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Combined effect of prebiotics and probiotics on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*).

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Abstract

Citric acid and *Bacillus subtilis* are widely used in diets of fish as prebiotic and probiotic for improved growth performance and enhanced immune response. This study aims to investigation of used *Bacillus subtilis* and combination between Citric acid and Bacillus subtilis in diets of Nile tilapia (*O. niloticus*) juveniles and affected on Histopathology and morphometric assessment of intestine, growth, feed utilization and body composition. Nile tilapia were fed control diet (D1) without addition, additive *Bacillus subtilis* (BS) at a rate 10cm/kg diet (D2), 10cm (BS)+5g citric acid/kg diet (D3) and 10cm (BS)+10g (CA)/kg diet (D4) for 90 days. Results showed that morphometric assessment of intestine improved and significant difference in duodenum, jejunum and ileum in mucosal length and villi length between all treatments except mucosal length in duodenum. Also, these results showed the highest values and best group D4 to contain (10cm BS+10g CA) followed by D3 to contain (10cm BS+5g CA) and the lowest values showed in control group (D1). Also, histopathology of intestine showed that duodenum, jejunum and ileum of fish from group control and D2 showing normal villi and normal mucosal lining. On the other hand, results of intestine of fish from group D3 and D4 showing increase the villi length in duodenum, increase the villi length and branches in jejunum and normal mucosal lining in ileum. Results of body weight gain, SGR, condition factor, FCR, FE, PER, PPV and chemical composition of fish showed improvement and significant differences ($P \geq 0.05$) between all treatments contain citric acid and *Bacillus subtilis* compared to control. This suggests that citric acid and *Bacillus subtilis* in diet had a

synergistic effect on histopathology and morphometric assessment of intestine, growth performance, feed utilization and body composition of Nile tilapia (*O. niloticus*) juveniles.

Keywords: Nile tilapia, morphometric intestine, citric acid, *Bacillus subtilis.*

INTRODUCTION

While tilapia has been cultured since the early 1950s, it has become increasingly popular recently and is currently second only to carp in terms of total world production. For this reason, it is often referred to as 'aquatic chicken' or the 'poor man's fish'. Tilapia farming has now spread across a wide range of culture systems – from small ditches to large ponds and reservoirs, in fresh and seawater, from peri-urban to rural areas. Tilapia plays a significant role in food security because it is consumed by the poor, especially those residing in rural areas, as well as by the inhabitants of urban areas, who buy their foods from supermarkets. Tilapia has therefore become 'everyone's fish'. It now directly or indirectly contributes to the livelihood of these people by supplying cheap animal protein, providing employment and generating income. Although tilapia is originally from Africa, they have now been accepted in most countries of Asia and Latin America. Nile tilapia (*Oreochromis niloticus*) became a focus species although there are over 200 species available for culture in different parts of the world. There were several underlying reasons for this. In Thailand, Nile tilapia has a special history **(Ram C. Bhujel, 2014)**. The protection and cure of the infectious aquatic animal-diseases, in Egypt, include a limited number of Government-approved antibiotics. However, the use of antibiotics can lead to the evolution of antibiotic-resistant bacterial strains (**FAO, 2006**) and may modified the immune response of fish **(Lundén *et al.* 2002)**. Probiotics, known as beneficial microbes, are being proposed as an effective and eco-friendly alternative to antibiotics. They were first applied in aquaculture species more than three decades ago, but considerable attention had been given only in the early 2000s. Probiotics is one of the idefinition alternatives that can lessen the reliance of the fish production to antibiotics (**Verschuere *et al.* 2000; Nayak, 2010; Lazado & Caipang, 2014a, 2014b; Akhter *et al.* 2015**). Bacillus subtilis (*B. subtilis*) has been shown to possess antitumor and immunomodulatory activities (**Cohen *et al.* 2003**). Some studies have demonstrated that B. subtilis and spores of B. subtilis act as probiotics by promoting the growth and viability of the beneficial lactic acid bacteria in the intestinal tracts of humans and some animals (**Hoa *et al.* 2000**). Citric acid (CA) is an organic acid widely applied in food and pharmaceuticals industry. CA can enhance

