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USING OF PROBIOTICS AND SPICE OILS AS FEEDING STIMULANTS IN DIETS OF NILE TILAPIA, *OREOCHROMIS NILOTICUS*

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ABSTRACT

The present study was carried out in order to investigate the response of Nile tilapia fry to some commercial probiotics (Biogen, Megalo and yeast) and some spices oils (garlic and fennel oils) and the combinations of Megalo and each of garlic and fennel as dietary feed additives. Therefore, 8 diets were prepared, the 1st diet (without any supplementation) as control (D1) and the others were supplemented by 0.2% Biogen (D2), 0.3% yeast (D3), 0.2% Megalo (D4), 0.1% Garilc oil (D5), 0.1% fennel oil (D6), 0.1% garlic oil+0.2% Megalo (D7) and 0.1% fennel oil+0.2% Megalo (D8). The feeding trial lasted for 90 days.

At the termination of the experiment, D1(control diet) released the lower body weight (12.15 g) compared to 20.80, 21.45, 21.40, 20.50, 20.72, 21.95 and 19.56 g for the different experimental diets D2, D3, D4, D5, D6, D7 and D8, respectively, and the differences between body weight for the control group (D1) and each of the other treatments were significant ($P<0.05$) while the differences between these treatments (D2, D3, D4, D5, D6, D7 and D8) were not significant, and the same trend was also observed for weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER).

Supplementation of the basal diet of Nile tilapia with probiotics or spices oils did not significantly ($P>0.05$) affected hematocrite (Ht) percentages and liver transferase enzymes (AST and ALT) values while hemoglobin (Hb) level showed some variations. Probiotics or spices oils had no significant effect on proximate analysis of Nile tilapia.

Key words: probiotics, spices, Nile tilapia, growth performance, feed utilization

INTRODUCTION

The demand for animal protein for human consumption is currently on the rise and is largely supplied from terrestrial farm animals. Aquaculture, however, is an increasingly important option in animal protein production. This activity requires high-quality feeds with a high protein content, which should contain not only the necessary nutrients but also complementary additives to keep organisms healthy and promote favourable growth. Some of the most utilized growth-promoting additives include hormones, antibiotics and some salts **(Klaenhammer and Kullen, 1999).** Although these do promote growth, their improper use can result in adverse effects in the animal and the final consumer and can lead to resistance of pathogenic bacteria in case of antibiotics **(El-Haroun *et al.,* 2006)**. An alternative to the antibiotics is the use of beneficial bacteria (probiotic) to fight the pathogenic bacteria by competitive exclusion, which is an acceptable practice in animal husbandry **(Sissons, 1989).**

The direct nutritional benefits of probiotic bacteria include vitamin production, availability of minerals and trace elements and production of important digestive enzymes. Probiotics increase growth, improve efficiency, prevent intestinal disorders and stimulate pre-digestion of anti-nutritional factors present in the ingredients (**Holzapfel *et al.,* 1998**).

Biogen contains *Bacillus licheniformis* and *B. subtilis*. The advantage of these spore-forming bacteria is that they are able to survive the pelletization process. After transit passage through the stomach, they germinate in the intestine and use a large number of sugars (carbohydrates) for their growth and produce a range of relevant digestive enzymes, amylase, protease and lipase (**El-Haroun *et al.,* 2006)**. Garlic (*Allium sativum L.*) is widely cultivated in Egypt and used as flavoring agent, a common feed and popular remedy. Because the thyroxin like activity of garlic, it is suggested that it has growth stimulating effect. Garlic stimulated growth by increasing the inflow of glucose into tissues and thyroid like activity **(El-Nawawy, 1991)**. Sulfur compounds in garlic are considered as active antimicrobial agents and improve immunity and therefore stimulate growth (**El-Afify, 1997)** and have a mode of action similar to antibiotics **(Ibrahim *et al.,* 2004).**

The present study was designed to investigate the effect of some commercial probiotics (Biogen, Megalo and yeast) and some spices oils (garlic and fennel oils) as dietary feed additives on growth performance, feed utilization, some hematological parameters, liver function and proximate composition of *O. niloticus*.

