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Supplementation of Herbal Essential Oil Mixture to diet of Nile Tilapia and Evaluating Growth Performance, Health Status and Intestinal Histology

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ABSTRACT

The aim of the present study was to investigate the effect of dietary supplementation with Herbal oil mixture (HEOM) on growth performance, body composition, some blood parameters and intestine histology of Nile tilapia fish fingerlings. One hundred and Eighty Nile Tilapia (*Oreochromios Niloticus*) fish fingerlings were randomly divided into three treatment groups of 60 fish each (negative control and Herbal oil mixture (HEOM) at two levels). Each treatment group was further sub-divided into three replicates of 20 fish per replicate. The groups fed the diet as the following, group I fed basal diet (control). Group II fed basal diet + 0.05% HEOM. Group III fed basal diet + 0.1% HEOM for 12 weeks.

The obtained results confirmed the significant gradually (with increasing the feed additive level) showed improvements in fish final weight, weight gain, specific growth rates, feed utilization (feed intake and feed conversion) by increasing herbal oil mixture levels. These feed additives, also, significantly improved fish carcass composition (protein, ether extract, energy content). Total and differential leukocytic count was improved. Herbal oil mixture reduced blood Cholesterol, triglycerides, glucose and liver enzymes. These additives improved intestine histology. So, it could be recommended the addition of 0.1% herbal oil mixture to Nile tilapia fish diet.

INTRODUCTION

Recently, medicinal herbs proved to be effective in different fish species, including gopher, rainbow trout, tilapia, carp and others. Chemical analysis of these herbs has shown its constituents to be principally carvacrol and thymol *(1)*, several studies have been conducted on the effect of dietary essential oils or combinations on the performance of fish and poultry but with varying and often conflicting results. Some reports suggested that dietary herbal essential oils improved growth performance *(2,3)*. Ching *(4)* reported that dietary oregano oil improve not only growth and feed utilization but also survival rate of shrimp.

Bay laurel *(Laurus Nobilis* l. (LN)), a species held in high esteem since, ancient times and evergreen tree is cultivated in many warm parts of the world, particularly in the Mediterranean area is natively cultivated on the coastal up to an altitude of 600-800 M *(5)*. Methanolic extracts of the plant contain polar compounds (such are phenols, flavones and flavonols) and show antioxidative activity against lipid peroxidation *(6)*. Conforti et al.,*(7)* determined that the amounts of phenolics in ln leaves were 210 and 219 mg g^{-1} for wild and cultivated plant extracts, respectively.

Fennel (Foeniculum vulgare) is claimed to have an effect in relieving inflammation. In an in vivo study with mice, oral administration of f. Vulgare fruit methanolic extract exhibited

inhibitory effects against acute and subacute inflammatory diseases and type IV allergic reactions and showed a central analgesic effect *(8)*. An effect of oil of f. Vulgare on hemostasis has been evidenced, with a significant correlation with its phenylpropanoid content *(9)*.

Sage (Salvia Officinalis) extracts contain active antioxidative factors such as phenolic diterpenes, flavonoids and phenolic acids *(10)*. Terpenoids in the dried green leaves in sage (salvia officinalis l.) Were regarded as the most promising sources of natural anti-oxidants, where sage protected against h2o2-induced DNA damage *(11)*.

MATERIALS AND METHODS

This experiment was done to evaluate the effect of dietary inclusion of herbal essential oil mixture of Bay Laurel (Laurus Nobilis l. (LN)) oil, fennel (Foeniculum Vulgare) oil and sage (Salvia Officinalis l.) Leaf oil (0.05% and 0.1%) in the diet of Nile tilapia fingerlings in compare with control diets and its effect on growth performance, body composition, some immunological and biochemical parameters and intestine histology.

