

## Influence different levels of Oregano extract (*Origanum vulgare*) as Food Additives on growth performance, chemical composition and blood components for *Oreochromis niloticus* fingerlings under semi-intensive culture conditions

## El-Moghazy, M. M.<sup>1</sup>; A. F., Fath El-Bab<sup>1</sup>; M. T. Shehab El-Din<sup>2</sup>; and Eslam, A. Mohameed<sup>1</sup>

1- Animal production, Faculty of Agriculture, Damietta University, Egypt.

2- Central laboratory for Aquaculture Research at Abbassa, Fish Health, (Sakha Unit), Egypt.

\*Corresponding Author (Ahmed Farouk) E. Mail Address: ah\_farouk74@yahoo.com, Received: Feb. 27, 2018; Accepted: March 29, 2018 Vol.8 (1):13-30

## ABSTRACT

The aim of this study is to investigate the effect of oregano (Origanum vulgare) by different levels (control, 0.05, 0.10, 0.15 and 0.20% Origanum vulgare) in Oreochromis niloticus diets on Growth performance parameters, histology and chemical analysis of sex reversed males of Nile tilapia O. niloticus. Ten concrete ponds  $3x7 \times lm (21 \text{ m}^3 \text{ for each} - \text{semi intensive culture})$ were used in this study. The ten ponds represented 5 treatments (with 2 replicates for each treatment), and each pond was stocked with lo fish /  $m^3$  (210 fish for each pond) with an initial weights average ranged between 4.90 and for five treatments. 5.02 +0.67gm respectively. Fingerlings were fed at a rate of 5% of the total fish mass until the end of experimental period (16 weeks). All diets contained 30% crude protein and about 360 Kcal/100 g digestible energy. fish fed these diets twice daily for six days a week. The duration of the experiment was 16 weeks. The results obtained from this experiments showed that: -

The highest rate of final body weight (FBW), daily weight gain (DWG), final body length (FBL), specific growth rate (SGR) and protein efficiency ratio (PER), were recorded for the third treatment (0.10% *Origanum* 

*vulgare*). while, the first treatment (control) was recorded the lowest growth parameters (FBW, DWG, FBL, SGR and PER).

The chemical composition for the whole fish, the second treatment was recorded the highest rate of protein% and the third treatment recorded the highest rate of fat% and the first treatment recorded the highest rate of ash%. As for the edible part of the fish, the second treatment was recorded the highest rate of protein and ash % and the third treatment recorded the highest rate of fat%. With regard for the Blood components, the fifth treatment had the highest rate of hemoglobin and creatinine and the third treatment was recorded the highest rate of hemoglobin and treatment recorded the highest rate of highest rate of red blood cells and the first treatment recorded the highest rate of liver functions and cholesterol. The second treatment recorded the highest rate of total protein and albumin.

Based on the results obtained in this study, it can be concluded that the use of 0.10% oregano extract was best for growth, liver function and chemical composition under semi-intensive culture.

Key words: oregano, *Origanum vulgare*, intensive culture, growth performance, *O. niloticus* 

#### Introduction

Phytogenics are a class of natural growth promoters (NGPs) or non- antibiotic growth promoters utilized as feed additives, obtained from herbs, spices or other plants (**Yang** *et al.*, **2015**; **Yitbarek**, **2015**). They are normally considered as suitable alternatives to antibiotic growth promoters (AGPs) in livestock production (**Costa** *et al.*, **2013**). For many years phytogenics have been used for medical purposes, as food preservatives, and spices for human food (**Steiner**, **2009**). They derive their names from the plants from which they are obtained and are of different forms. They can be from dried and ground seeds, roots, leaves, peels, twigs, barks, stems, flowers and fruits from herbs and spices or extracts from different plant parts in the form of essential oils (Eos) (**Gabor** *et al.*, **2012**). Thymol and carvacrol have been evaluated because they are commercially available and economically feasible. They are major compounds of Eos derived from thyme, oregano and citrus, respectively. Thymol and carvacrol are constituents of Eos from plants belonging to the family *Lamiaceae* (Michiels *et al.*, 2012). Carvacrol is a clear liquid with a molecular weight of 150.21 and its density is 0.975 g cm<sup>-3</sup> at 25 °C with a boiling point of 237 - 238 °C (Lee *et al.*, 2003; Suganthi and Manpal, 2013; Alagawany *et al.*, 2015).

The genus *Origanum* provides the well-known oregano spice source Greek and Turkish guys. oregano The name is derived from the Greek Oros Ganos a mountain and the joy. Thus, it is often known locally as "The joy of the mountains". The Greeks and Romans used the oregano, but that the species has never been clear to them and confusion began in the beginning of its history. In the Middle - age in Poland, the oregano was used against a number of diseases. The twenty - the eighth century herbalist K 'Eogh describes the oregano as having "a hot character and dry". It is good against the pain of stomach and heart and also useful for coughs, pleurisy, and obstruction of the lungs and stomach, and also comforts the head and nerves **Colombo** *et al.*, (2014).

Oregano is coming in America North with various colonists and escaped from gardens in the wild. The oregano quickly is part of the traditional medicine in the States United. It was not until the Second World War that the oregano grown in importance as a flavoring. The military returned the Mediterranean brought the oregano taste, and once the pizza has become ingrained in the American consciousness, oregano became every American. (**Ferreira** *et al.*, 2014).

The aim of the present experiment was for study the effect of *Origanum vulgare* on growth performance parameters and chemical composition liver and Kidney functions for *O. niloticus* under semi-intensive culture conditions.

