Effect of probiotics and some spices as feed additives on the performance and behaviour of the Nile tilapia, *Oreochromis niloticus*

[1]Magdy A. Soltan and [2]Said M. El-Laithy
[1]Department of Animal Production, Faculty of Agriculture, Benha University, Egypt
[2] Department of Hygiene, Animal Behavior and Management. Fac. of Veterinary Medicine, Benha University, Egypt

ABSTRACT

Probiotic microbial feed supplements are gaining wide acceptance in livestock production, and may be applicable to aquaculture production systems. The present study was designed to investigate the effect of incorporation of probiotics *(Bacillus subtilis* and Biogen®), spices (garlic or fennel) and also a combination of *B. subtilis* with garlic or fennel in the diets of Nile tilapia *Oreochromis niloticus*. A total of 420 Nile tilapia fry were divided into seven treatments (3 replicates for each treatment) and fed the experimental diets for 90 days. The basal diet was fomulated to contain 30% CP and 2700 Kcal ME kg^{-1} and divided into 7 diets. The 1^{st} one as a control diet (D1). The other diets were supplied by the diffeent additives, *B. subtilis* (D2), 0.2% Biogen® (D3), 1% garlic (D4), 1% fennel (D5), *B. subtilis*+1% garlic (D6) and *B. subtilis*+ 1%fennel (D7)

Results of the present experiment indicated that, supplementation of the basal diets with probiotics *B. subtilis* or Biogen® and spices (garlic or fennel) significantly ($P<0.01$) improved survival rate of Nile tilapia. A combination of *B. subtilis* and garlic or fennel showed the best fish survival rate but did not significantly differ from those recorded for the diets supplemented with probiotic or spices alone.

Feed intake (FI), feed utilization and growth performance of Nile tilapia including final body weight (BW), final body length (BL), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER), were significantly ($P<0.01$) higher in all treatments than the control diet. The combination of *B. subtilis* and fennel in tilapia diets showed the best feed utilization (FCR and PER) and the highest growth performance (BW, BL, WG, and SGR) compared to the other treatments.

The highest and optimum feeding behaviour among all diet treatment was observed in fish fed the diet D7 while the lowest feeding behaviour was recorded by fish fed the control diet (D1). Also, the combination of *B. subtilis* with garlic or fennel improved the feeding behaviour than that observed for fish fed the diet supplemented with *B. subtilis* alone. There was no significant difference in agonistic activities amongst fish fed the different diets supplemented with probiotics or spices and the control diet.

Probiotics (*B. subtilis* or Biogen®) or spices (garlic or fennel) or the combinations of *B. subtilis* and garlic or fennel in the diets of Nile tilapia significantly (P<0.01) decreased hematocrite (Ht) and reduced the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while hemoglobin (Hb) showed some variation (but not significant). Proximate composition of fish whole-body indicated that, dry matter (DM), crude protein (CP) and ether extract (EE) were significantly (P<0.05) affected by the different treatments compared to control fish group.

Key words: Nile tilapia, probiotics, *Bacillus,* Biogen®, garlic, fennel, 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 (Gongora, 1998; 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).

Schrezenmeir and de Vrese (2001) defined probiotics as a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial health effects in this host'. Probiotic protection can be due to mechanisms such as nutritional competition and/or production of antibacterial substances. Therefore, probiotics must be considered as potentially useful for the control of fish diseases (Irianto and Austin, 2002).

In aquaculture industry, several probiotic species were used, including *Saccharomyces* spp. (Surawicz *et al.*, 1989), *Lactobacillus acidopilus* (Venkat *et al.,* (2004), *B. subtilis* (Kumar *et al.,* 2006, Ghosh *et al.,* 2007 and Keysami *et al.,* 2007) and mixed cultures (Lessard and Brisson, 1987).

There has been increasing interest in the possible use of probiotics in aquaculture, including application in black tiger shrimp, *Penaeus monodon* (Rengpipat *et al.,* 2000), salmonids *Oncorhynchus nerka* and rainbow trout, *Salmo gairdneri* (Irianto and Austin 2002), white shrimp, *Litopenaeus vannamei* Boone (Venkat *et al.,* (2004), Indian major carp, *Labeo rohita* Ham. (Kumar *et al.,* 2006) and *Penaeus japonicus* and *P. semisulcatus* (Saleh, 2007).