digestive function and alleviate stress, which has been reported in land animals such as pig (**Partanen and Mroz 1999**; **Øverland *et al.* 2000, 2008; Partanen *et al.* 2002**) and broiler chick (**Brenes *et al.* 2003; Gauthier 2005**), but relatively few studies were reported in aquatic animals. The improvement in growth and nutrients digestibilities by dietary CA was also reported in tilapia, *Oreochromis niloticus x O. aureus* (**Pan *et al.* 2004**), allogynogenetic crucian carp, *Carassius auratus gibelio* (**Leng *et al.* 2006**), rainbow trout, *Oncorhynchus mykiss* (**Sugiura *et al.* 2001; Pandey and Satoh 2008**) and red sea bream, *Pagrus major* (**Sarker *et al.* 2005**). Therefore, the present study aimed to investigate the effect of probiotic and citric acid supplementation on Histopathology and morphometric assessment of intestine, growth, feed utilization and body composition in Nile tilapia (*O. niloticus*) juveniles.

MATERIAL AND METHODS

Aimed and location:

The present study was carried out at the Fish Experimental Station belonging to the Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The aim of the experiment is to investigate the effect of probiotic (*Bacillus subtilis*) and prebiotic (citric acid) on diets of Nile tilapia in growth performance, feed utilization and body composition.

Description of experimental aquaculture units:

Fish were reared in cement ponds (1 X 4 x $1m^3$) with a uniform size and weight were at a density of 20 fish/m^3 (each pond $4m^3$). All experimental ponds were supplied with air through an aeration system which connected with air pump (5hp). In the present study two additive feed in diets *Bacillus subtilis* (BS) at a rate 10cm/kg diet, 10cm (BS)+5g citric acid/kg diet, 10cm (BS)+10g (CA)/kg diet and control.

Experimental fish:

The fish used in this study were Nile tilapia (*O. niloticus*) juveniles, were purchased from the Prof**.** Dr**.**Ismaiel Radwan fish Farm, Kafr El-Sheikh Governorate., Egypt. The experimental fish were transported at early morning using a special fish transport car with aeration facilities. They were acclimated to 14 days before starting the experiment thereafter. 960 fish were randomly distributed in four experimental dietary groups in 4 ponds and stocked at a density 20 fish /m^3 with an average initial weight of 0.5g / fish.

Feeding rate and techniques:

Feeding ration amounted to 4% of total body weight daily at the experiment period (12 weeks). Diets were fed to each group in the form of dried suitable to the fish size. Fish were fed 6 days/week and the amount of feed was divided into two equal portions at 9 am and 2 pm. Every fourteen days, the fish in each tank was weighed and the amount of feed was corrected according to the new fish biomass throughout the experimental period (**Annet, 1985**).

Experimental diets:

Four experimental diets were formulated to contain about 30% crude protein and 4700 kcal/kg gross energy. The first diet Table (1) had served as a control diet (D1) and contained mainly of herring meal; soybean meal and wheat bran. Elsewhere, the experimental diets 2 (D2) contained the same diet of control and additive probiotic *Bacillus subtilis* (BS) at a rate 10cm/kg diet. Also, the experimental diets 3 (D3) contained 10cm (BS)+5g citric acid/kg diet the experimental diets 4 (D4) contained 10cm (BS)+10g (CA)/kg diet to investigate the effect of probiotics and citric acid on growth performance and feed utilization of red hybrid tilapia (*O. niloticus x O. mosambicus*) fingerlings.

Chemical analysis of fish:

At the beginning of feeding trial, a total number of 10 fingerlings were netted, weighed and immediately kept in a deep freezer (-18°C) for chemical analysis (as zero group). A similar procedure was applied at the end of such experimental period (five fingerlings as final samples of each treatment). Samples of each treatment were separately and dried at 65°C for 24 hrs. Then ground in a mixer. Representative samples were chemically analyzed according to **A.O.A.C. (2005)** methods, while their energy contents were calculated according to **NRC (1993)**.

