EFFICIENCY OF USING LOW PLANT PROTEIN DIETS CONTAINING SOME FEED ADDITIVES ON GROWTH PERFORMANCE AND BODY COMPOSITION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS.

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SUMMARY

The present experiment was carried out to evaluate the possible impact of thyme as a natural antioxidant, citric acid and sodium sulphate on improving the utilization of plant protein diets fed to Nile tilapia (*Oreochromis niloticus*) fingerlings. Seven experimental diets were formulated and fed to mono-sex Nile tilapia (*Oreochromis niloticus*) fry with average initial weight of 0.80 ± 0.03g. The control diet (T_1) was formulated to contain 30% CP (5% fish meal) and (4598 kcal GE/Kg). To investigate the effects of using low protein diets with or without adding some feed additives, the others six diets were formulated almost iso-nitrogenous (25% CP) and iso-caloric (4470 ± 37 Kcal GE) and no fish meal was used in their formulations. The second diet (T_2) presented as basal diet where no feed additives were added, while the rest diets; from T_3 to T_7; were added with 0.3, 0.3, 0.6, 0.5 and 1.1% of thyme, citric acid, 1:1 thyme + citric acid, sodium sulphate (Na_2SO_4) and mixture of thyme + citric acid + sodium sulphate; respectively. The results concluded that addition of either thyme, citric acid or sodium sulphate improved the utilization of low protein diets and addition mixture of the three additives significantly increased weight gain and protein content in fish carcass by 43.82 and 7.01%, respectively, compared to basal diet. These results showed that mixture of thyme, citric acid and sodium sulphate is the most successful additives for improving performance of Nile tilapia fingerlings fed low protein diets.

Keywords: *tilapia, low protein, feed additives, thyme, citric acid, sodium sulphate*

INTRODUCTION

Fish meal (FM) is considered the major animal protein ingredient in aquaculture diets for its nutritional quality (Hardy and Tacon, 2002). However, FM is one of the most expensive macro-ingredients in fish diets because it is used in high proportions. Moreover, its availability is limited due to various factors such as climatic phenomena, the increasing demand for FM use in aquaculture diets, and the overexploitation and decline of fish stocks that are used to produce FM (Dong *et al.* 1993). Therefore, it has become necessary to find substitutes for fish meal in the aquaculture feeds. Numerous studies have been focused on plant protein (PP) sources as the most suitable alternatives protein for fish meal in fish diets. Unfortunately, attempts to use these ingredients to replace the FM component in farmed fish diets had limited responses because most PP alternatives suffer from deficiencies in essential amino acids, minerals, palatability /acceptability; the presence of complex carbohydrates (NRC, 1993; Ogunji, 2004) and the presence of a wide variety of anti-nutritional factors (Francis *et al.* 2001).

On the other hand, these PP are not only considered cheaper than fish meal but also enjoy high availability and may be containing valuable antioxidants. Guo *et al.* (2002) demonstrated *in vitro* that the main flavonoids present in soy bean had a higher antioxidant activity.

Growth rate, one of the most important parameters determining the economic efficiency of commercial fish culture, is influenced by several factors (Brett and Groves, 1979). Numerous studies have been conduct to ascertain the dietary protein requirements of tilapia *(Oreochromis niloticus)* with reported dietary protein requirements ranging from 30% CP in the case of pond reared fish and juveniles (Omar, 1994a; Siddiqui *et al.* 1988) to over 45% CP in the case of fry and fingerlings reared within indoor aquaria (Omar, 1994b; El-Sayed and Teshima, 1992). It is clear from previous studies that superoxide radicals and hydrogen peroxide (H_2O_2) as by-products of oxidative metabolism has been generated continuously from aerobic tissues (Halliwell and Gutteridge, 1989) whereas the oxidative stress occurs when reactive oxygen species (ROS) generation exceeds its removal (Sies, 1986). Reactive oxygen species are able to start the lipid peroxidation process which is potentially dangerous in fish, since they contain a high percentage of polyunsaturated fatty acids (Halliwell and Gutteridge, 1989; Sargent *et al.* 1999). The deleterious effects of ROS include oxidation of proteins, DNA, steroid components as well as peroxidation of unsaturated lipids in cell membranes (Martínez-Álvarez *et al.* 2005).