MATERIALS AND METHODS

All-male Nile tilapia, *O. niloticus* L. fry were obtained from fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, Egypt. Fish were kept under the same environmental conditions at the wet lab and placed in a fiberglass tanks for 2 weeks as an acclimation period to the laboratory conditions. Acclimated fish were distributed randomly at a rate of 20 fish/100 L aquarium. The initial weight of fish ranged from

1.78 to 1.93±0.04 g. Each aquarium was supplied with compressed air via air-stones from air pumps. Settled fish wastes with one half of water were siphoned daily and water volume was replaced by aerated tap water from the storage tank.

Feed and feeding:

The basal diet was formulated to contain 30% crude protein and 3000 Kcal (metabolizable energy) and divided into 8 parts. The first part prepared without feed additives as control diet (D1) and the other seven parts prepared to contain 0.2% Biogen (D2); 0.3% yeast (D3); 0.2% Megalo (D4); 0.1% garlic oil (D5); 0.1% fennel oil (D6); 0.1% garlic oil+0.2% Megalo (D7) and 0.1% fennel oil+0.2% Megalo (D8) in 3 replicates, therefore 24 aquaria were used in this study.

Diet formulation and proximate analysis are shown in Table (1). All ingredients homogeneously and 150 ml of water was added. Afterwards, the mixture (ingredients and water) were mixed using kitchen mixer to make a paste of each diet. Pelleting of each diet was carried out by passing the mixture through ring die pelleting machine with a (3mm) diameter matrix. The pellets were air dried for three days and stored in plastic bags which were kept in a refrigerator at (-2^{o}C) during the experimental period to avoid rancidity. The diets were prepared palletized. Experimental diets were formulated to meet the nutritional requirements of fish (**NRC, 1993).**

Fish were fed the experimental diets at a rate of 5% of their biomass twice daily (at 9.00 and 14.00). Fish in each aquarium were sampled biweekly and feed amounts were adjusted according to the new fish biomass. The feeding period in the experiment lasted for 90 days.

Water quality parameters:

Water in situ temperature was measured by oxygen-thermometer apparatus. Different methods of chemical analysis were carried out according to **Boyd, (1984)** and **APHA, (1985).** pH was measured using glass electrode pH meter. Dissolved oxygen was measured in situ using of oxygen-meter.

Proximate analysis of diet and fish:

At the end of the experiment, the basal diet and fish samples from each treatment were chemically analyzed according to the standard methods of Association of Official Analytical Chemists **AOAC, (1990).**

Growth performance and feed utilization parameters:

The following equations were used to measure the different growth and feed utilization prameters:

Weight gain (WG) = W2 – W1;

Specific growth rate (SGR) = 100 (ln W2 – ln W1) / T

Where W1 and W2 are the initial and final weights, respectively, and T is the number of days of the experimental period;

Feed conversion ratio (FCR) = Feed intake (g) / weight gain (g);

Protein efficiency ratio (PER) = Weight gain (g)/protein intake (g),

Hematological parameters and liver enzymes:

At the end of experimental period, 10 fish from each treatments and control group were taken for physiological measurements. Fish were fasted for 24 h prior to blood sampling. Fish were anaesthetized by buffered tricaine methane sulfonate (20mg ∕ L) and blood was collected from the fish caudal vein by a sterile syringe. The blood samples were divided into two parts, the first part was put in clean and dry tubes then centrifuged for 15 min for separation of serum (collected serum was stored at -20°C) for determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The second part was taken on dry and clean tube with EDTA solution for measuring hemoglobin (Hb) and hematocrite (Ht) in blood after good mixing. Hemoglobin (g%) was determined according to the method described by **Stopkopf (1983).** Serum AST and ALT were determined colorimetrically using spectrophotometer using specific kits according to **Reitman and Frankel (1975)**.

Table (1): Composition and proximate analysis of basal diet.

Ingredients	%
Fish meal (65%)	28.0
Soybean meal (40%)	18.0
Yellow corn	24.0
Wheat flour.	13.0
Wheat bran.	9.0
Corn oil	4.0
Vit. & Min. mix.[1]	4.0
Sum	100.0
Proximate analysis	
Dry matter	95.23
Protein	30.12
Lipid	5.32
Ash	8.45
ME (Kcal/Kg diet)[2]	3019
P/E ratio[3]	99.78

1 Vitamin & mineral mixture / kg premix: Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g, Bl. 0.4 g; Riboflavin. 1.6g: B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, *22* g; Zn, *22* g; Fe, 12 g; Cu, 4 g; I, 0.4 g. Selenium. 0.4 g and Co. 4.8 mg.