Growth performance parameters:

Fish growth performance, weight gain, average body weight gain, condition factor and specific growth rate were determined according to **Recker, (1975)** and **Castell & Tiews, (1980)** as following equations:

1. Body weight gain (BWG) = (W1) – (W0)

Where:

W1: mean final weight W0: mean initial weight

2. Condition factor (K) = FW / FL3 x 100

Where:

FW: Final body weight (g) FL3: Final body length (cm^3)

3. Specific growth rate (SGR, % / day) = [Ln W1 – Ln W0 / T] 100
Where:
Ln : the natural log W1 : final weight at the certain period (g)
W0: initial weight at the same period (g)
T: experimental period (day)

Table (1): The composition and proximate analyses of basal diet

Items	D1	D2	D3	D4
Fish meal	8	8	8	8
Glutein	20	20	20	20
Soybean meal	25	25	25	25
Wheat bran	7	7	7	7
Corn	35	35	35	35
*Vit & Min .Mix	2	2	2	2
Citric acid (g/kg diet)	0	0	5	10
Bacillus subtilis (cm/kg diet)	0	10	10	10
Linseed Oil	6	6	6	6
(CMC) carboxy methyl cellulose	2	2	2	2
Total	100	100	100	100
proximate analysis of diets				
Dry matter (DM) %	91.27	90.92	90.82	90.68
Crude protein(CP) %	30.09	30.05	30.03	30.01
Ether extract(EE) %	12.99	13.38	13	13.22
Crude five(CF) %	4.44	4.42	4.43	4.41
Ash%	8.32	6.89	7	8.18
**NFE%	44	45.2	45.5	44.1
***GE(Kcal/100g)3	470.51	479.11	476.2	472.53
****DG(Kcal/100g)3	352.88	359.33	357.15	354.40

* Vitamin & mineral mixture/kg premix: Vitamin D, 0.8 million IU; A, 1.33g; D3, 1.68g; E, 6.66g; C, 16.8g; k, 0.8g; B1, 0.4g; Riboflavin 3.75g; B6 2.45g; B12, .33mg; NI, 9.42g; Pantothenic acid, 12.42g; Folic acid, 0.68g; Biotin, 16.6mg; BHT, 0.5g; Mn, 14.7g; Zn, 31.6g; Fe, 18.3g; 1, 0.62g; Selenium, 0.22g and Co, 6.8mg.

** Calculated by differences [Nitrogen free extract (NFE) = [100-(CP+ EE+ CF+ Ash)].

*** Gross energy value was calculated from their chemical composition, Estimated according to Jobling, (1983). As 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

**** Digestible energy, estimated according to Jobling, (1983), using digestible energy = gross energy X 0.75.

Feed efficiency parameters:

1. Feed intake (FI)= fish weight x (feeding level/100) x number of days

2. Feed conversion ratio (FCR) (**Tacon, 1987**): The feed conversion ratio (FCR) was expressed as the proportion of dry food consumed per unit live weight gain of fish.

FCR = Feed intake (g) / weight gain (g).

3. Protein efficiency ratio (PER) (**Davies & Morries 1997**):

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

4. Feed efficiency (FE)

(FE %) = [Weight gain (g) / Feed intake (g)]

5. Protein productive value (PPV) (**Marias and Kissil, 1979**): (PPV %) = [PR1– PR0 / PI] 100

Where:

PR1 : is the total fish body protein at the end of the experiment. (On dry matter basis)

PR0: is the total fish body protein at the start of the experiment. (On dry matter basis)

PI: Protein intake.

Histopathology and morphometric assessment of intestinal villi absorptive capacity:

Samples from the different intestinal portions were collected from fish of different groups, and then fixed in 10% neutral buffered formalin. After dehydration and clearance, the tissues were embedded in paraffin and sectioned in 5 µm thickness. The serial sections were subjected to staining with hematoxylin and eosin (**Bancroft *et al.*, 2013**).

The morphometric analysis was performed using Image J analysis software (National Institutes of Health, MD, USA), whereas the villus height (measured from the tip of the villus to the villus- crypt junction), villus width from the mid of the villus and crypt depth (measured from the crypt-villus junction to the base of the crypt).

Statistical analysis:

Results are the mean values of duplicates. SPSS 20.0 INC., Chicago, IL, USA (**SPSS, 2011**) was used to perform statistical calculations. All data were subjected to one-way analysis of variance (ANOVA) followed by the Duncan's post hoc multiple test at a 5% probability level (**Duncan, 1955**).

RESULTS AND DISCUSSION

Growth performance:

Results revealed that, the average values of initial weights and lengths at the start of the experiment were 5.6±0.02, with insignificant differences among the experimental period, indicating the complete randomization of individual fish among the experimental trials at the start of the experiment and were homogenous.

