Effect of different sources and levels of some dietary biological additives on: IV- immunity and haematology of Nile Tilapia, *Oreochromis niloticus*

Abdelhamid, A. Mohamed[1]; Refaey, M. M. Ali[1]; Seden, M. E. Abdelfatah[2] and Osama A.Zenhom[2]

1- Anim. Prod. Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt.
2- Cent. Lab. For Aqua. Res., Abbasa, Abo-Hammad, Egypt.

ABSTRACT

This study aimed to investigate the effect of dietary graded levels of three pre- and probiotics on the immunity and haematology of all-males mono-sex Nile tilapia fish. The experimental period was 16 weeks after 2 weeks adaptation period. In the pathogenicity test, dietary inclusion of Aqua Superzyme at 0.01% of the diet, Garlen Allicin at 0.01% of the diet, and Diamond V (XPC) at 0.5% of the diet, generally reduced the mortality rate (%) and therefore increased the survival rate (%). Yet, the only significant ($P<0.05$) positive effect was due to Aqua Superzyme at 0.01% of the diet in general and after 48h whereas the other supplements did not significantly ($P>0.05$) affect the pathogenicity. The comparisons among substances and their levels clear that the best survival was occurred in the treatment 1:1, i.e the fish group fed Aqua Superzyme (at 0.01% of the diet) diet. The haematological parameters' values increased significantly ($P<0.05$) by using Aqua Superzyme, particularly at 0.01% of the diet; Garlen Allicin, particularly at 0.01% of the diet; and Diamond V (Original XPC), particularly at 0.4% of the diet). Yet, the overall significantly ($P<0.05$) best valus for all tested parameters among additives and their levels was that contained Aqua Superzyme at 0.02% of the diet. So, it is recommended to supply Nile tilapia's diet with the prebiotic Aqua Superzyme at 0.01-0.02% of the diet to improve the immunity system of the fish.

Keywords: Nile tilapia, pathogenicity, haematology (Hb, RBCs, PCV% and WBCs).

INTRODUCTION

Antibiotics have been used for many years as growth promoting agents in addition to their antipathogenic bacterial function which reduce growth and feed conversion. These growth promoters were linked to emergence of multiple drug resistant bacteria and antibiotic residues in animal products (Wary and Davies, 2000). Therefore, using natural feed additives to substitute antibiotics has become an area of great interest (Kumar *et al*., 2003).

Probiotics, unlike antibiotics, imply the use of live micro-organism rather than specific products of their metabolism. Probiotics, which means for life in Greek (Gibson and Fuller, 2000) has been defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance. The mechanism of probiotic effect on animal health was conclusively reviewed by Patterson and Burkholder (2003). They reported that probiotics enable the host animal to return to animal state competition for substrates, production of compounds that inhibit pathogenes, and competition for attachment sites. Results showed that dietary supplementation of *B. subtilis* at a dose of 1.35×10^{7} cfu g^{-1} improved non-specific immune responses and disease resistance of juvenile large yellow croaker, *L. crocea* (Ai *et al*., 2011). The present investigation aimed to study

the effect of dietary graded levels of three pre-and probiotics on the immunity and haematology of all-males mono-sex Nile tilapia fish.

MATERIALS AND METHODS

All the experimental conditions, diets, and facilities were as mentioned before in Abdelhamid *et al*. (2013). The 1st and 2nd additives used were the prebiotic Aqua Superzyme and the probiotic Garlen Allicin, each at 0.01, 0.02, and 0.03% of the diet. The 3rd additive used was the probiotic Diamond V-Original XPC at 0.4, 0.5, and 0.6% of the diet, besides the control without additives.

Pathogenicity:

After 16 weeks of feeding on experimental diets, the fish of each experimental group were divided into two subgroups, each containing 10 fish and were placed into 150 L tank each. The feeding rate was 3% of biomass per day during the 10 days trail. The challenge experiment was carried out using the strain *Aeromonas hydrophila* isolated previously in the laboratory of fish disease Department, Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammad, Sharkia, Egypt, The first subgroup was challenged intraperitoneally (IP) with pathogenic *Aeromonas hydrophila* (0.2 ml of 5 x 10^5 CFU /ml). The second subgroup was injected IP with 0.2 ml of saline solution as a control. Both subgroups were kept under observation for 10 days to record the daily mortality rate as described by Schaperclacus *et al*., (1992). All the experimental infected fish were daily noted for any abnormal clinical signs and mortalities. The dead and clinically diseased fish were subject to bacterial re-isolation.

Haematology:

At the end of the experimental period, 10 fish from each of the treatments and control group were taken for physiological investigations. Fish were not fed for 24 h prior to blood sampling. Fish were anaesthetized using buffered tricaine methane sulfonate (20 mg /L) and blood was collected from fish caudal vein by a sterile syringe. Blood samples were transferred into dry and clean tube with EDTA solution for measuring hemoglobin (Hb), red blood cells (RBCs), and hematocrite (PCV%) in blood after good mixing.

Total erythrocytic were performed simultaneously using the improved neubauer chamber and Natt & Herrick, solution as diluting fluid according to the method described by Natt and Herrick (1952), where the total number of red cells in five secondary squares were determined and multiplied by 10,000. This value represents the total number of erythrocytes per cubic millimeter of blood.

Number of erythrocytes /mm^3 = (N x 200 x 400 x10)/80, where:

N = Number of erythrocytic cells counted in 80 small squares

200 = Dilution factor

400 = Area of each small squares (tertiary squares)

80 = Number of small squares (tertiary).