2 Metabolizable energy was calculated from ingredients based on **NRC (1993)** values for tilapia.

3 Protein to energy ratio in mg protein/Kcal ME.

Statistical analysis:

Statistical analysis of the obtained data was analyzed according to **SAS (1996).** Differences between means were tested for significance according to Duncan's multiple rang test as described by **Duncan (1955).** The following model was used to analyze the obtained data: $\mathbf{Y_{ij} = \mu + \alpha_i + e_{ij}}$

Where: Y_{ij} = the observation on the ij^{th} fish eaten the i^{th} diet; μ = overall mean, α_i= the effect of i^{th} diet and e_{ij} = random error assumed to be independently and randomly distributed (0, δ^2 e).

RESULTS AND DISCUSSION

Water quality parameters:

As described in Table (2) water temperature ranged from 27.20 to 29.25°C; dissolved oxygen (DO) ranged between 3.32 and 3.81 mg/l and pH values ranged between 8.04 and 8.30 for the different treatments during the entire experimental period (90 days) of the study (Table 2) and the differences in water temperature and pH between the different treatments were not significant (P>0.05).

As shown in Table (2), all tested water quality criteria (temperature, pH value DO) were suitable and within the acceptable limits for rearing Nile tilapia *O. niloticus* fingerlings **(Boyd, 1990)**. These positive findings in water quality criteria related with good growth performance since there were no mortalities among all treatments.

Table (2): Water quality parameters for aquaria used for the different treatments.

Diets	Water temperature (°C)	Dissolved oxygen (mg/l)	pH
D1 (Control)	27.60	3.59 bc	8.13
D2 (0.2% Biogen)	28.10	3.49 dc	8.26
D3 (0.3% Yeast)	28.05	3.32 d	8.04
D4 (0.2 Megalo)	27.85	3.41 dc	8.08
D5 (0.1% Garlic oil)	27.20	3.44 dc	8.20
D6 (0.1% Fennel oil)	29.10	3.72 ab	8.29
D7 (0.1% Garlic oil + 0.2% Megalo)	29.25	3.44 dc	8.09
D8 (0.1 Fennel oil +0.2% Megalo)	27.40	3.81 a	8.30
Standard error	±0.85	±0.06	±0.07

Averages followed by different letters in each column are significantly different (P<0.05)

2. Growth performance of Nile tilapia *O. niloticus*:

The initial body weight (BW) for fish received the different treatments ranged between 1.78 and 1.93 g. At the experiment termination, D1(control diet) released the lower BW (12.15 g) compared to 20.80, 21.45, 21.40, 20.50, 20.72, 21.95 and 19.56 g for the experimental diets D2, D3, D4, D5, D6, D7 and D8, respectively and the differences between

BW for the control group and each of the other treatments were significant (P<0.05) while the differences between these treatments (D2, D3, D4, D5, D6, D7 and D8) were not significant (P>0.05) indicating that the different feed additives used in the present experiment showed relatively the same BW which was significantly higher than that showed for the control diet (without probiotics or spices oils) and the same trend was also observed for weight gain (WG) and specific growth rate (SGR) as shown in Table (3).

The obtained results indicated that, supplementation of the basal diet by 0.2% Biogen significantly (P<0.05) improved final BW, WG and SGR of Nile tilapia compared to the control diet and these results are in accordance with those reported by **Bayoumi (2004); Khattab *et al.*, (2004)**; **Elam (2004); EL-Haroun *et al.* (2006)**; **Mohamed *et al.* (2007); Eid and Mohamed (2008) and Soltan *et al.*, (2010)** who indicated that using of Biogen at level of 0.1-0.2% significantly improved growth performance of Nile tilapia *O. niloticus* fingerlings. In the this respect, **Mehrim (2011)** indicated that the inclusion of Biogen at a level of 3g Kg^{-1} diet for Nile tilapia, at stocking density rate of 30 fish/m^3 is useful to get the best fish performance (final BW, WG and SGR) with friendly effects on the environment.

