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EFFECT OF DIFFERENT LEVELS OF BASIL AND GINGER OILS ON GROWTH PERFORMANCE, FEED UTILIZATION, AND HEMATOLOGICAL INDICES OF RED TILAPIA *Oreochromis* spp. FINGERLINGS

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ABSTRACT

Seven experimental diets containing 0, 1, 2, and 3% of Basil and Ginger oils were used to study their effects on growth performance, survival, feed consumption, body composition, and hematological indices on Red tilapia *Oreochromis* Spp. fingerlings. Fourteen glass aquaria (60×40×40 cm, 96-L capacity) were used, two aquaria per treatment. Ten fish with the same average weight (39.4±0.3 g/fish) were stocked in each aquarium. Fish were fed 3% of their body weight, twice a day for 45 days. Higher significance ($P<0.05$) was found in final body weight, weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio , and productive protein value ‘between groups of fish were fed diets containing basil and ginger oils and control diet (0.0% oil). Increasing levels of basil and ginger oils led to enhancing growth, feed conversion ratio and nutrient retention efficiencies. Basil oil diets at different levels led to an increase in growth performance and feed consumption and were better than Ginger oil diets, while ginger oil diets recorded an increase in the number of white blood cells compared to the control diet. The inclusion of 3% basil oil in the diet exhibited superior results compared to other tested levels, suggesting its recommendation for use in red tilapia diets.

INTRODUCTION

Medicinal and Aromatic Herbs are added to fish diets in several forms such as powder, oils, and extracts. Herbs are cheap and do not have harmful effects on the environment **(Shakya, 2017).** Also, they have stimulants for digestion and improve immunity **(Alcicek *et al.*, 2003).** Some herbs, such as basil (*Ocimum basilicum*) enhance growth **(Sivaram *et al.*, 2004),** and improve feed conversion ratio **(Shalaby, 2004).** Also, Ginger (*Zingiber officinalis*) improves immunity **(Citarasu *et al.*, 2002).** Essential oils have many benefits when added to fish diets, increasing weight **(Zheng *et al.*, 2009; Acar *et al.*, 2015).** Basil leaves (*Ocimum basilicum*) contain essential oils and active substrates such as Ocimene, Methyl Chavecol, and Linalool **(El-Dakr *et al.*, 2008).** One of the most common active ingredients in the basil plant is Estragole with linalool **(Ekundayo *et al.*, 1987; Yayi *et al.*, 2001).** On the other hand ginger (*Zingiber officinale*) is used as a powerful medicinal plant in the world and has many active ingredients like gingerol and gingerdiol which act as antioxidants **(Kikuzaki and Nakatani, 1996).** Ginger roots have active ingredients including ginger oil and gingerol **(Chang *et al.*, 2013).** Some of the most important essential volatile oils in ginger are p-cineole, R-terpineol, and zingiberene **(EL-Ghorab *et al.*, 2010).**

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This study aimed to evaluate the growth performance and immunity of Red tilapia, *Oreochromis* Spp. by using cheap sources of Aromatic plant oils (basil and ginger oils) at different levels.

MATERIALS AND METHODS

This study was conducted at the Fish Research Center, Al-Arish University, North Sinai, Egypt. Fourteen glass aquaria (60× 40 × 40 cm, 96-L net water volume) in duplicate were arranged in series and received brackish water (5.3 ppt). Air blower (5 HP) was used for aeration. Red tilapia, *Oreochromis* Spp. Fingerlings Were used and obtained from the Fish Research Center. Ten fish of the average initial weight (39.4 ± 0.3 g) were selected and randomly distributed in the experimental aquaria. Fish were fed the control diet for one week.

Seven experimental diets (30% crude protein) containing 0, 1, 2, and 3% of basil and ginger oils obtained from the commercial market in North Sinai were used. Fish fed 3% of the body weight daily, 9 AM and 2 PM for 45 days. Weight measurements were taken every two weeks and the amount of feed was determined according to the new weight. Fish were reared at 29.1 ± 1.2^{o}C, 5.3±0.1 mg l^{-1}. and 8.2±0.1 for temperature, salinity, and pH, respectively. Diets, and fish samples were analyzed according to **AOAC (1995)** for dry matter, crude protein, ether extract, crude fiber, nitrogen-free extract (NFE), and ash. Energy values were calculated according to **NRC (1993)** and **Garling and Welson (1976)** for gross energy, and digestible energy, respectively.