#### **Material and Methods**

#### Location:

The present study was carried out at a private farm in Tollumbat No. 7 in Riyad City, Kafr El-Sheikh governorate, Delta district at the Northern part of Egypt. The aim of this study is to investigate the effect of *Origanum vulgare* on Growth performance parameters, histology and chemical analysis for mono sex Nile tilapia (*O. niloticus*) fingerlings.

#### **Experimental design:**

The experiment began at the 5<sup>th</sup> July 2016 and continued to the 25<sup>th</sup> of October of the same year (112 days). Ten concrete ponds 3x7x1m (21 m<sup>3</sup> for each – semi intensive culture) were used in this study. The ten ponds represented 5 treatments (with 2 replicates for each treatment), and each pond was stocked with lo fish / m<sup>3</sup> (210 fish for each pond) with an initial weight averaged 4.90, 5.60, 5.49, 5.01 and 5.02 ± 0.67gm for five treatments, respectively.

#### Fish source:

The experimental mono sex *O. niloticus* fingerlings were obtained from the same farm in Tollumbat No. 7 in Riyad City, Kafer El-sheikh Governorate. The experimental fish were transported in a tank to the ponds, and after arrival to the ponds fish were adapted and distributed randomly into ten ponds represents the five treatments studied with replicates.

#### **Tested diets:**

The tested diets were formulated to contain 30% crude protein and about 360 Kcal/100 g digestible energy. Formulation of the experimental diets and the chemical composition are illustrated in table (1).

#### **Feeding practices:**

Fish were fed at a daily rate of 3% of total biomass until the end of experimental period for 6 days/week (twice daily at 9.00 am and 3.00 pm). Every biweekly, thirty fish randomly obtained from each pond then weighed and the amount of feed was adjusted according to the changes in body weight throughout the experimental period.

Feed ingredients	Experimental diets				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal (72%)	15	15	15	15	15
Yellow corn (9.6%)	28	28	28	28	28
Soybean meal (40%)	41	41	41	41	41
Wheat bran (11.4%)	10.5	10.5	10.5	10.5	10.5
Vegetable oil	2.50	2.45	2.40	2.35	2.30
Vit. & Min. mixturel <sup>1</sup>	3.00	3.00	3.00	3.00	3.00
oregano ( <i>Origanum</i> vulgare)	00	0.05	0.10	0.15	0.20
Sum	100	100	100	100	100
Crude protein (CP)	28.141	28.27	28.11	27.98	27.91
Ether extract (EE)	4.44	4.63	4.87	4.96	4.98
Crude fiber (CF)	9.33	10.22	10.10	10.19	10.14
Ash	10.12	10.14	10.33	10.44	10.39
Digestible energy (Kcal/kg)	3602	3616	2590	2627	2621

Table 1: Food composition and proximate chemical analysis of the experimental diets.

### **Growth parameters:**

Records of live body weight (g) and body length (cm) of individual fish were measured in 30 fish for each pond and registered every 14 days (two weeks) in the experiment. Growth performance parameters were measured by using the following equations:

## A- Growth performance:

-Condition factor (K): it expresses the relationship between weight and length of fish. Condition factor was obtained using the following formula: -

 $K = (W/L^3) \ge 100$ 

Where: -

W = weight of fish in "grams"

L= total length of fish in "cm"

-Specific growth rate (SGR): it is one of the most important methods of growth expression, which are related to time and estimated by the following equation.

$$SGR = \frac{LnW2 - LnW1}{t} \times 100$$

Where: -

ln = the natural log

W1= first fish weight

W2 = the following fish weight in "grams" and

t = period in days.

- Daily weight gain = final weight – initial weight (g)/ Period (day).

B- Feed efficiency parameters:

-Feed conversion ratio (FCR):

one of the parameters used to determine the value of feeds for providing the necessary food amount required for one unit of growth.

A lower value indicates an improved outcome. Feed conversion ratio was calculated by the equation

FCR = Feed ingested (g)/Weight gain (g)

- protein efficiency ratio (PER):

PER is probably the most widely method used for evaluating protein quality in fish. PER was measured by the following equation.

PER = Weight gain (g)/Protein ingested (g)

## - Blood sampling:

At the end of the experiment, ten fish were chosen at random from each pond and scarified with a sharp knife. Blood samples of five fish were collected from the caudal vein in clean tube with 10% EDTA solution to determine hematocrit (Ht) and hemoglobin (Hb). Blood samples of the other five fish were collected also from the caudal vein in clean dry centrifuge tubes, kept for 15 minutes and centrifuged at 3000 rpm for lo minutes, then kept frozen at -20°C for determination of blood chemistry, aspartate aminotransferase (AST) and alanine aminotransferase (AlT), creatinine, glucose. Total protein was determined by the method of **Bradford** (1976), albumin content by the method of **Doumas** *et al.*, (1971). Serum cholesterol and triglycerides were measured by the method of **Trinder** (1969) method.

## **Hematological Parameters:**

Hematocrit (Ht) was determined by the microhematocrit method as described by **Reitman and Frankel** (1957). Whereas, (Hb) was determined by the total hemoglobin kit which is a standardized procedure of the cyanomethemoglobin method.

## **Blood chemistry:**

Aspartate aminotransferase (AST) and alanine aminotransferase (AIT) activities were determined according to the method described by **Reitman and Frankel (1957)**. Serum creatinine and uric acid were measured by calorimetric method and enzymatic determination methods, respectively as described by **Henry (1974)**.

## Chemical analysis of whole fish body and experimental diets:

Chemical composition of fish and diet samples were determined according to procedures of **AOAC** (**1990**). Dry matter was determined after drying the samples in an oven (105°C) for 24h. Ash by incineration at 550°C for 12 h. Crude protein was determined by micro-Kjeldhal method, N%  $\times$  6.25 (using Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40 - 60°C).