On the other hand, **Abdel-hamid *et al.*, (2002)** found that Biogen supplementation (2 and 4 g/kg $^{-1}$ diet) did not significantly improve fish growth performance. Also, **Diab *et al.*, (2002)** reported that Biogen addition to fish diet at 0.5, 1.0 and 1.5% gave insignificant increase in fish growth performance.

Table (3): Effect of some probiotics and spices oils on growth performance of Nile tilapia.

Diets	BW (g) Initial	BW (g) Final	WG (g)	SGR (%)
D1 (Control)	1.78	12.15 b	10.37 b	2.14 b
D2 (0.2% Biogen)	1.88	20.80 a	18.92 a	2.67 a
D3 (0.3% Yeast)	1.86	21.45 a	19.59 a	2.72 a
D4 (0.2 Megalo)	1.82	21.40 a	19.58 a	2.74 a
D5 (0.1% Garlic oil)	1.80	20.50 a	18.70 a	2.70 a
D6 (0.1% Fennel oil)	1.87	20.72 a	18.85 a	2.67 a
D7 (0.1% Garlic oil + 0.2% Megalo)	1.93	21.95 a	20.02 a	2.70 a
D8 (0.1 Fennel oil +0.2% Megalo)	1.90	19.56 a	17.66 a	2.59 a
Standard error	0.04	1.59	1.55	0.07

* Means followed by different letters in each column are significantly (P<0.05) different.

Generally, results of Table (3) showed that, supplementation of the basal diet with 0.2% Biogen improved all growth parameters (BW, WG and SGR). The main ingredients of Biogen are allicien, ginsieng, *B. subtilis* and high units of hydrolytic enzymes. Allicin which is one of the garlic by-product stimulate growth because of its thyroid like activity (**El-Nawawy 1991**) also it has anti-microbial properties. Ginsieng which could have a growth promoting ability via prevention and treatment of sub-clinical infections, the same findings were observed by **Galal *et al.,* (1997)**. *B. subtilis* have been shown to produce digestive enzymes as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes or its effect in improving digestive activity by synthesis of vitamins and co-factors or enzymatic improvement **(Gatesoupe, 1999).** Probiotics inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and space, and alteration of the microbial metabolism. It also improves the nutrition by detoxifying the potentially harmful compounds in feeds, by producing vitamins such as biotin and vitamin B_{12} (**Hoshino *et al.,* 1997**), and by stimulating host immunity (**Gibson *et al.,* 1997**). Hydrolytic enzymes of Biogen (amylolytic, lipolytic, proteolytic and cell separating enzymes) when reached a certain level it act as a growth promoter through the increase in digestibility and absorbability of different nutrients in the gastrointestinal tract of fish.

Biogen can enhance the metabolism and energy of fish body cells, raise the efficiency of feed utilization, Biogen has a particular good flavor and appetizing function which can increase the palatability of feed, promote the secretion of digestive fluids and stimulate the appetite **(Mehrim, 2001).** Moreover, it increases the vitality of cells by supplying oxygen to whole body and improves the immune responses, helps to excrete heavy metals, inhibits aflatoxin and stimulates the normal endocrine system (**Diab *et al.,* 2002**).

Compared to control fish group, supplementation of the basal diet with 0.3% yeast or 0.2% Megalo (commercial probiotic) significantly ($P<0.05$) improved BW, WG and SGR of fish (Table 3). These results may be attributed to the action to adherence of *S. cerevisiae* cells to the gut and the secretion of amylase enzymes which shared in the increased digestibility of the diet. Whereas, the increased growth performance of *O. niloticus* treated with commercial product Megalo containing living *S. cerevisiae* with *B. subtilis* could be also attributed to the inhibition of some intestinal bacteria flora and increasing the non-specific immunity of the treated *O. niloticus*. The adherence capacity of *S. cerevisiae and B. subtilis* to the intestinal mucosa inhibits the attachment of the other intestinal bacteria to these binding sites and so preventing the disease occurrence with its negative impact on the fish growth. In this respect, **Abd El-Halim *et al.,* (1989)** found that the addition of living yeast in diet improve the performance of *O. niloticus*. **Abdel-Tawwab *et al.,* (2008)** indicated that, after 6 weeks feeding period Galilee tilapia fed 10.0 g yeast/Kg diet exhibited the highest final BW, WG and SGR than those fed the control diet ($P<0.05$). **Hoseinifar *et al.,* (2011)** reported that, dietary supplementation of 2% inactive brewer's yeast (*S. cerevisiae*) significantly improved final BW, WG and SGR of juvenile beluga sturgeon (*Huso huso*) compared to the control treatment. In another recent study**, Mansour (2011)** studied the effect of supplementation of a new patent local probiotic (T-Protphyt 2000) at a rate of 0, 1, 2, 3 g/Kg diet for African catfish *Clarias gariepins* brood stock and they found that, the 4th treatment (3 g/Kg diet) realized significantly (P<0.05) the best values of final BW, WG and SGR.