Experimental Fish and Culture

Fingerlings of Nile tilapia, *Oreochromis niloticus* , were obtained from the fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia. 180 Nile Tilapia fingerlings were divided into equal 3 triplicate groups (each replicate contained 20 fish), and each replicate of the fish groups was stocked in its corresponding glass aquaria for two weeks to be acclimatized before the start of the experiment. The fish in this experiment were located in 9 rectangular glass aquaria (30 x 40 x 150 cm) filled with dechlorinated tap water which continuously aerated by a small air compressor. The water temperature, dissolved oxygen, PH, ammonium (NH4), nitrite and nitrate were measured and found to be 27-28 oC, 5.8 mg/l 702, 0.3 mg/l, 0.027 mg/ l,7 mg/ l, respectively.

Experimental diets and feeding trial

The fish were fed nearly isonitrogenous and isocaloric diet according to *(12)* containing essential oil mixture of laurel oil (*Laurus Nobilis* l. (LN)), fennel (Foeniculum Vulgare) oil and sage (Salvia Officinalis) oil as pellet for 12 weeks. GroupI. Fed control diet without additives, group II. Fed control diet + HOM 0.05% and group III. Fed control diet + HOM 0.1%. Table (1 and 2) shows the formulation and composition of experimental diets. The amount of feed (on dry matter basis) delivered per day was adjusted once every three weeks after weighing of fish groups and calculated as percentage of body weight *(13)*. Fish groups were manually fed their respective diets 3 times/day (at 09:00 am, 11:00 am and 13:00 pm), at a level of 3% of body weight, there was nothing left in the bottom of glass aquarium, so the amount of the given diets was also the amount of intake of diets. The feed was dispersed by hand in each glass aquarium. To minimize stress after weighing, feeding was discontinued for 24 hours *(14)*. Nine fish were taken at the beginning and at end of the experiment from each group to measure their body length to determine the condition factor.

Proximate chemical analyses for diet ingredients and fish body composition was done according to standard methods *(15)*.

Indices for evaluation of experimental results

(a) Evaluation of growth performance

Average body weight measured according *(16)*. Weight gain (*Average weight gain (AWG, g. Fish^{-1}) = final body weight - initial body weight*). Specific growth rate % (SGR) was calculated according to *(17)*.

(B) Evaluation of feed utilization

Feed intake (FI) was calculated as the total weight diet offered in a given period divided by

the number of survival fish. Feed conversion ratio (FCR) was calculated according to *(18)*.

(C) Measurement of condition factor (k):- was calculated according to *(19)*.

Blood sampling

At the end of the experiment blood samples, were obtained from the caudal vessels, and collected from a random sample of 3fish/group, into a clean sterilized tube containing EDTA for determination of total leukocytic count (TLC) and differential leukocytic count. Another sample was taken without anticoagulant for determination of some blood biochemical parameters.

Immune response measurements

Total leukocytic count (TLC) was determined according to *(20)*. Differential leukocytic count was calculated according to *(21)*.

Blood biochemical measurements

Serum glucose was measured according to *(22)*. Plasma total proteins were determined by Biuret method as described by *(23)*. Plasma albumin concentration was determined according to *(24)*. Plasma globulin was obtained by subtracting the albumin value from the value of total for each sample. Plasma total cholesterol was determined according to the method described by *(25)*. Plasma triglycerides were determined according to the method described by *(26)*. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to *(27)*.

Histological examination of intestine

Samples of intestine were immersed in 10% neutral buffer formalin, and then processed using the usual histological technique.

Statistical analysis

The obtained data were subjected to one-way ANOVA analysis of variance *(28,29)*. Differences among treatment means were determined by Duncan's multiple range test at a ($P< 0.05$) level of significance *(30)*.

Table 1. Proximate chemical composition of feedstuffs used in formulation of diets in the experiment (analyzed).