## Statistical analysis:

The statistical analysis of date was carried out by applying the computer program, **Sas (1996)** by adopting the following model: -

 $Yijk = \mu + R_i + B_j + E_{ijk}$ 

Where:

 $Y_{ijk}$  = the K-<sup>th</sup> observation of the J-<sup>th</sup> treatment and i-<sup>th</sup> replicate.

 $\mu$  = overall mean

 $R_i$  = the effect of i-<sup>th</sup> replicate

 $B_i$  = the effect of j-<sup>th</sup> treatment

 $E_{ijk}$  = random error assumed to be independently randomly distributed (0,  $\delta^2 e$ )

Differences among means were tested for significance according to **Duncan's multiple range test (1955).** 

#### **Results and Discussion**

# Effect of oregano (*Origanum vulgare*) levels for fish diets on growth performance of Nile tilapia *O. niloticus*:

As presented in table (2) averages of initial body weight ranged between 4.9 to 5.6 with no significant differences among the experimental groups table (2) indicating the complete randomization of fish distribution among the five treatments. at the final experimental period the results indicated that, compared to un food additives in diets (controls), diets supplemented with 0.5 and 0.10% thymol and carvacrol had significant growth-promoting effects on Nile tilapia.

The results were agreement with Lee *et al.*, (2012) found that, phytogenics perform their initial activity in feeding as a flavor and thereby influence eating patterns, the secretion of digestive fluids and total feed intake. The stimulation of digestive secretions including saliva, digestive enzymes, bile and mucus is considered to be an important action of feed additives. In another way, olfactory feed ingredients enhance the growth through their ability to act as feeding enhancers for fish to eat more feed than normal (Adams, 2001).

Results of DWG illustrated in Table (2) indicated that, the increasing percentages of oregano at level 0.05 and 0.10% significantly (P < 0.05) increased daily weight gain of tilapia fish compared with 0.15 and 0.20% of Origanum vulgare. The final mean weight of Nile tilapia fed on diets 0.05, 0.10, 0.15 and 0.20% Origanum vulgare were higher than those of fish fed the control diets, these diets enhanced daily weight gain and % fish survival compared with the control. Growth- promoting effects of Origanum vulgare have also been reported in other fish species and monogastric animals (Juin et al., 2003; Acar et al., 2015; Dalkilic et al., 2015; Ngugi et al., 2016). With Japanese quails, Eos containing 92.3 % thymol and carvacrol enhanced WG of quails from day 14-35 compared to the control (Dalkilic et al., 2015). With Mozambique tilapia, Acar et al., (2015) reported that a diet supplemented with 1000 ppm of an Eos extract from sweet orange peels containing 83 % limonene and thymol improved the % WG of the fish compared to the control. In addition, 1000, 2000, 5000 and 8000 ppm of bitter lemon (Citrus limon) Eos containing 81.4 % limonene and thymol improved FW and WG of ningu (*labeo victorianus*) fingerlings (**Ngugi et al., 2016**).

**Table (2):** Effect of increasing percentage of oregano (*Origanum vulgare*) in prepared diets on growth performance and feed utilization of Nile tilapia.

Diets	N 0.	D1 contro1	D2 0.05%	D3 0.10%	D4 0.15%	D5 0.20%
Initia1 weight	60	4.9±0.67	5.6±0.67	5.49±0.67	5.01±0.67	5.02±0.67
Fina1 weight	60	132.64±1. 85c	158.81±1.8 5ab	161.13±1. 85a	140.94±1. 85b	137.82±1.8 5bc
DWG	60	1.14±0.07 4b	1.37±0.074 a	1.39±0.07 4a	1.21±0.07 4b	1.19±0.074 b
Initia1 1ength	60	7.50±0.13	7.92±0.13	7.89±0.13	7.50±0.13	7.38±0.13
Fina1 1ength	60	21.40±0.6	21.90±0.6	22.10±0.6	21.30±0.6	21.60±0.6
Initia1 K	60	1.18±0.20	1.17±0.20	1.16±0.20	1.20±0.20	1.27±0.20
Fina1 k	60	1.56±0.37 c	1.75±0.37a	1.72±0.37 a	1.68±0.37 bc	1.57±0.37c
Specific growth rate	60	1.75±0.01 8b	3.01±0.018 a	3.04±0.01 8a	2.99±0.01 8a	2.97±0.018 a
Protein efficienc y ratio	60	2.52±0.02 2b	2.76±0.022 a	2.80±0.02 2a	2.67±0.02 2ab	2.47±0.022 b
Feed conversi on ratio	60	1.72±0.48 a	1.41±0.48c	1.37±0.48 c	1.62±0.48 b	1.64±0.48b

Averages within each row having different letters are significantly different (P<0.05), otherwise they are not.

Average of fish body length (cm) as affected by *Origanum vulgare* in fish diets are illustrated in Table (2). Results presented in this table indicate that, differences in initial body length among the

experimental fish groups were insignificant which indicate that the experimental groups at the start were homogenous and randomly distributed. and the end of experimental period The third treatment (T2) (0.10% oregano) gave the heaviest body lengths and the control (o% oregano) gave the lowest body lengths.

In accordance with **Rajat and Panchali**, (2014), *O. niloticus* fed diet supplemented with phytogenic showed the highest Bl compared with fish fed control diet. on the other hand, **Jarolowicz** *et al.*, (2012) reported that, Juvenile European pikeperch, *Sander lucioperca* fed commercial feed supplemented with phytogenic did not have an impact on final body length.