Most probiotics are supplied as live supplements in the diet, which has the ability to survive the passage through the intestinal tract (Verschuere *et al,*. 2000). The direct nutritional benefits of probiotic bacteria include vitamin production, availability of minerals and trace elements and production of important digestive enzymes. Probiotics increases 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 probiotics (*B. subtilis* and Biogen®); some spices (garlic and fennel) or the combination of *B. subtilis* and each of garlic or fennel on viability, growth performance, feed utilization, liver function, feeding and aggressive behaviour and proximate composition of *O. niloticus*.

MATERIALS AND METHODS

Nile tilapia fry were obtained from The World Fish Center at Abbassa, Sharkya Governorate, Egypt and acclimated to laboratory conditions in 1700-L fibreglass tanks. The feeding trial was performed at the Fish Nutrition Lab (Faculty of Agriculture, Benha University, Egypt).

Bacteial mixture preparation

B. subtilis strain was obtained in powdered form (7×10^9 cell per gram) from National Organization for Drug Control and Research, Cairo, Egypt. A bacterial mixture was prepared by mixing 10 mg of *B. subtilis* powder with 990 mg of wheat flour to give approximately 7×10^7 cell per gram.

Diets and feeding regime

The basal diet was fomulated to contain 30% CP and 2700 Kcal ME kg^{-1} and divided into 7 diets. The 1st one as a control diet while the other diets were supplied by the diffeent additives as shown in Table (1). In preparing the diets, dry ingredients were first ground to a small particle size and mixed thoroughly with added water to obtain a 30% moisture level. Diets were passed through a mincer with diameter of 2 mm and were sun-dried for 3 days.

Culture conditions

At the beginning of the experiment, 21 glass aquaria (100 × 50 × 40 cm) were supplied with freshwater (180 L for each) at a rate of 1L min^{-1} with supplemental aeration and each aquarium was stocked by 20 fish (weight ranged from 2.60 to 2.65 g). Fish were fed the diets at a daily rate of 10% (during the 1^{st} month), then reduced to 7% (2^{nd} month) and 4% (3^{rd} month) of total biomass. Fish were fed 6 days/week (twice daily at 9.00 am and 3.00 pm). The amount of feed was bi-weekly adjusted according to the changes in body weight throughout the experimental period (90 days).

Table (1): Composition of the basal diet used in the experiment.

Ingredients	Diets						
	D1	D2	D3	D4	D5	D6	D7
Fish meal (72% CP)	16	16	16	16	16	16	16
Soybean meal (44%CP)	35	35	35	35	35	35	35
Yellow corn	32	32	32	32	32	32	32
Wheat bran	10	9	9.8	9	9	8	8
Vegetable oil	4	4	4	4	4	4	4
Vit. & Min. Mixture[1]	3	3	3	3	3	3	3
B. subtilis (7×10^{7} cell g^{-1})	-	1	-	-	-	1	1
Biogen®	-	-	0.2	-	-	-	-
Garlic	-	-	-	1	-	1	-
Fennel	-	-	-	-	1	-	1
Sum	100	100	100	100	100	100	100
Proximate analysis *(determined on dr. matter basis)*							
Dry matter (DM)	97.12	96.68	97.00	97.00	98.32	97.65	97.98
Crude protein (CP)	29.76	30.67	29.91	29.58	29.45	29.95	29.88
Ether extract (EE)	6.47	7.05	7.32	7.76	6.67	7.00	7.16
Crude fiber (CF)	8.45	7.98	8.04	8.45	8.44	7.95	8.34
Ash	9.00	7.66	7.25	8.13	9.11	7.87	8.11
NFE[2]	46.32	46.64	47.50	46.08	46.33	47.20	46.51
ME[3] (Kcal/kg diet)	2710	2760	2770	2785	2708	2730	2740
P/E ratio[4]	109.82	111.12	107.98	106.21	108.75	109.71	109.05

[1] Vitamin & mineral mixture/kg premix : Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; 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] Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)

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

[4] Protein to energy ratio in mg protein/kcal ME.