Grune *et al.* (1997) showed that the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack. In addition, Abd El-Hakim *et al.* (2009) used *Curcuma longa* plus thyme as natural antioxidants supplement in broiler diet to decrease the damage of protein by free radical and decrease the loss of protein in feces. They obtained significant increase in nitrogen retention by 13.25% compared to control diet and indicated that natural antioxidants may decrease the damage of protein by free radical and decrease the loss of protein in feces. Addition of organic acids in aquafeed may act as a chelating agent and bind with various cations along the intestine (Ravindran and Kornegay, 1993), resulting in increasing minerals absorption in intestinal. Ali *et al.* (2008) found with rabbits that the addition of citric acid plus *Curcuma longa* decreased both the harmful microorganisms in the caecum and plasma globulin and also they indicated that this combination of additives saved protein which needed for immunity and directly towards growth. Also, Zheng *et al.* (2009) found that commercial products containing natural oregano essential oil can act as a growth promoter, increase antioxidant activity, enhance muscle protein sedimentation and also improve disease resistance to pathogens when added to channel catfish feed. Falany (1991) showed that sulfating has evolved as a key step in xenobiotic metabolism. It is known that not all nature antioxidants are absorbed in intestine. In this respect, Azuma *et al.* (2000) found that absorbed flavonoids are present in the common blood circulation in the form of glucuronide, sulphate and methylate conjugates are excreted via urine or bile while chlorogenic acid (important phenolic antioxidants) remained intact in the small intestine.

Ali *et al.* (2007) found that combination of thyme and sulphate is the most successful additive for laying hen. Ali *et al.* (2010) found that the *Cuminum cyminum* L plus sulphate seemed to be the best additive under the heat stress condition. They indicated that addition of sulphate to some extent increase the activity of natural antioxidants. Since the fish meal contain high digestible protein, mineral and other nutrients, its presence in the diet will alleviate the bad effect of low protein diet.

The aim of this research is to evaluate the possibility impact of thyme as a natural antioxidant, citric acid and sodium sulphate as feed additives on improving Nile tilapia (*Oreochromis niloticus*) fingerlings performance under the using of plant protein diets.

MATERIALS AND METHODS

The present experiment was implemented at Fish laboratory, Utilization of by-Products Department, Animal Production Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Experimental fish and culture technique:

Mono sex Nile tilapia (*Oreochromis niloticus*) fry were obtained from private hatchery, Wady El-Natroun District, El-Behair Governorate. The average initial body weight of Nile tilapia fry was 0.80 ± 0.03g. The fish were acclimated to the laboratory conditions for 2 weeks in $1m^3$ fiberglass tank and fed commercial diet containing 30% crude protein. Before starting the experiment, a batch of fish was randomly selected, weight recorded and stored as zero group at -4°C for proximate chemical analysis thereafter. The fish were stocked at a density of 10 fish / aquarium (70 l each) in duplicates via water recalculating system. Water exchange rate for the system was approximately 10% of total volume per day. All fish were fed their respective diets at a level of 6% of body weight 6 days a week. The daily ration was divided into three equal portions and offered three times a day at 9.00, 12.00 and 15.00 hrs. The fish in each replicate were weighed weekly and the amount of daily diet was adjusted accordingly. The actual experimental period extended for 91 days.