Ingredients	DM	CP	EE	CF	Ash	*Ca	*AP	*Lysine	*Methionine	NFE
Fish Meal, 66%	93.80	63.80	8.40	0.80	18.20	5.19	2.88	4.75	1.75	2.63
Poultry By-Products, 60%	92.60	60.40	12.60	2.20	14.50	3.51	1.81	3.06	1.10	3.20
Soy Bean Meal, 44%	90.60	43.75	1.20	6.10	6.50	0.30	0.65	2.85	0.57	33.05
Yellow Corn	89.20	8.75	3.80	2.10	1.20	0.03	0.28	0.25	0.17	73.25
Corn Gluten, 60%	91.60	60.37	2.20	1.50	2.20	0.07	0.44	1.11	1.63	25.33
Wheat Bran	89.30	16.62	4.30	11.40	6.10	0.13	1.16	0.58	0.19	50.98
Calcium Dibasic Phosphate	96.70	-	-	-		26.00	18.00	-	-	-
Lysine, Hcl, 78%	98.50	118.00	-	-	-	-	-	78.00	-	-
DL-Methionine, 98%	98.20	58.00	-	-	-	-	-	-	98.00	-
Vit Mineral-Premix	97.80	-	-	-	-	-	-	-	-	-
Vegetable oil	-	-	98	-	-	-	-	-	-	-

*Calculated According To *(12)* **NFE= DM - (EE + CP + ASH + CF).

Table 2. Physical composition and calculated analysis of the diets used in the experiment

Ingredients	%
Fish Meal, 66%	18.00
Poultary-Byproducts, 60%	14.00
Soy Bean Meal, 44%	18.00
Yellow Corn	31.70
Corn Gluten, 62%	5.20
Wheat Bran	6.00
Vegetable Oil	5.00
L-Lysin HCL 98%	0.20
D L- Methonine	0.20
Calcium Dibasic Phosphate	0.50
Vitamin. Mineral Premix*	1.20
Total	100
Calculated Composition	
DM, %	91.60
CP, %	35.08
EE, %	10.00
CF, %	2.98
Ash, %	7.29
NFE, %	34.50
Ca, %	1.42
P, %	1.07
Lysin, %	2.12
Methonine, %	0.92
DE, Kcal/ Kg**	2900.16

*Vitamin And Mineral Mixture (Alfakema): - Each 1 Kg Contains: - Vit. A 580000 I.U, Vit. D3 8600 I.U, Vit. E. 720mg, Vit. K3 142mg, Vit C 0.1mg, Vit B1 58mg, Vit B2 34mg, Vit. B6 34mg , Vit.B12 58mg , Folic Acid 86 Mg , Pantothenic Acid 8mg , Manganese Sulfate 65mg , Zinc Methionine 3000mg , Iron Sulfate 2000mg , Copper Sulfate 3400mg , Cobalt Sulfate 572mg , Sodium Selenite 25mg , Calcium Iodide 25mg , Calcium Carbonate (Carrier Substance) Till 1000gm.

** Digestible Energy Calculation Based On Values Of Protein 3.5 Kcal/Gm, Fat 8.1 Kcal/Gm, NFE 2.5 Kcal/Gm. According To *(31)*

RESULTS AND DISCUSSION

Growth indices

The results for allover growth performance are presented in (Table 3).The fish group fed on diet contained 0.1 % herbal oil mixture recorded the best final body weight, body gain (g), FRC, SGR% followed by fish group fed on diet contained 0.05% herbal oil mixture in comparison with the control group. Feed intake significantly ($p < 0.05$) increased in fish groups fed on diet contained herbal oil mixture in comparison with the control group. These results are in agreement with *(32)* who stated

that herbal mixture extract improve olive flounder growth rate, and this improvement in growth performance by herbal mixture could be related to better nutrient digestibility and absorption, improved digestive enzymes and maintaining the function and structure of the small intestine, leading to an increased digestive capacity Similarly, *(33)* mentioned that use of 0.25% thyme oil significantly increase growth rate, feed intake and feed utilization of fish. Conversely, *(34)* stated that essential oil had no significant (P>0.05) effect on body weight, weight gain, specific growth rate, feed intake, feed conversion ratio, mortality rate of White leg Shrimp.