At the start of the experiment average values of condition factor (K) ranged between 1.16 and 1.20 with insignificant differences between these means which indicate the random distribution of fish groups (table, 2). while the final condition factor The first treatment (control-0% oregano) gave the heaviest condition factor and third treatment (Tl) (0.10% oregano) the gave the lowest condition factor. these results are in agreement with, **Jarolowicz** *et al.*, (2012) reported that, Juvenile European pikeperch, *Sander lucioperca* fed commercial feed supplemented with phytogenic had no impact on final condition factor.

on the other hand, **Mehrabi** *et al.*, (2012) reported that the addition of phytogenic to the feed produced the better K with values significantly (P<0.05) higher than the control in rainbow trout (*oncorhynchus mykiss*) fingerlings.

Results of SGR illustrated in Table (2) indicated that, the increasing percentages of *Origanum vulgare* at level 0.05 and 0.10% significantly (P < 0.05) increased daily weight gain of tilapia fish compared with 0, 0.15 and 0.20% of *Origanum vulgare*. Zheng *et al.*, (2009) he found that, channel catfish of 50 g attained a significantly higher final weight than the control with diets formulated with 500 ppm of carvacrol and a blend of carvacrol- thymol (. The reason for the discrepancy in results could not be explained. Humer *et al.*, (2014) reported that post larvae of *Penaeus monodon* had significantly higher weight gain and specific growth rate when fed with herbal appetizer, *Zingiber officinalis* enriched Artemia.

Contrary to this study, **Yilmaz** *et al.*, (2015) did not observe an improvement in WG and SGR of rainbow trout fed on diets supplemented with 1000, 3000 and 5000 ppm carvacrol.

# Effect of addition of oregano (*Origanum vulgare*) for fish diets on feed utilization of *o. niloticus*:

#### Feed conversion ratio (FCR):

Average FCR (table, 2) during the whole experimental period for the experimental diets control, 0.05, 0.10, 0.15 and 0.20% *Origanum vulgare* were 1.72, 1.41, 1.37, 1.62 and 1.64, respectively. Analysis of variance show insignificant differences between FCR averages which indicate that, the addition of *Origanum vulga*re in tilapia diets had improved (FCR) which the best FCR was recorded for treatment 3 (0.10%) and lowest FCR was recorded for control. This suggested that the enhanced somatic growth of Nile tilapia found in this study with thymol and carvacrol supplemented diets was due to efficient utilization of nutrients in the diets as a result of other nutritional factors like enhanced nutrient transportation and absorption, lipid and energy metabolism and antioxidant activity among others (**Hashemi and Davoodi, 2010; Steiner and Syed, 2015; Zeng et al., 2015; Abd El-Hack, et al., 2016**).

## Protein efficiency ratio:

Average PER during the whole experimental period (Table, 2) for the experimental diets control, 0.05, 0.10, 0.15 and 0.20% *Origanum vulgare* were 2.52, 2.76, 2.80, 2.67 and 2.47, respectively. Analysis of variance show insignificant differences between PER averages which indicate that, the addition of origanum vulgare in tilapia diets improved PER which treatment 3 (0.10%) recorded the best PER and the lowest PER was recorded for treatment 5 (0.20%) and control.

Loum et al., (2013) found that, the higher PER values found with thymol-fed fish compared to those fed on carvacrol and limonene supplemented diets is attributed to a physiological age/size- related effect with smaller fish utilizing protein more effectively than bigger fish because they are in an active growth stage. In addition, PER is known to be lower with higher quantity of protein in the diet (Sweilum et al., 2005).

## Chemical composition of fish:

Chemical composition for whole fish, flesh and by products:

Results of chemical composition of whole fish body indicated that, addition of 0.15 and 0.20% of *Origanum vulgare* decreased protein content of whole fish from 61.95 and 61.68% but the differences between these means were not significant however as the addition level reached at 0.05 and 0.10% *Origanum vulgare*, protein content of whole fish decreased significantly to 63.57 and 64.42%, respectively.

Results of chemical composition of whole fish body indicated that, moisture content of fish flesh was 81.31, 81.32, 80.38, 82.02 and 79.96 %, respectively. fat percentages in fish flesh of fish fed the different experimental diets Dl, D2, D3, D4 and D5 were 4.77, 5.57, 5.39, 4.10 and 4.13 %, respectively. protein percentages were 86.19, 84.75, 88.57, 87.28 and 84.38 %, respectively. Ash values were 7.68, 8.07, 7.62, 7.20 and 7.05 % respectively and the differences between these means of the groups except for fat were significant.

Average of moisture percentages in by-products for fish fed the experimental diets (control, 0.05, 0.10, 0.15 and 0.20 *Origanum vulgare*) were 74.84, 72.82, 72.15, 71.87 and 71.54 %, fat 6.51, 7.55, 7.83, 6.02 and 5.85% and ash 36.34, 38.36, 37.77, 41.59 and 42.18%, protein 5.51, 52.07, 52.92, 51.18 and 51.9, respectively and the differences between average of protein, fat and ash were significant (P> 0.05).

Results of the present study are in close agreement with those obtained by **Yang** *et al.*, (2015) and **Zeng** *et al.*, (2015) indicated that, *O. niloticus* fed diet supplemented with phytogenic recorded high level of dry matter and lipid content than other control group and had no effect on ash content. Also, **Dalkilic** *et al.*, 2015; Ngugi *et al.*, (2016) found that, the dietary inclusion of thymol and carvacrol increased protein content and lowered fat content of the whole fish body, without significant differences in ash content of Nile tilapia.

