OUTDOOR STUDY ON THE USE OF ECHINACEA (*ECHINACEA PURPEREA*), MARJORAM (*ORIGANUM MAJORANA*) AND YEAST (*SACCHAROMYCES CEREVISIAE*) AS FEED ADDITIVES FOR *OREOCHROMIS NILOTICUS*

DIAB, A. S., Y. M. ABDEL-HADI, M. H. AHMAD, S. F. SAKR AND M. E. ABO EL-ATTA

Central Laboratory for Aquaculture Research (CLAR), Abbassa, Sharkia, Egypt

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Abstract

A number of 2400 *Oreochromis niloticus* were fed on ration containing Echinacea, Marjoram and yeast at different levels as feed additives to a basal ration (25% protein). There were 10 treatments including: treatment 1 (E1) contained Echinacea in the rate of 0.25% in ration, treatment 2 (E2) had Echinacea 0.5%, treatment 3 (E3) had Echinacea 1%, treatment 4 (M1) had Marjoram 1%, treatment 5 (M2) had Marjoram 2%, treatment 6 (M3) had Marjoram 3%, treatment 7 (Y1) had Yeast 0.5‰, treatment 8 (Y1) had Yeast 1‰, treatment 9 (Y1) had Yeast 2‰ and treatment 10 (control) had fish fed on basal ration only. The fish were distributed into a number of 40 hapas in the rate of 60 fish per hapa and 4 hapas were designated for each treatment as 4 replicates. The experiment lasted for 3 months, where it was divided into 3 stages, the period of each stage was 1 month. The feeding rate was 10% of the total fish biomass in each hapa in the 1st month then decreased to 5% in the 2nd month and 3% in the 3rd month. At the end of each stage, the fish in each hapa were collected, counted, weighed and then transferred to aquaria for liver sampling to conduct total and fractionation protein analyses. Challenge test was performed via I/P injection of 10 fish from each treatment with a virulent strain of *Aeromonas hydrophila*. Samples of fish were also taken before starting the experiment and at the end of the 3 months for proximate analyses. Results revealed that, the best treatments in this study were ranked as Echinacea 1% (E3) followed by Marjoram 1% (M1), Marjoram 3% (M3), Echinacea 0.5% (E2) and then by Echinacea 0.25% (E1) via compiling their results obtained from different tools, which were used for assessment of immune response of experimented fish. Although yeast gave the least results compared with Echinacea and Marjoram, yeast 1‰ was relatively the best rate among the 3 levels of yeast used in this study.

INTRODUCTION

Fish Diseases have a great economic impact on the continuously developing aquaculture in Egypt. Diseased fish lose appetite and don't utilize antibiotics mixed with feed rendering these antimicrobials useless during a disease outbreak. Besides, most antimicrobials are chemicals and harmful for both aquatic ecosystem and the human consumer of the treated fish with the resulting drug resistance and hypersensitivity. Thus, there is a mandate to seek for biological and environmentally friendly substances as alternatives for the control and prevention of fish diseases.

In the last two decades, many substances have proved their usefulness in fish culture because of their properties to stimulate the immune system and increase disease resistance (*Rodriguez et al., 2003*). One of the herbal stimulants of the immune system is the medicine plant *Echinacea purpurea L.* (Moench), which is used already in human medicine. Echinacea preparations are ascribed to stimulate various non-spëcific immunological parameters such as phagocytosis or activity of lymphocytes (*Wagner et al., 1986*). There are no newer reviewed data available for the application of Echinacea in productive livestock and veterinary medicine (*Maass et al., 2005*).

Origanum plants are widely used allover the world as a very popular spice, under the vernacular name 'oregano'. They are of great economic importance which is not only related to their use as a spice. In fact, as recent studies have pointed out, oregano is used traditionally in many other ways as its essential oils have antimicrobial, cytotoxic and antioxidant activity (*Lagouri et al. 1993, Sivropoulou et al. 1996*).

Saccharomyces cerevisiae cell walls are constructed almost entirely of β-1,3-D-glucan, mannoproteins and chitin, bound together by covalent linkages (*Cabib et al., 1982*). In vertebrates, each one of these purified compounds is known to increase innate (non-specific) defense mechanisms and/or disease resistance (*Pietrella et al., 2001*). However, very little data exists concerning the use of whole yeasts as immunostimulants (*Ortuno et al., 2002*), although their use would make the administration of such substances in fish farms cheaper and easier compared with the use of commercial isolated compounds (*Ortuno et al., 2002 and Rodriguez et al., 2003*). Therefore, the present work was planned to study the efficacy of such herbs and yeast as immunostimulant feed additives for *Oreochromis niloticus*.

