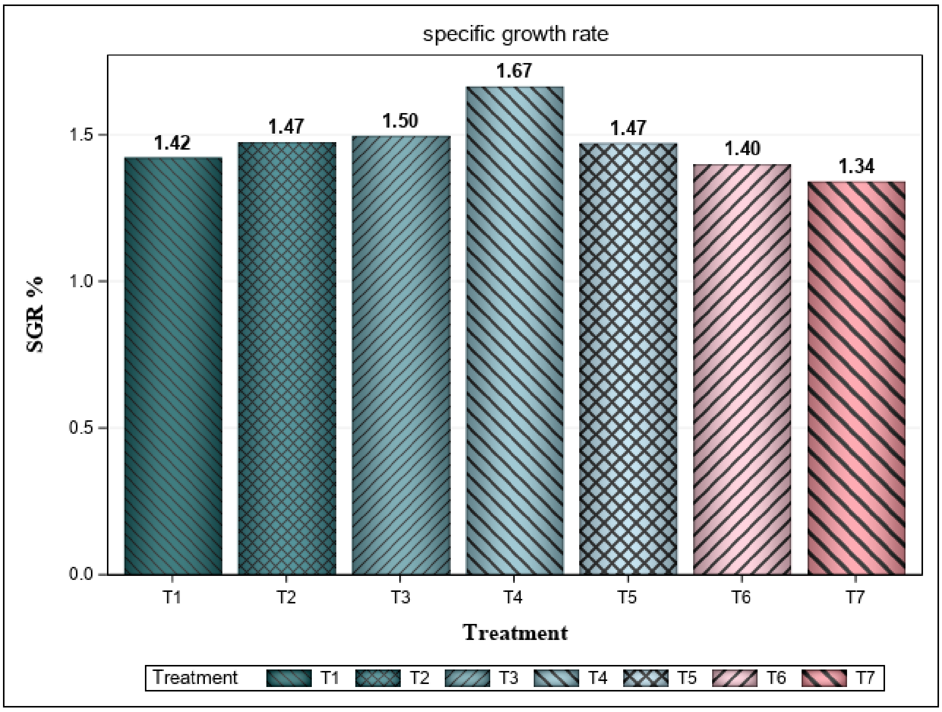


Fig. 2. Effect of adding medicinal plants to the diets for red Tilapia (*Oreochromis* sp.) on the specific growth rate

Table 3. Feed utilization of red tilapia as affected by Rosemary (*Rosmarinus officinalis*) leaves and Ginger (*Zingiber officinale*) rhizome with different concentrations in fish diets

	Experimental Diets						
***Item**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Feed intake (g fish^{-1})	40.9 ± 2.03^{b}	41 ± 0.14^{b}	44.6 ± 0.743^{ab}	47.4 ± 1.14^{a}	43.5 ± 1.63^{ab}	42 ± 2.31^{ab}	40.1 ± 1.94^{b}
Protein intake (g fish^{-1})	12.3 ± 0.603^{b}	12.4 ± 0.0504^{b}	13.4 ± 0.206^{ab}	14.3 ± 0.335^{a}	13.1 ± 0.509^{ab}	12.7 ± 0.71^{ab}	12.1 ± 0.582^{b}
Fat intake	3.63 ± 0.221^{b}	3.67 ± 0.00843^{b}	4 ± 0.0622^{ab}	4.26 ± 0.107^{a}	3.89 ± 0.141^{ab}	3.75 ± 0.202^{ab}	3.59 ± 0.19^{b}
Energy intake	187 ± 9.53^{b}	187 ± 0.565^{b}	203 ± 3.34^{ab}	216 ± 5.33^{a}	199 ± 7.54^{ab}	192 ± 10.6^{ab}	183 ± 8.89^{b}
FCR[1]	2.19 ± 0.0811^{ab}	2.12 ± 0.117^{ab}	2.09 ± 0.00339^{ab}	1.96 ± 0.0593^{b}	2.21 ± 0.113^{ab}	2.32 ± 0.178^{ab}	2.44 ± 0.203^{a}
FE[2]	45.8 ± 1.7^{ab}	47.3 ± 2.6^{ab}	47.8 ± 0.0775^{ab}	51.2 ± 1.55^{a}	45.3 ± 2.32^{ab}	43.3 ± 3.32^{ab}	41.3 ± 3.44^{b}
PER[3]	1.53 ± 0.0556^{ab}	1.57 ± 0.0871^{ab}	1.59 ± 0.00467^{ab}	1.7 ± 0.0503^{a}	1.5 ± 0.0787^{ab}	1.43 ± 0.111^{ab}	1.37 ± 0.113^{b}
PPV[4] (%)	27.5 ± 1.22^{abc}	27.3 ± 1.58^{abc}	28.1 ± 0.00929^{ab}	30.1 ± 0.927^{a}	24.8 ± 1.31^{bcd}	23.2 ± 1.53^{cd}	21.8 ± 1.79^{d}
EPV[5] (%)	15.2 ± 0.633^{ab}	14.2 ± 0.936^{abc}	14.6 ± 0.0651^{abc}	15.9 ± 0.543^{a}	13.1 ± 0.764^{bcd}	12 ± 0.932^{cd}	11.2 ± 1.03^{d}