Condition Factor (K)

The results of condition factor (k) at the end of the experiment are present in (Table 3). The highest values for body condition factors are recorded in fish group fed on diet contained 0.1% herbal oil mixture in comparison with control and the other group. These results are supported by *(19)* who reported that the high condition factor is an indicator to the good nutritional (healthy) state of fish.

Whole body composition

The results of whole body composition are presented in (table 4). The results showed that DM and CP content were significantly (P <0.05) increased by herbal oil mixture addition when compared with the control group, while the body composition of EE and Ash content of the group fed herbal oil mixture significantly (P <0.05) decreased when compared with other fish groups. While *(34)* stated that herbal essential oil have no significant (P>0.05) effect on meat composition of White leg Shrimp.

Effect on immunological parameters

As shown in Table 5 Total Leukocyte Counts (TLC), neutrophils%, lymphocytes % and monocytes % recorded the higher values after dietary inclusion of herbal oil mixture when compared with the control, the best results was recorded in the fish group fed diet supplemented with 0.1% herbal oil mixture. On other hand, Plasma total protein and globulin levels were not significantly (p> 0.05) increased by increasing the amount herbal oil mixture in the diet when compared to the Control group. While *(33)* stated that total protein and globulin concentrations of Tilapia Nilotica in plasma were significantly increased by 0.1 and 0.25% thyme essential oil. Also *(35)* mentioned that dietary inclusion of EO to laying hens diet had no significant effect on total or differential leukocyte count except monocyte count was higher in fennel-included compared to ginger-included and control dietary group.

Effect on some blood parameters:

The results of some biochemical parameters are presented in (table 6). Dietary supplementation of 0.1% herbal oil mixture significantly (P<0.05) decreased blood glucose level. Moreover addition of 0.05% and 0.1% herbal oil mixture significantly (p> 0.05) decreased blood cholesterol and triglycerides level, and addition of herbal oil mixture significantly decreased ALT and AST in comparison with the control group. The reduction of the plasma level of triglycerides, AST and ALT and cholesterol concentration by herbal essential oil indicated that they have a strong Hepatic-protective effect as mentioned by *(36)*.

Effect on intestine histology

The effect of experimental diets on the intestine histology in different fish groups were presented in Fig. 1, 2 and 3. Herbal oil mixture improved intestinal villus length; also there was dose related increase in thickness of tunica mascularlis in comparison with the control group. Similarly, addition of feed additives to Rainbow Trout (*Oncorhynchus mykiss*) diets significantly (P<0.05) improved intestinal villus length, width and intestine villus goblet density *(37)*.

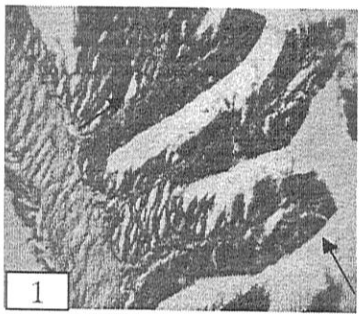

Fig. 1(control)
Obj. x40 : Oc.x10

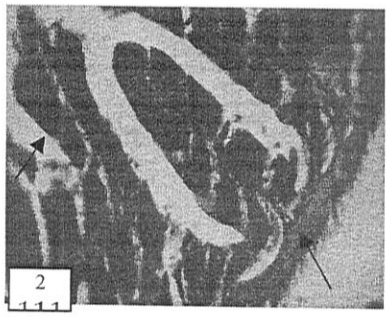

Fig. 2 (0.05%, HEOM)
Obj. x40 : Oc.x10

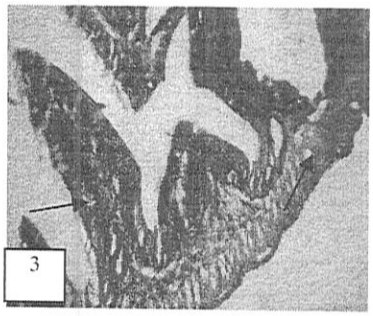

Fig. 3 (0.1%, HEOM)
Obj. x40 : Oc.x10

In conclusion, dietary supplementation of herbal oil mixture improved the growth performance, body composition, enhanced the immune status and intestine histology of Nile tilapia. Also, addition of herbal oil mixture reduced fish glucose, cholesterol, triglycerides and liver enzymes under Egyptian condition.