For determination of hemoglobin (Hb) the blood was treated with a reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide reacts with blood forms methoglobin which was converted to cyanomethemoglobin by cyanide. The color obtained was measured spectrophotometerically at wave length 546 nm according to the method described by Stoskopf (1993*)*. Concentration of blood heamoglobin was calculated from the following equation:

Blood heamoglobin concentration = A x 36.8 = g/dl, Where**:**

A = Absorbance of sample.

The packed cell volume (haematocrite %) was measured using the microhaematocrit method (Schalm, 1975). The volume of the red cells as a percentage of the total volume of the blood was recorded as PCV%.

Statistical analysis:

Data obtained were analyzed using one-way analysis of variance which was performed according to SAS (2006). Differences were subjected to Duncan's (1955) multible range test.

RESULTS AND DISCUSSION

Immunity (pathogenicity against *Aeromonas hydrophila*):

In the pathogenicity test, dietary inclusion of Aqua Superzyme (A_1), Garlen Allicin (G_1), and Diamond V (XPC_2), (Tables 1, 2, and 3, respectively) generally reduced the mortality rate (%) and therefore increased the survival rate (%). Yet, the only significant ($P \leq 0.05$) positive effect was due to A_1 in general and after 48h (Table 1) whereas the other supplements (Tables 2 and 3) did not significantly ($P \geq 0.05$) affect. The comparisons among substances and their levels clear that the best survival was occurred in the treatment 1*1, i.e the fish group fed Aqua Superzyme (at 0.01% of the diet) diet (Table 4).

Table 1: Bacterial challenge test (pathogenicity, %) of *O. niloticus* fed the Aqua Superzyme experimental diets.

Treat.	$Mortality_{24}$	$Mortality_{48}$	Mortality	Survival
Control	80.0^{ab}	0.0^{b}	80.0^{ab}	20.0^{bc}
A_1	60.0^{bc}	0.0^{b}	60.0^{c}	40.0^{a}
A_2	53.33^{c}	13.33^{a}	66.66^{bc}	33.34^{ab}
A_3	86.67^{a}	0.0^{b}	86.66^{a}	13.34^{c}
P > F	0.039	0.052	0.014	0.014
±SE	7.45	3.33	4.71	4.71

a-c: means in the same column having different letters are significantly ($P \leq 0.05$) fifferent.

Table 2: Bacterial challenge test (Pathogenicity, %) of *O. niloticus* fed the Garlen Allicin experimental diets.

Treat	$Mortality_{24}$	$Mortality_{48}$	Mortality	Survival
Control	80.0^{a}	00.0^{a}	80.0^{a}	20.0^{a}
G_1	7555^{a}	00.0^{a}	73.34^{a}	26.66^{a}
G_2	82.22^{a}	00.0^{a}	86.66^{a}	13.34^{a}
G_3	82.22^{a}	00.0^{a}	86.66^{a}	13.34^{a}
P > F	0.330	00.0	0.363	0.363
±SE	2.721	00.0	1.925	1.925

a: means in the same column having the same letter are significantly ($P \geq 0.05$) not fifferent.

Table 3: Bacterial challenge test (Pathogenicity, %) of *O. niloticus* fed the Diamond V (Original XPC) experimental diets.

Treat	$Mortality_{24}$	$Mortality_{48}$	Mortality	Survival
Control	80.0^{a}	0.0^{a}	80.0^{a}	20.0^{a}
XPC_1	64.33^{a}	17.66^{a}	64.33^{a}	35.67^{a}
XPC_2	60.0^{a}	0.0^{a}	66.66^{a}	33.34^{a}
XPC_3	77.66^{a}	0.0^{a}	80.0^{a}	20.0^{a}
P > F	0.001	0.0001	0.0397	0.00.11
±SE	2.461	1.666	3.975	7.45

a: means in the same column having the same letter are significantly ($P \geq 0.05$) not fifferent.

Table 4: Comparison of bacterial challenge test (pathogenicity, %) of *O. niloticus* fed the Aqua Superzyme, Garlen Allicin, and Diamond V (Original XPC) experimental diets.

T*L	$Mortality_{24}$	$Mortality_{48}$	Mortality	Survival
Control	80.0^{ab}	0.0^{b}	80.0^{a}	20.0^{b}
1*1	60.0^{cd}	0.0^{b}	60.0^{c}	40.0^{a}
1*2	53.33^{d}	13.33^{a}	66.66^{bc}	33.34^{a}
1*3	86.66^{a}	0.0^{b}	86.66^{a}	13.34^{b}
2*1	75.55^{abc}	0.0^{b}	77.77^{ab}	22.23^{b}
2*2	82.22^{a}	0.0^{b}	82.22^{a}	17.78^{b}
2*3	82.22^{a}	0.0^{b}	82.22^{a}	17.78^{b}
3*1	64.33^{bcd}	17.66^{a}	64.33^{c}	35.67^{a}
3*2	60.0^{cd}	0.0^{b}	66.66^{bc}	33.34^{a}
3*3	77.66^{ab}	0.0^{b}	80.0^{a}	20.0^{b}
P > F	0.0014	0.0001	0.0011	0.0001
±SE	5.254	2.233	4.085	3.576

a-c: means in the same column having different letters are significantly (P≤0.05) fifferent.