*Means followed by different letters in each row are significantly different $P < 0.05$. 1- FCR= feed conversion ratio, 2- FE= feed efficiency, 3- PER= protein efficiency ratio, 4- PPV= protein productive value, EPV= energy productive value.

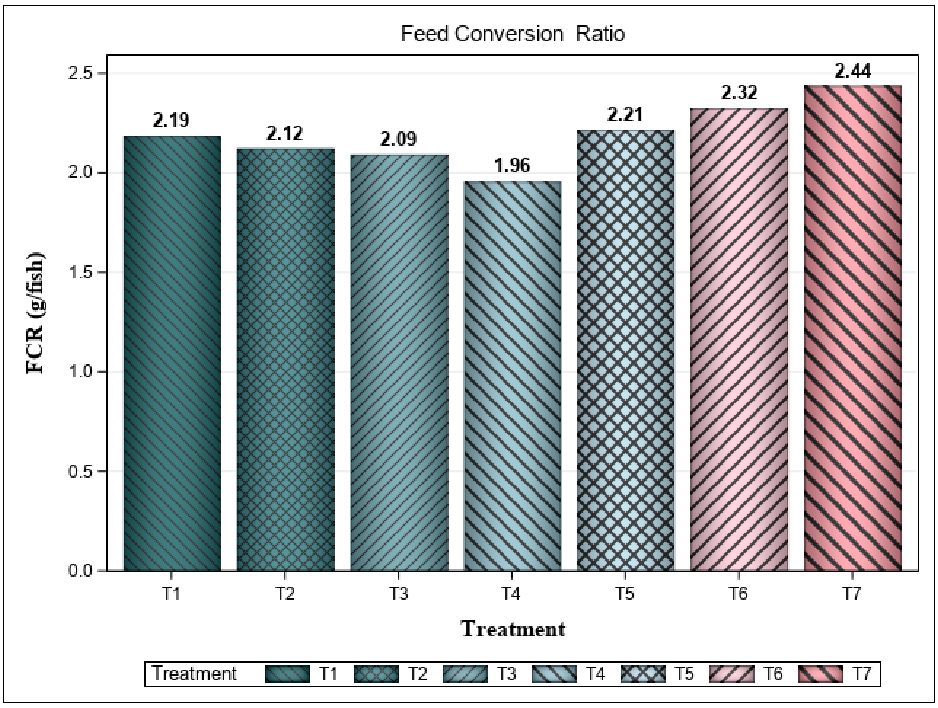


Fig. 3. Feed conversion ratio (FCR) for all treatments

Table 4. Chemical composition and energy content of whole body of red tilapia as affected by different diets on dry matter basis

