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EFFECTS OF SOME HERBAL PLANT SUPPLEMENTS ON GROWTH PERFORMANCE AND THE IMMUNE RESPONSE IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

Using of natural plants as immunostimulant in fish is more useful than antibacterial drugs that cause adversely side effects for fish, environment and consumers. Therefore, the present study was to investigate the effects of dietary turmeric (Curcuma longa), rosemary (Rosmarinus officinalis) and thyme (Thymus vulgaris) as feed additives on growth performance, proximate composition and some immuno-hematological parameters on Nile tilapia. Four nitrogenous (31.43-31.56 % crude protein) and isocaloric (17.90-18.04 kJ/g) diets were formulated to contain 0% (control), 1% of turmeric, rosemary or thyme, respectively. The results reveled that, supplementation with medicinal herbs significantly increased (P<0.05) weight gain, specific growth rates and protein efficiency ratio (PER) compared to those in the control group. Moreover, the medicinal herb additives had insignificant effect on carcass composition and some biochemical composition. Hematocrit and leukocrit levels in the fish fed with turmeric, rosemary and thyme added diet were found to be significantly (P <0.05) higher than the control group. However, leukocyte level among the experimental groups was not significantly differed (P >0.05). In neutrophils of experimental fish groups, there were higher numbers of NBT (+) cells fixed on lamellas than those of the control group. So, based on the current findings together with the low cost and the immunostimulative effect of turmeric, rosemary and thyme it can be recommended to be used in fish feed to minimize the mortalities caused by some pathogens.

Keywords: *carcass composition, growth, immuno-hematologic, medicinal herbs, Nile tilapia.*

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (L.) is an important species for freshwater aquaculture. Improving fish performance and disease resistance of cultured organisms are major challenges facing fish culturists (Nicolae et al., 2016). Intensive Nile tilapia culture usually requires balanced diets which are formulated with several ingredients, especially additives that have effectiveness of fish dynamic and physiological functions.

Medicinal plants have different activities such as antistress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and antimicrobial properties due to the active principles such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids and essential oils. Since many of their derivatives are known as growth stimulants and/or immunostimulant agents, the use of these compounds has increased in finfish and shellfish diet in recent decades. In the aquaculture sector, the use of medicinal plants (phytochemicals) has increased significantly over the past decade for different purposes such as sex reversal compound Gholipour et al, 2011), growth enhancer (Asadi et al., 2012), immune-stimulant, and antipathogenic (Yılmaz et al., 2013). One of the relatively new practiced ways to improve health conditions for cultivated aquatic organisms is using a

medicinal herb as an immune-stimulator or growth enhancer (Citarasu, 2010).

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae* (Chan et al., 2009). It is native in southeast India. Curcumin, the active ingredient from the spice turmeric is a potent antioxidant (El-Bahr et al., 2007). It is also strongly alleged that turmeric can improve digestion and nutrient metabolism. Turmeric feeding may elevate the non-specific immune system and give long term of protection (Sahu et al., 2008). Sivagurunathan et al., (2011) enhanced the nonspecific immune response of *Cirrhinus mrigala* by feeding diet containing *Zingiber officinale* and *Curcuma longa* when exposed to *Pseudomonas aeruginosa*.

Thyme has strong antimicrobial and antioxidant activity due to its very high contents of thymol, p-cymene, carvacrol, eugenol and 4-allylphenol (Rota et al., 2008). Carnosic acid and rosmarinic acid are the main chemical constituents of rosemary, and they are particularly high antioxidants (Erkan et al., 2008).

The objective of this study was to determine the effects of turmeric, rosemary and thyme on growth performance, proximate composition and some immuno-haematological parameters on Nile tilapia.

MATERIALS AND METHODS

Experimental Fish and Culture Technique Nile tilapia (*Oreochromis niloticus*) fingerlings with an initial body weight of 21.7 ± 0.2 g were obtained from Arab Fisheries Hatchery, El-Khashah, Kafer El-Shekh Governorate, Egypt. The fingerlings were stocked into four cement ponds (each with 45 m3) at private farm in Nubaria city, Beheira Governorate, Egypt. Each cement pond was divided into five equal compartments (net pens) by netting (each of 9 m3) and each pen was stocked with 50 fish. Three replicate pens were randomly assigned to each treatment. Prior to the start of experiment, fish were acclimated to the experimental conditions for two weeks, during this period fish were fed a control diet at 3% of body weight. The daily ration was divided into two equal portions and fed two times a day (08:00 and 15:00). The fish were fed one of four experimental diets for 140 days (20 weeks). The cement ponds were supplied with freshwater from the Naser Irrigation Branch, Beheira Governorate where the water turnover rate was 0.3 m3 pond day-1 and fish were held under natural light (12:12 light: dark schedule).