Table 3. Effect of experimental diets on all over growth performance of Nile tilapia fingerlings after (12 weeks) at the end of the experiment

Parameters	Experimental groups		
	Control	Herbal oil mixture (0.05%)	Herbal oil mixture (0.1%)
Initial BW (g)	9.53 ± 0.04	9.46 ± 0.1	9.35 ± 0.1
Final BW (g)	31.16 ± 0.13 ^{c}	39.85 ± 0.26 ^{b}	42.94 ± 0.62 ^{a}
Body gain (g)	21.63 ± 0.09 ^{c}	30.39 ± 0.21 ^{b}	33.59 ± 0.71 ^{a}
Specific growth rate (%)	1.41 ± 0.04 ^{b}	1.71 ± 0.01 ^{a}	1.79 ± 0.03 ^{a}
Feed intake (g)	35.23 ± 0.19 ^{c}	46.25 ± 0.41 ^{a}	37.47 ± 0.56 ^{b}
Feed conversion ratio	1.63 ± 0.08 ^{a}	1.52 ± 0.01 ^{a}	1.12 ± 0.04 ^{b}
Condition factor	1.75 ± 0.1 ^{b}	1.87 ± 0.01 ^{b}	2.54 ± 0.1 ^{a}

^{abc} Mean in the same row with different superscripts are significantly different at ($P < 0.05$)

Table 4. Effect of experimental diets on body composition of Nile tilapia fish fingerlings after (12 weeks) at the end of the experiment

Nutrient (%)	Experimental groups		
	Control	Herbal oil mixture (0.05%)	Herbal oil mixture (0.1%)
Dry matter	21.00 ± 0.29 ^{c}	22.89 ± 0.11 ^{b}	23.88 ± 0.05 ^{a}
Protein	54.87 ± 0.38 ^{c}	64.53 ± 0.20 ^{a}	62.83 ± 0.55 ^{b}
Fat	8.70 ± 0.1 ^{a}	7.5 ± 0.12 ^{b}	7.69 ± 0.14 ^{b}
Ash	24.33 ± 0.17 ^{a}	22.43 ± 0.47 ^{b}	23.00 ± 0.29 ^{b}

^{abc} Mean in the same row with different superscripts are significantly different at ($P < 0.05$)

Table 5. Effect of experimental diets on some total and differential leukocyte count of Nile tilapia fish fingerlings after (12 weeks) at the end of the experiment

Parameters	Experimental groups		
	Control	Herbal oil mixture (0.05%)	Herbal oil mixture (0.1%)
Total leukocytic count (10cells/ml)	29.67 ± 0.44 [c]	32.73 ± 0.47 [b]	36.40 ± 0.58 [a]
Neutrophils (%)	22.70 ± 0.1 [b]	24.67 ± 0.12 [a]	24.33 ± 0.12 [a]
Eosinophils (%)	5.18 ± 0.11 [a]	2.00 ± 0.11 [b]	0.34 ± 0.12 [c]
Baisophils (%)	6.06 ± 0.11 [a]	2.33 ± 0.12 [b]	0.67 ± 0.12 [c]
Lymphocytes (%)	61.36 ± 0.11 [c]	64.36 ± 0.11 [b]	66.33 ± 0.11 [a]
Monocytes (%)	4.70 ± 0.11 [c]	6.64 ± 0.04 [b]	8.33 ± 0.12 [a]

[abc] Mean in the same row with different superscripts are significantly different at ($P < 0.05$)