As described in Table (3) supplementation of the basal diet with 0.1% fennel oil or 0.1% garlic oil significantly (P<0.05) improved growth performance (BW, WG and SGR) of Nile tilapia compared to fish group fed the basal diet (D1) and the same trend was also observed for the combinations of Megalo with garlic oil or with fennel oil (D7 and D8).

El-Dakar *et al.,* (2004) found that fennel seed meal used in tilapia diets improved significantly SGR than those fed the control diet.

Khalil *et al.,* (2001) showed that garlic contains allicin, which promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the utilization of energy, leading to improved growth. Garlic has been used for centuries in many societies against parasitic, fungal, bacterial and viral infections. The chemical characterization of their sulphur compounds has promoted claims that such compounds are the main active antimicrobial agents (**Rose *et al.,* 2005**).

Soltan and El-Laithy (2008) indicated that supplementation of basal diet with each of *B. subtilis,* Biogen, garlic, fennle or the combinations of *B. subtilis* with each of garlic or fennel significantly (P<0.001) improved BW, WG and SGR of *O. niloticus* and the best growth parameters were recorded for fish fed the basal diet supplemented by a combination of *B. subtilis* and fennel and the worst one was recorded for fish fed the control diet. They also added that fennel was better than garlic when used as feed additive alone or in a combination with *B. subtilis* in Nile tilapia diets.

3. Feed intake and feed utilization

During the entire experimental period (90 days), results of Table (4) indicated that fish consumed relatively the same amount of diets supplemented with the different probiotics or spices oils and the differences in feed intake between the different feed additives (D2, D3, D4, D5, D6, D7 and D8) were not significant while the differences between each of these treatments and the basal diet (D1) were significant (P<0.05).

Feed conversion ratio (FCR) for the control diet showed the highest (worst) values compared to the other experimental diets (Table 4). It is interest to note that Biogen feed additive supplemented diets did not only have an effect on FI and subsequently WG, but also affected FCR, indicating an overall increase in the efficiency of utilization in the deposition of tissues. **Elam (2004)** with Nile tilapia *O. niloticus* and *Mugil cephalus* and **Bayoumi (2004)** and **El-Haroun *et al.,* (2006)** with *O. niloticus* reported that total FCR was significantly (P<0.05) improved if the diet was supplemented with 1 or 2 g of Biogen/Kg feed**.** Also, **Soltan *et al.,* (2010)** indicated that, FCR was improved with increasing Biogen level in the diet up to 2 g Biogen/Kg diet whereas the highest level of Biogen 3 g Biogen/Kg diet re-increased feed required for the production of the same unit of weight gain. **Mehrim (2011)** reported that the inclusion of Biogen at a level of $3g/Kg^{-1}$ diet significantly ($P \leq 0.05$) improved FCR of Nile tilapia, *O. niloticus*. **Hoseinifar *et al.,* (2011)** indicated that, dietary supplementation of 2% inactive brewer's yeast *S. cerevisiae* significantly improved FCR of *Huso huso* compared to the control treatment.

Table (4): Effect of some probiotics and spices oils on feed intake, feed utilization of Nile tilapia

Diets	Feed intake (g)	Feed conversion ratio	Protein efficiency ratio
D1 (Control)	16.69 b	1.61 a	2.08 b
D2 (0.2% Biogen)	25.28 a	1.34 b	2.49 a
D3 (0.3% Yeast)	24.50 a	1.25 b	2.67 a
D4 (0.2 Megalo)	25.09 a	1.28 b	2.60 a
D5 (0.1% Garlic oil)	24.64 a	1.32 b	2.53 a
D6 (0.1% Fennel oil)	25.48 a	1.36 b	2.47 a
D7 (0.1% Garlic oil + 0.2% Megalo)	25.95 a	1.30 b	2.57 a
D8 (0.1 Fennel oil +0.2% Megalo)	24.70 a	1.42 b	2.38 a
Standard error	±1.21	±0.66	±0.12

* Means followed by different letters in each column are significantly (P<0.05) different.