Growth and feed utilization parameters

Growth performance and feed utilization parameters were determined according to Cho and Kaushik (1985) as follows:

Specific growth rate (SGR) = [(LnW2-LnW1)/t]×100 Where:- Ln = the natural log, W1= initial fish weight; W2 = the final fish weight in "grams" and t = period in days.

Feed conversion ratio (FCR) = feed intake (g)/wet weight gain (g),

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g),
Water quality: Parameters of water quality were determined according to the methods of APHA (1992). Ammonia and nitrite were measured at weekly intervals while water temperatures were recorded daily in each tank using a mercury thermometer suspended at 30-cm water depth. Also, dissolved oxygen was measured daily by oxygen meter and pH by pH meter.

Behavioural observations

Behavioural observations were performed as follows: each treatment group was observed two times daily 15 minutes/time (5 minutes/aquarium) for 3 days/week at circularly predetermined time from 8 am to 5 pm (Fraser and Broom, 1990) for recording the feeding and aggressive behaviour. Any fish showed scraping feed on the water surface with opened mouth or fed on feed particles suspended at water column and on particles at the bottom of the aquarium was recorded to show feeding behaviour (Lovell, 1989) while any fish showed chasing toward other fish with opened mouth or biting other fish was recorded to show aggressive behaviour (Turner *et al.,* 1988).

Blood samples and liver functions

Blood samples were obtained from fish at the end of experimental period. Five fish per tank were randomly chosen and anaesthetized by ethylene glycol mono-phenol ether. Blood samples were collected from the caudal vein using heparinized 27-gauge needles and tuberculin syringes. Hematocrite (Ht) was determined using the micro-Ht method described by Brown (1988). Hemoglobin (Hb) was determined using the total Hb kit (Sigma Diagnostics, Sigma, St Louis, MO. USA) which is standardized procedure using the cyanomethemoglobin method. Liver was removed, homogenized and assigned for determination of Aspartate transaminase (AST) and Alanine transaminase (ALT) according to Reitman and Frankel (1957).

Chemical analysis

At termination of the experiment, three fish were randomly sampled from each tank and subjected to the chemical analysis of whole fish body. Chemical analysis of fish, diets and feces were determined according to the methods of AOAC (1990).

Statistical analysis

The statistical analysis of data was carried out by applying the computer program, SAS (1996) by adopting the model: $Y_{ij} = \mu + \alpha_i + e_{ij}$ Where, Y_{ij} = the observation on the j^{th} fish eaten the i^{th} diet; μ = overall mean, α_i = the effect of i^{th} diet and e_{ij} = random error.

RESULTS AND DISCUSSION

Water quality

During the whole experimental period, water temperature ranged from 26.45 to 29.33°C, dissolved oxygen fom 4.16 to 6.67 mg L^{-1}, pH from 7.60 to 7.90 and total ammonia from 0.11 to 0.14 mg L^{-1}. There were no significant (P<0.05) differences in water quality parameters among treatments during the whole experimental period indicating that, the experimental diets had no detrimental effects on the surrounding water quality of experimental fish.

Survival rate

As described in Table (2), survial rate for fish fed the control diets was found to be 90%. Supplementation of the control diet with *B. subtilis,* 0.2% Biogen®, garlic or fennel significantly increased survival rates to 95.00, 96.67, 95.00 and 95.00%, respecively. The combinations of *B. subtilis* with garlic or fennel also increased surival rates to 98.33%. No significant differences (P>0.05) were observed among fish fed garlic or fennel in survival. At the same time, incorporation of bacteria with garlic or fennel in the diets increased survival rate to 98.3% but did not significantly different from those recorded for fish fed the diets supplemented by garlic or fennel alone. El-Dakar and Goher (2004) used *B. subtilis* in micro-binding diets for *Penaeus japonicus* post-larvae. They found that the level of survival in response to bacterial challenge was high in shrimp fed the diet containing *B. subtilis,* while survival rate declined in the control shrimp fed the basal diet.

Superiority of *B. subtilis* in survival, growth and health status due to its effect as biocontrol or bacterial antagonism effect and production of antimicrobial agents such as antibiotic, antimicrobial peptide substances. Also, stimulation of the immune system using probiotic strains has been reported by Rengpipat *et al.*, (2000). Several mechanisms have been suggested as modes of action for probiotic bacteria. The competitive exclusion mechanism, based on the substitution of pathogen by the beneficial population, has been considered to be important by many authors (Moriarty, 1998; Gatesoupe, 1999; Li and Galtin, 2004).