In probiotics, the bacteria produce lactic thereby lowering the pH that accordingly weakens the growth of most pathogenic bacteria and favour acid producers. Lactic acid bacteria are characterized as Gram positive usually nonmotile, non sporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism. Member of this group containing both rods (*Lactobacillus* and carnobacteia) and Cocci (Streptococci); they are generally catalase negative and they usually lack cytochromes (Sneath, 1986).

The spcies currently being used in probiotic preparation are *L. bulgaricus, L. acidophilus, L. casi, L. helveticus, L. lactis, L. salivarvis, L. plantarum, Streptococcus thermopillus, Enterococcus faccium, Ent. falacalis, Bifidobacterium* spp. *and E. Coli.* Consumption of *Lactobacillus* products and supplements cpntaining viable lactic bacteria results in their establishment in the gastrointestinal tract. Their presence in the intestinal tract has been suggested to be prophylactic, they may reduce risk associated with dietary onset of carcinogensis directly due to the prodution of procarcinogenic substances or indirectly due to the reduction in the levels of enzymes that convert procarcinogens to carcinogens. The suppression is probably medidated by lactic acid bacteria through activation of host defense system (Fernandes and Shahani, 1990).

Perdigon *et al.* (1990) indicated that feeding with *Lactobacillus casei* and *Streptococcus salivsius* spp. protect against *Salmonells tyhimurium*, the protective effect of *L. casei* against *S. typhimurium* was associated mainly to 1 gA production in intestinal secretions. However, Probiotics (specific microbial feeds with potential benefits to the host), and prebiotics (dietary components such as complex carbohydrates able to change the colonic microenvironment fostering colonization with non-enteropathogens) are areas of current interest because they offer alternatives for the management of the growing problem of multiple antibiotic resistance and overwhelming infections in the hospitalized patient (Josephlevy, 1998).

The research of probiotics for aquatic animals is increasing with the demand for environment friendly aquaculture. The probiotics were defined as live microbial feed supplements that biocontrol and antagonist pathogens. Many other beneficial effects may be expected from probiotics, e.g., stimulation of the immune system. The most promising prospects are sketched out, but considerable efforts of research will be necessary to develop the applications to aquaculture (Gatesoupe, 1999 and Castillo, 2008).

In other investigation, Verschuere *et al.* (2000) reviewed the probiotic bacteria as biological control agents in aquaculture. They cited that in aquaculture system, the immediate ambient environment has a much larger influences on the health status than with terrestrial animals or humans; since in the aquatic environment, hosts and microorganisms share the ecosystem. Much more than terrestrial animals, aquatic farmed animals are surrounded by an environment that supports their pathogens independently of the host animals, and so (opportunistic) pathogens can reach high densities around the animal. Surrounding bacteria are continuously ingested either with the feed or when the host is drinking. They concluded that probiotics enhance the immune response. So, Poole *et al*. (2007) recorded that the emergence of multidrug resistant pathogens has stimulated a need to find alternatives to antimicrobials. Thus, Abdelhamid *et al*. (2008) used the *Trichoderma viride* for biological control of fungal (*Saprolegnia* sp. and *Aspergillus ochraceus*) diseases of fish.

Abd El-Rahman and El-Bana (2006) used *Micrococcus luteus* as a bacterial probiotic which presented *in vitro* and *in vivo* antagonistic effects against the pathogenic bacteria *Aeromonas hydrophila*. Also, Taoka *et al*. (2006) indicated that probiotics treatment is promising as an alternative method to antibiotics for disease prevention in aquaculture. However, dietary supplementation of probiotic and vitamin C improved the immune response of fish (Nayak *et al*., 2007 and Panigrahi *et al*., 2007) as well as fish health (Panigrahi and Azad, 2007). Moreover, Abdel-Tawwab *et al.* (2008); Aly *et al*. (2008c); El-Ashram *et al.* (2008); Marzouk *et al.* (2008); Wang *et al*. (2008 a and b); Abd El-Aziz *et al.* (2009) and El-Nobi *et al.* (2009) studied the effect of probiotics on survival and resistance of fish.

Moreover, Aly *et al.* (2008a) found that some *Bacillus* and *Citrobacter* strains isolated from Nile tilapia (*B. pumilus, B. firmus*, and *C. freundii*) showed inhibitory effects against *A. hydrophila*. To evaluate the use of commercial live bakers' yeast, *Saccharomyces cerevisiae* as an immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.), fish (0.33 g) were fed a diet containing 0.0, 0.25, 0.50, 1.0, 2.0, or 5.0 g yeast/kg diet for 12 weeks. After the 12-week experimental period, fish of each treatment were challenged by pathogenic *Aeromonas hydrophila*, which was given by interperitoneal (IP) injection and kept under observation for 10 days to record clinical signs and the daily mortality rate. Total fish mortality 10-days after IP injection with *A. hydrophila* and its count after incubation with fish serum decreased with the increase of yeast level in fish diets. However, the lowest fish mortality and bacterial counts were obtained in fish fed 5.0 g yeast/kg. These results indicate that bakers' yeast supplement is promising as an alternative method to antibiotics for disease prevention in tilapia aquaculture, and the optimum level of live bakers' yeast is about 1.0 g per kg diet (Abdel-Tawwab *et al*., 2008).