Values of protein efficiency ratio (PER) for fish groups fed the supplemented diets (D2, D3, D4, D5, D6, D7 and D8) were significantly (P<0.05) improved compared to that recorded for control diet (D1) indicating the superiority of supplementation of the tested feed additives and probiotics to the basal diet. The differences between each of the different treatments and the control group were significant while the differences between supplemented diets were insignificant.

As described in Table (4) the best PER was recorded for fish group fed the diet supplemented by yeast (2.67) and these results are relativelly in agreement with those obtained by **Abdel-Tawwab *et al.,* (2008)** who found that, Galilee tilapia fed 10.0 g yeast/Kg diet exhibited the highest feed intake, FCR and PER and apparent protein utilization than those fed the control diet ($P<0.05$).

The better feed utilization with yeast supplementation may be due to that yeast might play a role in enhancing feed intake resulting in higher fish growth, due to the high feed intake, protein utilization, energy utilization and the high nutrient digestibility.

Soltan and El-Laithy (2008) found that, incorporation of probiotics (*B. subtilis* or Biogen, spices (garlic or fennel) and the combination of *B. subtilis* with garlic or fennel in the diets of of Nile tilapia *Oreochromis niloticus*, significantly ($P<0.01$) improved feed intake FI, FCR and PER. The highest FI and the best FCR were obtained for fish fed the diet supplied by a mixture of *B. subtilis* and fennel and the worst FI, FCR and PER were obtained for fish fed the control diet (basal diet).

From a nutritional point of view this means that supplementation of fish diets with probiotics or spices optimized protein use for the growth which can decrease the amount of feed necessary for fish growth, which could result in reducing production costs.

The present results revealed that the use of the probiotic Biogen, yeast or Megalo and garlic oil or fennel oil or their combinations with Megalo as a feed additive for Nile tilapia is recommended to stimulate productive growth performance (BW, WG and SGR) feed intake and nutrient utilization (FCR and PER). The superiority of probiotics/spices in enhancing growth parameters can be attributed to:

1) increasing palatability and maximize feed intake (**Sakr 2003**);
2) enhancing activities of pancreatic lipase, chymotrypsin and amylase (**Abou-Zeid, 1998**),
3) improving protein and energy digestibility (**Sakr., 2003**);
4) increasing fish vitality (**Abd El-Maksoud *et al.*, 2002**);
5) increasing fish immunity through its effects on the liver function (**Ozbek *et al.*, 2003**) and
6) reducing the feed waste that causing improvement of aquatic culture environment for fish (**El-Dakar *et al.*, 2004)**.

Another possible explanation for increased growth performance with added probiotic is the improvement in digestibility, which may in turn explain the better growth and feed efficiency observed with the supplemented diets. Otherwise, probiotics influence digestive processes by enhancing the population of beneficial microorganisms, microbial enzyme activity; improving the intestinal microbial balance, consequently improving the digestibility and absorption of food and feed utilization (**Bomba *et al.,* 2002**).

4. Some haematological variables and serum transaminases (AST&ALT):

The haematological variables haematocrit (Ht) and haemoglobin (Hb), as an indicator of the rate of haemoglobin synthesis to red cell formation, and erythrocyte sedmentation rate were determined because it is important measures **(Barraza, *et al.,* 1991).** Results of Table (5) indicated that, supplementation of the basal diet with probiotics and spices oils did not significantly (P>0.05) affected hematocrite (Ht), while hemoglobin (Hb) level showed some variation but without clear trend.

Fish fed the D8 diet showed the highest levels of Hb and Ht, while those fed diet D6 had the lowest ones. Similar results were obtained by **Kumar *et al.,* (2006).** They found that, serum Hb level of Indian major carp *Labeo rohita* (Ham) did not significantly differ when *B. subtilis* was supplemented to the diets. On the other hand, **Marzouk *et al.* (2008)** reported that both fish groups fed on diet supplemented with probiotics (dead *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis* and *S. cerevisae*) showed significant (P<0.05) increase in the hemoglobin level compared to fish fed the control diet.