Experimental diets:

Seven experimental diets were formulated in the present experiment. The control diet (T_1) was formulated to contain high protein diet (30% CP) and (4598 kcal GE/Kg) using fish meal as source of animal protein and no tested feed additives were used. To investigate the effects of using low protein diets with or without adding some feed additives, the others six diets were formulated almost iso-nitrogenous (25% CP) and iso-caloric (4470 ± 37 Kcal GE) and no fish meal was used in their formulations. The second diet (T_2) served as basal diet where no feed additives were added, while, the rest diets (from T_3 to T_7) were supplemented with 0.3, 0.3, 0.6, 0.5 and 1.1% of thyme, citric acid, thyme + citric acid (1:1), sodium sulphate (Na_2SO_4) and mixture of thyme + citric acid + sodium slphate (1:1:1); respectively. Diets were sufficient in essential vitamins, Ca, P, lysine and methionine and cystine (NRC, 1993). All the ingredients used in the experiment were purchased from the local market. The formulated diets were processed by blending the dry ingredients into a homogeneous mixture, and then passing the mixed of feed through a laboratory pellet mill. The proximate analysis of the feed ingredients used in formulating the experimental diets is shown in Table (1). The formulation and the proximate analysis of the experimental diets (on %DM) fed to Nile tilapia (*Oreochromis niloticus*) fish are presented in Table (2). Diets were kept in black plastic bags then stored in a refrigerator at 1°C throughout the whole experimental period.

Table (1): Proximate analysis (DM %) of the feed ingredients used in formulating the experimental diets.

Ingredients	Moist.	Crude protein	Ether extract	Crude fiber	Ash	NFE*	GE**
Fish meal	7.80	65.00	6.89	0.88	15.49	11.74	4828
Soybean meal	9.19	44.00	1.20	7.30	5.98	41.52	4552
Wheat bran	11.00	13.41	3.90	12.00	6.20	64.49	4187
Yellow corn	11.00	7.50	3.80	2.60	1.30	84.80	4280
Corn gluten	10.00	60.00	2.20	2.00	1.60	34.20	5046

* *Calculated by difference.,*

** *Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and 4.0 (Cal GE/g DM) for crude protein, ether extract, crud fiber and nitrogen free extract, respectively (Jobling, 1983).*

Water quality:

Water temperature was maintained at 24 ± 1 °C. Rates of water flow were adjusted to maintain oxygen saturation above 80%. The water quality parameters in the system were monitored every other day and the ranges were: dissolved oxygen 6.5-8.0 mg l^{-1}, total ammonia 0.1-0.3 mg l^{-1}, and pH 6.8-8.2. No critical values were detected for nitrite (NO_2) and nitrate (NO_3) radicals. Analytical methods were done according to American Public Health Association (APHA, 1992). The pH and water temperature values were determined by digital temperature and pH meter. Dissolved oxygen was monitored by using Oxymeter, Jan way model 9071.

Analytical methods:

The chemical analysis of the feed ingredients, the experimental diets and fish carcass were done to estimate moisture, crude protein (CP %), ether extract (EE %), crude fiber

Table (2): Formulation and proximate analysis of the experimental diets (on %DM) fed to to Nile tilapia (*Oreochromis niloticus*) fingerlings.