Also, Aly *et al.* (2008b) reported that the probiotic activity of two bacteria (*Bacillus subtilis* and *Lactobacillus acidophilus*) was evaluated by its effect on the immune response of Nile tilapia (*Oreochromis niloticus*), beside its protective effect against challenge infection. The *in-vitro* antimicrobial assay showed that *Bacillus subtilis* and *Lactobacillus acidophilus* inhibited the growth of *A. hydrophila*. The *B. subtilis* inhibited the development of *P. fluorescens* while *L. acidophilus* inhibited the growth of *Strept. iniae*. The *B. subtilis* and *L. acidophilus* proved harmless when injected in the *O. niloticus*. The serum bactericidal activity was high in the group that was given a mixture of the two bacteria. However, during the past two decades, the use of probiotics as an alternative to the use of antibiotics has shown to be promising in aquaculture, particularly in fish and shellfish larviculture (Tinh *et al*., 2008).

Moreover, Abdelhamid *et al*. (2009) showed the positive effect of the prebiotic "T-Protphyt 2000" at the two concentrations against all the tested bathogenic bacteria and showed nearly no clear difference between the two concentrations of prebiotic. Also, it has a similar effect of that of the antibiotic (OTC), especially with the pathogenic bacteria, *Aeromonas* and *Pseudomonas*, which showed sensitivity towards prebiotic, while the *Vibrio* sp. showed resistance to OTC at the two concentrations. The prebiotics T-Protphyt 2000 and Bio-Mos® (Abdelhamid *et al*., 2009 and 2012) improved also fish performance as a consecoence of the positive effects of the prebiotics on fish health and resistance.

Probiotic bacteria Micrococcus species isolated from the gonads of apparently healthy *Oreochromis niloticus* had antagonistic effect against the pathogenic *Aeromonas hydrophila in vitro*. The inhibition zone to *A. hydrohpila* was 47 mm in diameter due to M. species (Osman *et al*., 2010). Algedawy *et al*. (2011) concluded that the probiotic Biogen® is superior to the multienzyme mixture Natuzyme® for improving the cellular and humoral immune responses. Moreover, Dietary supplementation of *B. subtilis* at a dose of 1.35×10^{7} cfu g^{-1} improved non-specific immune responses and disease resistance of juvenile large yellow croaker, *L. crocea* (Ai *et al.*, 2011).

Some pre-and pro-biotics as powders (mainly T-Protphyt 2000, Bio-Mos®, European Instand Dry Baker's Yeast, P ROBAX, and Bio BUDS) were tested in laboratory for their efficacy as bactericides. The bactericidal activity of these pre-and pro-biotics was tested *in vitro* against nine of pathogenic strains of Gram –negative and positive bacteria (*Aeromonas hydrophilla, Pseudomonas aeruginosa, Pseudomonas fluorescence, Vibrio* spp., *Klebsiella* spp., *Shigella* spp., *Salmonella* spp., *Proteus* spp., and *Escherichia coli*) at two concentrations compared with oxytetracycline. The results showed positive effect of these pre-and pro-biotics at the two concentrations against all the tested bacteria (Abdelhamid and El-Barbar, 2013).

Gut microbiology and indigenous gut histology of Atlantic salmon (*Salmo salar* L.) was investigated following feeding of a control and a prebiotic (EWOS prebiosal®) diet and *ex vivo* exposure to saline or the probiotic bacterium *Carnobacterium divergens*. The results showed that *ex vivo* exposure of *C. divergens* at 10^{8} CFU ml^{-1} did not cause cell damage to the intestine tract of Atlantic salmon. Furthermore, prior provision of dietary prebiotic elevated the ability of *C. divergens* to adhere to the epithelium or mucus layer in the proximal intestine, where culturable heterotrophic bacterial levels (which were identified as *C. divergens*) were elevated by 234% compared to the control. This effect was not apparent in the distal intestine. The ability of isolated carnobacteria from the *ex vivo* experiment to inhibit growth of two fish pathogenic bacteria (*Yersinia rückeri* and *Aeromonas salmonicida* ssp. salmonicida) was assessed. Extracellular products from all 11 of the isolated carnobacteria strains, plus the type strain *Carnobacterium inhibens* CCUG 31728, inhibited the *in vitro* growth of *Y. rückeri*. However, only extracellular products from *C. divergens* isolate 57 inhibited the growth of *A. salmonicida* ssp. Salmonicida (Kristiansen *et al*., 2013).

Haematology:

The haematological parameters' values increased significantly ($P\leq0.05$) by using Aqua Superzyme, particularly A_1 (Table 5); Garlen Allicin, particularly G_1 (Table 6); and Diamond V (Original XPC), particularly XPC_1 (Table 7). Yet, the overall significantly ($P\leq0.05$) best valus for all tested parameters among additives and their levels was 1*2, i.e. Aqua Superzyme at 0.02% of the diet (Table 8).

The research of probiotics for aquatic animals is increasing with the demand for environmentfriendly aquaculture. The extension of the probiotic concept is pertinent when the administered microbes survive in the gastrointestinal tract. Otherwise, more general terms are suggested, like biocontrol when the treatment is antagonistic to pathogens, or bioremediation when water quality is improved. Three main characteristics have been searched in microbes as candidates to improve the health of their host. 1. The antagonism to pathogens was shown *in vitro* in most cases. 2. The colonization potential of some candidate probionts was also studied. 3. Challenge tests confirmed that some strains could increase the resistance to disease of their host. Many other beneficial effects may be expected from probiotics, e.g., competition with pathogens for nutrients or for adhesion sites, and stimulation of the immune system. The most promising prospects are sketched out, but considerable efforts of research will be necessary to develop the applications to aquaculture (Gatesoupe, 1999).