Mehrim (2011) indicated that mono-sex Nile tilapia *Oreochromis niloticus* fed the basal diet supplemented with 3g Biogen Kg^{-1} diet for 14 weeks significantly ($P \leq 0.05$) increased hematological parameters (hemoglobin, RBCs count, blood platelets and WBCs count).

Estimation of serum enzyme activities of AST and ALT were taken as an indication of the amount of liver damage, as the elevated serum enzyme levels might be related to the degree of liver injury. Liver disease causes an increase in some serum enzymes by blocking their elimination into the blood **(Barraza *et al.,* 1991)**.

Results of Table (5) outlined liver transferase enzymes (AST and ALT). As observed in this table, liver transferase enzymes AST and ALT values ranged between 10.95-12.40 and 8.0–10.9 u/l for AST and ALT, respectively. The differences of AST and ALT levels were not significant between control and any of the other feed additives. On the contrary, **Marzouk *et al.* (2008)** found that, fish groups fed on diet supplemented with probiotics (dead *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis*+*S. cerevisae*) revealed significant (P<0.05) decrease in ALT and AST compared to the control group fed on probiotic-free diet. Similarly, **Soltan and El-Laithy (2008)** found that, ALT and AST levels were significantly decreased when Nile tilapia fed diets supplemented with probiotics or spices compared to diet fish group. Similar results were obtained by **El-Dakar *et al.* (2004)** who showed a significant lower (P<0.05) levels of ALT and AST activities with Nile tilapia fed fennel seed meal in diets. Whereas, **Abdel-Tawwab *et al.,* (2008)** found that serum AST and ALT levels of Galilee tilapia fed the basal diet supplemented by 10 g yeast/Kg diet were not significantly affected.

Table (5): Effect of some probiotics and spices oils on some haematological variables and serum transaminases (AST&ALT) of Nile tilapia.

Diets	Haematological variabl		Serum transaminases	
	Ht (%)	Hb (g/dl)	AST (u/l)	ALT (u/l)
D1 (Control)	18.90	6.80 ab	11.80	10.35
D2 (0.2% Biogen)	16.45	5.90 b	11.90	10.05
D3 (0.3% Yeast)	17.05	6.45 ab	12.35	11.15
D4 (0.2 Megalo)	17.30	6.15 ab	12.05	10.90
D5 (0.1% Garlic oil)	17.50	6.25 ab	10.95	8.35
D6 (0.1% Fennel oil)	16.05	5.65 b	11.20	8.00
D7 (0.1% Garlic oil + 0.2% Megalo)	18.35	6.35 ab	12.15	9.10
D8 (0.1 Fennel oil +0.2% Megalo)	23.00	8.80 a	12.40	9.05
Standard error	±2.26	±0.78	±0.50	±1.02

Averages followed by different letters in each column are significantly different (P<0.05)

6. Proximate analysis of whole fish:

Chemical analysis of whole fish at the end of the study are illustrated in Table (6). As described in this table dry matter content of whole fish are relatively the same and ranged between 26.52-27.22% for the different diets and the same trend was also observed for protein (63.30-63.89%), fat (25.05-26.23%), and the differences in dry matter, protein, fat and ash were not significant.

Chemical analysis at the end of a feeding trial is frequently used to determine the influence of feed on fish composition. According to **Hepher (1990),** endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish. It should be noted that within endogenous factors, the composition of the feed is only the factor, which could have influenced the chemical composition of fish, as other endogenous factors were maintained uniform during the study.

Results in the present study are in close agreement with those obtained by **Khattab *et al.* (2004); EL-Haroun *et al.* (2006)**; **Mohamed *et al.* (2007); Eid and Mohamed (2008); Mehrim (2011)** for tilapia and **EL-Haroun, (2007)** for catfish**.** On the other hand, **Abdel-Hamied *et al*., (2002)** found that the dietary inclusion of Biogen increased the protein content and lowered the fat content of the whole fish body, without significant differences in ash content of Nile tilapia.

Abdel-Tawwab *et al.,* (2008), found that Galilee tilapia fed the diet supplemented by 10.0 g yeast/Kg diet exhibited the highest significantly (P<0.05) whole-body contents of protein and ash, and lower lipid content than fish fed the basal diet (P<0.05).