MATERIALS AND METHODS

1- The used substances:-

- Echinacea (Sikum) was used in the experiment (E) at levels of E1 (0.25 %), E2 (0.5 %) and E3 (1%) of fish feed.
- Sweet Marjoram (Egyptian herbs' shops) was used in the experiment (M) at levels of M1 (1%), M2 (2%) and M3 (3%). It's known as *Origanum majorana or Majorana hortensis*.
- Yeast (BioBuds 2-X, Al-Gharieb for import and export- produced by Brookside AGRA L.C., USA) was used in the experiment (Y) at levels of Y1 (0.5‰), Y2 (1‰) and Y3 (2‰).

2- Fish:-

A total number of 2400 fingerlings of all male tilapia, *Oreochromis niloticus* were used. The fish were of an average body weight of 2±0.1 gram and an average total length of 5±0.1 cm. Fish was acclimatized for 2 weeks in 4 hapas.

3- Hapas and gill nets:-

A number of 40 hapas (1.5 x 1 x 1 meter) were fixed using steal rods in an earthen pond with an area of 1 acre at Abbassa fish farm. Two kilograms of gill nets (each kilo was 150 long X 10 meters width) were used to cover hapas in order to protect the fish from predation by the aquatic birds.

4- Feeding regime:-

The fish were fed in the rate of 10% of body weight in the 1st month then in the rate of 5% during the 2nd month and 3% in the 3rd month. The feed was placed in 20 clean plastic feeders (used for chicks), which were immersed into the water of the hapas with the aid of stones and a plastic rope held between 4 metal rods fixed into the ground of the 4 corners of the earthen pond. Feeding was once daily using a basal ration (25 % protein) for the fish in the control group and mixed with different levels of herbal and yeast additives as shown above.

5- Experimental design:- The experiment was conducted for 3 months during the summer of 2005. Each of the 10 treatments was represented by 4 hapas as 4 replicates. The fish were distributed from the 4 store hapas into their 40 respective hapas in the rate of 60 fish per hapa after dawn of mid June. The fish were then fed daily on powdered feed during the 1st month and on crushed pelleted feed during the 2nd and 3rd months using different treatments.

The first fish sample was taken 1 month after starting of the experiment and feeding on different rations. The fish were collected from all hapas and then weighed totally and the average body weights were calculated. Five fish were taken from each hapa (20 fish from each treatment) for conducting the challenge test and taking liver samples for total and fractionate protein analyses. A fixed weight for the 5 fish was deducted from each hapa not to impair the similarity of the treatments.

Challenge test was carried out as I/P inoculation of 0.5 ml/fish of 24 hours broth culture of *Aeromonas hydrophila* according to Enany et al. (1995).

Liver samples were prepared by taking 0.2 gram of liver from each fish to be put in an epindorph tube and then 1 ml of distilled water was added before closing the tubes. The liver samples were emulsified using ultrasound homogenizer for just few seconds and finally were kept under deep freezing until conducting the analyses. The 2nd and the 3rd fish samples were taken in the same manner, 2 and 3 months after

starting the experiment respectively. Liver samples were analyzed using the method described by Laemmli, 1970.

A number of 40 fingerlings were taken before the experiment and another 40 fish were sampled (4 fish from each treatment) at the end of the experiment for conducting proximate analyses to examine the effect of used substances on meat quality of the treated fish according to methods of AOAC (1984).

The data were statistically analyzed on the computer using the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Results revealed that average body weight (ABW) of the treated fish and that of control fish (control group) showed no significant difference after 1, 2 and 3 months from start of the experiment except treatments (Y1) and (Y2), which showed significantly higher ABW of fish when compared with that of fish of other treatments and those of control. However, results of ABW of fish in both treatments (Y1 and Y2) were discarded as the fish escaped from hapas of these treatments after 2 months of the experiment leaving few fish in each hapa, which consumed more food, resulting finally in larger fish and false results than other treatments. Thus, herbs under study had no effect on average body weight of the treated fish. These results were supported by those recorded by *Bagni et al., 2005* in sea bass over the long-term application of dietary yeast β-glucan, which induced no significant body weight difference in treated and control fish. Similarly, but in pigs, the growth performance of the pigs in the different experiments was not significantly influenced by the supplementation of Echinacea (*Maass et al., 2005*). On the other hand, there was no significant difference regarding survival rate between treated and control fish in the 3 months except for fish in treatments of Echinacea (E1, E2 and E3), which had relatively the highest survivability than of other treatments and control group. The treated fish in general, demonstrated the least survival rate in the 2nd month and the highest in the 1st month followed by the 3rd month (Table 1). These results were supported with those reported by *Bagni et al. (2005)* in sea bass (*Dicentrarchus labrax*) over the long-term application of dietary yeast β-glucan, which elicited no significant difference in treated and control fish with respect to survival rate.