Table 5: Mean immunity index values for Nile tilapia (*O. niloticus*) fed the Aqua Superzyme containing experimental diets.

Treat.	Hb, g/dl	RBCs× 106/mm3	HCT,%	P. $\times10^3$/mm^3	WBCs $\times10^3$/mm^3	N. $\times10^3$/mm^3	L. $\times10^3$/mm^3	M. $\times10^3$/mm^3
Control	4.60b	1.70 c	13.20 c	25.0 c	19.06 c	7.10 c	11.50b	1.0 b
A1	6.53 a	2.20 ab	20.76 ab	37.0 a	24.30 a	9.16 a	13.56 a	1.20 a
A2	6.70 a	2.23 a	21.23 a	34.66 ab	22.66 b	8.46b	12.63ab	1.10ab
A3	6.0a	1.90bc	18.56b	32.66b	22.30b	8.20b	12.43ab	1.03b
P > F	0.005	0.010	0.0002	0.0001	0.0001	0.0004	0.016	0.052
±SE	0.314	0.092	0.754	0.942	0.412	0.186	0.336	0.044

a-c: means in the same column having different letters are significantly (P≤0.05) fifferent.
Hb: haemoglobin, RBC_s: red blood cells, HCT: haematocrit, P: Platelets, WBC_s: white blood cells, N: Neutrophil, L: Lymphocyte, M: Monocyte.

Table 6: Mean immunity index values for Nile tilapia (*O. niloticus*) fed the Garlen Allicin containing experimental diets.

Treat.	Hb, g/dl	RBCs× 10^6/mm^3	HCT, %	P. $\times10^3$/mm^3	WBCs $\times10^3$/mm^3	N. $\times10^3$/mm^3	L. $\times10^3$/mm^3	M. $\times10^3$/mm^3
Control	4.60b	1.70 b	13.20 c	25.0 b	19.06 c	7.10 b	11.50a	1.0 a
G_1	6.20 a	2.03 a	19.66 a	29.0 a	21.93 a	8.06a	12.06 a	1.03 a
G_2	5.16 b	1.80 b	16.20 b	27.0 ab	21.06 ab	7.96a	11.80a	1.03a
G_3	5.23b	1.80b	16.36b	28.66a	20.43bc	8.03a	11.36a	1.03a
P > F	0.015	0.039	0.002	0.010	0.009	0.001	0.35	0.915
±SE	0.26	0.06	0.76	0.66	0.43	0.12	0.28	0.04

a-c: means in the same column having different letters are significantly (P≤0.05) fifferent.
Hb: haemoglobin, RBC_s: red blood cells, HCT: haematocrit, P: Platelets, WBC_s: white blood cells, N: Neutrophil, L: Lymphocyte, M: Monocyte.

Table 7: Mean immunity index values for Nile tilapia (*O. niloticus*) fed the Diamond V (Original XPC) containing experimental diets.

Treat.	Hb, g/dl	RBCs× 10^6/mm^3	HCT,%	P. $\times10^3$/mm^3	WBCs $\times10^3$/mm^3	N. $\times10^3$/mm^3	L. $\times10^3$/mm^3	M. $\times10^3$/mm^3
Control	4.60^a	1.70 ^{a}	13.20 ^{b}	25.0 ^{a}	19.06 ^{b}	7.10 ^{b}	11.50^a	1.0 ^{a}
XPC_1	5.76 ^{a}	1.83 ^{a}	16.20 ^{a}	26.66 ^{a}	20.03 ^{ab}	7.83^a	11.57 ^{a}	1.03 ^{a}
XPC_2	4.73 ^{a}	1.73 ^{a}	14.16 ^{ab}	24.83^a	21.26^a	7.23^{ab}	12.30^a	1.03^a
XPC_3	4.73^a	1.70^a	14.16^{ab}	26.0^a	20.53^a	7.46^{ab}	12.03^a	1.03^a
P > F	0.015	0.50	0.090	0.12	0.037	0.126	0.20	0.91
±SE	0.35	0.068	0.72	0.53	0.43	0.20	0.27	0.041

a-b: means in the same column having different letters are significantly (P≤0.05) fifferent.
Hb: haemoglobin, RBC_s: red blood cells, HCT: haematocrit, P: Platelets, WBC_s: white blood cells, N: Neutrophil, L: Lymphocyte, M: Monocyte.

Table 8: Comparison among mean immunity index values for Nile tilapia (O.niloticus) fed the Aqua Superzyme, Garlen Allicin, and Diamond V (Original XPC) containing experimental diets.