Water temperature, dissolved oxygen, pH, and ammonia were monitored during the study, to maintain water quality at optimal range for Nile tilapia. Water temperature was recorded daily at 13:00 using a mercuric thermometer suspended at 30 cm depth. Dissolved oxygen (DO) was measured at 05:00 using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH at 09:00 by using pH meter (Orion pH meter, Abilene, Texas, USA). Ammonia was measured three times a week according to APHA (1985)

Experimental herbs and diets

Turmeric (*Curcuma longa*), rosemary (*Rosmarinus officinalis*) and Thyme (*Thymus vulgaris*) were obtained from a local market. The herbs were added to the feed at 1%. Additionally, a control group was fed a diet without herbal supplementation. Four experimental diets were formulated to supply 32% crude protein and 3000 kcal digestible energy (DE)/kg diet according to NRC, 1993. The feed components of the diets are presented in Table 1. The ingredients were finely ground and mixed. About 400 ml of cold water/kg diet was added to obtain stiff dough. The obtained dough was passed through (2 mm) die of a meat mincer, pelleted then air dried by electric fan at room temperature for 24 h. The pellets were packed in plastic bags and refrigerated at 4 ºC until use.

Energy calculated according to 23.6 kJ g-1 protein, 39.5 kJ g-1 lipid, and 17.0 kJ g-1 NFE

The ingredients were mixed in a blender and the mixture was then pressed through a 2-mm die in a pelleting machine. The pellets were dried in a drying cabinet (40°C) until moisture dropped well around 10% and stored in bags in a deep freeze at -20°C until used.

Growth performance and proximate composition

Growth performance and feed utilization were calculated according to the formulae given below. Proximate analyses of the diets and fish

fillets were performed using standard methods. Dry matter was analysed by drying at 105°C in an oven to a constant weight, crude fat by Dietheyl ether extraction, crude protein by the Kjeldahl method, and crude ash by incineration at 525°C in a muffle furnace for 12 h.

Table 1. Ingredient and composition of experimental diets

Ingredients (%)	Control	Turmeric	Rosemary	Thyme
Fish meal	27	27	27	27
Soybean meal	12	12	12	12
Ground corn	36	36	36	36
Wheat bran	13	12	12	12
Corn gluten meal	8	8	8	8
Vegetable oil	2	2	2	2
Vitamins & minerals premix (1)	0.7	0.7	0.7	0.7
NaCl	0.3	0.3	0.3	0.3
Turmeric	0	1	0	0
Rosemary	0	0	1	0
Thyme	0	0	0	1
Chemical analyses (%)				
Dry matter	91.64	91.89	91.77	91.82
Crude protein	31.56	31.49	31.51	31.43
Crude fiber	3.06	3.11	3.09	3.14
Crude fat	6.46	6.33	6.38	6.31
Ash	11.65	12.18	11.89	11.97
NFE	47.27	46.89	47.13	47.15
Energy (kJ/g)	18.04	17.90	17.97	17.93

Data analysis

Growth performance was determined and feed utilization was calculated as following:

(1) Daily weight gain (DWG, g) = [mean final body weight (g) - mean initial body weight (g)]/number of days.
(2) Body weight gain (BWG, %) = [(final body weight (g) - initial body weight (g)) /initial body weight (g)] ×100.
(3) Specific growth rate (SGR, % day− 1) = [(Ln final weight- Ln initial weight) ×100]/ duration in days.
(4) Condition factor (CF) = (fish mass / fish total length3) ×100.
(5) Feed conversion ratio (FCR) = [dry weight of feed (g)/ wet weight gain (g)].
(6) Protein efficiency ratio (PER) = Increment in body weight (g)/ protein intake (g).
(7) Daily feed intake (FI, g d− 1fish− 1) = diet consumed × 100 / duration in days /fish number per tank
(8) Survival rate percentage = 100x (total number of fish at the end of the experiment/ total number of fish at the start of the experiment).

Blood Samples and Analysis Haematological studies

By the end of the feeding experiment, fish of all groups of the blood sets were anesthetized using tricaine methanesulfonate (MS-222, Argent Chemical Labs, Redmond, Washington, USA), and blood samples were collected from the caudal vein and the samples were divided into 2 portions. Sodium citrate (3%) was added to one part for differential leukocytic count and phagocytic assay. The total WBC counts were determined according to the method of Stoskopf (1993). Haematocrit (Hct) were determined by using microhematocrit-heparinized capillary tubes and a microhematocrite centrifuge (10,000 g for 5 min). The values of Hct were determined within 30 min alter bleeding. The second part of blood sample was allowed to clot, centrifuged for serum separation. Total proteins and albumin were determined by “Protein” and “Albumin” kits (Spectrum, Egyptian Company for Biotechnology, Obour City, Cairo, Egypt) according to manufacture's recommendation. Globulin levels were calculated mathematically by subtracting albumin value from total protein value and expressed in g/dl according to Khalil, (2000).