Results (Table 2) showed that, at the end of the experimental period (12 weeks), the maximum final weights were achieved in the experimental fish fed diets containing 10cm Bacillus subtilis and 10g citric acid and had a significantly (P>0.05) higher total weight gain compared to the control diet and the rest of the experimental diets. However, the lowest growth performance was observed in the experimental fish fed the experimental diet (D1) control diet with an average final weight of 64.95±0.15 (g) compared to the other of the experimental diets. Final fish weight and growth performance indicated that, additive Bacillus subtilis and citric acid in diets of fish showed positive effects on growth performance of Nile tilapia (*O. niloticus*) fingerlings and improvement in body weight. The present study showed that, the mean final weight, weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) and condition factor (K) of the fish fed diets containing Bacillus subtilis and citric acid were significantly (p<0.05) than that of fish fed with the control diet. Found significant differences (P>0.05) in survival rate were group (1) (97%), group (2) (98%), group (3) (99%) and group (4) (100%). experimental groups.

Feed utilization:

Feed conversion ratio (FCR), feed efficiency (FE), Protein retention (PR), protein efficiency ratio (PER), Protein retention (PR) and protein productive value (PPV) are shown in table (3). All variables related to feed utilization efficiencies such as feed conversion ratio and protein efficiency ratio in all experimental diets were influenced by dietary treatments. Feed utilization of Nile (*O. niloticus*) fingerlings was improved slightly when fed with diets containing *Bacillus subtilis* and citric acid without significant differences among them (P>0.05) except the control diet, while significant increased were obtained with the increase in Bacillus subtilis and citric acid rate.

Table (2): Effect of additive Bacillus subtilis and citric acid in diets on growth performance of the **Nile tilapia (*O. niloticus*).**

Parameters	D1 (Control)	D2 (10cm BS)	D3 (10cm BS+5g	D4 (10cm BS+10g
Initial fish weight (g)	**5.6±0.03**	**5.1±0.05**	**5.2±0.02**	**5.4±0.01**
Final fish weight (g)	**64.95±0.15^{c}**	**67.30±0.10^{b}**	**68.35±0.25^{ab}**	**69.70±0.90^{a}**
Total weight gain (g)	**59.95±0.15^{c}**	**62.30±0.10^{b}**	**63.35±0.25^{ab}**	**64.70±0.90^{a}**
AV. Daily gain (g)	**0.67±0.01^{c}**	**0.69±0.03^{b}**	**0.71±0.02^{ab}**	**0.72±0.01^{a}**
SGR (%/ day)	**2.39±0.09^{c}**	**2.42±0.2^{b}**	**2.44±0.09^{ab}**	**2.46±0.07^{a}**
Condition factor (K)	**1.83±0.05^{a}**	**1.62±0.02^{bc}**	**1.58±0.01^{c}**	**1.72±0.01^{ab}**
No. of fish at start.	**240**	**240**	**240**	**240**
No. of fish at end.	**235**	**236**	**238**	**240**
Survival ratio (SR %)	**97**	**98**	**99**	**100**

Table (3): Effect of additive Bacillus subtilis and citric acid in diets on the feed utilization of the Nile tilapia (*O. niloticus*)

Parameters	D1 (Control)	D2 (10cm BS)	D3 (10cm BS+5g	D4 (10cm BS+10g
Feed intake (g diet/fish) (FI)	**74.66±0.23^{b}**	**75.76±0.95^{ab}**	**76.09±0.12^{ab}**	**76.78±0.32^{a}**
Feed conversion ratio (FCR)	**1.25±0.01^{a}**	**1.22±0.02^{ab}**	**1.20±0.01^{b}**	**1.19±0.01^{b}**
Feed efficiency (FE) (g/ fish)	**0.80±0.01^{b}**	**0.82±0.01^{ab}**	**0.84±0.03^{a}**	**0.84±0.01^{a}**
Protein efficiency ratio (PER)	**2.68±0.03^{b}**	**2.74±0.04^{ab}**	**2.78±0.01^{a}**	**2.81±0.02^{a}**
Protein retention (PR %)	**8.66±0.19^{b}**	**10.29±0.13^{a}**	**10.74±0.27^{a}**	**11.06±0.71^{a}**
Protein productive value	**38.63±0.76^{b}**	**45.28±1.15^{a}**	**47.03±1.23^{a}**	**48.01±1.91^{a}**