Ingredients (%)	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Fish meal (65% cp)	5.00	-	-	-	-	-	-
Soybean meal (44%	37.00	29	29	29	29	29	29
Wheat bran	7.00	6.	6.	6.	6.	6.	6.
Yellow corn	35.00	47	46	46	46	46	46
Corn gluten	12.00	12	12	12	12	12	12
Corn oil	3.00	2.	2.	2.	2.	2.	2.
Vit. & Min. premix	1.00	1.	1.	1.	1.	1.	1.
Di Ca phosphate	-	0.	0.	0.	0.	0.	0.
DL- lysine	-	0.	0.	0.	0.	0.	0.
DL- methionine	-	0.	0.	0.	0.	0.	0.
Calcium carbonate	-	0.	0.	0.	0.	0.	0.
Thyme (1)	-	-	0.	-	-	-	-
Citric acid (2)	-	-	-	0.	-	-	-
Citric acid + Thyme	-	-	-	-	0.	-	-
Na_2SO_4 (3)	-	-	-	-	-	0.	-
Mixture (1+2+3)	-	-	-	-	-	-	1.
Total	100	10	10	10	10	10	10
Proximate analysis (%)							
Moisture	10.20	10	10	10	10	10	10
Crude protein (CP)	30.71	25	25	24	26	25	25
Ether extract (EE)	5.15	3.	4.	3.	4.	4.	4.
Crude fiber (CF)	8.57	6.	6.	5.	7.	5.	5.
Ash	4.74	3.	4.	4.	3.	4.	4.
NFE*	50.83	60	59	61	58	59	59
GE (Kcal/Kg) **	4598	44	44	44	45	44	44
Ca	0.401	0.	0.	0.	0.	0.	0.
P	0.602	0.	0.	0.	0.	0.	0.
Lysine	1.59	1.	1.	1.	1.	1.	1.
Meth. + Cystine	1.11	1.	1.	1.	1.	1.	1.

¶ *Contains per kg: vitamin A, 4.8 m. I.U; vit D3 , 0.8 m.I.U; vit E, 4.0 g; vit. K, 0.8 g; vit B1, 0.49, vit. B2, 1.6 g; vit. B6, 0.6 g; vit. B12, 4 mg; Pantothenic acid 4 g; Nicotinc acid 8 g; Folic acid, 400 mg; Biotin, 20 mg; Choline chloride, 200 mg; Copper, 4.0 g ; Iodine, 0.4g ; Iron, 12 mg ; Manganese, 22 g; Zinc 22 g and Selenium 0.04 g.*

* *Calculated by difference.*

** *Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and 4.0 (Cal GE/g DM) for crude protein, ether extract, crud fiber and nitrogen free extract, respectively (Jobling, 1983).*

(CF %) and ash contents according to the methods of A.O.A.C. (2000), while nitrogen free extract (NFE %) was calculated by difference. Gross energy (Kcal GE/Kg) contents of all the samples were calculated according to Jobling (1983). Calcium (Ca), phosphorus (P), lysine (Ly) and methionine plus cystine (Meth. + Cystine) in ingredients used were calculated according to (NRC, 1993).

Statistical analysis:

Biological data obtained from the treatments were subjected to statistical evaluation using one-way analysis of variance (ANOVA) of the general liner model (GLM) using SAS (1990) statistical package. Duncan's multiple range test (Duncan, 1955) was used to test the significance ($P<0.05$) of differences among means.

RESULTS AND DISCUSSION

Growth performance:

Effect of the experimental diets on growth performance parameters of Nile tilapia are shown in Table (3). Body weight and weight gain decreased significantly ($P<0.01$) with decreasing the protein content of diets from 30 to 25 %. These results confirm those found previously by (Omar, 1994a; Siddiqui *et al.* 1988) who showed that the 30% CP diet produced the best growth performance of *O. niloticus* fingerlings.

To explain the lower performance of fish fed low protein diet (basal diet) which contain only plant protein source (T_2) that contain phytic acid and limited P availability whereas, phytic acid chelates with various cations like Na, K, Ca, Mg, Mn, Zn, Fe and Cu which become unavailable to fish (Leiner, 1994). In the present study, fish meal was not used in basal diet to avoid its beneficial effects on performance of low protein diets. So, addition of thyme (T_3), citric acid (T4) or both (T5) numerically increased body weight, weight gain, average daily gain and specific growth rate compared to basal diet (T_2). The beneficial effect of thyme may be due to its contents of active materials whereas Miguel *et al.* (2004) reported thyme species have higher antioxidant capacity. Also, Basilico and Basilico (1999) reported that its phenolic compounds (carvacrol and thymol) which present in its essential oil exhibit considerable antimicrobial and antifungicidal effects. On the other hand, beneficial effect of citric acid may be due to the increase the digestibility of phosphorus and other minerals as observed by Sugiura *et al.* (2001) with other species like rainbow trout and Boiling *et al.* (2000) with chicks. Also, Vielma *et al.* , (1999) reported that dietary acidification by citric acid significantly increased whole body iron in fish. On the other hand, several amino acids can chelate with polyvalent cations and these linkages may aid in the passage of the metal ions across cell membranes (Scott *et al.* 1982).

