

Effect of Herbal Plants “Thymus Vulgaris and Rosmarinus Officinale” on Growth Performance and Protection of *Oreochromis Niloticus* against *Aeromonas hydrophila* Infection.

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Abstract

The present study was carried out to investigate the effects of thyme and rosemary as feed additives on growth performance, cellular immunity, and protection of *Oreochromis niloticus* against *Aeromonas hydrophila*. Fish were divided into 3 groups and fed on three experimental diets (basal diet 0%, 1% thyme, and 1% rosemary). Fish received thyme and rosemary treated diets showed a significant increase ($P < 0.05$) in weight gain rate, specific growth rate, and average body weight at 2nd and 4th weeks. Feed conversion ratio revealed significant increase at 4th weeks for thyme treated group compared to the control. Intestino-somatic index was significantly increased ($P < 0.05$) in thyme fed group at 6th weeks while Hepato-somatic index exhibited significant increase ($P < 0.05$) at 2nd weeks post feeding for rosemary treated fish. Blood parameters showed that total erythrocytes, packed cell volume, hemoglobin concentration, total leucocytes and differential leukocytic count were significantly increased in thyme treated group at 6th weeks. Fish fed on rosemary-supplemented diet revealed significant increase in total leukocytic counts at 4th and 6th weeks, while differential leukocytic count had significant increase along the experimental period. Serum lysozyme activity was significantly increased in thyme fed group along the whole period and in rosemary treated group at 2nd and 4th weeks in relation with control. Nitric oxide was significantly lower in thyme fed group than the control group. Antioxidant enzymes Superoxide Dismutase, Catalase and Glutathione Reductase revealed significant increase ($P < 0.05$) with significant decrease of malondialdehyde in thyme treated group in relation with control. Challenge infection by *A. hydrophila* showed highly significance protection with survivable rate of 90 % in groups fed with rosemary and thyme for 6 weeks. The results suggested that thyme and rosemary can be recommended as a supplementary for *O. niloticus* increasing the protection against *A. hydrophila*.

Keywords: Thyme, Rosemary, Growth performance, Immunity, Nile tilapia, *Aeromonas hydrophila*.

1. Introduction

In aquaculture bacterial diseases are the cause of significant economic losses in tilapia farms and the main mean for controlling of it, is antibiotics but using antibiotics as feed additive or therapeutics lead to appearance of resistant bacterial strain and accumulation of residues in fish and environment may reach to human [1]. On the other hand, vaccination has some disadvantages as difficult application, more labor required, high cost and impossible to develop pro-active prophylactic strategies in new diseases emerging from time to time [2]. Therefore, the application of herbal plant products as plant extract is more useful as they are safe and eco-friendly, directly taken by humans as food or medicine. They act as a substitution for feed, growth promoter, immune-stimulant, antimicrobial and as fertilizer in aquaculture with no adverse effect on natural ecosystem [3]. Thyme is used since ancient times, in the kitchen, cosmetics and for medicinal purposes. It includes thymol (44-60%) an essential oil having strong antiseptic properties, rich in antioxidants, potassium, magnesium and vitamins A, C and E [4]. The therapeutic properties of thyme in aquaculture includes anesthetic, antioxidant, digestion stimulant, antibacterial and immune

enhancement [5;6]. The carnosic acid and rosmarinic acid are the main chemical constituents of rosemary and they have antioxidant and antimicrobial activity [7]. The use of thyme and rosemary increase the protection against *Streptococcus agalactiae*, *S. iniae* and *A. hydrophila* in *O. niloticus* [8], their use enhance the health condition of sea bass [9] and improved the growth performance, disease resistance and immunity of *O. mossambicus* [10] and *O. niloticus* [11]. The aim of this study was to evaluate the effect of thyme and rosemary on the growth performance, Immuno-hematological parameters, antioxidant activity and the protection of *Oreochromis niloticus* against *Aeromonas hydrophila* infection.