Blood samples were drawn from fish and placed in tubes containing Na2EDTA. The number of red blood cells and white blood cells was calculated according to the method of **Dacie and Lewis (1991)**. While, hemoglobin was calculated following the method of **Drabkin and Austin (1932)**. The hematocrit value was calculated according to the method of **Sorrell-Raschi and Tomasic (1998).** The number of platelets was calculated by using **Brecher *et al*. (1953)** method. Values of MCV; MCH and MCHC were calculated by using the method of **Jain (1993)**.

The data was statistically analyzed by one–way analysis of variance (ANOVA) using (SPSS version 26). Duncan's multiple range test (**Duncan, 1955**) were used to compare between mean differences at a significance level of 5%

RESULTS

Chemical analysis of all tested diets and composition of fish are shown in Tables 1 and 2. Results indicated that all experimental diets were isonitrogenous and isocaloric. Red tilapia fish were fed levels of Basil and Ginger oils during the experimental period. Results showed that groups of fish that were fed on diets containing levels of Basil oil gave higher body weight than those fed on the Ginger oil and control diet. Table 3 shows that add of Basil oil at levels of 1.0%, 2.0, and 3.0% in red tilapia diets led to an improvement in the final body weight, weight gain, and specific growth rate compared to the Ginger oil and the control diets. Fish group fed diet containing 3% Basil has recorded the highest body weight gain and SGR compared to the other fish groups.

The highest 'SGR' was recorded in the fish group fed 3% Basil oil followed by 2%. Fish fed on the control diet recorded the lowest growth. The highest 'FCR' was found when fish that fed diet with 3% Basil oil, the same trend was found in FE%. Protein efficiency ratio 'PER' was significantly high ($p<0.05$) for inclusion on Basil oil levels (1, 2 and 3%) than the tested levels of Ginger and the control diet (Table 3). However, no significantly differences ($p> 0.05$) were noticed in Survival rate in all tested in groups of fish (100%).

Table 1. Composition of the Basil and Ginger tested diets used in the study

Ingredients	Experimental Diets						
	Control	Basil oil levels			Ginger oil levels		
	1	2	3	4	5	6	7
	(0.0%)	(1.0%)	(2.0%)	(3.0%)	(1.0%)	(2.0%)	(3.0%)
Grams per 100 g.							
Fish meal	15	15	15	15	15	15	15
Soybean meal	40	40	40	40	40	40	40
Corn meal	18	18	18	18	18	18	18
Barley	10	10	10	10	10	10	10
Wheat bran	10	9	8	7	9	8	7
Starch	3	3	3	3	3	3	3
Linseed oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Basil oil	-	1.0	2.0	3.0	-	-	-
Ginger oil	-	-	-	-	1.0	2.0	3.0
Min and Vit. Premix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100	100	100

1-Vitamins and mineralsmixture per/1kg contained: vit (A): 10.000.000"IU", vit (D_3): 10.000.000"IU", vit (E): 1 "mg", vit (P_1): 400 "mg",vit (P_2): 600 "mg", vit (p_6): 1.200 "mg", vit(P_{12}):4.50 "mg" vit (PP):6500 "Mg", vit (K_3): 6500 "mg", vit(c):1200 "mg", inositol: 50"mg", Biotin:80 "mg" ,d-pentotholic: 325 "mg", folic asid: 50"mg", choline HI:175 "mg", Cobalt (Co): 120 "mg", Iron (Fe):15 "mg", Manganese(Mg):35 "mg", Copper (Cu): 1.250 "mg", Zink (Zn):31.250 "mg", Selenium (Se):50 "mg", Iodine:50 "mg", BHT:125"mg".

Table 2. Chemical analysis of the Basil and Ginger diets used in the study

Ingredients	Experimental Diets						
	Control	Basil oil levels			Ginger oil levels		
	1	2	3	4	5	6	7
	(0.0%)	(1.0%)	(2.0%)	(3.0%)	(1.0%)	(2.0%)	(3.0%)
Dry matter	82.04	82.04	82.04	82.04	82.04	82.04	82.04
% of dry matter basis							
Crude protein	30.72	30.38	30.25	30.13	29.99	30.28	30.40
Ether extract	5.71	5.78	5.82	6.04	5.74	5.76	6.71
Crude fiber	6.13	6.25	6.26	6.28	6.70	6.30	6.02
Nitrogen free extract	49.73	49.61	49.60	49.56	49.53	49.48	48.88
Ash	7.71	7.98	8.07	7.99	8.04	8.18	7.99
GE[1] (Kcal /100g)	436	435	434	435	432	433	437
DE[2] (Kcal/100g)	593	591	591	591	589	589	591
P/E[3] ratio mg CP/Kcal	70.46	69.84	69.70	69.25	69.42	69.93	69.57

1- Gross energy (GE); was calculated according to the method of NRC, 1993.