In the present study, supplementation of the basal diet by Biogen® resulted in higher survival rate than fish fed the control diet. Mehrim (2001) reported that addition of 0.3 % Biogen® to the diet increased the survival rate of tilapia compared with the control diet (without Biogen®). El-Barbary (2002) showed that survival rate of Nile tilapia was increased as Biogen® level increased from 0 to 0.4 %. The positive effect of Biogen® may be due to its spices (garlic and ginger) and the probiotic effects which serve as antitoxic, antibacterial and antifungal agents, which may lead to improve the survival rate. *B. subtilis* may play a vital role to control pathogen. Ghosh *et* *al.* (2007) indicated that incorporation of pobiotics (*B. subtilis*) in fish diets significantly increased survival and decreased fry mortality.

Feed intake and feed utilization

Results of Table (2) indicated that, incorporation of probiotics (*B. subtilis* or Biogen®), spices (garlic or fennel) and the combination of *B. subtilis* with garlic or fennel 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 (D7) supplied by a mixture of *B. subtilis* and fennel and the worst FI, FCR and PER were obtained in fish fed the control diet (D1).

In practical terms, 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. Ringo and Gatesoupe (1998) showed a similar improvement in the biological value of the diets supplemented with probiotics.

Probiotic diet including *B. subtilis* or Biogen® and spices in the present study showed better feed intake and feed utilization compared to control diet. *B. subtilis* may serve in this case as a co-feeding of inert feed and may help to maximize the diets efficiency through stimulating digestive tract. Co-feeding not only stimulates the ingestion of feed particles, but also promotes digestion and assimilation of diets by fish (Koven *et al.,* 1998). Biogen® compounds (garlic, ginger, *B. subtilis* and digestive enzymes) increased palatability of the diets containing Biogen® through its attractive fragrant.

From a nutritional point of view and in agreement with the data of Shelby *et al.* (2006), the present results revealed that the use of the probiotic Biogen® as a feed additive for Nile tilapia is recommended to stimulate productive growth performance and nutrient utilization (FCR and PER).

The superiority of some medicinal plants/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 a and b). About third of feed introduced to aquatic cultured animals is wasted into the water (Axler *et al.,* 1996) causing adverse effects on water quality. Reduction of the feed waste is very important especially in culture systems depending on the reuse of rearing water. In this context, incorporation of some natural plants may caused a reduction of uneaten feed by aquatic cultured animals (Sakr, 2003).

Table (2): Survival rate (SR), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) as affected by incorporation of probiotics and spices in the diets of Nile tilapia.

Experimental diets	Survival rate %	FI (g/fish)	Feed utilization	
			FCR	PER
D1(Control)	90.00 b	36.22 c	1.70 a	1.98 c
D2 *B. subtilis*	95.00 a	44.65 b	1.52 b	2.22 c
D3 (0.2% Biogen®)	96.67 a	50.97 a	1.35 c	2.48 b
D4 (1% garlic)	95.00 a	37.45 c	1.33 c	2.53 b
D5 (1% fennel)	95.00 a	38.83 c	1.16 d	2.92 a
D6 (*B. subtilis* +1% garlic)	98.33 a	41.89 b	1.34 c	2.50 b
D7 (1% *B. subtilis* +1% fennel)	98.33 a	50.72 a	1.19 d	2.83 a
± *Standard error*	±0.55	±1.48	±0.06	±0.03
Probability	0.0043	0.0035	0.0021	0.0021

Means followed by the different letters in each row for each trait are significantly different (P<0.05).

Growth performance

As described in Table (3), supplementation of control 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 growth parameters (BW, BL, WG and SGR). The best growth parameters (BW, BL, WG and SGR) were recorded for fish fed the diet D7 in which the basal diet was supllied by a combination of *B. subtilis* and fennel and the worst one was recorded for fish fed the control diet.

Results indicated that fennel was better than garlic when used as feed additives alone or in a combination with *B. subtilis* in Nile tilapia diets. In agreement with the obtained results, El-Dakar *et al.,* (2004 b) found that fennel seed meal used in tilapia diets improved significantly SGR than those fed the control diet.