2. Materials and methods

2.1 Fish

Nile tilapia, *O. niloticus* weighted (18±1.5 g) and average body length (11±0.5 cm) were obtained from Private fish farm at Kafr EL-Sheikh Governorate, Egypt and transported in double walled polyethylene bags to the wet lab at Faculty of Veterinary M-edicine Benha University. The health conditions of fish were examined for any disease condition (parasitic, bacterial) as described by Austin and Austin [12]. Fish was placed in well-

prepared fiberglass (750L) tanks filled with de-chlorinated water. The water temperature was adjusted to $25\pm 2^{\circ}\text{C}$ and the oxygen level was maintained at optimal level using aerators. Fish was fed basal diet at a rate of 3% body weight twice daily. The uneaten food and excreta were siphoned and water exchange of about its third volume was done daily.

2.2 Preparation of experimental diets

Herbal plants (*Thymus vulgaris*) and (*Rosmarinus officinales*) were purchased from El-abad hypermarket (El Khateep spices department), Toukh, El-Kalubia Governorate. The dry whole plant of thyme and rosemary were grinded to fine powder by electrical blinder. The basal diet was divided into three parts, the first part was kept as control diet where the 2nd part was incorporated with thyme 1%, and remaining part was supplied with 1% rosemary. Suitable amount of water was added to them to form wet dough then pelleted, kept to dry at room temperature, then packed in clean dry plastic container and kept tightly closed at 4°C .

2.3 Feeding experiment

Oreochromis niloticus were acclimated to lab conditions for two weeks. Fish then divided into three groups, one control and two treated groups in two replicates. The control group was fed on basal diet and two treated groups were fed on the basal diet incorporated with thyme and rosemary at a rate of 1% respectively. Fish was fed twice daily at a rate 3% of body weight for a period of six weeks from start of feeding.

2.4 Determination of growth performance and somatic indices

2.4.1 Determination of growth parameters

Sample of Ten fish were taken from each group in two replicates at the end of 2nd, 4th and 6th weeks post feeding for determination the growth performance parameters. Weight Gain Rate (WGR), length Gain Rate (LGR), Feed Conversion Ratio (FCR), and Feed Efficiency Ratio (FER) were calculated according to Bo liu *et al.*, [13]. Moreover, Specific Growth Rate (SGR) was determined as described by Laird and Needham [14]. Total body weight gain and average daily gain were also estimated according to Jauncey and Ross [15].

2.4.2 Determination of bio-somatic indices

At the end of 2nd, 4th and 6th weeks from start of feeding, samples of ten fish from each group in two replicates were used. Liver, spleen, and intestine were taken after de-fatting. The Hepato-somatic (HSI) and Spleno-somatic (SSI) indices were calculated according to Yun *et al.*, [16] while

Intestino-somatic index (ISI) was estimated according to Zhang *et al.*, [17].

2.5 Determination of hematological parameters

At the end of 2nd, 4th and 6th weeks blood samples were taken from control and treated groups. Blood was withdrawn from the caudal blood vessels in two portions; one with anticoagulant for measuring blood parameters and the second portion without anticoagulant for separation of serum by allowing the blood to clot at room temperature. Then the tubes were centrifuged at $3000 \times g$ at 4°C for 15 min, the serum was collected and stored at -20°C . Blood elements (RBCs and WBCs) were counted according to Kanaev [18] using Neubauer-improved haemocytometer (Neubar, improved, Germany). Hemoglobin concentration was measured using the cyanomethoglobin method and differential leukocytes count (DLC) was carried out according to Stoskopf [19]. The Packed cell volume (PCV %) and blood indices (MCV, MCH and MCHC) were estimated after the method described by Dacie and Lewis [20].

2.6 Determination of non-specific immune-parameters

Pooled serums were used for estimation of lysozyme activity according to Schlitz [21] and serum nitric oxide was assessed according to Rajaraman *et al.*, [22].

2.7 Determination of antioxidant enzymes activity

For determination of antioxidant enzymes activity, weighted liver tissues from thyme treated groups were homogenized using cool phosphate buffer saline (PBS) with PH (7.4), centrifuged at 4°C at $4000 \times g$ for 15 min and keeping the supernatants at -20°C , where Superoxide Dismutase (SOD) and Catalase (CAT) were measured according to Fossati *et al.* [23]. Glutathione Reductase (GSH-Rx) and Malondialdehyde (MDA) were estimated according to Satoh [24] using commercial kits (Bio-diagnostic, Egypt).