Table (3): Growth performance of Nile tilapia (*Oreochromis niloticus*) as affected with the experimental diets (Mean ± SE).

Item	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Initial body weight, IBW (g/fish)	0.76 ±0.01	0.76 ±0.003	0.77 ±0.003	0.76 ±0.003	0.76 ±0.003	0.77 ±0.005	0.83 ±0.03
Final body weight, FBW (g/fish)	13.74^{ab} ±0.43	10.56^{d} ±0.68	11.62^{cd} ±0.27	11.05^{d} ±0.34	11.71^{cd} ±0.54	12.84^{bc} ±0.06	14.91^{a} ±0.73
Weight gain, WG (g/fish)	12.98^{ab} ±0.41	9.79^{d} ±0.68	10.84^{cd} ±0.28	10.28^{d} ±0.34	10.95^{cd} ±0.54	12.08^{bc} ±0.06	14.08^{a} ±0.69
Average daily gain, ADG (g/fish/day)	0.14^{ab} ±0.005	0.10^{d} ±0.008	0.11^{cd} ±0.003	0.11^{cd} ±0.003	0.12^{cd} ±0.005	0.13^{bc} ±0.00	0.15^{a} ±0.008
Specific growth rate, SGR (%/day)	3.18^{a} ±0.06	2.87^{c} ±0.06	2.97^{c} ±0.03	2.93^{c} ±0.03	2.99^{bc} ±0.04	3.10^{ab} ±0.01	3.17^{a} ±0.008

a, b,..etc.: Means within the same row with different superscripts are significantly different ($P < 0.05$).
WG(g)=FBW–IBW; ADG (g fish/ day)=(AWG(g)/days); SGR (%/day)= ((LnFBW– LnIBW)/feeding days)×100

Under low protein diets may be the passage of metal ions decrease, thus citric acid is very useful under these conditions. Weight gain increased by 11.85% with addition of thyme plus citric acid (T_5) compared to basal diet (T_2). Ali *et al.* (2008) and Abd El-Hakim *et al.* (2009) attributed the beneficial effect of *Thymus vulgaris or Curcuma longa* and organic acid (citric acid) on disrupt effect of essential oils on Gram-positive bacteria and citric acid on Gram-negative bacteria and the addition of both of them have a synergetic effect. It is surprising that weight gain increased by 23.39% with addition of sulphate alone (T_6) compared to the basal diet. The positive effect of sulphate may due to its role in transport mineral from elementary channel. Also, sulphate has another beneficial effects and roles in nutrition like detoxification of aflatoxin B1 (Ali *et al.* 2006), detoxification of phenolic compounds (Ali, 2005) and partial substitute of methionine if the later is not adequate (Miller, 1974). However, it was mentioned before, the metal ions passage decrease under low protein diet. Possible reason for the increased weight gain may be related to the high availability of minerals with addition of sulphate under this condition. In this respect, Wedekind *et al.* (1994) observed that Zn from $ZnSO_4$ was more available than from zinc–lysine and zinc–methionine. . Also, Ali *et al.* (2010) found that with growing chicks under heat stress condition, sulphate addition increased ash retention by 61.63% compared to control diet. From previous discussion, it could be indicated that sulphate has a role in increasing ash retention under low protein diet. The retention of minerals is very important for their role in antioxidant defense such as Mn or Zn which play a role in antioxidants enzyme and manganese is a crucial component of the metalloenzyme (Mn superoxide dismutase, MnSOD). Luo *et al.* (1992) determined that MnSOD functioning as a free radical scavenger is by far the most important Mn-containing enzyme. Also, one of the most significant functions of zinc is related to its antioxidant role and its participation in the antioxidant defense system (Powell, 2000). On the other hand, antioxidant enzymes are crucial in the effort to counteract oxygen toxicity when the supply of other antioxidant compounds is scarce or depleted (Ahmad, 1995).