The obtained results may be due to the effect of probiotics that 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 B12 (Hoshino *et al.,* 1997), and by stimulating host immunity (Gibson *et al.,* 1997). 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).

As described in Table (3), supplementation of the control diet with Biogen® improved all growth parameters (BW, BL, WG and SGR). The main ingredients of Biogen® are allicien (the product of garlic), ginsieng, *B. subtilis*, high units of hydrolytic enzymes (amylolytic, lipolytic, proteolytic and cell separating enzymes). The high unit hydrolytic enzyme group of Biogen® my make the starch, fat, protein of feeds to be entirely dissociated and absorbed in gastrointestinal tracts of the poultry and domestic animals.

Biogen® can enhance the metabolism of fish body cells, raise the efficiency of feed utilization and balance the secretion of various secretary glands. Moreover, it increases the vitality of cells by supplying oxygen to whole body and improves the immune responses (Diab *el al.,* 2002), helps to excrete heavy metals, inhibits aflatoxin and stimulates the normal endocrine system. Elam (2004) found that BW and WG of Mugil and tilapia fish significantly increased when the diets were supplemented by 0.2% Biogen®. Saleh (2007) showed that there was a significant increase in body weight of shrimp as Biogen® level increased till 0.1 - 0.2 then it decreased at level of 0.3.

Table (3): Parameters of growth performance, as affected by incorporation of probiotics and spices in the diets of Nile tilapia.

Experimental diets	No.	BW (G) Initial	BW (G) Final	BL (cm) Initial	BL (cm) Final	WG (g/fish)	SGR
D1(Control)	60	2.61	23.98 c	5.41	10.74 d	21.37 d	2.46 e
D2 *B. subtilis*	60	2.60	32.10 b	5.12	12.27 b	29.50 c	2.79 c
D3 (0.2% Biogen®)	60	2.62	40.49 ab	5.40	13.87 a	37.87 ab	3.04 ab
D4 (1% garlic)	60	2.59	30.79 b	5.44	12.15 b	28.20 c	2.75 d
D5 (1% fennel)	60	2.61	33.80 b	5.30	12.60 b	31.19 b	2.85 c
D6 (1% *B. subtilis* +1% garlic)	60	2.60	35.99 b	5.34	12.92 b	33.39 b	2.92 b
D7 (1% *B. subtilis* +1% fennel)	60	2.63	45.43 a	5.28	13.93 a	42.80 a	3.17 a
± Standard error		+0.11	±0.99	0.04	±1.67	±0.92	±0.02
Probability		0.8472	0.0008	0.9002	0.0005	0.0010	0.0010

Means followed by the different letters in each row for each trait are significantly different (P<0.05).

Feeding and aggressive behavioure

Results of Table (4) indicate that the highest and optimum feeding behaviour (98.33%) among all diet treatment was observed in fish fed the diet D7 while the lowest feeding behaviour (76.67%) was recorded by fish fed the control diet (D1). Also, the combination of *B. subtilis* with garlic or fennel improved the feeding behaviour than that observed for fish fed the diet supplemented with *B. subtilis* alone. The obtained results may be due to the increasing palatability of diets (Sakr, 2003) and increasing the vitality of fish fed the diets containing probiotics and spices (Abd El-Maksoud *et al*., 2002)

The results of Table (4) also indicate that, there was no significant difference in agonistic activities amongst fish fed the different diets supplemented with probiotics or spices and the control diet. The reduction in the percent of agonistic activities between fish groups fed the different diets may be due to that all fish groups in the present study did not reach the age of sexual maturity and most cichlid fish of both sexes do not hold feeding territories and are non aggressive until the establishment of breeding territories (Schwank, 1987). Turner *et al.* (1988) reported that, for *Tilapia mariae* reared in the laboratory, non-breeding fish in large groups are generally non-aggressive and non-territorial.

Table (4): Feeding and aggressive behaviour of Nile tilapia as affected by incorporation of probiotics and spices in the diets.