Treat.	Hb, g/dl	RBCs× 10^6/mm^3	HCT,%	P. ×10^3/mm^3	WBCs ×10^3/mm^3	N. ×10^3/mm^3	L. ×10^3/mm^3	M. ×10^3/mm^3
Control	4.60^{d}	1.70^{c}	13.20e	25.0^{d}	19.06^{g}	7.10^{e}	11.50^{de}	1.0 ^{b}
1*1	6.53^{a}	2.20^{a}	20.76^{ab}	37.0^{a}	24.30^{a}	9.16^{a}	13.56^{a}	1.20^{a}
1*2	6.70^{a}	2.23^{a}	21.23^{a}	34.66^{ab}	22.66^{b}	8.46^{b}	12.63^{b}	1.10^{ab}
1*3	6.0^{ab}	1.90^{bc}	18.56^{bc}	32.66 ^{b}	22.30^{bc}	8.20^{bc}	12.43^{bc}	1.033^{b}
2*1	6.20^{ab}	2.03^{ab}	19.66^{ab}	29.0^{c}	21.93^{bcd}	8.06^{bcd}	12.06^{bcde}	1.033 ^{b}
2*2	5.16^{bcd}	1.80^{bc}	16.20^{cd}	27.0 ^{cd}	21.06^{def}	7.96^{bcd}	11.80^{bcde}	1.033 ^{b}
2*3	5.23^{bcd}	1.80^{bc}	16.36^{cd}	28.66 ^{c}	20.43^{ef}	8.03^{bcd}	11.36^{e}	1.033 ^{b}
3*1	5.76^{abc}	1.83^{bc}	16.20^{cd}	26.66^{cd}	20.03^{fg}	7.83^{cd}	11.56^{cde}	1.033 ^{b}
3*2	4.73^{cd}	1.73^{c}	14.16^{de}	24.83^{d}	21.26^{cde}	7.23^{e}	12.30^{bcd}	1.033 ^{b}
3*3	4.73^{cd}	1.70^{c}	14.16^{de}	26.0^{d}	20.53^{ef}	7.46^{de}	12.03^{bcde}	1.033 ^{b}
P > F	0.0005	0.0004	0.0001	0.0001	0.0001	0.0001	0.0005	0.0622
±SE	0.320	0.080	0.743	0.804	0.368	0.188	0.271	0.0380

a-g: means in the same column having different letters are significantly (P≤0.05) fifferent.

Hb: haemoglobin, RBC_s: red blood cells, HCT: haematocrit, P: Platelets, WBC_s: white blood cells, N: Neutrophil, L: Lymphocyte, M: Monocyte.

Kobeisy and Hussein (1995) found that dietary live yeast may improve the haematological picture in Nile tilapia. Also, Irianto and Austin (2002) cited that probiotics actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competetion for ntrients and/or space, alteration of microbial metabolism, and/or by the stimulation of host immunity. Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of indigestible components. Moreover, Sahan and Duman (2008) found that haematocrit, leucocytes, monocytes, and neutrophils were increased in common carp fed with beta glucan. Also, Aly *et al.* (2008c) showed significant increase in haematocrit values in group of Nile tilapia fed the mixture of *B. subtilis* and *L. acidophilus* comparing with the control.

Aquaculture is one of the fastest developing growth sectors in the world. However, disease outbreaks are constraint to aquaculture production, thereby affects both economic development of the country and socio-economic status of the local people in many countries. Disease control in aquaculture industry has been achieved by following different methods using traditional ways, synthetic chemicals and antibiotics. However, the use of such expensive chemotherapeutants for controlling diseases has been widely criticized for their negative impacts like accumulation of residues, development of drug resistance, immunosuppressants and reduced consumer preference for aqua products treated with antibiotics and traditional methods are ineffective against controlling new diseases in large aquaculture systems. Therefore, alternative methods need to be developed to maintain a healthy microbial environment in the aquaculture systems there by to maintain the health of the cultured organisms. Use of probiotics is one of such method that is gaining importance in controlling potential pathogens. This review provides a summary of the criteria for the selection of the potential probiotics, their importance and future perspectives in aquaculture industry (Sahu *et al.*, 2008).

Amer (2012) concluded that Diamond V XP (inactive yeast a commercial product containing 100% dried *Saccharomyces cerevisae* distributed by DIAMOND V mils, Cidar Rapids, IOWA, and USA) could be used successfully as feed additive for feeding Nile tilapia *Oreochromis niloticus* without any adverse effects on their productive performance. It could be suggested that dietary supplement with Diamond V XP (1.5g) is useful in the intensive production system of fish.

Hassan (2013) obtained results concerning the effects of the probiotic Hydroyeast Aquaculture® on adult male *O. niloticus* showed that the 15 g/kg diet realized best significantly ($P \leq 0.05$) values for RBCs count, PCV %, and WBCs count, followed by 10 g probiotic/kg diet compared with the control group. However, about adult females' *O. niloticus*, the 10 g probiotic/kg diet was the best treatment concerning the significantly ($P \leq 0.05$) improvement of the hematological parame ters (Hb content, RBCs count, PCV% and WBCs count). So, based on the obtained results, the optimum level of the tested probiotic Hydroyeast Aquaculture® was depinding on fish sex.

REFERENCES

Abd El-Aziz, M.; Ali, M. Yassin, S. Raafat, A. Moustafa, M. and Rashad, S. (2009). Effect of some probiotics on Oreochromis niloticus experimentally infected by Aeromonas hydrophila. Global Fish. & Aquacult. Rese. Conference, Cairo, p: 24.

Abdelhamid, A.M. and El-Barbary, M.I. (2013). An in vitro study on the effect of some pre and probiotics on some pathogenic bacteria. Engormix.com, Aquaculture Technical articles, Nutrition, 20p.

Abdelhamid, A. M.; El-Barbary, M.I. and Hasan, M. M. M. (2012). Effect of dietary supplementation with Bio-mos® or T-protphyt 2000 with and without hormone treatment on performance, chemical composition, and hormone residues of mono-sex Nile tilapia. J. Anim. & Poul. Prod. Mansoura Univ., 3: 99-113.

Abdelhamid, A. M.; Mehrim, A. I.; El-Barbary, M. I.; Ibrahim, S. M. and Abdel-Wahab, A. I. (2009). Evaluation of a new Egyptian probiotic by African catfish fingerlings. J. Environ. Sci. & Technol., 2(2):133–145 (engormix.com, Aquaculture Technical Articles, 13 p).