2.8 Challenge infection

A. hydrophila pathogenic strain was obtained from fish diseases and management department Faculty of Veterinary Medicine, Benha University, the bacterium was grown overnight in tryptone soy broth at 28°C , then centrifuged at $3000 \times g$ for 10 minute at 4°C ; the pelleted cells were washed twice and resuspended in sterile Physiological saline and adjusted to 1.5×10^8 cell/ml⁻¹ by spectrophotometer (620 nm).

At the end of 2nd and 4th and 6th weeks post feeding, ten fish (in two replicates) from each treated and control groups were intra-peritoneally

(IP) injected with 0.2 ml suspension of *A. hydrophila* in 0.9 % saline (W/V). Mortalities were monitored over 2 weeks. Clinical signs and post mortem findings in dead and moribund fish were recorded.

2.9 Statistical Analysis

The data was analyzed by one-way analysis of Variance (ANOVA) and Duncan's multiple range tests to determine significant differences between groups using the statistical package for the social sciences (SPSS) software (Version 17.0). A value of $P < 0.05$ was considered significant. Antioxidant enzymes activity was analyzed by independent-sample T. Test.

3. Results and discussion

3.1 Effect of dietary thyme and rosemary on growth performance of *O. niloticus*

The current study showed significant increase in average body weight (ABW), weight gain rate (WGR), weight gain (WG), specific growth rate (SGR), length (L) and length gain rate (LGR) along the whole periods in *O. niloticus* fed on thyme, while groups fed on rosemary revealed a little positive effect on growth performance Table (1). Similar enhancement in growth parameters of Nile tilapia fed on thyme treated diet than other groups fed on rosemary supplied diet were recorded [25]. In addition, Yilmaz *et al.*, [9] observed an increase in FCR of sea bass fed on 1% thyme than fish fed on 1% rosemary treated diet and Dorojan *et al.*, [26] recorded an improvement in growth performance of Stellate Sturgeon fed with thyme. These results also came in accordance with Zaki *et al.*, [27] who recorded significant increase of WGR and improving of the tissue nature in tilapia fed on thyme. In the same respect, Marzouk *et al.*, [28] recorded significant increase of growth and hematological parameters in Nile tilapia fed on barely and onion supplemented diets. Moreover, using of HBP enhanced the weight gain and other growth performance parameters of Nile tilapia [29]. These observations may be due to stimulation of pancreatic enzymes secretion that necessary in nutrient digestion and adsorption [30] or presence of active compounds of thyme which act as antioxidants and hence decrease the action of stressors and enhance the aquatic welfare around the fish. Also other elements such as potassium, magnesium, ferrous, vitamins A, C and E in thyme are essential for fish growth [4].

3.2 Effect of dietary thyme and rosemary on bio-somatic indices of *O. niloticus*

The bio-somatic indices are considered as environmental stress indicators of fish [31]. The results in the current study revealed significant increase of intestino-somatic index in fish received

thyme-supplemented diet for 6 weeks and significant increase of HSI in group fed rosemary treated diet for 2 weeks Table (2). In the same respect, significant increase of ISI in *O. niloticus* fed on 2% ginger [32] and HBP [29] were recorded. Moreover, a little positive effect on bio-somatic indices of African catfish fed with rosemary extract was observed by Funda and Yiğitarlan [33]. The increase of intestino-somatic index may be due to an increase in thickness of intestinal tract villi [34], while the increase of HSI could be attributed to hypertrophy and hyperplasia of liver cell in fish exposed to stress [35].