Experimental diets	Feeding behaviour %	Aggressive behaviour %
D1(Control)	76.67 c	3.33
D2 (*B. subtilis*)	88.33 b	3.33
D3 (0.2% Biogen®)	96.67 a	1.67
D4 (1% garlic)	88.33 b	1.67
D5 (1% fennel)	88.33 b	3.33
D6 (1% *B. subtilis* +1% garlic)	96.67 a	1.67
D7 (1% *B. subtilis* +1% fennel)	98.33 a	3.33
± *Standard error*	±1.78	0.32
Probability	0.0007	1.0904

Means followed by the different letters in each row for each trait are significantly different ($P<0.05$).

Blood parameters and liver functions

Results of Table (5) indicate that, supplementation of the basal diet with feed additives (probiotics or spices) significantly ($P<0.01$) decreased hematocrite (Ht) and hemoglobin (Hb) level showed some variation (but not significant). Fish fed the control diet showed the highest levels of Hb and Ht while fish fed the diet supplemenmted with garlic showed the lowest ones followed by those fed the diet supplemented by Bioen®. 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 as *B. subtilis* supplemented to the diets.

Compared to control serum level, transferase enzymes (ALT and AST) were significantly decreased when the other diets were supplemented with all treatments specially those fed the diet enriched by garlic or with Bigoen®. These results indicate that probiotics or spices removed the toxic factors of the diets and therefore improved liver function. Shalaby *et al.* (2003) found that activity of liver enzymes (ALT and AST) were markedly decreased in tilapia fed diets containing licorice roots than those fed the control diet. El-Dakar *et al.* (2004b) showed significant lower ($P<0.05$) ALT and AST activities with fish fed fennel seed meal in diets.

Garlic inhibits the fatty acids synthesis and other lipid components in liver and reduces the level of fat accumulation in liver, which leads to a decrease in liver weight (Ibrahim *et al.,* 2004). Garlic contains a variety of organosulphur compounds, amino acids, vitamins and minerals (Block, 1985). Sulphur compounds of garlic are responsible for inhibition of cholesterol synthesis (Liu and Yeh, 2000). Garlic is reported to have hypoglycemic (Al-Salahy, 2002). The possible mechanism in hypoglycemic effects of garlic was due to enhancement of insulin level in blood either by stimulating secretion of insulin cells or converting proinsulin to insulin.

Table (5): Blood parameters and liver functions of Nile tilapia as affected by incorporation of probiotics and spices in the diets.

Experimental diets	Hb (g dL^{-1})	Ht(%)	ALT	AST
D1(Control)	7.19	27.48 a	45.77 a	48.24 a
D2 (*B. subtilis*)	7.35	26.22 a	41.33 b	43.82 b
D3 (0.2% Biogen®)	6.47	23.29 b	42.17 b	42.36 b
D4 (1% garlic)	6.89	20.00 c	42.02 b	42.22 b
D5 (1% fennel)	7.93	26.03 a	45.00 a	43.57 b
D6 (1% *B. subtilis* +1% garlic)	7.43	25.32 a	43.24 a	44.54 b
D7 (1% *B. subtilis* +1% fennel)	7.78	26.62 a	44.91 a	40.98 b
± *Standard error*	±0.83	±1.32	±1.56	±1.25
Probability	0.0629	0.0043	0.0020	0.0031

Means followed by the different letters in each row for each trait are significantly different (P<0.05).

Proximate analysis of fish whole-body

Concerning proximate whole-body composition, results of Table (6) indicate that, DM, CP and EE contents of Nile tilapia were significantly (P<0.05) influenced by the different treatments, while ash content showed some variations (but not significant) among the different teatments. This expected as fish in all treatments did not grow essentially at the same rate. Barros *et al.,* (2000) and Yildirim *et al.* (2003) 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.

Fish fed the diet D7 showed the highest DM and EE and the lowest CP contents of the whole body, while the opposite trend was recorded for fish fed the control diet which showed the lowest DM, EE, ash and the highest CP of the whole body.

Conclusion

Based on the obtained results, it is recommended to supplement tilapia diets with probiotics or spices and apply different combinations of probiotics and spices as natural feed additives. Further research is still needed to detect the mode of action of probiotics on digestibility, immune response and stress resistance. Also, it is important to define the probiotic levels administered to fish to avoid over-dosing and under-dosing with resultant lower efficacy and unnecessary costs.