Contrary, **Farahi** *et al.*, (2012); Kim *et al.*, (2013) reported that carcass composition was not affected by phytogenics inclusion for tilapia fish, phytogenics. Lee *et al.*, (2012); Guroy *et al.*, (2014) reported that body fat content is closely related to weight gain and inversely related to body moisture content and this agreed with the obtained results of the present study.

Table (3): Effect of increasing percentage of oregano (*Origanum vulgare*) in prepared diets on Chemical composition of Nile tilapia.

Diets		D1 contro1	D2 0.05%	D3 0.10%	D4 0.15%	D5 0.20%	Polled SE		
Chemical composition for whole fish									
Moisture	6	80.53±1.29a	77.41±1.29ab	79.21±1.29a	79.07±1.29a	77.38±1.29b	$\pm 1.29$		
Protein	6	63.26±0.42b	64.57±0.42a	64.42±0.42a	61.95±0.42ac	61.68±0.42c	$\pm 0.42$		
Fat	6	22.18±0.18a	21.63±0.18b	21.71±0.18b	20.82±0.18c	20.48±0.18c	$\pm 0.18$		
Ash	6	16.41±0.58a	14.91±0.58b	13.82±0.58c	14.67±0.58b	14.93±0.58b	$\pm 0.58$		
Chemica1	cor	nposition fish	f1esh						
Moisture	6	82.31±0.75	$81.32 \pm 0.75$	$80.38 \pm 0.75$	$82.02 \pm 0.75$	$79.96 \pm 0.75$	±0.75		
Protein	6	86.19±3.80b	84.75±3.80c	88.57±3.80a	87.28±3.80a	84.38±3.80c	±0.83		
Fat	6	4.77±0.23b	5.57±0.23a	5.39±0.23a	4.10±0.23b	4.13±0.23b	±0.23		
Ash	6	$7.68 \pm 0.49$	$8.07 \pm 0.49$	$7.62 \pm 0.49$	$7.2 \pm 0.49$	$7.15 \pm 0.49$	±0.49		
Chemica1	Chemica1 composition for fish by products								
Moisture	6	74.84±0.94a	72.82±0.94b	72.15±0.94b	71.87±0.94b	71.54±0.94b	±0.94		
Protein	6	51.51±0.91	52.07±0.91	52.92±0.91	51.18±0.91	51.09±0.91	±0.91		
Fat	6	6.51±0.29ab	7.55±0.29a	7.83±0.29a	$6.02 \pm 0.29b$	5.85±0.29b	$\pm 0.29$		
Ash	6	36.34±0.64b	38.36±0.64b	37.77±0.64b	41.59±0.64a	42.18±0.64a	±0.64		

Averages within each row having different letters are significantly different (P<0.05), otherwise they are not.

Effect of addition of oregano (*Origanum vulgare*) for fish diets on some hematological indices, liver functions, total protein, albumin, creatinine triglyceride and cholesterol of *O. niloticus*.

Hemoglobin (Hb) as affected by *Origanum vulgare* levels increased from 10.61 to 10.88, 11.20, 11.09 and 11.75g/dl and (Ht) 14.72, 14.87, 15.43, 16.85 and 15.65% for fish fed the diets supplemented with the five *Origanum vulgare* levels control, 0.05, 0.10, 0.15 and 0.20% *Origanum vulgare*.

Red blood cells (RBCs) significantly (P<0.05) increased with each increase in *Origanum vulgare* and fish T3 (addition of 010% *Origanum vulgare*) recorded the highest value of RBCs. Also, rates of MCV followed the same trend (table, 4).

Hashemi and Davoodi, (2010) found that red tilapia fed diet supplemented with phytogenics showed no significant differences between Hb and Ht values of fish fed the diet supplemented with phytogenics and Hb and Ht of control group. However, Yang *et al.*, 2015 and Yitbarek, (2015) indicated that found phytogenics elevated Ht for *O. niloticus*.

As shown in table 4, ALT values decreased with increasing the *Origanum vulgare* levels, being 87.43, 84.60, 84.60, 84.77 and 86.85u/l for fish fed the diets supplemented with control, 0.50, 0.10, 0.15 and 0.20% *Origanum vulgare*, respectively.

Results in table 4 showed that, AST values were 17.49, 16.25, 15.97, 15.90 and 16.74u/l for fish fed on experimental diets (control, 0.05, 0.10, 0.15 and 0.20% *Origanum vulgare*, respectively). These results indicated that AST values decreased by increasing in *Origanum vulgare* levels. The values of ALP increased with increasing the *Origanum vulgare* levels, being 125.77, 130.93, 132.43, 131.77 and 129.43u/l for fish fed diets supplemented with control, 0.05, 0.10, 0.15 and 0.20%, respectively. And the differences in ALT, AST and ALP values attributed to *Origanum vulgare* levels effect were significant (P<0.01).

Diets		D1 contro1	D2 0.05%	D3 0.10%	D4 0.15%	D5 0.20%	Polled SE
Hematological indi	ces						
Hb (g/d1)	6	10.61c	10.88b	11.20a	11.09a	11.75a	0.041
Ht (%)	6	14.72c	14.87c	15.43b	16.85a	15.65b	0.064
<b>RBC</b> (10 <sup>16</sup> )/cmm	6	1.92d	1.95c	2.02a	1.98b	1.96e	0.0054
MCV	6	59.83d	61.50c	64.00a	62.83b	62.13a	0.31
Liver functions							
ALT (µ/1)	6	87.43a	84.60b	84.60b	84.77b	86.85a	0.11
AST (µ/1)	6	17.49a	16.25b	15.97b	15.90b	16.74a	0.14
ALP (µ/1)	6	125.77b	130.93a	132.43a	131.77 a	129.43	1.04
<b>Blood parameters</b>							
Total protein g/dl	6	3.45c	3.85a	3.72b	3.63b	3.51b	0.047
Albumin g/dl	6	1.48c	1.74a	1.58b	1.27d	1.48b	0.027
Creatinine mg/dl	6	0.84b	0.83b	0.83b	0.94ab	1.07a	0.0037
Triglyceride mg/d1	6	86.23a	85.93b	85.43b	87.10b	88.98a	2.54
Cholesterol mg/d1	6	86.60a	77.93b	71.10c	76.10ab	81.10a	1.81

Table (4): Effect of increasing percentage of oregano (Origanum vulgare) in prepared diets on blood components of Nile tilapia.