		Experimental Diet						
***Item**	**Initial sample**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Dry matter	31.36	28.1 ± 0.0901^{a}	26.4 ± 0.0409^{bc}	26.5 ± 0.025^{b}	26.6 ± 0.04^{b}	26.1 ± 0.035^{c}	25.8 ± 0.186^{d}	25.7 ± 0.02^{d}
Crude protein	63.52	63.1 ± 0.232^{b}	65.6 ± 0.268^{a}	65.9 ± 0.0417^{a}	66 ± 0.005^{a}	63.4 ± 0.0858^{b}	63.2 ± 0.153^{b}	62.9 ± 0.055^{b}
Either extract	13.5	22.1 ± 0.318^{a}	20.3 ± 0.0283^{bc}	20.2 ± 0.0433^{bc}	20.2 ± 0.08^{bc}	20.3 ± 0.0458^{b}	20 ± 0.0133^{bc}	19.8 ± 0.15^{c}
Ash	22.98	14.8 ± 0.0858^{c}	14.2 ± 0.297^{d}	14 ± 0.00167^{d}	13.8 ± 0.085^{d}	16.3 ± 0.132^{b}	16.8 ± 0.167^{ab}	17.3 ± 0.205^{a}
Gross energy	549.31	565 ± 0.0168^{a}	561 ± 0.0178^{a}	562 ± 0.00172^{a}	563 ± 0.0078^{a}	549 ± 0.00915^{b}	545 ± 0.00992^{c}	541 ± 0.0172^{c}

*Means followed by different letters in each row are significantly different ($P<0.05$).

Table 5. Protein, fat, ash and energy retained of whole-body composition for red tilapia at the end of the experiment

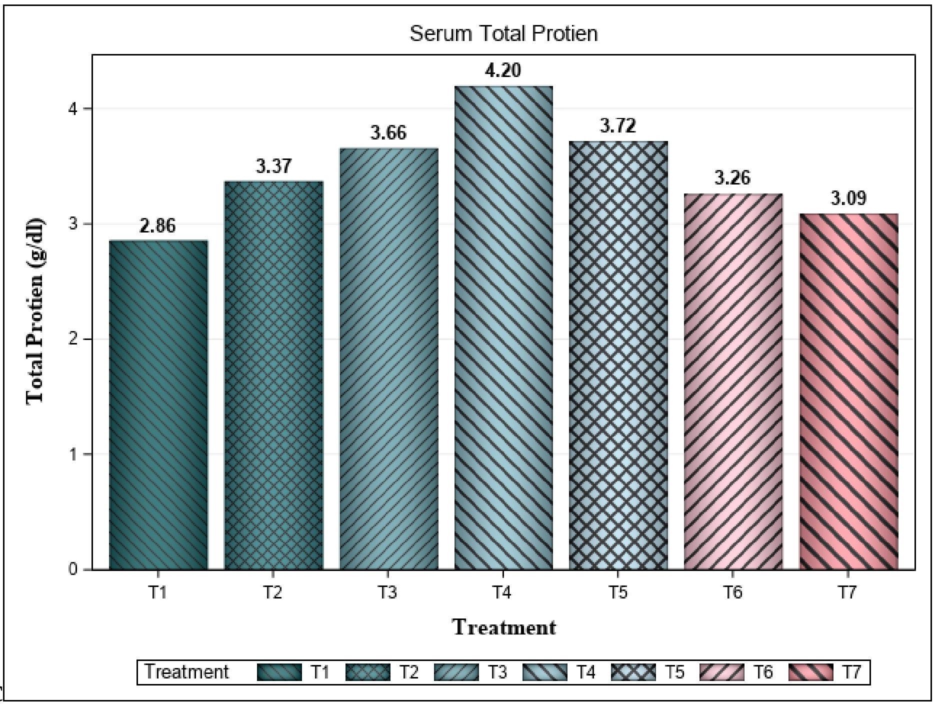
	Experimental Diet						
***Item**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Protein retained (g)	3.18 ± 0.00135^{c}	3.2 ± 0.195^{c}	3.57 ± 0.0654^{b}	4.11 ± 0.041^{a}	3.06 ± 0.0565^{c}	2.73 ± 0.0496^{d}	2.44 ± 0.106^{d}
Fat retained (g)	1.29 ± 0.0409^{ab}	1.1 ± 0.0488^{c}	1.21 ± 0.0204^{b}	1.37 ± 0.000684^{a}	1.11 ± 0.0118^{c}	0.99 ± 0.00449^{d}	0.89 ± 0.0156^{e}
Ash retained (g)	0.586 ± 0.0134	0.518 ± 0.0299	0.567 ± 0.0204	0.679 ± 0.0246	0.657 ± 0.0115	0.606 ± 0.0386	0.564 ± 0.0507
Energy retained (g)	30.1 ± 0.393^{bc}	28.4 ± 1.56^{c}	31.6 ± 0.562^{b}	36.1 ± 0.238^{a}	27.7 ± 0.43^{c}	24.8 ± 0.322^{d}	22.2 ± 0.748^{e}

*Means followed by different letters in each row are significantly different (P<0.05).