Statistical analysis

Data are expressed as mean and mean standard error. A one-way analysis of variance (ANOVA) was used to determine differences among some different herbal plant, using SPSS 11.5 statistical software. Differences were considered significant at an alpha of 0.05 ($P<0.05$). Tukey's HSD multiple comparison test was used to determine significant differrences among means.

RESULTS AND DISCUSSIONS

Growth performance and feeding efficiency

Tilapia fed turmeric, rosemary and thyme had significantly higher ($P < 0.05$) weight gain, specific growth rates and protein efficiency ratio compared to those in the control group. Meanwhile, the condition factor and feed conversion ratio were significantly better ($P<0.05$) in the turmeric, rosemary and thyme groups compared to the control group (Table 2). Survival remained high in all treatments and ranged from 96.67 to 98.67 %, and no significant differences ($P < 0.05$) were detected among treatments.

Whole body compositions

The proximate composition was significantly different ($p < 0.05$) among the four experimental fish groups (Table 3). Tilapia fed turmeric, rosemary and thyme had significantly higher ($P < 0.05$) crude protein compared to those in the control group. Meanwhile, the moisture and crude lipid were significantly low ($P < 0.05$) in the turmeric, rosemary and thyme groups compared to the control group. But, there were no significant differences ($P > 0.05$) among all groups in ash content.

Table 2. Growth performance and feed utilization of Nile Tilapia (*Oreochromis niloticus*) fingerlings fed the tested herbal plants

Parameters	Treatment					
	Control	Turmeric	Rosemary	Thyme	SEM	P Value
Initial Weight (g)	21.80	21.59	22.02	21.76	0.09	0.852
Final Weight (g)	182.44 ^{b}	212.73 ^{a}	216.38 ^{a}	208.46 ^{a}	7.69	0.015
Weight gain (g)	160.64 ^{b}	191.14 ^{a}	194.36 ^{a}	186.70 ^{a}	7.68	0.011
DWG (g)	1.15 ^{b}	1.37 ^{a}	1.38 ^{a}	1.33 ^{a}	0.06	0.001
SGR (% day^{-1})	1.52 ^{b}	1.64 ^{a}	1.64 ^{a}	1.61 ^{a}	0.02	0.018
Final CF	2.93 ^{a}	2.73 ^{b}	2.67 ^{b}	2.62 ^{b}	0.12	0.021
FCR	3.16 ^{a}	2.71 ^{b}	2.74 ^{b}	2.82 ^{b}	0.14	0.029
PER	1.00 ^{b}	1.17 ^{a}	1.17 ^{a}	1.13 ^{a}	0.03	0.011
DFI (g d^{-1}fish^{-1})	3.63	3.71	3.78	3.75	0.19	0.531
Survival (%)	96.67	98.33	98.67	98.33	2.83	0.644

a and b Means in the same row with different superscripts are significantly different ($p\leq 0.05$).
DWG (Daily weight gain), SGR (Specific growth rate), CF (Condition factor), FCR (Feed conversion ratio), PER (Protein efficiency ratio), DFI (Daily feed intake), PPV (Protein productive value).

Immune Response

Groups treated with turmeric, rosemary and thyme showed a significant increase ($P<0.05$) in haematocrit, leukocrit levels and nitroblue tetrazolium activity (NBT) in comparison with control one (Table 4). While, the leukocyte number was insignificantly differences ($P<0.05$) among treatments were detected. Serum total protein, albumin and globulin values were significantly higher ($P<0.05$) in fish supplemented with turmeric, rosemary and thyme when compared to the control group. Higher value of globulins was observed in fish supplemented with turmeric.

Table 3. Proximate composition (on Dry matter basis) of whole body of Nile Tilapia (*Oreochromis niloticus*) fingerlings fed some different herbal plant

Parameters	Treatment					
	Control	Turmeric	Rosemary	Thyme	SEM	P Value
Moisture	73.85 ^{a}	73.17 ^{b}	72.99 ^{b}	73.05 ^{b}	0.31	0.039
Crude protein	55.83 ^{b}	56.94 ^{a}	57.14 ^{a}	56.96 ^{a}	0.16	0.012
Crude lipid	23.66 ^{a}	22.73 ^{b}	22.88 ^{b}	22.71 ^{b}	0.09	0.032
Ash	21.18	21.21	21.29	21.19	0.14	0.639

a-b Means in the same row with different superscripts are significantly different ($p\leq 0.05$).