Chemical composition of the whole body experimental fish:

At the start and end of the feeding trial, proximate composition of whole fish body values for fish fed with experimental diets is given in Table (4). At the end of the experiment, significant differences were detected in the whole body dry matter (26.88 - 27.42 %). Results showed significant difference in protein (52.98 - 63.11 %; dry matter), lipid (21.42 - 28.85 %; dry matter), ash no significant difference (14.74 – 17.63 %; dry matter) contents of fish fed with the different experimental diets ($P>0.05$).

Morphometric assessment of intestinal villi absorptive capacity

Results of morphometric assessment of intestine showed that improved and significant difference in duodenum, jejunum and ileum in mucosal length and villi length between all treatments except mucosal length in duodenum. Also, these results showed the highest values and best group D4 to contain (10cm BS+10g CA) followed by D3 to contain (10cm BS+5g CA) and the lowest values showed in control group (D1)

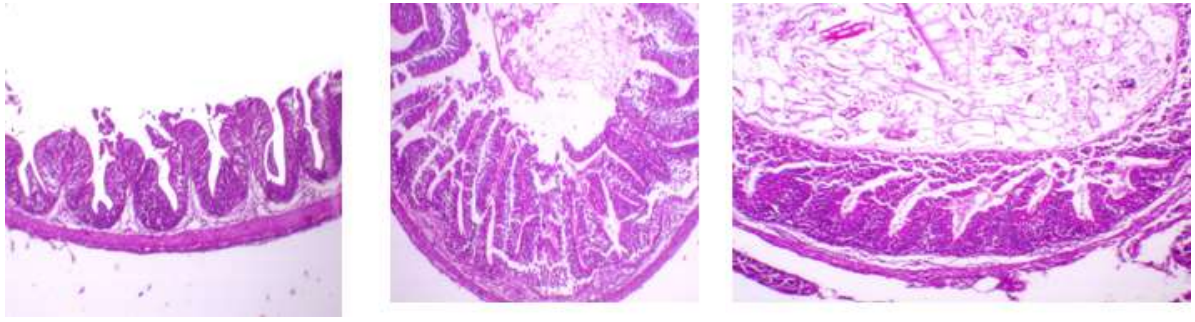
Histopathology assessment of intestinal villi absorptive capacity

Results of histopathology of intestine showed that duodenum, jejunum and ileum of fish from group control and supplemented with 10cm BS diet showing normal villi and normal mucosal lining (Fig. 1and Fig. 2). On the other hand, results of intestine of fish from group supplemented with 10cm BS+5g CA and 10cm BS+10g CA showing increase the villi length in duodenum, increase the villi length and branches in jejunum and normal mucosal lining in ileum (Fig. 3 and Fig. 4).

Table (4): Effect of additive Bacillus subtilis and citric acid in diets on Whole body chemical composition of the Nile tilapia (*O. niloticus*)

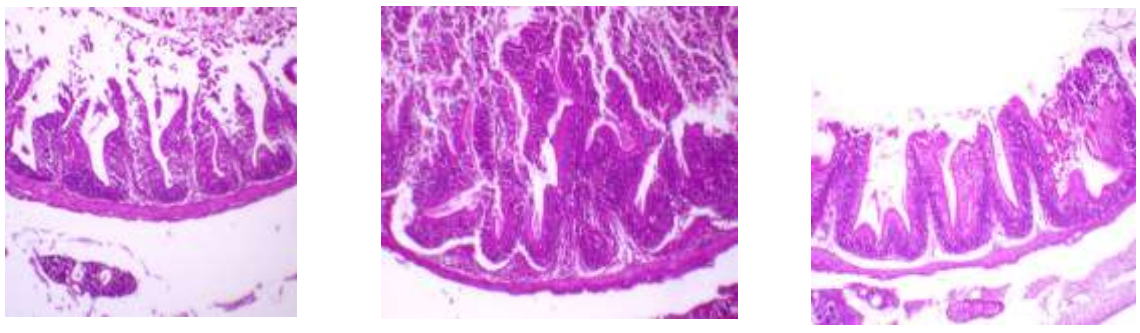
Proximate	Initial	D1 (Control)	D2 (10cm BS)	D3 (10cm BS+5g	D4 (10cm BS+10g
Dry matter (DM)	**78.52±0.54**	**27.42±1.03^{b}**	**27.86±0.46^{b}**	**28.17±0.96^{b}**	**26.88±1.03^{b}**
Crude protein (CP)	**54.44±0.78**	**52.98±1.0^{e}**	**59.03±0.34b^{c}**	**59.81±0.88^{b}**	**63.11±0.57^{a}**
Ether extract *(*EE)	**20.87±0.93**	**28.85±0.66^{a}**	**24.31±0.30^{c}**	**23.36±0.52^{c}**	**21.42±0.53^{d}**
Ash (%)	**24.23±1.08**	**17.63±1.63^{ab}**	**15.97±0.20^{ab}**	**15.92±0.94a^{b}**	**14.74±0.29^{b}**