2- DE Digestible energy (DE) was calculated according to Garling and Wilson, 1976.

3- Protein to Energy ratio (P/E ratio) = (mg CP/Kcal gross energy).

Table 3. Growth performance and feed consumption of Red tilapia *Oreochromis* Spp. fed on diets containing levels of Basil and Ginger oils

Ingredients	Experimental Diets							SE*
	Control	Basil oil levels			Ginger oil levels			
	1	2	3	4	5	6	7	
	(0.0%)	(1.0%)	(2.0%)	(3.0%)	(1.0%)	(2.0%)	(3.0%)	
Initial wt. (g/fish)	39.50^{a}	39.60^{a}	39.50^{a}	39.20^{a}	39.60^{a}	39.50^{a}	39.20^{a}	0.04
Final wt. (g/fish)	43.40^{d}	46.30^{ab}	47.85^{b}	51.65^{a}	44.20^{cd}	44.90^{cd}	45.50^{cd}	0.73
Gain[1] (g/fish)	3.90^{d}	6.70^{bc}	8.35^{b}	12.45^{a}	4.60^{cd}	5.40^{cd}	6.30^{bc}	0.75
ADG[2] (g/day)	0.08^{d}	0.14^{bc}	0.18^{b}	0.27^{a}	0.10^{cd}	0.12^{cd}	0.14^{bc}	0.01
SGR[3]%./day[1]	0.08^{d}	0.14^{bc}	0.17^{b}	0.25^{a}	0.10^{cd}	0.10^{cd}	0.13^{bc}	0.01
SR[4] (%)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	0.00
(K)[5] factor	1.50^{a}	1.36^{a}	1.65^{a}	1.70^{a}	1.45^{a}	1.63^{a}	1.52^{a}	0.03
FI (g/fish)	36.46^{d}	38.10^{b}	38.22^{b}	38.97^{a}	36.85^{cd}	37.26^{c}	37.54^{bc}	0.85
FCR[6]	9.44^{a}	5.73^{cd}	4.57^{de}	3.16^{e}	8.02^{ab}	6.90^{bc}	5.99^{cd}	0.55
FE	10.69^{d}	17.57^{bc}	21.84^{b}	31.91^{a}	12.48^{cd}	14.49^{cd}	16.77^{bc}	1.88
PER[7]	0.35^{d}	0.58^{bc}	0.72^{b}	1.06^{a}	0.41^{cd}	0.48^{cd}	0.55^{bc}	0.06
PPV[8]	644^{a}	609^{a}	604^{a}	592^{a}	628^{a}	626^{a}	620^{a}	6.53

Values containing a common superscript in the row have not significantly (P>0.05).
*SE refers to standard error.

1- Gain (g) = Final weight (g) – Initial weight (g).
2- ADG = Gain (g) / Time (day) .
3- SGR (%/day) = {100 x (ln final weight – ln initial weight)}/days.
4- Survival rate (%) = (Final fish number /initial fish number) x 100
5- Condition factor = {(Final weight) /(Final length)3} x 100
6- Feed conversion ratio = dry matter intake / gain
7- Protein efficiency ratio = weight gain / protein intake
8- Protein productive value = 100 x protein gain/protein intake

Table 4. Whole composition of Red tilapia *Oreochromis* Spp. fed diets contain levels of Basil and Ginger oils

No.	Experiment No.								SE*
	Initial fish	Control	Basil oil levels			Ginger oil levels			
		(0.0%)	(1.0%)	(2.0%)	(3.0%)	(1.0%)	(2.0%)	(3.0%)	
Dry matter	64.84	67.11	69.11	70.23	70.51	71.80	72.05	73.11	0.51
% of dry matter basis	16.46	18.56	19.56	19.32	18.74	18.50	18.27	17.97	0.14
Crude protein	64.84	68.11	69.11	70.23	70.51	71.80	72.05	73.11	0.44
Ether extract	18.70	15.33	11.33	10.45	10.75	9.70	9.68	8.92	7.35
Ash	16.46	17.56	19.56	19.32	18.74	18.50	18.27	17.97	0.26

Values containing a common superscript in the row have not significantly (P>0.05).
*SE refers to standard error.

Table 5. Hematological indices of Red tilapia *Oreochromis* Spp. fed diets contain levels of Basil and Ginger oils.