Averages within each row having different letters are significantly different (P<0.05), otherwise they are not.

**Chakraborty** *et al.*, (2014) found that, ALT and AST levels were significantly decreased when Nile tilapia fed diets supplemented with phytogenics or spices compared to control group. Similar results were obtained by **Hippenstiel** *et al.*, (2011) who showed a significant lower (P<0.05) levels of ALT and AST activities with Nile tilapia fed fennel seed meal in diets. Whereas, **Suntres** *et al.*, (2013) found that serum AST and AlT levels of Galilee tilapia fed the basal diet supplemented by thymol and carvacrol were not significantly affected. Similarly, **Oluremi** *et al.*, (2007) observed a decrease in activity of the enzymes, like AST, ALT and lactate dehydrogenase (LDH) in *O. niloticus* after being fed with a diet containing thymol. and a mixture of thymol and carvacrol. Similar results were also observed in *Cyprinus carpio* fed thymol (**Zeng** *et al.*, 2015).

Table 4 showed that total protein (TP) contents was significantly increased with the first level of *Origanum vulgare* 3.85 g/dl supplementation and then decreased with increasing *Origanum vulgare* level and the same trend was also observed for albumin and the differences among values are significant (table, 4). As described in table 4, TP and albumin recorded the highest values for fish fed the diet supplemented with *Origanum vulgare* 0.05 % than those fed the other diet. As described in table 4, creatinine values increased significantly by the increasing of *Origanum vulgare* at levels 0.15 and 0.20% *Origanum vulgare* and the same trend was also observed for triglycerides.

Results in table 4 showed also that cholesterol values decreased with each increase in *Origanum vulgare* level up to 0.10% and the differences in cholesterol. Increase in the serum protein and albumin levels is thought to be associated with a stronger innate response in fish (**Suntres** *et al.*, **2013**). In addition, **Brenes and Roura**, (**2010**) reported that diet supplemented with phytogenics increase the serum protein and albumin and globulin level of rainbow trout. **Michiels** *et al.*, (**2008**) reported that application of thymol and carvacrol as feed additives enhanced the immune and health status of tilapia (*O. niloticus*).

#### References

Abd El-Hack, M. E; Alagawany, M; Farag, M. R; Tiwari, R; Karthik, K; Dhama, K; Zorriehzahra, J. and Adel, M. (2016). Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. Journal of Essential Oil Research 28, 365-382.

- Acar, U; Kesbi, O. S; Yilmaz, S; Gultepe, N. and Turke, A. (2015). Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against Streptococcus iniae. Aquaculture 437, 282-286.
- Adams, R.P., (2001). Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Academic Press. New York.
- Alagawany, M; El-Hack, M. E. A; Farag, M. R; Tiwari, R. and Dhama, K. (2015). Biological effects and modes of action of carvacrol in animal and poultry production and health - a review. Advances in Animal Veterinary Science 3, 73-84.
- AOAC (1990). official Methods of Analysis. 15th edition. Association of official Agricultural Chemists, Washington, D.C.
- Bradford, M. M., (976). A rapid and sensitive method for the quantification of microgram quantities of protein. Annual Review of Biochemistry, 72: 248.
- Brenes, A. and Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. Animal Feed Science and Technology 158, 1-14.
- Chakraborty, S. B; Horn, P. and Hancz, C. (2014). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture 5, 1-19.
- Colombo, M; Priori, D; Gandolfi, G; Boatto, G; Nieddu, M; Bosi, P. and Trevisi, P. (2014). Effect of free thymol on differential gene expression in gastric mucosa of the young pig. Animal 8, 786-791.
- Costa, L. B; luciano, F. B; Miyada, V. S. and Gois, F. D. (2013). Herbal extracts and organic acids as natural feed additives in pig diets. South African Journal of Animal Science 43, 181-193.
- Dalkilic, B; Simsek, U. G; Ciftci, M. and Baykalir, Y. (2015). Effect of dietary orange peel essential oil on physiological, biochemical and metabolic responses of Japanese quails as affected by early age thermal conditioning and fasting. Revue Medecine Veterinaire 166, 154-162.