Surai (2002) showed that to achieve optimum protection, the tissues deploy an integrated antioxidant system that consists of a diverse array of lipid-soluble (e.g. vitamin E, carotenoids), water-soluble (e.g. ascorbic acid, glutathione) and enzymic (e.g. glutathione peroxidase, superoxide dismutase) components. The key feature is that these various components act in synergy. It is known that (carvacrol and thymol) which present in thyme are lipid soluble while conjugation with sulphate make them water soluble (Williams, 1959)

Thus, the addition of thyme, citric acid and sulphate (T_7) significantly improved weight gain by 43.82% compared to basal diet.

From the all aforementioned results, it seemed that mixture of the three tested additives has a synergetic effect on fish growth and the fish under low protein diet may need to:

1- Decrease the degradation of protein in tissues by free radical with addition natural antioxidants like thyme.
2- Increase the mineral absorption by increasing mineral availability with addition of citric acid.
3- Increase activity of natural antioxidants and make them water soluble with addition of sulphate.

Feed utilization:

The data in Table (4) indicated that there were significant ($P<0.01$) differences between values of feed intake recorded by different treatments. Decreasing protein level from 30 to 25% decreased significantly feed intake and the feed conversion became worse except in fish group fed T_7. Plant protein as a sole source of protein in low protein diet contains phytic acid and other antinutritional factors whereas, phytic acid inhibits activities of some digestive enzymes such as pepsin, trypsin and alpha-amylase (Liener, 1994) which impair feed conversion. Also (Krogdahl *et al.* 2003) with Atlantic salmon, found that soybean meal produced a decrease in mucosal enzymes, which were coincidental with an impaired feed conversion. However, in the present study, addition of thyme (T_3) to the basal diet increased feed intake and improved feed conversion while addition of citric acid alone (T_4) or with thyme (T_5) increased only feed intake. These results indicate that free radical production is accompanied by low feed utilization. In this respect, Iqbal *et al.* (2004, 2005) with broiler, found that increasing H_2O_2 production and higher protein oxidation were consistently observed in duodenum with low feed efficiency, breast muscle and liver mitochondria. Thus, the addition of thyme improved feed conversion as a result of decreasing free radical which destroy protein and lipid. Addition of sulphate (T_6)

significantly increased feed intake and improved feed conversion that may due to the increase in the minerals availability and the later are participated in antioxidants defense. Feed intake increased by 33.29% and the feed conversion ratio was improved by 6.98% in fish group fed diet (T_7) compared to basal diet (Table 4). Decreasing protein level of the diet increased significantly protein efficiency ratio and numerically protein productive values (PPV) compared to control diet. Addition of sulphate alone or in mixture with thyme and citric acid increased utilization of protein. Fish fed diets contained sulphate or mixed additives ($T_{6,7}$, respectively) were superior in protein utilization compared with those fed the other treatments. Reducing protein content in the diet decreased significantly the energy utilization and all feed additives that used in this study improved the energy utilization compared to basal diet.

Table (4): Feed utilization of Nile tilapia (*Oreochromis niloticus*) as affected with the experimental diets (Mean ± SE).