Intestine part	Duodenum		Jejunum		Ileum	
Measure / TRT	mucosal length	villi length	mucosal length	villi length	mucosal length	villi length
D1 (Control)	**399.88±19.19 c**	**301.32±12.33 d**	**522.71±29.47 c**	**424.15±34.59 c**	**294.20±14.82 c**	**164.41±19.37 b**
D2 (10cm BS)	**474.39±7.92 b^{c}**	**386.91±3.67^{b} c**	**798.14±90.21 ab**	**612.12±22.42 b**	**518.56±26.10 a**	**378.53±19.98 a**
D3 (10cm BS+5g CA)	**561.02±19.50 a**	**449.93±10.11 b**	**873.00±70.47 ab**	**713.19±12.50 a**	**460.66±21.14 ab**	**380.60±42.39 a**
D4 (10cm BS+10g CA)	**569.75±6.77^{a}**	**496.01±14.45 a**	**896.58±46.54 a**	**739.07±19.99 a**	**503.37±37.04 a**	**374.82±33.47 a**



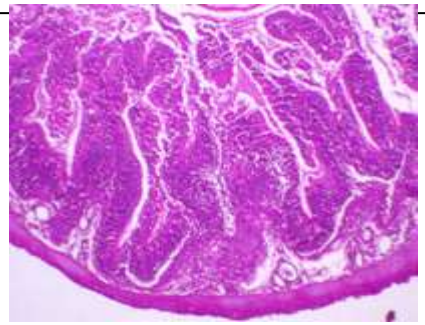
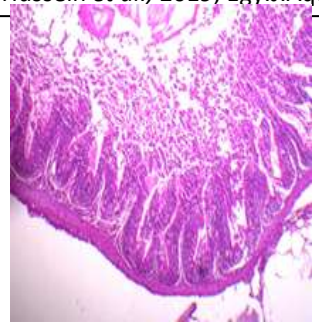
1- Duodenum normal vili length 2- jejunum normal vili length 3- Ileum normal mucosal lining

Fig. (1): showed normal duodenum, jejunum and ileum in control.



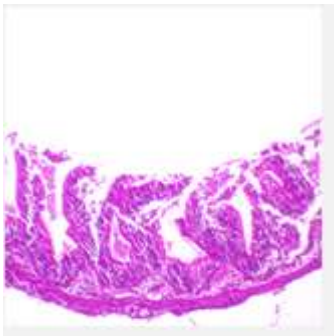
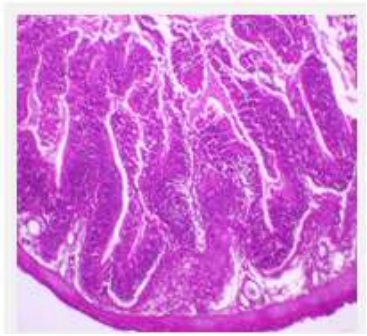
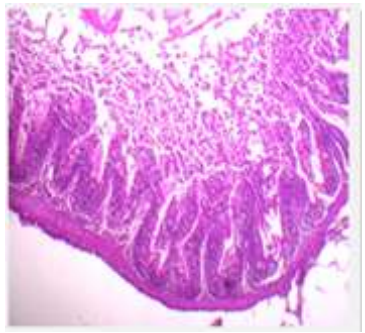
1- Duodenum normal vili length 2- jejunum normal vili length 3- Ileum normal mucosal lining

Fig. (2): showed normal duodenum, jejunum and ileum in D2 supplemented with10 cm *Bacillus subtilis*.