Indices	Experiment No.							SE*
	Control	Basil oil levels			Ginger oil levels			
	1	2	3	4	5	6	7	
	(0.0%)	(1.0%)	(2.0%)	(3.0%)	(1.0%)	(2.0%)	(3.0%)	
RBCs[1] × 10^6	1.50[a]	1.80[a]	1.23[a]	1.26[a]	1.06[a]	1.73[a]	1.22[a]	0.10
WBCs[1]× 10^3	27.92[c]	30.71[bc]	34.14[ab]	28.49[c]	27.50[c]	29.90[bc]	38.33[a]	1.07
Hb[2] (g dl^{-1})	4.50[a]	5.40[a]	4.45[a]	4.93[a]	4.25[a]	5.20[a]	6.35[a]	0.25
Hct[3]%	13.50[a]	16.20[a]	12.75[a]	14.25[a]	11.75[a]	15.60[a]	14.60[a]	0.73
PLT[4]	53.50[a]	62.50[a]	59.50[a]	36.50[a]	44.50[a]	46.00[a]	59.50[a]	6.00
MCV[5]	109.10[a]	121.10[a]	120.75[a]	125.50[a]	116.85[a]	115.35[a]	119.30[a]	2.92
MCH[5] (pg)	45.45[a]	48.25[a]	45.50[a]	47.25[a]	51.40[a]	48.80[a]	52.15[a]	1.15
MCHC[5%]	30.50[c]	38.85[ab]	33.65[bc]	34.45[bc]	36.15[bc]	44.10[a]	39.75[ab]	1.27

Values containing a common superscript in the row have not significantly (P>0.05).
*SE refers to standard error.
1- Red blood cells and white blood cells were calculated according to **Dacie and Lewis (1991)**
2- hemoglobin was calculated according to **Drabkin and Austin (1932)**
3- Hematocrit was calculated according to **Sorrell-Raschi and Tomasic (1998)**
4- The number of platelets according to **Brecher *et al.* (1953)**.
5- MCV, MCH, and MCHC were calculated according to **Jain (1993)**.

Hematological indices of red tilapia showed by Table 6. No significantly differences in the count of red blood cells 'RBCs'; hemoglobin 'Hb' and hematocrit 'HCT' in the Basil and Ginger oil diets, but significant differences were observed in the count of white blood cells 'WBCs' between the treatments. 3% Ginger oil diet gave an increase in the count of 'WBCs'.

DISCUSSION

In the current study, the results showed that the diets containing basil oil were better than the diets containing ginger oil in terms of growth performance and feed consumption. **de Souza *et al.* (2019)** revealed that adding basil oil at a level of 3 ml to fish diets led to enhanced growth performance. Also, **Chung *et al.*(2020)** revealed the effects of levels of basil oil on the diets of young arapaima fish, as the basil oil diets at the level of 2 ml recorded the highest 'WG' and 'SGR', while FCR decreased. On the other side, **Amirkhani and Firouzbakhsh (2015)** explained that carp fish were fed on the basil extract diet with 400ml/kg recorded the highest growth rate. While the feed conversion ratio recorded the lowest growth rate (P<0.05) at 400 and 800ml/kg basil extracts.

Brum *et al.* (2017) showed that the basil oil diet at a level of 0.5, which the Nile tilapia fish were fed, showed the best increase in growth and was better than the ginger oil diet. However, **Chung *et al.*(2021)** found that Fish diets supplemented with 0.5 ml level of ginger oil resulted in improved growth and feed conversion rate. A group of Nile tilapia fish were fed a diet with 0.5% ginger oil showed a significant increase in growth performance, But gave the lowest feed conversion ratio **(Abu-Alya *et al.*, 2022). Alsaiad and Al-Zayat (2019)**

showed that the group of Nile tilapia fish fed with a 1% level of ginger extract gave the highest growth performance, while ginger extract with 1% and 0.5% levels recorded that the highest rate of feed conversion and protein efficiency. On the other side, Diets supplemented with garlic or onion oil extract that were fed to Nile tilapia fish at a level of 1% recorded the highest weight and growth rate, while no increase($P>0.05$) in feed conversion rate was recorded **(Hussein *et al.*, 2016)**.