3.3 Effect of dietary thyme and rosemary on haematological parameters of *O. niloticus*

Haematological parameters are an important index of the health status of fish, hence infection, stress and nutritional deficiency are always accompanied with low level of them [36;37]. The present work showed enhancement of RBCs, PCV and Hb for *O. niloticus* fed on thyme and significant increases in leukocytes counts and differential leukocytic counts in both treated groups compared with control Table (3). These results were supported by Gültepe *et al.*, [25] who recorded significant increase of haematological parameters in fish fed on 1% thyme and rosemary and by Zaki *et al.*, [27] who showed positive effect on blood parameters in tilapia fed on 1% thyme seeds meal. In addition, Marzouk *et al.*, [28] recorded significant increase in WBCS count in tilapia fed on herbal supplemented diet. The red blood cells indices (MCV and MCH) have a wide range of physiological variation and not accurate for assessment the hematological status of fish due to manual RBC count lacks the precision necessary for the accurate assessment for calculating accurate MCH values [38]. Also Blaxhall and Daisley [39] recorded that PCV, Hb and MCHC may be better parameters for the assessment of blood status in fish than red blood cell indices (MCV and MCH). Our work revealed that rosemary treated group showed slight significant increase in MCH at 4th weeks compared to the control. Meanwhile, the red blood indices (MCV, MCH and MCHC) showed non-significant value in thyme treated group along the whole period Table (3). In the same manner, Ahmadifar *et al.*, [40] and Yilmaz *et al.*, [41] recorded non-significant values in MCV, MCH and MCHC in trout fed with carvacrol treated diets compared to the control. The increases in these values may be due to macrocytic anemia, while the decreases are considered as detection of hypochromic anemia [42].

3.4 Effect of dietary thyme and rosemary on non-specific immunity of *O. niloticus*

Immunological parameters (non-specific) and (adaptive-immunity) are considered the two wings of fish to resist any infection and any suppress of it led to diseases occurrence, from this parameters lysozyme activity and peroxidase activity [43]. Lysozyme is one of the most important immunity factor used by fish to resist pathogenic infection [44]. In the present study, fish fed with thyme and rosemary showed significant increase ($P < 0.05$) of lysozyme activity in first 4 weeks post feeding Table (4). Nearly similar findings were observed by Zaki *et al.*, [27] who recorded significant increase of lysozyme activity in tilapia fed on thyme seed meal and by Giannenas *et al.*, [45] who revealed significant increase of lysozyme activity in trout fed on carvacrol and thymol treated diets. In addition, Bilen *et al.*, [46] reported increase of lysozyme activity of trout fed on 1% tetra (*Cotinus coggyria*). The increase of lysozyme activity may be due to increase of the neutrophils and lymphocytes and hence their secretion (lysozymes and hydrolytic enzymes). Nitric oxide is an intracellular mediator produced in various live cells if exposed to stress factors, while the unregulated production of nitric oxide can cause nitrosative stress, leading to damages of proteins/DNA, cell injury and death. NO concentration in serum can be used as an inflammatory marker for disease status and progression [47]. The present study showed reduction of NO production in Nile tilapia fed on 1% thyme along the whole time of experiment and at 6th week in rosemary fed group Table (4). This result coincide with result of Giannenas *et al.*, [45] who revealed significant decrease of NO in trout fed with thymol treated diets and Guerreiroa *et al.*, [48] who observed lower NO level in sea bream fed with galactooligosaccharides diet. These findings could be attributed to the scavenger action of anti-oxidant compounds of thyme against any free radical and due to increase of macrophage and neutrophils cells count with humeral acidic secretions and their peroxidase pathways as inhibitory precursors that prevent synthesis of any harmful and un stable compounds in live tissues [49].

3.5 Effect of dietary thyme on antioxidant enzymes activity of *O. niloticus*

In aquaculture, antioxidants play two key roles. 1st role is, they protect the lipid in the diet from oxidative damage and the 2nd role is protective to living tissues from destructive action of free radicles produced during metabolic process by scavenger actions [50]. Malondialdehyde is a marker of oxidative stress and considered as one of toxic byproduct of polyunsaturated fatty acid peroxidation leading to membrane and DNA

damage [51]. Antioxidant enzymes including SOD, CAT and GSH-RX are representing the first line of defense against oxidative stress [52]. The present study exhibited significant increase of antioxidant enzymes, GSH-RX, SOD, CAT and significant decrease of MDA level in thyme treated groups Table (5). Several studies have been recorded decrease in MDA levels and significant increase of SOD and CAT as in rainbow trout fed on herbal treated diet [53], trout fed on thyme oils [54] and Nile tilapia fed on immunostimulant [55]. Moreover, Xie *et al.*, [56] observed higher hepatic catalase, SOD and GSH-RX in common carp fed Chinese herbal plant. The increase in antioxidant enzymes activities could be attributed to increase the neutrophils as secretory cell of thus compounds, and ability to prevent free radical to destruct the other cells which appeared in low level of MDA. This indicated that dietary thyme activates the antioxidant enzymes, which provide tissue protection and eliminate free radicals. This was supported by the fact that when MDA appeared lower than normal level indicated good fish health condition [57].