Table 4. Haematocrit, leukocrit, leukocyte levels, glass-adherent NBT (+) cell activation and total protein of Nile Tilapia (*Oreochromis niloticus*) fingerlings fed some different herbal plant

Parameters	Treatment					
	Control	Turmeric	Rosemary	Thyme	SEM	P Value
Hematocrit level (%)	21.7 [b]	23.8 [a]	23.1 [a]	23.5 [a]	0.84	0.009
WBC counts ($\times 10^3/mm^3$)	3.5 [b]	4.8 [a]	4.2 [a]	4.4 [a]	0.06	0.005
Leukocrit level (%)	1.3 [b]	2.1 [a]	1.8 [a]	1.9 [a]	0.21	0.002
Leucocyte number (10^3)	41.7	39.1	40.7	40.2	2.84	0.641
NBT (+) cell activation (number)	25.6 [b]	29.8 [a]	27.9 [a]	28.6 [a]	1.52	0.017
Total protein, mg/dl	3.28 [b]	4.12 [a]	3.78 [a]	3.89 [a]	0.33	0.001
Albumin, mg/dl	1.13 [b]	1.65 [a]	1.43 [a]	1.47 [a]	0.13	<0.001
Globulin, mg/dl	2.15 [b]	2.47 [a]	2.35 [a]	2.42 [a]	0.16	<0.001

a-b Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

This study was to investigate the pathways in the somatic growth and nutritional physiology of Nile tilapia that could be affected by turmeric, rosemary and thyme additive. The three phytogenic compounds had been shown to be a promote growth in monogastric animals (Hafeez et al., 2016), as it confirmed herein with Nile tilapia. Given that it remained unclear which pathways are involved in promoting growth. This study focused on genes regulating appetite, nutrient digestion, absorption and transport, lipid metabolism, antioxidant enzymes and growth in Nile tilapia. The expression of selected genes involved in the above pathways was assessed in major tissues where they exert their action, namely brain, fore intestine and liver. More specifically, the brain is a key site for stimulating appetite through the action of appetite regulating peptides (Pierce et al., 2012).

It appears that the different herbal plants improve the nutrient utilization of better growth of the fish. Most probably fat was used for energy, and protein was used for growth in different herbal plants diets. It has been shown that herbs stimulate the secretion of pancreatic enzymes, important factors in nutrient digestion and assimilation (Frankic et al., 2009). This trend can be related with the higher significant, weight gain, SGR, PER and better FCR obtained with different herbal plants supplementation. The results of the present study investigated that some medicinal herbs can be effective immune-stimulators. Their effectiveness has been demonstrated in the rearing of fish (Yin et al., 2006). Moreover, some studies have confirmed that the application of a diet with herbal adjutants has a positive impact on the health and resistance of the fish, and also improves their conditions and growth rate (Sivaram et al., 2004).

Some recent studies have exhibited that feeding fish with different herbal plants resulted in an elevated disease resistance and improved survival, which may be attributed to an improvement of immune functions (Yılmaz et al., 2013). Yılmaz et al. (2012) also evaluated the use of thyme (*T. vulgaris*) in a diet for sea bass and they demonstrated that 1% (1000 mgkg-1) thyme powder produced the highest protein and energy retentions, and slightly improved growth performance.

The enhanced growth response indicated by turmeric supplementation in this study may be due to improved feed consumption, improved feed utilization, which is an indication of increased nutrient digestibility and antioxidant activity of Turmeric (Osawa et al., 1995) that stimulates protein synthesis by enzymatic system. This is in accordance to Pransin (2006) who reported that goldfish fed turmeric supplemented diets, had highest acid protease, alkaline protease and lipase activity, enhanced growth rate. Also, Manal et al., (2014) results showed that all turmeric extract fed fish had significant higher specific activities of digestive enzymes and indicated that growth rate will be enhanced in follow up. Although a number of studies have reported beneficial effects of curcumin on growth rates, none have reported specific need for initial acclimatization of curcumin supplemented feed (Cao et al., 2015). These results are in accordance with (Amany Diab et al., 2014) who demonstrated a

reduction in mortality of curcumin fed *Oreochromis niloticus* (Nile tilapia) post-challenge with *Pseudomonas fluorescens*.