In the current study, no significant differences were observed in the count of red blood cells, hemoglobin, and hematocrit in the basil and clove oil diets but, significant differences were observed in the count of white blood cells between the treatments, ginger oil diets were better than basil oil diets in number of white blood cells. Although, **Mohammadi *et al.* (2020)** reported that carp fish that were fed on (0.2% and 0.4%) ginger extract in diets recorded an increase in the count of red blood cells, white blood cells, hemoglobin level, and value of hematocrit. **El-Ashram *et al.*,(2017)** showed that Nile tilapia fish fed on basil oil at levels of (0.25%, 0.5%, and 1%) in diets recorded the highest percentages in hematocrit, respectively. the group of common carp fish fed 0.5% ginger oil in their diet recorded a superiority in the number of Red blood cells(1.14×106 cells) and white blood cells (146.74×103 cells) **(Al-Hussaini and Salman, 2022)**. Also, **Chung *et al.* (2021)** found that Increasing levels of ginger oil in Nile tilapia fish diets led to an increase in white blood cells ($y = 157.324 + (15.342\times)$).

Acar *et al.* (2019) noted that there were no significant differences between treatments of sea bass fed bergamot peel oil diets in the count of red blood cells; hematocrit; and hemoglobin concentration. **Gaber (2000)** found that Nile tilapia fish fed on diets containing clove oil showed significant changes in hemoglobin, as hemoglobin values increased when clove oil levels increased to 80 ml, as hemoglobin values were affected by clove oil and white blood cells increased**.**

In the current study, the results revealed that PLT, MCV, and MCH had no significant differences between the treatments ($P>0.05$) but, MCHC value had significant differences ($P<0.05$) between the ginger oil and basil oil treatments, as the ginger oil diets showed the higher increase in MCHC. **Chung *et al.* (2021)** reported that blood measurement parameters (MCV, MCH, and MCHC) and platelets in the Nile tilapia fish group fed on diets with levels of ginger oil had no significant differences. However, **Shokr and Mohamed (2019)** showed that the Nile tilapia fish group were fed diets containing levels of ginger powder led to an increase ($p<0.05$) in PLT and MCHC and a decrease in MCV and MCH.

Conclusion

The results of the current study showed that using Basil oil in the diets of fish can increase growth performance and feed consumption better than Ginger oil which can enhance the immune response in Red tilapia, *Oreochromis* Spp. to evaluate the effect of mixing the Basil and Ginger oils, more studies are recommended.

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الملخص العربي

تاثير مستويات مختلفة من زيوت الريحان والزنجبيل علي اداء النمو واستخدام العلف ومؤشرات الدم على اصبعيات البلطي الاحمر

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تم تصنيع 7 علائق تجريبية تحتوى على (0.0%)، (1.0%)، (2.0%)، (3.0%) من زيت الريحان وزيت الزنجبيل بهدف دراسة تأثير هذه النسب المختبرة على أداء النمو ومعدل البقاء وكفاءة استهلاك العلف والتأثير على مكونات الجسم ومؤشرات الدم لاصبعيات سمك البلطي الأحمر *Oreochromis* Spp . تم استخدام ستة عشر حوضًا زجاجيًا (60 × 40 × 40 سم، بسعة 96 لترلكل حوض)، بواقع حوضين لكل معاملة. وتم تخزين عشرة أسماك بنفس متوسط الوزن (39.4±0.3 جم/سمكة) في كل حوض أسماك. تم تغذية الأسماك بنسبة 3% من وزن الجسم مرتين يومياً لمدة 45 يوماً. تبين وجود فرق معنوى كبير (P<0.05) في الوزن النهائي والزيادة ومعدل النمو النوعي، ومعدل التحويل الغذائي، وكفاءة البروتين والقيمة الحيوية للبروتين الإنتاجي بين الأسماك التى تم تغذيتها على علائق تحتوى على زيت الريحان وزيت الزنجبيل عن عليقة المقارنة (بلا اى اضافة لاى زيت مختبر). أدت زيادة مستويات زيوت الريحان والزنجبيل إلى تحسن في النمو ونسبة تحويل الأعلاف وكفاءة الاحتفاظ بالمغذيات. أدت زيادة زيت الريحان بمستويات مختلفة فى العلائق إلى زيادة في أداء النمو واستهلاك العلف وكانت أفضل من علائق زيت الزنجبيل، بينما أظهرت علائق زيت الزنجبيل زيادة في عدد خلايا الدم البيضاء مقارنة بالعليقة الكنترول. وكان العليقة التى تحتوى على 3% من زيت الريحان متفوقة على باقى المستويات المختبرة الأخرى ويوصى باستخدامها في علائق البلطي الأحمر.

الكلمات الإسترشادية : زيت الريحان، زيت الزنجبيل، البلطي الاحمر، الاضافات الغذائية، تغذية الأسماك.

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