1-duodenum increase the villi length 2- jejunum increase the villi length 3- ilium normal mucosal lining

Fig. (3): showed increase in length of villi in duodenum and jejunum and normal mucosal lining in ileum D3 supplemented with 10 cm *Bacillus subtilis*+5g acetic acid.

1- Jejunum increase the villi length 2- ilium normal mucosal lining 3- duodenum increase the villi length

Fig. (4): showed increase in length of villi in duodenum and jejunum and normal mucosal lining in ileum (D4) supplemented with 10 cm *Bacillus subtilis*+10g acetic acid.

DISCUSSION

In the present study addition of *Bacillus subtilis* (BS) at a rate 10cm/kg diet, 10cm (BS)+5g citric acid/kg diet, 10cm (BS)+10g (CA)/kg diet alleviated some of these negative effects. Adding of *Bacillus subtilis* and citric acid in the diets increased the growth performance such as Weight gain, Average daily gain, Specific growth rate and Condition factor, also, enhanced feed utilization such as, Feed efficiency, Protein retention, Protein efficiency ratio, Protein productive value and reduced the FCR, also, this addition in diets showed improvement in body chemical composition in fish. The obtained results are similar to those reported by **(He *et al* (2013)** in hybrid tilapia, **Ai *et al* (2011)** in *Larimichthys crocea*, **Bairagi *et al* (2004)** in Labeo rohita (*Hamilton*), **Telli *et al* (2014)** in Nile tilapia, **Ng *et al* (2014)** in red hybrid tilapia, **Baruah *et al* (2005)** in Labeo rohita (*Hamilton*), **Khajepour and Hosseini (2012)** in Beluga (*Huso huso*) and **Su *et al* (2014)** in white shrimp. Results of **Liu *et al.* (2012)** indicated that feed of fish in diets contain 10^4, 10^6, and 10^8 CFU g-1 *B. subtilis* showed improvement significant in growth performance and feed conversion ratio. Results of **Ai *et al.* (2011)** showed that at each dietary FOS level and dietary supplementation of 1.35×107cfu g^{-1}*B. Subtilis* significantly increased the specific growth rate (SGR) ($P<0.01$) and feed efficiency ratio (FER) ($P<0.05$) compared with the groups without *B. subtilis* supplementation. **Bairagi *et al.* (2004)** indicated that diets formulated with 40% Leucaena leaf meal and bacteria *B. subtilis* resulted in improvement growth performance and feed utilization, (feed efficiency ratio, protein efficiency ratio, apparent net protein utilization) of rohu fingerlings (*Labeo rohita* Ham.). **Daniels *et al.* (2010)** demonstrated that used diet containing *Bacillus spp*+MOS in feed of larval *Homarus gammarus* had improved significantly ($P<0.01$) weight gain, carapace length, weight to carapace length ratio, specific growth rate (SGR), food conversion ratio (FCR) and post-larval condition, compared to all other groups. **Lin *et al.* (2012)** who revealed that additive chitosan oligosaccharides and *Bacillus coagulans* in diets of *Cyprinus carpio koi* lead to improvement of final weight, specific growth rate (SGR) and feed conversion ratio (FCR). In contrast, **Khajepour and Hosseini (2012)** revealed that additive citric acid in diets of Beluga, *Huso huso* showed increasing in growth performance (final weight, weight gain and specific growth rate) and feed utilization (Protein efficiency ratio and decreased feed conversion ratio and increased the protein and phosphorus digestibility. **Su *et al.* (2014)** indicated that used citric acid in diets of white shrimp lead to increase of weight gain and decreased of feed conversion