Item	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Feed intake, FI (g/fish)	20.71^{b}	16.85^{d}	18.13^{cd}	18.61^{c}	18.71^{c}	19.22^{bc}	22.46^{a}
	±0.85	±0.53	±0.13	±0.45	±0.40	±0.01	±0.63
Feed conversion ratio, FCR	1.59^{a}	1.72^{ab}	1.67^{ab}	1.81^{b}	1.71^{ab}	1.59^{a}	1.60^{a}
	±0.01	±0.07	±0.03	±0.03	±0.05	±0.005	±0.05
Protein efficiency ratio, PER	2.04^{c}	2.28^{ab}	2.31^{ab}	2.24^{b}	2.23^{b}	2.46^{a}	2.46^{a}
	±0.01	±0.10	±0.04	±0.03	±0.07	±0.01	±0.07
Protein productive value, PPV (%)	33.19^{c}	36.05^{ab}	36.61^{ab}	35.25^{bc}	36.12^{abc}	39.04^{ab}	40.34^{a}
	±0.70	±2.42	±0.85	±0.47	±0.60	±0.98	±1.72
Energy utilization, EU (%)	49.72^{a}	35.60^{c}	39.09^{bc}	38.06^{bc}	40.36^{bc}	43.44^{ab}	47.97^{a}
	±1.36	±3.30	±1.31	±1.46	±0.86	±0.75	±3.73

a, b... etc.: Means within the same row with different superscripts are significantly different (P <0.05). FI (g) = Total dry matter feed intake, FCR = FI / WG, PER = WG / protein intake, PPV (%) = (Retained protein /protein intake) × 100, EU (%) = (Retained energy / energy intake) × 100.

Proximate analysis of fish whole-body:

Average percentages of moisture, protein, fat, and ash on a dry matter basis of tilapia fish carcass are shown in Table (5). There were significant differences (P<0.01) between treatments in values of protein. Decrease level of protein in basal diet did not affect the protein in fish carcass.

Table (5): Whole body composition (%DM) of Nile tilapia (*Oreochromis niloticus*) as affected with the experimental diets at the end of experiment (Mean ± SE).*

Item	Treatments						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Dry matter (DM%)	29.31	28.59	28.33	28.73	28.53	28.36	27.89
	±0.24	±0.65	±0.78	±0.33	±0.86	±0.68	±1.27
Crude protein (CP%)	54.68^{bc}	54.18^{bc}	55.11^{bc}	53.83^{c}	55.73^{b}	55.17^{bc}	57.98^{a}
	±0.89	±0.41	±0.27	±0.37	±0.25	±0.75	±0.59
Ether extract (EE%)	24.69	22.74	23.21	24.03	24.54	22.33	22.95
	±0.19	±0.18	±0.41	±0.23	±0.18	±0.26	±0.45
Ash (%)	9.31^{e}	10.56^{bc}	10.75^{b}	10.38^{b}c	10.02^{cd}	9.66^{de}	11.85^{a}
	±0.11	±0.16	±0.18	±0.09	±0.31	±0.16	±0.35
Gross energy (Kcal GE/kg)	5870^{a}	5710^{b}	5747^{b}	5783^{b}	5857^{a}	5743^{b}	5553^{c}
	±28.87	±20.82	±26.03	±12.02	±18.56	±24.04	±26.03

a, b,..etc.: Means within the same row with different superscripts are significantly different (P < 0.05).
** Fish analysis as zero group was: DM, 22.76%; CP, 53.48%; EE, 20.13%; Ash, 18.92% and GE, 5220 Kcal/Kg.*

Addition of thyme alone or with citric acid numerically increased the value of the whole-body protein contents compared to basal diet. These data are in consistent with those of Zheng *et al.* (2009) who found that commercial product containing natural oregano essential oil increase antioxidant activity and enhance muscle protein sedimentation. Also, the whole-body protein contents were increased by myo-inositol (as an antioxidants) supplementation (Jiang *et al.* 2009). Addition of sulphate alone numerically increased the whole-body protein contents compared to basal diet.

Hence the mixture of thyme, citric acid and sulphate (T_7) recorded the highest value of the whole-body protein contents which increased by 7.01 % compared with basal diet (Table 5).