Table 6. Effect of dietary levels of Rosemary (*Rosmarinus officinalis*) leaves and Ginger (*Zingiber officinale*) rhizome with different concentrations in red tilapia fingerlings

	Experimental Diet						
***Item**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Total Protein (g/dl)	2.86 ± 0.095^{d}	3.37 ± 0.12^{bc}	3.66 ± 0.09^{b}	4.2 ± 0.08^{a}	3.72 ± 0.05^{b}	3.26 ± 0.14^{c}	3.09 ± 0.11^{cd}
Albumin (g/dl)	1.32 ± 0.1^{c}	1.54 ± 0.07^{bc}	1.65 ± 0.09^{ab}	1.88 ± 0.09^{a}	1.87 ± 0.09^{a}	1.39 ± 0.08^{bc}	1.24 ± 0.11^{c}
Globulin (g/dl)	1.54 ± 0.195^{b}	1.83 ± 0.05^{ab}	2.01 ± 0.16^{ab}	2.32 ± 0.01^{a}	1.85 ± 0.14^{ab}	1.87 ± 0.22^{ab}	1.85 ± 0.22^{ab}
Albumin/globulin ratio (A/G)	0.882 ± 0.177^{a}	0.841 ± 0.0153^{a}	0.821 ± 0.0448^{a}	0.811 ± 0.0423^{a}	1.02 ± 0.126^{a}	0.759 ± 0.132^{a}	0.687 ± 0.141^{a}
lysozyme (µg/ml)	1.52 ± 0.12^{d}	2.78 ± 0.04^{c}	3.25 ± 0.12^{b}	3.62 ± 0.09^{a}	3.23 ± 0.08^{b}	2.73 ± 0.04^{c}	2.48 ± 0.11^{c}
AST (u/l)	47.8 ± 0.66^{a}	47.4 ± 0.86^{a}	43.1 ± 1.52^{b}	40.8 ± 1.6^{b}	35.2 ± 0.41^{c}	36.6 ± 1.17^{c}	41.3 ± 1.44^{b}
ALT (u/l)	23.5 ± 0.86^{a}	22.5 ± 0.93^{ab}	21.6 ± 0.16^{ac}	19.7 ± 0.66^{c}	21 ± 0.08^{bc}	20.4 ± 0.12^{bc}	20.5 ± 0.88^{bc}

*Means followed by different letters in each row are significantly different (P<0.05)



C

Fig. 4. Serum total protein (g/dl) in growing red Tilapia as affected by medicinal plants