3.6 Challenge infection

Herbs are rich sources of immune-enhancing substances, herbal Immunostimulant in contrast to vaccines; it can be modulating the innate or non-specific immune response. Herbal plant extracts have anti-bacterial activity against *Aeromonas hydrophila* and *Pseudomonas* [58]. The present study revealed that *O. niloticus* fed on 1% thyme and rosemary showed reducing in mortality rates (10%) after 6th weeks feeding compared with (90%) mortality for controls Fig (1). These observation supported by result of Gültepe *et al.*, [25] and Ergün *et al.*, [10] on *O. mossambicus* fed on thyme and rosemary incorporated diets which exhibited reduction in cumulative mortality in treated groups. Rosemary also increases resistance against *Streptococcus agalactiae*, *S. iniae* and *Aeromonas hydrophila* in *Oreochromis sp.* [8] and *O. niloticus* [59]. Similarly high level of protection against *A. hydrophila* was reported in Nile tilapia fed HBP [29] and fructooligosaccharides [60]. In the same respect an increase of the resistance against *Vibrio alginolyticus* infection was observed in Nile tilapia fed on Curcumin [61] and *Vibrio harveyi* in Asian sea bass fed on ginger containing diet [62]. They attributed this improvement in protection and reduction in mortalities to enhancement in cellular, humeral immune parameters and increases of antioxidant enzymes activities [63, 64, 65].

4. Conclusion

Dietary supplementation of thyme and rosemary enhance the cellular immunity and beneficial for controlling *A. hydrophila* infection in *O. niloticus*.

Thyme improves growth performance of fish, while rosemary has a little effect on growth of Tilapia fish.

Fig (1) Mortality % of *O. niloticus* treated with thyme and rosemary following infection with *A. hydrophila*

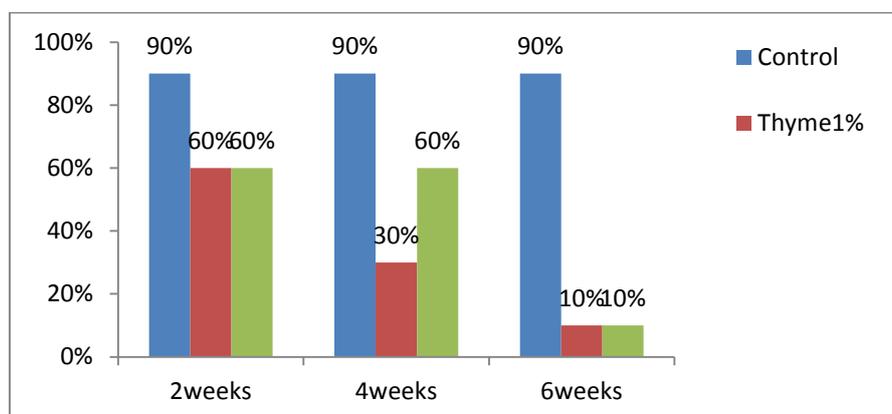


Table (1) Effect of dietary supplementation of thyme and rosemary on growth performance of *O. niloticus*.