- Doumas, B. T. Watson, W. A., and Biggs, H. G., (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clinica Chimica Acta, 31: 87–96.
- Duncan, M.B. (1955). Multiple ranges and multiple F-tests. Biometrics, 11:1-42.
- Farahi, A; Kasiri M; Sudagar, M; Soleimani, I. M. and Zorriehzahra, S. M. J. (2012). Effect of dietary supplementation of *Melissa* officinalis and Aloe vera on hematological traits, lipid oxidation of carcass and performance in rainbow trout (Oncorhynchus mykiss). online Journal of Animal Feed Research 2, 1-5.
- Ferreira, P. M. F.; Nascimento, L. S.; Dias, D. C.; Moreira, D. M. V.; Salaro, A. L.; Freitas, M. B. D.; Carneiro, A. P. S.; Zuanon, J. A. S. (2014). Essential oregano oil as a growth promoter for the yellowtail tetra, Astyanax altiparanae. Journal of the World Aquaculture Society, New Jersey, v. 45, n. 1, p. 28-34.
- Fishbase (2016). World Wide Web electronic publication. Froese, R. and Pauly. D (eds). www.fishbase.org.
- Gabor, E; Sara, A; Bentea, M; Creta, C. and Baciu, A. (2012). The effect of phytoadditive combination and growth performances and meat quality in rainbow trout (*Oncorhychus mykiss*). Scientific Papers: Animal Science and Biotechnologies 45 (2).
- Guroy, B; Mantoglu, S; Kayali, S. and Sahin, I. (2014). Effect of dietary Yucca schidigera extract on growth, total ammonia-nitrogen excretion and haematological parameters of juvenile striped catfish *Pangasianodon hypophthalmus*. Aquaculture Research 45, 647-654.
- Hashemi, S. R. and Davoodi, H. (2010). Phytogenics as new class of feed additive in poultry industry. Journal of Animal and Veterinary Advances 9, 2295-2304.
- Henry, R. J. (1974). Clinical Chemistry Principles and Techniques, 2nd ed. Harper and Row. Publ, New York, p. 525.
- Hippenstiel, F; Abdel-Wareth, A. A. A; Kehraus, S. and Sudekum, K-H. (2011). Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers - a review. Arch.Geflügelk 75, 226-234.
- Humer, E; Rohrer, E; Windisch, W; Wetscherek, W; Schwarz, C;

Jungbauer, L. and Schedle, K. (2014). Gender-specific effects of a phytogenic feed additive on performance, intestinal physiology and morphology in broiler chickens. Journal of Animal Physiology and Animal Nutrition 99, 788-800.

- Jarolowicz, S., Zakes, Z., Siwicki, Z., Kowalska, A., Hopoko, M, Glabski, E., Demsk- Zakes, K. and Partyka, P. (2012). Effect of brewer yeast extract on growth performance and health of juvenile pikeperch, *Sander lucioperca* (l.) Aquacuture Nutrition, doi: 10.1111/j.1365-2095.2011.00915.x
- Juin, H; Elgaard, T. and Chicoteau P. (2003). Effect of a citrus extract (NoR-SPICE AB) on broiler performances. British Poultry Science 44, 810-811.
- Kim, K. T; Jeon, G. H; Cho, S. H; Lim, S. G; Kwon. M. and Yoo, J. (2013). Effects of dietary inclusion of various concentrations of *Scutellaria baicalensis* Georgi extract on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurus asotus*). Aquaculture Research 44, 1502-1510
- lee, K. W; Everts, H; Kappert, H. J; Frehner, M; losa, R. and Beynen, A. C. (2003). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science 44, 450-457.
- Lee, D; Ra, C; Song, Y; Sung, K. and Kim, J. (2012). Effects of dietary garlic extract on growth, feed utilisation and whole body composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). Asian-Australian Journal of Animal Science 25, 577-583.
- loum, A; Sagne, M; Fall, J; Ndong, D; Diouf, M; Sarr, A. and Thiaw, O. T. (2013). Effects of dietary protein level on growth performance, carcass composition and survival rate of fry monosex Nile tilapia *Oreochromis niloticus* reared under recirculating system. Journal of Biology and life Science 4, 13-22.
- Mehrabi, Z., Firouzbakhsh, F. and Jafarpour, A. (2012). Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Journal of Animal Physiology and Animal Nutrition, 96: 474–481.
- Michiels, J; Missotten, J; Dierick, N; Fremaut, D; Maene, P. and De Smet, S. (2008). In- vitro degradation and in vivo passage kinetics

of carvacrol, thymol, eugenol and trans-cinnaldehyde along the gastrointestinal tract of piglets. Journal of Science of Food and Agriculture 88, 2371-2381.

- Michiels, J; Missottenb, J; ovynb, A; Dierick, N; Fremauta, D. and De Smet, S. (2012). Effects of dose thymol and supplemental flavours or camphor on palatability in a choice feeding study with piglets. Czech Journal of Animal Science 57, 65-74.
- Ngugi, C. C; oyoo-okoth, E. and Muchiri, M. (2016). Effects of dietary levels of essential oil (Eo) extract from bitter lemon (*Citrus limon*) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in juvenile *Labeo victorianus* fingerlings challenged with Aeromonas hydrophila. Aquaculture Research. doi:10.1111/are.13062.
- Oluremi, O. I. A; Ngi, J. and Andrew, I. A. (2007). Phytonutrients in citrus fruit peel meal and nutritional implication for livestock production. livestock Research for Rural Development 19 (7).
- Rajat, G. and Panchali, D. (2014). A study on antioxidant properties of different bioactive compounds. Journal of Drug Delivery and Therapeutics 4, 105-115.
- Reitman, S., and S. Frankel. (1957). Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Journal of Clinical Pathology, 28: 56-59.
- SAS. Statistical Analysis System (1996). SAS Procedure Guide version 6.12 Ed. SAS Institute Inc., Cary, NC, USA.
- Steiner, T. (2009). Phytogenics in Animal Nutrition: Natural Concepts to optimize gut health and performance. Nottingham University Press, Nottingham. ISBN: 978-1-904761-71-6.
- Steiner, T. and Syed, B. (2015). Phytogenic feed additives in animal nutrition. In: Medicinal and aromatic plants of the world: Scientific, production, commercial and utilization aspects. Springer, Dordrecht, The Netherlands. Page 403-423.
- Suganthi, R. U. and Manpal, S. (2013). Biological and pharmacological actions of carvacrol and its effects on poultry: An updated review. World Journal of Pharmacy and Pharmaceutical Science. 2, 3581-3595.