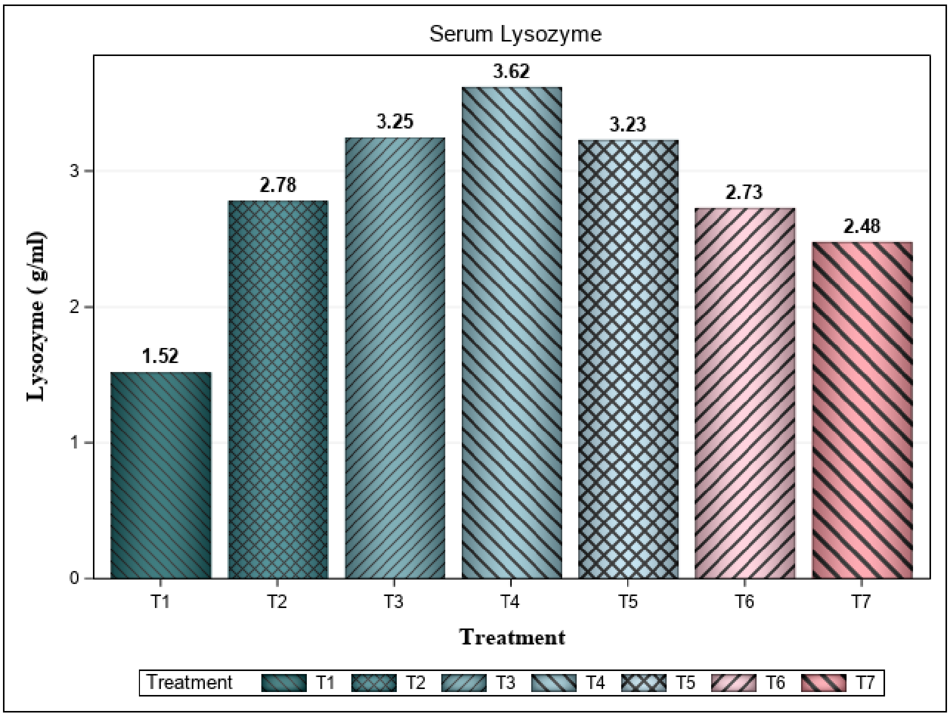


Fig. 5. Serum lysozyme (µg/ml) in growing red Tilapia (*Oreochromis* sp.) as affected by medicinal plants

However, feeding fish on T2 and control diets T1 lead to increase ($P \leq 0.05$) AST (47.4 and 47.8 respectively) while, it was noticed that the higher significant was recorded the control group which was the best treatment when compared with other groups in ALT (23.5u/l).

DISCUSSION

In spite of the medicinal uses of herbs, to date, there is still insufficient knowledge of the pharmacological properties and safe use of rosemary leaf powder for culturing tilapia against bacterial diseases. Many natural plant products have shown improved performance of fish when used as a dietary supplement **(Ayyat *et al.*, 2018; Ayyat *et al.*, 2020; Mohamed *et al.*, 2020)** .The current experiment used the dietary inclusion of Rosemary (*Rosmarinus officinalis*) leaves powder and Ginger (*Zingiber officinale*) rhizome in the diet with different concentrations. When compared to the control group, Rosemary leaves powder in the diets improved tilapia growth rates, feed consumption, and body composition (crude protein and ash %). On the opposite, adding Ginger rhizome in the diets did not improve the tilapia growth rates, feed consumption, and body composition (ash% and cp %). Moreover, the group fed 2 percent and 1.5 percent Rosemary leaves powder had improved growth performance and body composition compared with all groups. Several research on Nile tilapia, *O. niloticus* **(Ayoub *et al.*, 2019)**, sea bass (*Dicentrarchus labrax*) **(Yılmaz *et al.*, 2012)** and common carp (*Cyprinus carpio*) **(Yousefi *et al.*, 2019)** have found similar results when fish fed ditets containing Moringa (*Moringa oleifera*) and Rosemary (*Rosmarinus Officinalis*). Furthermore, **(Kubiriza *et al.*, 2019)** found that an arctic charr (*Salvelinus alpinus*) fish fed a rosemary-supplemented diet improved growth rates.

Some factors, such as fish species, feeding trial period, rosemary-supplemented form and level, could affect growth following the Rosemary-supplemented diet. **(Yousefi *et al.*, 2019)**. Though, dietary palatability and stimulated appetite led to increased feed intake **(Kubiriza *et al.*, 2019)**. Rosemary Leaves are also renowned for their usefulness in regulating nutrient absorption through the gastrointestinal tract **(Koga *et al.*, 2006)** ,because oxidative stress is one of the leading causes of intestinal mucosal damage and poor growth, **(Bhattacharyya *et al.*, 2014)**. **Mohamed *et al.* (2016)** showed that rosemary Leaves' strong antioxidant qualities may help to improve intestinal mucosal condition and hence growth. Rosemary (*Rosmarinus officinalis*) supplementation resulted in significant alterations in the WG, SGR, and PER in Nile Tilapia (*Oreochromis niloticus*) **(Hassan *et al.*, 2018)**. Though, adding 3% Rosemary leaf powder increased the final weight, WG, and SGR of common carp significantly **(Yousefi *et al.*, 2019).** Also, **Yılmaz *et al.* (2019)** stated that adding 0.1 percent, 0.25 percent, or 0.5 percent Rosemary extract to Tilapia (*Oreochromis* sp) diets had no effect on the fish meat's, growth performance, biometric indexes, or chemical composition. This may be due to the properties of Rosemary(*Rosmarinus officinalis*) is rich in carnosic and rosmarinic acid, compounds that were reported to have significant antioxidant features **(Thorsen and Hildebrandt, 2003; Erkan *et al.*, 2008).** In other studies, conducted with fish, it was seen that plants generally do not change the condition factor **(Dugenci *et al.*, 2003; Mesalhy *et al.*, 2008)**. Also, **Turan and Yigitarslan (2016)** reported that addition of Rosemary extract in ratios of 0.25% and 0.5% to the feed of carp (*Cyprinus carpio*) resulted in a weight increase and decreased FCR. This difference may be related to the variance in the diets of carnivorous and omnivorous fish.