Abdelhamid, A.M.; Seden, M.A.L. and Zenhom, O. A. (2013). Effect of different sources and levels of some dietary biological additives on: I- growth performance and production economy of Nile tilapia fish. J. Anim. & Poul. Prod. Mansoura Univ., 4 (11): 615-634.

Abd El-Rahman, A.M.M. and El-Bana, L.F.A. (2006). Using of Micrococcus luteus as a probiotic bacteria among cultured Oreochromis niloticus, SCVMJ, X, (1): 73 – 82.

Abdel-Tawwab, M.; Abdel-Rahman, A.M. and Ismael, N.E.M. (2008). Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with Aeromonas hydrophila. Aquacult., 280: 185–189.

Ai, Q.; Xu, H., Mai, K., Xu, W., Wang, J. and Zhang, W. (2011). Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture, 317: 155–161.

Algedawy, S. A., Soliman, K. K., Elashmawy, I.M. And El-Sayed, Y. S. (2011). Effect of commercial probiotic and exogenous enzymes on the growth performance, immune response, and hemato-biochemical parameters of broiler chickens. The 4th Sci. Conf. of Animal Wealth Research in the Middle East & North Africa, p: 23 (Abstract).

Aly, S. M.; Abdel Atti, N. M. and Mohamed, M. F. (2008a). Effect of garlic on the survival, growth, resistance and quality of *Oreochromis niloticus*. 8th Inter. Symp. on Tilapia in Aquacul.,Cairo, pp: 277-296.

Aly, S.M., Abd-El-Rahman, A.M. John, G. and Mohamed, M.F. (2008b). Characterization of some bacteria isolated from *Oreochromis niloticus* and their potential use as probiotics. Aquacult., 277: 1 – 6.

Aly, S.M., Ahmed, Y.A. Ghareeb, A.A. and Mohamed, M.F. (2008c). Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish and Shellfish Immunology, 25: 128 – 136.

Amer, A.A.A.M. (2012). Effect of different levels of protein and probiotics on productive performance of fresh water fishes. M.Sc., Fac. Agric., Kafrelsheikh Univ.

Castillo, M. (2008). Blocking pathogens with *Bacillus* strains. Feed-Mix, 16 (5): 19.

Duncan, D. (1955). Multiple range and multiple F-tests. Biometrics, 11: 1-42.

El-Ashram, A.M.M., Mohammed, M.F. and Aly, S.M. (2008). Effect of Biobuds® as a commercial probiotic product in cultured tilapia. 8th International Symposium on Tilapia in Aquaculture, Oct., Cairo, Egypt, pp: 1089 – 1096.

El-Nobi, G. A.; Reda, R. M. Hassanin, M. E. and Elmowalid, G. (2009). The impact of applications of probiotic *Bacillus subtilis* on health and growth of koi carp *Cyprinus carpio*. Global Fisheries & Aquaculture Research Conference, Cairo, p: 38.

Fernandes, C.F. and Shahani. K.M. (1990). Aiiiicarcinogcnic and inimunological properties of dietary *Lactobacilli*. J. Food protect, 53: 704-710.

Gatesoupe, F.J. (1999). The use of probiotics in aquaculture. Aquacult., 180:147-165.

Gibson, G.R. and Fuller, R. (2000). Aspects of *iu vitro* and *in vivo* research approaches directed towards identifying probiotics and preluolics lor human me.J. Nutr, 130:391-395.

Hassan, M.E.M. (2013). Physiological Studies on Fish Production. M.Sc. Thesis, Fac. Agric., Al-Mansoura Univ.

Irianto, A. and Austin, B. (2002). Review, Probiotics in aquaculture. Journal of Fish Diseases, 25: 633 – 642.

Josephlevy, M. D. (1998). Immunonutrition: The Pediatric Experience. Nutrition, 14 (7/8): 641– 647.

Kobeisy, M.A. and Hussein, S.Y. (1995). Influence of dietary live yeast on growth performance and some blood constituents in *Oreochromis niloticus* Proc.5th Sci. Conf. Animal Nutrition, 12-12 Dec., P 417-425, Ismailia, Dec.

Kristiansen, M.; Ringø, E., Merrifield, D., Vecino, J.G. and Myklebust, R. (2013). Evaluation of Prebiotic and Probiotic Effects on the Intestinal Gut Microbiota and Histology of Atlantic salmon (*Salmo salar* L.). Engormix.com, Aquaculture Technical articles, Nutrition, 14.

Kumar, U.S.; Vijaysarnthi, S.K. and Kaq, S. (2003). Effect ol feeding probiotics on He performance of broiiejs in. cxpcrimculal fowl typhoid. Indian vet.80: 52-55.

Marzouk, M.S., Moustafa, M.M. and Mohamed, N.M. (2008). The influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, pp. 1059-1071.

Natt, M.P. and Herrick, C.A. (1952). A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poul. Sci., 31:735–738.

Nayak, S. K.; Swain, P. and Mukherjee, S. C. (2007). Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeo rohita* (Ham.). Fish & Shellfish Immunology, 23: 892-896.

Osman, H. A. M.; Ibrahim, T. B., Soliman, W. and Aboud, O. (2010). Improvement growth and immune status using a potential probiotic bacteria *Micrococcus* species among cultured *Oreochromis niloticus*. New York Sci. J., 3(10): 5-11.