ratio. The enhanced in growth performance and nutrients digestibility's by dietary citric acid was also reported in tilapia, *O. niloticus x O. aureus* (**Pan *et al.* 2004**). Adding citric acid a rate 10.0 g kg^{-1} in diet of *O. niloticus x O. aureus* showed increased pepsin activity by 29.6 % (**Li *et al.* 2009**). Improvement in growth performance and feed utilization in this study due to *Bacillus subtilis* and citric acid increased the villi length in duodenum and jejunum in intestine this lead to increased the absorption surface and therefore increased utilization of food and reflected on the growth and health of fish. Also, may have contributed to the increase on protease and amylase activity in intestine. This is likely because of the changes in intestinal pH by citric acid, as it has been reported that pH of digest is also a factor to influence CCK release and exocrine pancreatic secretion. In addition, the effect of citric acid on metal ion release may account for the changes of amylase in tilapia intestine (**Li *et al.* 2009**). Also, organic acids inhibited the harmful microorganisms to improve microecosystem (**Øverland *et al.* 2008; Partanen and Mroz 1999**) and reduced the intestinal pH value to enhance the reproduction of beneficial bacteria (**Knarreborg *et al.* 2002; Partanen and Mroz 1999**). It has been proved that CA can inhibit the adhesion of Escherichia coli with disturbing DNA synthesis in nucleus (**Gedek *et al.* 1993**).

Conclusion

In the present study was best treatment D4 (10cm BS+10g CA) increasing in growth performance (final weight, weight gain and specific growth rate) and feed utilization.

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التأثير المشترك للبريبيوتك والبروبيوتك على أداء النمو والإستفادة من الغذاء فى أسماك البلطى النيلى

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الملخص العربى

حمض الستريك وبكتيريا الباسيلس سابتليس كثيراً ما يتم استخدامهما فى علائق الأسماك كبريبيوتيك وبروبيوتيك حيث يتم تحسين النمو والإستفادة الغذائية وتزيد معدلات الإستجابة المناعية.

وتهدف هذه الدراسة إلى معرفة استخدام البكتيريا مضافة إلى حمض الستريك والبكتيريا وحدها مضافة إلى علائق زريعة البلطى النيلى وتأثيرها على الخلايا الطلائية المبطنة لنسيج الأمعاء وتقدير الطول فيها والنمو والإستفادة من الغذاء والتركيب الكيماوى للجسم .الأسماك المستخدمة فى التجرب هى أسماك البلطى النيلى وتم تقسيم المعاملات على النحو التالى :- عليقة رقم (١) وهى الكنترول بدون أى اضافات , عليقة رقم (٢) البكتيريا مضافة إلى العليقة بمعدل (١٠سم٢) / كجم علف , عليقة رقم (٣) ١٠سم من البكتيريا + ٥جم من حمض الستريك / كجم علف والعليقة رقم (٤) ١٠ سم من البكتيريا + ١٠ جم من حمض الستريك / كجم علف وذلك لمدة (٩٠يوماً) .

وكانت النتائج كالتالى:- لوحظ تحسن فى خملات الأمعاء وكانت هناك فروق معنوية فى الإثنا عشر والزوائد الأعورية واللفائفى فى الطول وفى طول الخملات بين كل المعاملات ما عدا طول الإثنى عشر . كما أوضحت هذه الدراسة أن هناك قيم عالية وأفضلية فى المجموعة الرابعة والتى تحتوى على (١٠ سم من البكتيريا + ١٠ جم من حمض الستريك / كجم علف) , ويليها المجموعة الثالثة والتى تحتوى على (١٠سم من البكتيريا + ٥جم من حمض الستريك / كجم علف), وأقل المستويات كانت فى المجموعة الأولى وهى مجموعة الكنترول . كما لوحظ بالنسبة للتغيرات النسيجية فى عليقة المجموعة الثانية انها طبيعية فى نسيج الخلايا الطلائية المبطنة للإثنى عشر واللفائفى والزوائد الأعورية وايضا طول الخملات .

كما أوضحت النتائج أن هناك زيادة فى وزن الجسم ومعدل تحويل الغذاء ومعامل الحالة والإستفادة من الغذاء والغذاء المأكول والبروتين المحتجز والتركيب الكيماوى للأسماك , كما لوحظ تحسن وفروق معنوية ($P \geq 0.05$) بين كل المعاملات التى تحتوى على البكتيريا وحمض الستريك وذلك مقارنة بمجموعة الكنترول .

لهذا ولكل ما سبق نوصى بإضافة حمض الستريك وبكتيريا الباسيلس فى علائق الأسماك لأنها ساعدت على تحسن تأثير التغيرات النسيجية للأمعاء , تطور النمو , الإستفادة من الغذاء والتركيب الكيماوى لإصبعيات البلطى النيلى .