Ginger (*Zingiber officinale*) is a powerful antioxidant that helps to inhibit the formation of free radicals **(Kim *et al.,* 2007)**. Ginger's phenolic components (shogaols, gingerols, volatile oils, phenolic ketone derivatives and flavonoids) promote antioxidant activity and inhibit lipid peroxidation **(Lebda *et al.,* 2012)**. Ginger promotes the production of pancreatic enzymes and bile from the liver, resulting in faster food digestion and intestinal bacteria balance **(Platel and Srinivasan, 2004)**. As a result, the consumption of energy improves, resulting in increased growth. Rhizome of the ginger plant (*Zingiber officinale*) includes a high concentration of proteolytic enzymes and lipolytic plants, which aid in the digestion of food protein and lipids **(Venkatramalingam *et al.,* 2007).**

In this study, Groups of fish fed 0.5%, 1% and 1.5% Ginger diets recorded the negative effect in body weight, weight gain and ADG when compared with other groups. These results didn't agreement with **Talpur *et al.* (2013)** .where as they stated that growth was dose-dependent and that the highest supplementation of Ginger (*Zingiber officinale*) at 5 and 10 g/kg feed was most beneficial for Asian sea bass growth and survival. While, these study in an agreement With **(Mahmoud *et al.,* 2019)** who discovered that there were no significant differences in final body weight (FBW) between experimental and control groups of fish, but that body weight gain (BWG) and specific growth rate (SGR) were significantly (p 0.05) lower in Nile Tilapia (*Oreochromis niloticus*) fish fed garlic and ginger (*Zingiber officinale*) powder supplemented diets compared to the control group. In addition, as compared to other experimental groups, the feed conversion ratio (FCR) of Nile Tilapia (*Oreochromis niloticus*) fish fed control basal diet improved. **Vahedi *et al.* (2017)** showed that the effects of adding dietary garlic and ginger on growth performance were mixed, and that this could be attributed to or depend on differences in fish species, size, age, sex, feeding programme, additive dose, diet precursors, fish nutritional/physiological status, and ambient culturing conditions. The increased growth after ginger (*Zingiber officinale*) supplementation can be linked to the host's increased release of intestinal proteases, which improves digestion and absorption of the feed's proteins components. Furthermore, ginger rhizomes, which are high in proteinase, aid in the digestion of proteins and amino acid absorption in the gastrointestinal tract **(Hashim *et al.,* 2011).**

Biochemical and haematological indicators are now widely utilised in aquaculture to assess a fish's growth and health **(Fazio *et al.,* 2013a; Fazio, 2019).** In previous investigations Total serum protein, globulins, albumins, ALT, AST, creatinine, and urea have all been measured assessed fish health. **(Fazio *et al.,* 2013b; Abd El-Rahman *et al.,* 2019)**. The addition of 1.5 percent RLP to the feed diet considerably boosted total protein and albumin in this study. These findings were investigated by **Yousefi *et al.* (2019)**, and it was found that the highest rosemary concentration in the feed diet had an influence on blood parameters, confirming the link between improved growth performance and rosemary's favourable effects on fish health. Continuing in the same vein, numerous studies give support to the obtained results presented here **(Bilen *et al.,* 2011; Akrami *et al.,* 2015; Hoseini *et al.,* 2018a; Hoseini *et al.,* 2018b)**