Pangrahi, A. and Azad, I. S. (2007). Microbial intervention for better fish health in aquaculture: the Indian scenario. Fish Physiol. Biochem., 33: 429-440.

Pangrahi, A.; Kiron, V. Satoh, S. Hirono, I.Kobayashi, T. Sugita, H. Puangkaew, J. and Aoki, T. (2007). Immune modulation and expression of cytokine genes in rainbow trout *Oncorhynchus mykiss* upon probiotic feeding. Development and Comparative Immunology, 31: 372-382.

Patterson, J A. and Burkholder, K.M. (2003). Application of probiotics and probiotics in poultry production. Poult. Sri., 82: 627-631

Perdigon, G.; Alvarez, S.; Nader Be Marias, M. It..; Roux. M.S and Holgado, A.P. (1990): The oral administration of Lactic acid bacteria increase Ihc mucosal intestinal immunity iu response to Enleropalhogens. I. food Protect., 53: 404-410.

Poole, T., Callaway, T. and Nisbet, D. (2007). Alternatives to Antimicrobials. Infectious Disease: Foodborne Diseases. Edited by: S. Simjee© Humana Press Inc.,Totowa, NJ (p: 419).

Sahan, A. and Duman, S. (2008). Effects of diet with β-1,3/1,6 glucan on some haematological parameters in common carp (*Cyprinus carpio* L., 1758) infected by ecto-parasites. Proc. Of Middle East & North Africa Conf. for Future of Animal Wealth, Cairo, Egypt, 16-18 Oct.

Sahu, M.K.; Swarnakumar, N.S., Sivakumar, K., Thangaradjou, T. and Kannan, L. (2008). Probiotics in aquaculture: importance and future perspectives. Indian J. Microbiol., 10 p.

SAS (2006). SAS statistical guide for personal computer, SAS Institute Inc. Cary, NC.

Schalm O. W. (1975). Veterinary Haematology 3rd Ed, Bailliere, Tindall and Cassel Ltd., London.

Schaperclaus, W.; Kulow, H.and Schreckenbach, K. (1992). Fish diseases volume 1-5th edition.

Sneath, P.H.A. (1986). Endo spore- forming gram- positive rods and cocci. P, 1104-l207.In: J .G. Holt (ed.), Bergey's manual of systematic bacteriology vol.2. Williams and Wikins, Baltimore, Md.

Stoskopf, M.K. (1993). Fish Medicine. W.B. Saunders Company Harcour Brace Lovanovish, Inc.

Taoka, Y., Maeda, H., Jo, J.Y., Jeon, M.J., Bai, S.C., Lee, W.J., Yuge, K. and Koshio, S. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fish Sci., 72: 310–321.

Tinh, N.T.N., Direckens, K., Sorgeloos, P. and Bossier, P. (2008). A review of the functionality of probiotics in the larviculture food chain. Mar. Biotechnol., 10: 1-12.

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology review, 64 (4): 655-671.

Wang, Y.B., Li, J. and Lin, J. (2008a). Probiotics in aquaculture: Challenges and outlook. Aquacult., 281: 1–4.

Wang, Y.B., Tian, Z., Yao, J. and Li, W. (2008b). Effect of probiotics, Enteroccus faecium, on tilapia (*Oreochromis niloticus*) growth performance and immune response. Aquacult., 277: 203-207.

Wary, C., and Davits, It. II. (2000). Competitive exclusion an alternative lo antibiotics. Vet. J., 59: 107-103.

ARABIC SUMMARY

تأثير اختلاف مصدر ومستوى بعض الإضافات الحيوية العلفية على : ٤- مناعة وخصائص دم أسماك البلطي النيلى

عبد الحميد محمد عبد الحميد[١]- محمد معاز على رفاعى[١]- مدحت السعيد عبد الفتاح سيدين[٢]
أسامة عوض زينهم[٢]
١- قسم إنتاج الحيوان، كلية الزراعة، جامعة المنصورة، المنصورة، مصر
٢- المعمل المركزى لبحوث الثروة السمكية، مركز البحوث الزراعية، العباسة، أبو حماد، مصر

استهدفت هذه الدراسة بحث تأثير مستويات علفية متدرجة لثلاث مركبات حيوية على المناعة وصورة دم أسماك بلطي نيلي وحيد الجنس كله ذكور. امتدت التجربة لستة عشر أسبوعا بعد أسبوعين فترة أقلمة. وثبت من نتائج اختبار المرضية (المناعة) أن أفضل مستوى من Aqua Superzyme و Garlen Allicin هو ٠,٠١% من العليقة، أما المستوى الأفضل من Diamond V (XPC) فكان ٠,٥% من العليقة، لكن على مستوى مختلف المواد والتركيزات فكنت المعاملة المحتوية على Aqua Superzyme بمستوى ٠,٠١% من العليقة هي الأفضل معنويا على الإطلاق. ومن ناحية صورة الدم، فكانت تتحسن معنويا باحتواء العليقة على ٠,٠١%، ٠,٠١% و ٠,٤% من العليقة من المواد الثلاثة على الترتيب، إلا أن أفضل معاملة على الإطلاق كانت المحتوية على Aqua Superzyme بمستوى ٠,٠٢% من العليقة. وعليه ينصح باحتواء علائق أسماك البلطي النيلى على البريبيوتيك Aqua Superzyme بمستوى ٠,٠١-٠,٠٢% من العلائق لتحسين جهاز المناعة للأسماك.