Feed conversion ratio (FCR) and protein efficiency ratio (PER) were affected, in the same trend, by the herbal additives. Best FCR and highest PER were observed ($P<0.05$) for fish groups fed different herbal plants compared with control group. It has been suggested that the herbal plants can improve the metabolism of fats and their utilization parameters (Jeong et al., 2007). High antioxidant activity was also recognized in medicinal plants which contain polyphenols that protect and reduce cellular damage by various radicals. It is also reasonable that antioxidants in herbs contribute to the activation of immune functions for various pathogens in fish. Immunostimulating activities of herbs has been reported in other fish (Jeong et al., 2007).

Herbal extracts have been shown to reduce mortality against pathogenic challenges in fishes (Jian & Wu, 2003). Immunostimulants can increase non-specific immunity by either enhancing the number of phagocytes or activating phagocytes or by increasing the synthesis of molecules involved in the innate immunity such as the complement lysozyme antiprotease. *Withania somnifera*, and extracts from *O. sanctum*, added to the diets have been shown to increase phagocyte activity and serum immunoglobulin level in *E. tauvina* and increased survival against *V. harveyi* infections (Sivaram et al., 2004).

The results of proximate analysis of whole body of tilapia hybrid for moisture, protein, ether extract and ash at the end of the study are shown in Table 3. Moisture content was decreased significantly in fish groups fed different herbal plants compared with control group. The protein contents in fish body were affected positively by additive of different herbal plants compared with control group. While lipid content was decreased ($P<0.05$).

On the same trend, feeding fish diets with herbal adjuvants can improve the lipid metabolism (Jeong et al., 2007) as fat is more effectively utilized as a source of energy, which means that protein ingested with the diets can be used more effectively for somatic growth.

Immune systems can be activated by the immunostimulants in several ways: enhancing the number of phagocytes, activating phagocytes or increasing the synthesis of the involved molecules. In our study, fish fed with turmeric, rosemary and thyme showed elevated phagocytic activity. Strengthening the defense mechanism of fish through prophylactic administration of natural plant products is one of the most promising methods of controlling diseases in aquaculture (Devasagayam and Sainis 2002). Using natural plants as immunostimulants in fish is more useful than using of antibacterial drugs that cause adverse side effects for fish, environment and consumers. Therefore, we investigated the effects of turmeric, rosemary and thyme, as herbal medicine plants on some immunological, hematological parameters and defense mechanisms of *Oreochromis niloticus* (Nile tilapia). Several types of leukocytes participate in the cellular immune response, including lymphocytes, monocytes, granulocytes (neutrophils, eosinophils and basophils), and cytotoxic cells (Nakanishi, 1999). Fernández et al. (2002) recorded that macrophages can be used as indicators to evaluate the health of fish. These cells play an important role in killing pathogens as in immune response.

Either increase or decrease in different leucocytes was pronounced in fish because all leucocytes were calculated as a percentage of the whole leucocytic count which constitutes 100 %. The decrease of the percentage of lymphocytes in fish groups may be attributed to the significant increase of other leucocytic cells.

Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. The serum protein level is an important indicator of humoral defense system of fish and increases especially in the fish fed with plant extracts (Misra et al., 2006; Marin et al., 2016). In the current study total protein in serum was significantly higher in fish fed on diet containing turmeric, rosemary or thyme when compared to basic diet. An investigation done by Toghyani et al. (2011) proved that serum total protein and globulin concentrations increased significantly in birds fed diet supplemented with thyme powder. Globulins

concentration is significantly higher in fish fed on turmeric supplement compared to the other groups. The increase in globulin levels may be due to the immune stimulant effect of turmeric, rosemary or thyme supplementation.

Abdel Zaher et al. (2009) who showed that, fish fed diet containing different levels as 0.5, 1 and 1.5 % of turmeric seeds meal, significantly increased the serum globulin. The increase in the serum globulin levels is thought to be associated with a stronger innate response in fish (Wiegertjes et al., 1996). The increase in globulin was suggested previously to indicate that, fish are immunologically strong (Nayak et al., 2004).

CONCLUSIONS

Supplementation with medicinal herbs significantly increased weight gain, specific growth rates and protein efficiency ratio compared to those in the control group.

The medicinal herb additives had insignificant effect on carcass composition and some biochemical composition.

Haematocrit and leukocrit levels in the fish fed with turmeric, rosemary and thyme added diet were found to be significantly higher than the control group. However, leukocyte level among the experimental groups was not significantly differed. In neutrophils of experimental fish groups, there were higher numbers of NBT (+) cells fixed on lamellas than those of the control group.

Based on the current findings together with the low cost and the immunostimulative effect of turmeric, rosemary and thyme it can be recommended to be used in fish feed to minimize the mortalities caused by some pathogens.

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