In recent study**, Mansour (2011)** used a new patent local probiotic (T-Protphyt 2000) supplemented at a rate of 0, 1, 2, 3 g/kg diet for African catfish *Clarias gariepins*. They found that, 3 g probiotic/Kg diet had the highest fat and the lowest ash content while protein content of carcasses was not significantly affected.

Table (6): Effect of some probiotics and spices oils on proximate analysis of Nile tilapia.

Diets	Dry matter (%)	Crude protein (%)	Fat (%)	Ash (%)
D1 (Control)	26.86	63.73	26.23	10.68
D2 (0.2% Biogen)	26.52	63.40	25.84	10.99
D3 (0.3% Yeast)	27.22	63.70	25.92	11.23
D4 (0.2 Megalo)	26.86	63.30	25.36	11.01
D5 (0.1% Garlic oil)	26.55	63.33	25.70	10.65
D6 (0.1% Fennel oil)	27.01	63.35	25.05	10.55
D7 (0.1% Garlic oil + 0.2% Megalo)	27.00	63.89	25.64	10.95
D8 (0.1 Fennel oil +0.2% Megalo)	26.52	63.69	25.52	10.80
Standard error	0.16	1.22	1.61	0.52

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الملخص العربى

إستخدام منشطات النمو الحيوية وزيوت التوابل كمحفزات نمو فى علائق أسماك البلطى النيلى

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أجريت هذه الدراسة لمعرفة إستجابة زريعة أسماك البلطى النيلى لإضافة بعض محفزات النمو الحيوية التجارية (البيوجين ، الميجالو، الخميره) وزيت الثوم وزيت الشمر وخلطات هذه الزيوت مع الميجالو. ولذلك تم تكوين ٨ علائق تجريبية حيث أستخدمت العليقة الأساسية بدون أى إضافات (مقارنة) أما العلائق السبعة الأخرى فقد أضيف إلى العليقة الأساسية ٠.٢% بيوجين (العليقة الثانية) ، ٠.٣ % ميجالو (العليقة الثالثة) ٠.٢% خميرة (العليقة الرابعة)، ٠.١ زيت الثوم (العليقة الخامسة) ، ٠.١ زيت الشمر (العليقة السادسة) ، ٠.١ زيت الثوم+٠.٢% ميجالو (العليقة السابعة) ، ، ٠.١ زيت الشمر+٠.٢% ميجالو (العليقة الثامنة) وقد تغذت الأسماك على العلائق التجريبية لمدة ٩٠ يوم.

فى نهاية التجربة أظهرت النتائج أن الأسماك التى تغذت على عليقة المقارنة قد أعطت أقل قيمة لوزن الجسم الحى (١٢.١٥ جم) مقارنة بالمعاملات الأخرى ٢٠.٨٠، ٢١.٤٥، ٢١.٤٠، ٢٠.٥٠، ٢٠.٧٢، ٢١.٩٥ ، ١٩.٥٦ جم التى تغذت على العلائق الأخرى على التوالى. وكانت الفروق فى وزن جسم الأسماك بين مجموعة المقارنة وأى من المعاملات السبعة الأخرى فروقاً معنويه فى حين كانت الفروق فى متوسطات أوزان الجسم بين مجموعات الأسماك التى تغذت على العلائق السبعة المضاف إليها بمنشطات النمو الحيوية وزيوت التوابل كانت فروقاً غير معنوية. وقد أظهرت باقى صفات النمو (زيادة الوزن ومعدل النمو) والغذاء المستهلك ومعدل تحويل الغذاء وكفاءة إستخدام البروتين إتجاهاً مشابهاً لوزن الجسم.

لم تؤثر الإضافات المستخدمة معنوياً على محتوى الدم من الهيموجلوبين أو الإنزيمات الناقلة لمجموعة الأمين (إنزيمات الكبد) بينما ظهرت بعض الفروق المعنوية فى متوسطات الهيماتوكريت.

أظهرت نتائج التحليل الكيميائى للأسماك أن الإضافات الغذائية المستخدمة فى تغذية أسماك البلطى النيلى لم تؤثر معنوياً على محتوى جسم السمكة من المادة الجافة والبروتين والدهن والرماد.