Groups LGR	W0	ABW	WGR	WG	SGR	FCR	FER	L0	L	
2weeks										
Control	18.71±0.29	26.38 ^a ±0.12	41.2 ^b ±1.55	7.67 ^b ±0.17	2.46 ^b ±0.08	2.705±0.11	0.37±0.02	11.24±0.035	11.36 ^b ±0.01	1.16 ^b ±0.45
Thyme1%	18.74±0.12	28.27 ^a ±0.24	50.95 ^a ±2.16	9.55 ^a ±0.35	2.94 ^a ±0.1	2.33±0.08	0.43±0.01	11.19±0.02	12.04 ^a ±0.01	7.59 ^a ±0.19
Rosemary1%	18.77±0.20	28.45 ^a ±0.01	51.63 ^a ±1.58	9.68 ^a ±0.19	2.98 ^a ±0.08	2.315±0.07	0.44±0.01	11.25±0.02	12.09 ^a ±0.09	7.46 ^a ±0.61
4weeks										
Control	18.71±0.29	34.54 ^c ±0.00	84.65 ^c ±2.86	15.83 ^c ±0.29	2.19 ^b ±0.06	3.44 ^b ±.15	0.29±0.01	11.24±0.035	12.81 ^b ±0.05	13.98 ^b ±0.23
Thyme1%	18.74±0.12	38.77 ^a ±0.02	106.98 ^a ±1.15	20.04 ^a ±0.1	2.6 ^a ±0.02	3.04 ^a ±0.03	0.33±0.01	11.19±0.02	13.1 ^a ±0.02	17.46 ^a ±0.44
Rosemary1%	18.77±0.20	36.83 ^b ±0.06	96.31 ^b ±2.46	18.06 ^b ±0.26	2.41 ^a ±0.05	3.22 ^{ab} ±0.07	0.31±0.01	11.25±0.02	12.8 ^b ±0.02	13.92 ^b ±0.34
6weeks										
Control	18.71±0.29	38.42 ^b ±0.19	105.39 ^c ±4.23	19.71 ^b ±0.49	1.71 ^b ±0.05	4.6±0.16	0.22±0.01	11.24±0.02	13.18 ^b ±0.05	17.31 ^b ±0.08
Thyme1%	18.74±0.12	43.45 ^a ±0.25	131.97 ^a ±2.74	24.72 ^a ±0.37	2.01 ^a ±0.03	4.15±0.06	0.24±0.01	11.19±0.02	13.96 ^a ±0.02	24.71 ^a ±0.36
Rosemary1%	18.77±0.20	38.96 ^b ±0.02	107.64 ^b ±2.36	20.12 ^b ±.23	1.74 ^b ±0.03	4.56±0.1	0.21±0.01	11.25±0.02	13.25 ^b ±0.02	17.78 ^b ±0.39

Values (means± SEM) with different letters in the same column are significantly different ($P < 0.05$, n=10)/replicate.

Table (2) Effect of dietary supplementation of thyme and rosemary on Bio-somatic indices of *O. niloticus*.

Groups ISI	HSI	SSI
2weeks		
Control		2.8 ^b ±0.02
Thyme1%		2.78 ^b ±0.05
Rosemary1%		3.13 ^a ±0.07
4weeks		
Control		2.95±0.03
Thyme1%		3.12±0.19
Rosemary1%		2.8±0.2
6weeks		
Control		3.28±.08
Thyme1%		3.02±0.05
Rosemary1%		3.1±0.11

Values (means± SEM) with different letters in the same column are significantly different ($P < 0.05$, n=10)/replicate.

Table (3) Effect of dietary supplementation of thyme and rosemary on hematological parameters of *O. niloticus*.