Table 6. Effect of experimental diets on some plasma biochemical parameters of Nile tilapia fish fingerlings after (12 weeks) at the end of the experiment

Parameters	Experimental groups		
	Control	Herbal oil mixture (0.05%)	Herbal oil mixture (0.1%)
Glucose (g/dl)	55.50 ± 0.17 [a]	55.62 ± 0.63 [a]	47.00 ± 0.30 [b]
Total protein (g/dl)	3.15 ± 0.05 [a]	3.38 ± 0.16 [a]	3.33 ± 0.16 [a]
Globulin (g/dl)	1.40 ± 0.12 [a]	1.76 ± 0.22 [a]	1.83 ± 0.19 [a]
Total albumin (g/dl)	1.75 ± 0.07 [a]	1.62 ± 0.07 [a]	1.50 ± 0.04 [a]
A/G ratio	1.25 ± 0.15 [a]	0.92 ± 0.13 [a]	0.82 ± 0.11 [a]
Cholesterol (mg/dl)	115.50 ± 0.62 [a]	84.25 ± 0.76 [b]	85.75 ± 0.25 [b]
TG (mg/dl)	86.50 ± 0.87 [a]	51.88 ± 0.68 [b]	44.75 ± 0.25 [b]
ALT (IU/L)	35.00 ± 0.73 [a]	25.63 ± 0.89 [b]	23.00 ± 1.00 [b]
AST (IU/L)	85.00 ± 0.58 [a]	72.00 ± 0.10 [b]	68.25 ± 0.25 [b]

[ab] Mean in the same row with different superscripts are significantly different at ($P < 0.05$)

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الملخص العربي

تاثير إضافة زيوت خليط من الاعشاب الى عليقة اسماك البلطى النيلى على اداء النمو و الحالة الصحية والحالة الهيستولوجية للامعاء

رانيا السيد محمود

قسم التغذية و التغذية الإكلينيكية – كلية الطب البيطري – جامعة الزقازيق – مصر

أجريت هذه الدراسة لإمكانية أستخدام خليط من زيوت الاعشاب (زيت الشمر, زيت اوراق الغار و زيت الميرامية) كأضافات أعلاف لتحسين اداء النمو و الحالة الصحية والحالة الهيستولوجيةلامعاء اسماك البلطي ، تم تقسيم عدد 180 سمكة إلى ثلاثة مجاميع متساوية الأوزان تقريبًا فى أحواض زجاجية مزودة بمضخات هواء ذات نوعية جيدة من المياه .تم تغذية هذه الأسماك على ثلاثة علائق، احتوت المجموعة الاولي علي العليقة الضابطة بدون أضافات والمجموعة الثانية أحتوت علي العليقة الضابطة مع إضافة ٠,٠٥% خليط زيوت الاعشاب و المجموعة الثالثة أحتوت علي العليقة الضابطة مع ٠,١% خليط زيوت الاعشاب. تم تغذية الأسماك علي العلائق المختلفة بنسبة ٣ % من الوزن الحى لمدة ١٢ أسبوعا.

وقد أوضحت نتائج هذه الدراسة على الآتى-:

- ارتفاع ملحوظ فى معدلات النمو وتحسين فى معامل التحويل الغذائي و الجسمي وكذلك بعض متغيرات الدم للأسماك التى غذيت على العليقة الضابطة مع إضافة خليط زيوت الاعشاب خاصة في المجموعة الثالثة (٠,١% خليط زيوت الاعشاب).
- حالة الأسماك الصحية ,و المناعية, و الحالة الهيستولوجية للامعاء قد تحسنت في المجموعات التي غذيت العليقة الضابطة مع إضافة خليط زيوت الاعشاب ,خاصة مع زيادة نسبة خليط زيوت الاعشاب إلي ٠,١%.

ويستخلص من نتيجة البحث أنه يمكن استخدام خليط زيوت الاعشاب في علائق أسماك البلطى لتحسين القيمة الغذائية ورفع الحالة المناعية لأسماك البلطي