Suntres, Z; Coccimiglio, J. and Alipour, M. (2013). The bioactivity

and toxicological actions of carvacrol. Critical Reviews in Food Science and Nutrition 55, 304-318.

- Sweilum, M. A; Abdella, M. M. and El-din, S. A. S. (2005). Effect of dietary protein- energy levels and fish initial sizes on growth rate, development and production of Nile tilapia, *Oreochromis niloticus* L. Aquaculture Research 36, 1414-1421.
- Trinder, P., (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry, 6: 24–27.
- Yang, C; Chowdhury, M. A. K; Hou, Y. and Gong, J. (2015). Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. Pathogens 4, 137-156.
- Yilmaz, E; Ergun, S. and Yilmaz, S. (2015). Influence of carvacrol on the growth performance, hematological, non-specific immune and serum biochemistry parameters in rainbow trout (*Oncorhynchus mykiss*). Food and Nutrition Sciences 6, 523-531.
- Yitbarek, M. B. (2015). Phytogenics as feed additives in poultry production: A review. International Journal of Extensive Research 3, 49-6
- Zeng, Z; Zhang, S; Wang, H. and Piao. X. (2015). Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of Animal Science and Biotechnology 6, 1-10.
- Zheng, Z. I; Tan, J. Y. W; liu, H. Y; Zhou, X. H; Xiang, X. and Wang, K. Y. (2009). Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). Aquaculture 292, 214-218.

الملخص العربى تأثير مستويات مختلفة من مستخلص الأوريجانو كإضافات غذائية على أداء النمو والتركيب الكيميائي ومكونات الدم لإصبعيات أسماك البلطى النيلى تحت ظروف الإستزراع شبه المكثف

مصطفى ماهر المغازى '، أحمد فاروق فتح الباب '، محمد تاج الدين شهاب الدين ' و إسلام أكرم ' ١- قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة دمياط ، مصر. ٢- قسم صحة الأسماك، وحدة بحوث سخا، المعمل المركزي لبحوث الثروة السمكية، مصر.

كان الهدف من هذه الدراسة هو دراسة تأثير بمستويات مختلفة من مستخلص نبات الاوريجانو والمحتوى على زيوت الثيمول والكارفاكرول على مقاييس أداء النمو، التحليل الكيميائي ومكونات الدم لأصبعيات أسماك البلطي النيلي وحيد الجنس تحت ظروف الاستزراع شبة المكثف. استخدمت في هذه الدراسة عشرة أحواض إسمنتية ٣ × ٧ × ١ م (٢١ م <sup>7</sup> لكل حوض). تمثل الأحواض العشرة ٥ معاملات (المقارنة، ٥٠,٠, ٠١, ٩, ١٠, و٠٢, من مستخلص الاوريجانو -٢ مكرر لكل معاملة) ، وتم تخزين الاسماك بواقع ١ سمكة / م<sup>7</sup> (٢١ سمكة لكل حوض اسمنتى) بمتوسط أوزان مبدئي ٤,٦, ٤,٩ م,٠٠ و ٢,٠٥ جرام للخمس معاملات ، على التوالي.

تم تغذية الإصبعيات بمعدل ٥٪ من وزن الجسم حتى نهاية الفترة التجريبية (١٦ أسبوعًا). تحتوي العلائق التجريبية على ٢٣ ٪ من البروتين الخام وحوالي ٣٦٠ كالوري / ١٠٠ جرام من الطاقة القابلة للهضم. وتم تغذية الأسماك مرتين يوميا لمدة ستة اسبوعيا. بدأت التجربة في ٥ يوليو ٢٠١٦ واستمرت حتى ٢٥ أكتوبر من نفس العام (١١٢ يومًا). كانت مدة التجربة ١٦ أسبوعًا. يتم تلخيص النتائج التي تم الحصول عليها في الآتي:

قياسات النمو: سجلت المعاملة الثالثة اعلى معدل لوزن الجسم النهائى والزيادة اليومية فى الوزن وطول الجسم النهائى ومعدل النمو النوعى ومعامل كفاءة البروتين، بينما سجلت المعاملة الاولى (المقارنة) أقل معدل لوزن الجسم النهائى والزيادة اليومية فى الوزن وطول الجسم النهائى ومعدل النمو النوعى ومعامل كفاءة البروتين.

التركيب الكيميائي: بالنسبة للسمكة الكاملة سجلت المعاملة الثانية اعلى معدل لنسبة البروتين والمعاملة الثالثة اعلى معدل لنسبة الدهن والمعاملة الاولى اعلى معدل لنسبة الرماد. أما بالنسبة للجزء المأكول من السمكة سجلت المعاملة الثانية اعلى معدل لنسبة البروتين ونسبة الرماد والمعاملة الثالثة اعلى معدل لنسبة الدهن.

أما بالنسبة لمكونات الدم: سجلت المعاملة الخامسة اعلى معدل لنسبة الهيموجلوبين والكرياتينين والمعاملة الثالثة اعلى معدل لكرات الدم الحمراء والمعاملة الاولى اعلى معدل لوظائف الكبد والكوليستيرول. وسجلت المعاملة الثانية اعلى معدل لنسبة البروتين الكلى والالبيومين.

استناداً إلى النتائج التي تم الحصول عليها في هذه الدراسة، يمكن الاستنتاج أن استخدام مستوى ١٠,١٠% من مستخلص الاوريجانو كانت هي الأفضل لأداء النمو ووظائف الكبد بالإضافة إلى التركيب الكيميائي وذلك تحت ظروف الإستزراع شبه المكثف في الاحواض الإسمنتية.