High levels of serum liver (AST and ALT) enzymes are indicative of cellular damage and leakage of hepatocellular membranes **(El-Moghazy *et al.,* 2014)**. The results of this study revealed that Rosemary Leaves Powder supplementation at 1% and 1.5 percent of the diet resulted in significant reductions in blood AST and ALT concentrations. **Naiel *et al.* (2019)** findings on tilapia supported our study results. Reduced serum liver enzymes of fish fed Rosemary Leaves Powder supplemented diets it is preventive action against liver illnesses related by high-fat diets, polluted diets, and conditions connected with environmental aquaculture stress could explain this **(Roncarati *et al.*, 2006)**.

The lysozyme is one of the most significant components of a fish's defensive system, and it works by activating the complement system and phagocytosis **(Magnadottir, 2006)**. Additionally, lysozyme possesses bactericidal activity **(Saurabh and Sahoo, 2008)**. **Ardo *et al.* (2008)** reported an increase in lysozyme activity in Nile tilapia fed *Astragalus membranaceus* and *Lonicera japonica* herbs separately or together after one week of feeding.

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الملخص العربي

تقييم الأعلاف غير التقليدية (أوراق نبات الحصالبان و ايزومات الزنجبيل) على أداء النمو، واستخدام الاعلاف والاستجابة المناعية لإصباعيات البلطي الأحمر (أوريوكروميس)

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أجريت الدراسة الحالية في مركز بحوث الاستزراع البحري، جامعة العريش، شمال سيناء، مصر. أجريت هذه الدراسة لتقييم تأثير إضافة مستويات مختلفة لعلائق الاسماك من أوراق الحصالبان وايزومات الزنجبيل على أداء النمو واستخدام العلف والاستجابة المناعية للبلطي الأحمر. تم توزيع 140 سمكة عشوائياً في 14 حوضا زجاجيا (120 لتر) لجميع التجارب بعدد عشرة أسماك لكل حوض بمتوسط وزن أولي 6.1 ± 0.2 جرام ومتوسط طول أولي 6.40 ± 0.5 سم. أجريت هذه الدراسة على سبع معاملات ولكل معاملة مكررتين. المجموعة الأولى كانت المجموعة الضابطة التي تغذت على العليقة الضابطة بدون إضافات، المجموعة الثانية والثالثة والرابعة تم تغذيتها على عليقة المقارنة المضاف اليها مسحوق أوراق الحصالبان الجاف (0.5%، 1% و1.5% على التوالي) تم تغذية المجموعة الخامسة والسادسة والسابعة على العليقة المضاف إليها مسحوق الزنجبيل الجاف (0.5%، 1%، 1.5% على التوالي) وتم تغذية الأسماك على علائق تحتوي على 30% بروتين خام مرتين في اليوم بمعدل 3% من وزن الجسم الكلي لمدة 14 أسبوعًا. في نهاية التجربة تم حساب وزن الجسم والطول والتحليل الكيميائي لكامل جسم اصباعيات البلطي الأحمر لتحديد معاملات النمو واستخدام العلف وبعض قياسات الدم. أشارت نتائج معاملات النمو إلى أن المجموعة رقم 4 سجلت أعلى قيم لمعظم معاملات النمو واستخدام العلف مقارنة بمجموعة التحكم. لكن المجموعة الضابطة سجلت أعلى معامل حالة (K) أظهرت قيم تكوين بروتين الدم ونشاط الليزوزيم اختلافات معنوية ($P < 0.05$) بين جميع مجموعات الأسماك. في الختام، نستنتج من ذلك انه من الممكن إضافة أوراق الحصالبان إلى علائق إصباعيات البلطي الأحمر لتحسين معدلات النمو والاستفادة من العلف والاستجابة المناعية وان اضافتها بالحدود المذكورة يكون سهل التطبيق وامن الاستخدام ومفيدا.

الكلمات الاسترشادية: البلطى الأحمر، أوراق الحصالبان، جذر الزنجبيل، اداء النمو، الاستفادة الغذائية، الاستجابة

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