Groups	RBCs	PCV%	Hb g/dl	MCV	MCH	MCHC	TLC	Monocytes	Lymphocytes	Neutrophil	Basophil	Eosinophil
2 weeks												
Control	3.37 ^a ±0.07	29.20 ^a ±0.58	8.54 ^a ±0.12	86.66±0.54	25.36±0.22	29.26±0.18	20.16±1.93	22.60 ^a ±0.51	39.20 ^a ±0.66	25.60 ^a ±0.93	0.60±0.24	0.60±0.24
Thyme1%	3.04 ^a ±0.13	26.20 ^a ±1.11	7.60 ^a ±0.34	86.63±0.63	24.68±0.44	29.0±0.71	19.72±1.59	25.80 ^a ±0.49	44.20 ^a ±0.37	27.00 ^a ±0.45	0.40±0.24	0.40±0.24
Rosemary1%	2.60 ^a ±0.05	22.00 ^a ±0.316	6.62 ^a ±0.22	84.68±0.96	25.09±0.54	30.07±0.76	19.98±1.45	24.80 ^a ±0.37	42.80 ^a ±0.37	28.00 ^a ±0.32	0.40±0.24	0.40±0.24
4 weeks												
Control	3.32 ^a ±0.15	28.40 ^a ±1.33	8.23±0.44	85.52 ^a ±0.32	24.75 ^a ±0.23	28.94±0.26	17.8 ^a ±1.86	23.00 ^a ±0.71	40.80 ^a ±0.66	26.40 ^a ±0.51	0.60±0.24	0.60±0.24
Thyme1%	3.49 ^a ±0.11	29.00 ^a ±0.89	8.54±0.25	83.23 ^b ±0.31	24.51 ^b ±0.11	29.46±0.13	14.99 ^b ±0.17	25.20 ^a ±0.37	45.00 ^a ±0.45	27.80 ^a ±0.37	0.40±0.24	0.40±0.24
Rosemary1%	2.96 ^a ±0.13	25.60 ^a ±0.87	7.42±0.43	86.68 ^a ±1.69	25.99 ^a ±0.27	29.91±0.72	21.8 ^a ±0.85	24.80 ^a ±0.49	43.80 ^a ±0.58	28.60 ^a ±0.24	0.40±0.24	0.40±0.24
6 weeks												
Control	3.41 ^b ±0.11	30.20 ^b ±0.58	8.77 ^b ±0.20	86.66±0.54	25.74±0.24	29.02±0.19	21.63 ^b ±0.44	24.00 ^a ±0.32	39.00 ^a ±0.71	23.60 ^a ±0.81	0.60±0.24	0.60±0.24
Thyme1%	4.23 ^b ±0.13	36.40 ^b ±1.12	10.84 ^b ±0.38	85.77±0.32	25.61±0.27	29.77±0.39	30.55 ^b ±0.58	25.80 ^a ±0.37	42.60 ^a ±0.51	28.20 ^a ±0.66	0.40±0.24	0.40±0.24
Rosemary1%	3.48 ^b ±0.26	31.20 ^b ±2.03	8.74 ^b ±0.79	85.54±0.69	25.01±0.38	29.24±0.36	31.02 ^b ±0.85	25.2 ^a ±0.37	44.60 ^a ±0.51	27.40 ^a ±0.51	0.40±0.24	0.40±0.24

Values (means± SEM) with different letters in the same column are significantly different ($P < 0.05$, n=5)/replicate.

Table (4) Effect of dietary supplementation of thyme and rosemary on lysozyme and nitric oxide of *O. niloticus*.

Groups	Lysozyme activity	Nitric oxide	
		2weeks	
Control		157.00 ^b ±1.00	6.33 ^a ±1.33
Thyme1%		177.90 ^a ±0.001	2.23 ^b ±0.00
Rosemary1%		177.9 ^a ±0.001	8.57 ^a ±0.70
4weeks			
Control		178.33 ^b ±0.33	26.67 ^a ±0.67
Thyme1%		200.04 ^a ±0.002	21.90 ^b ±0.80
Rosemary1%		200.04 ^a ±0.001	28.57 ^a ±0.73
6weeks			
Control		207.3 ^a ±7.33	41.67 ^a ±0.33
Thyme1%		200.04 ^a ±0.00	16.70 ^c ±0.00
Rosemary1%		177.90 ^b ±0.00	28.87 ^b ±2.07

Values (means± SEM) with different letters in the same column are significantly different ($P < 0.05$, n=3)/replicate

Table (5) Effect of dietary supplementation of thyme on antioxidant enzymes activity of *O. niloticus*.

Groups	SOD	CAT	GSH-Rx	MDA
Control	1086.10 ^b ±0.06	3.80±0.30	17.57 ^b ±0.27	355.00±0.001
Thyme1%	2120.35 ^a ±0.20	2.74±0.56	85.43 ^a ±0.25	509.19±0.11
4weeks				
Control	1362.07±0.03	3.13±0.23	67.20 ^b ±0.25	375.20 ^b ±0.10
Thyme1%	1155.06±0.06	1.78±0.12	109.68 ^a ±3.11	118.50 ^a ±0.002
6weeks				
Control	1224.07±0.03	1.47 ^b ±0.17	87.20 ^b ±4.95	493.90 ^b ±7.81
Thyme1%	1224.09±0.05	2.13 ^a ±0.047	113.55 ^a ±0.03	458.20 ^a ±0.11

Values (means± SEM) with different letters in the same column are significantly different ($P < 0.05$, n=3)/replicate

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