Table (6): Proximate analysis of fish whole-body (based on dry matter) of Nile tilapia as affected by incorporation of probiotics and spices in the diets.

Experimental diets	DM	CP	EE	Ash
D1(Control)	24.12 c	68.89 a	13.12 c	15.84
D2 (*B. subtilis*)	25.23 bc	66.72 ab	14.26 b	17.12
D3 (0.2 Biogen®)	25.54 b	66.82 ab	15.00 b	16.18
D4 (1% garlic)	25.87 bc	67.20 a	14.74 b	16.44
D5 (1% fennel)	26.46 b	66.76 ab	15.43 b	16.23
D6 (1% *B. subtilis* +1% garlic)	26.67 b	66.34 ab	16.89 ab	15.34
D7 (1% *B. subtilis* +1% fennel)	29.11 a	65.16 b	17.23 a	16.24
± *Standard error*	±1.88	±1.85	±0.86	±0.97
Probability	0.0541	0.0331	0.0253	0.0611

Means followed by the different letters in each row for each trait are significantly different (P<0.05).

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تأثير محفزات النمو الحيوية وبعض التوابل كإضافات غذائية على أداء وسلوك أسماك البلطى النيلى

[١]مجدى عبدالحميد سلطان و [٢] سعيد محمد مرسى الليثى

[١] قسم الإنتاج الحيوانى– كلية الزراعة – جامعة بنها

[٢]قسم الصحة وسلوكيات ورعاية الحيوان – كلية الطب البيطرى – جامعة بنها

تم تصميم هذه التجربة لدراسة تأثير إدخال بكتريا الباسيلاس والبيوجين وكذلك الثوم والشمر منفرداً أو مضافاً اليه بكتريا الباسيلاس وتأثير ذلك على أداء أسماك البلطى النيلى. وقد أجريت هذه الدراسة على ٤٢٠ سمكة تم توزيعها على سبعة معاملات وتم تكرار كل معاملة فى ثلاثة مكررات (احواض) وقد أستمرت التجربة ٩٠ يوماً وكان من أهم النتائج المتحصل عليها فى هذه الدراسة مايلى:

أظهرت النتائج أن إضافة بكتريا الباسيلاس أو البيوجين أو التوابل قد أدى إلى تحسن فى نسبة حيوية الأسماك كما أدى إضافة بكتريا الباسيلاس إلى الشمر فىعليقة واحدة إلى الحصول على أعلى نسبة حيوية وإن كان تأثير هذه الإضافة غير معنوى مقارنة بإستخدام التوابل منفردة فى العليقة.

أعطت العلائق التى أستخدمت فيها الإضافات الغذائية إلى الحصول على أعلى مقياس لكمية الغذاء المأكول، ومعامل تحويل الغذاء و كفاءة تحويل البروتين، وزن وطول الجسم والزيادة فى وزن الجسم وكذلك معدل النمو وكانت الفروق بين العاملات المختلفة والمجموعة الضابطة لهذه الصفات فروقاً معنوية.

أظهرت النتائج أن أفضل سلوك غذائى للأسماك تمت مشاهدته فى مجموعة الأسماك التى تغذت على المعاملة السابعة التى تم فيها إضافة بكتريا الباسيلاس مع الشمر فى العليقة الأساسية بينما أظهرت الأسماك التى تغذت على العليقة الإساسية (بدون إضافات) أضعف سلوك غذائى ولم يتأثر السلوك العدوانى للأسماك معنوياً بهذه الإضافات.

أدى إضافة محفزات النمو والتوابل إلى إنخفاض معنوى للهيماتوكريت ولم يتأثر الهيموجلوبين كثيراً بهذه المعاملات كما أدى إستخدام محفزات النمو والتوابل إلى إنخفاض معنوى فى مستوى بعض إنزيمات الكبد (الإنزيمات الناقلة لمجموعة الأمين) . وأظهرت النتائج كذلك أن محتوى جسم الأسماك من المادة الجافة والبروتين الخام قد تأثرت معنوياً بإدخال محفزات النمو والتوابل فى علائق أسماك البلطى النيلى فى حين لم تتأثر نسبة الرماد فى جسم السمكة بهذه الإضافات.