Since low molecular weight ligands such as amino acid may facilitate the uptake of elements in the intestinal tract (Davis and Gatlin, 1996). Protein deposition in muscle of fish at lower protein diet may need to be protected by natural antioxidants like thyme and mineral binding- protein like zinc and manganese which participate in the antioxidant defense system. For these reasons, the mixture of thyme, citric acid and sulphate seemed to make a good defense from free radical. The fat contents of Nile tilapia were not influenced by dietary supplements while ash values were significantly (P<0.01) different between treatments. Reducing protein level in fish diets significantly increase ash percentage in the fish carcass. These results can be explained on the base that fish increase ash content in their body to make defense from free radical under the deficiency of protein. Grune *et al.* (1997) showed that the degradation of proteins is an essential part of the overall antioxidant defenses against free radical attack. The addition of mixture of thyme, citric acid and sulphate (T_7) significantly increased ash percentage by 12.22% compared to basal diet (Table 5). However, Ali *et al.* (2010) found that with growing chicks under heat stress condition , sulphate addition increased ash retention by 61.63% compared to control diet. As we discussed before, the defense from free radical by natural antioxidants like thyme and factors like citric acid or sulphate increase mineral availability and resulted in increasing performance (Table 3) and both protein and ash content of fish carcass (Table 5). Reduce protein level in the diet significantly ($P<0.01$) increases gross energy of fish whole-body while the mixture of additives significantly decreased it compared to control diet or basal diet.

CONCLUSION

It was concluded that addition of either thyme, citric acid or sodium sulphate improved the utilization of low protein diets and addition of a mixture of the three additives significantly increased weight gain and protein content in fish carcass by 43.82 and 7.01%, respectively, compared to basal diet. Under the condition of this study, mixture of thyme, citric acid and sulphate is the most successful additives for improving performance of Nile tilapia fingerlings fed low protein diets.

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كفاءة إستخدام علائق نباتية منخفضة البروتين تحتوى على بعض الإضافات الغذائية على أداء النمو وتركيب جسم أصبعيات أسماك البلطى النيلى

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اجريت التجربة الحالية لتقييم التأثير المحتمل للزعتر كمضاد أكسدة طبيعي وحماض الستريك وكبريتات الصوديوم على تحسين إستفادة الأسماك المغذاه على عليقة منخفضة البروتين.

تم تركيب سبعة علائق تجريبية وغذيت لصغار أسماك البلطى وحيد الجنس بمتوسط وزن أولي (0.80 ± 0,03 جرام). تحتوى العليقة المقارنة (T_1) على (30 ٪ بروتين خام) و (4598 كيلوكالورى طاقة كلية/ كجم),وتحتوى العلائق الستة الأخرى تقريبا على (25 ٪ بروتين خام) وطاقة كلية حوالى (4470 سعر حرارى ± 37 كيلوكالورى / كجم) وكانت لا تحتوى على مسحوق الأسماك في تركيبها. تعتبر العليقة الثانية (T_2) عليقة قاعدية خالية من الإضافات وأضيف إلى العلائق من الثالثة إلى العليقة السابعة 0,3 ، 0,3 ، 0,6 ، 0,5 و 1,1 ٪ من الزعتر , حامض الستريك ,الزعتر + حامض الستريك (1:1) ، كبريتات الصوديوم وخليط من الزعتر + حامض الستريك + كبريتات الصوديوم على التوالي . وخلصت النتائج إلى أن إضافة أي من الزعتر أو حامض الستريك أو كبريتات الصوديوم أدى إلى تحسين الاستفادة من العلائق منخفضة البروتين وإضافة خليط من الثلاث إضافات أدى إلى زيادة الوزن وزيادة المحتوى البروتينى لجسم الأسماك بمقدار 43,82, 7,01 ٪ على التوالي مقارنة بالعليقة القاعدية. والخلاصة أن إضافة المخلوط المكون من الزعتر و حامض الستريك و الكبريتات من أفضل الإضافات لتحسين أداء أسماك البلطي النيلي المغذاه على علائق منخفضة فى البروتين.