Results of different immunity tests used to evaluate the immune response of fish under study, revealed that:-

Concerning results of the challenge test with *Aeromonas hydrophila*, there were significant lower mortalities among fish of treatments (M1), (M2) and (M3) (i.e. all levels of Marjoram) followed by yeast, (Y1), (Y2) and (Y3) than fish of the control group in the 1st month. Echinacea's treatments (E1 and E2) showed mortality as high

as control fish except in treatment E3, which had significantly lower mortality than control fish. However, there was no significant difference between treated and control fish regarding their mortality patterns in the 2nd month. On the other hand, there were significant lower mortalities among fish of treated groups than the control fish in the 3rd month (Table 2). This marked increase in disease resistance and survivability of the treated fish could be attributed to the immunostimulant effects of such herbs and yeast, which stimulate the immune response by promoting phagocytic cell function, increasing their bactericidal activity and/or non-specific cytotoxic cells and antibody production (*Sakai, 1999*). On the contrary, long-term oral administration of peptido-glucans decreased the immune response in rainbow trout when challenged with *vibrio anguillarum* (*Matzuo and Miyazono, 1993*), as well as in catfish (*Yoshida et al., 1995*).

With respect to total blood protein, results revealed that no significant differences between treated and control fish in the 3 months were recorded.

Blood albumin showed no significant differences between treated and control fish in 1st month. While in the 2nd month, albumin was significantly higher in fish of treatment (Y3) only than in fish of control. In the 3rd month, albumin was significantly higher in fish of treatment (E2) only than in fish of control.

Alpha globulin was significantly higher in blood of fish treated with all levels of Marjoram (M1, M2, and M3) than those of other treatments and control in 1st month. However, no significant difference was found between treated and control fish regarding Alpha globulin in the 2nd and 3rd months. These results may be attributed to the fact that essential oils of oregano (Marjoram) have antimicrobial, cytotoxic and antioxidant activity (Lagouri *et al.* 1993, Sivropoulou *et al.* 1996).

No significant differences were found between treated and control fish in estimates of Beta globulins in the 1st, 2nd and 3rd months. However, Beta globulin was significantly higher in the control fish than in fish of treatment (M1) only in the 1st month.

Gamma globulin was significantly higher in fish of treatments (E1) and (E3) than in control fish in the 1st month. However, no significant difference was found between treated and control fish in Gamma globulin in the 2nd and 3rd months.

Thus, no significant differences were found between treated and control fish concerning Alpha, Beta and Gamma globulins in the 2nd month. These results agreed with those reported by Bagni *et al. (2005)* and Maass *et al.* (2005). On the other hand, the results disagreed with those findings obtained by Rehman *et al.* (1999) who described an increase of specific antibodies in the plasma after application of Echinacea.

As a comparison between the 3 months' estimates of total and fractionation protein, no significant difference was found in all parameters between the 3 months in fish of the control group except in Gamma globulin only, which was significantly higher in fish of the 3rd month than of the 1st month.

In fish of treatments of Echinacea, (E1) and (E3), no significant difference was found in all parameters of total and fractionation protein between the 3 months. Similarly, in fish of treatment (E2) with the exception that albumin was significantly higher in fish of the 3rd month than in fish of the 2nd month.

With respect to Marjoram's treatments, fish of treatment (M1) had no significant difference between the 3 months in albumin, Alpha and Gamma globulins, while total protein was significantly lower in fish of the 1st month than those of the 2nd and 3rd months. Beta globulin was significantly lower in fish of the 1st month than those of the 2nd month. No significant differences were noticed between the 3 months concerning Beta and Gamma globulins in fish of treatment (M2), while albumin was significantly higher in fish in the 1st and 3rd months than in the 2nd month, but total protein was significantly higher in fish in the 3rd month than the 2nd one. In treatment (M3), no significant differences were found between fish in the 3 months in all parameters of protein except Alpha globulin, which was significantly higher in fish of the 1st month than 3rd and 2nd months. The essential oils of oregano have antimicrobial, cytotoxic and antioxidant activity (Lagouri *et al.,* 1993 and Sivropoulou *et al.*, 1996).

Concerning treatments of Yeast, fish of treatment (Y1) showed no significant difference between the 3 months in albumin and Beta globulin, while total protein and Gamma globulin were significantly higher in fish of the 3rd month than the 2nd and the 1st months. In treatment (Y2), no significant difference was observed between fish in the 3 months in all parameters of protein except Alpha globulin, which was significantly higher in fish of the 3rd month than of the 1st month. In treatment (Y3), no significant difference was noticed between fish in the 3 months in Beta and Gamma globulins but albumin was significantly higher in fish of the 2nd month than of the 1st month, while Alpha globulin was significantly higher in fish of the 3rd month than the 2nd and the 1st months as well as total protein, which was significantly higher in fish of the 3rd month than of the 1st month (Table 3). Dissimilar results with those of the 2nd and the 3rd months were recorded by Sakai (1999) and Bagni *et al.* (2005) who reported a negative feedback effect of the yeast β-glucan over the long-term oral administration in sea bass (*Dicentrarchus labrax*).

Results of proximate analyses showed that treated fish had significantly higher protein (except in fish of M3 and Y2) and moisture contents (in fish of treatments E1, M1 and M3) than control fish after 3-months. On the contrary, control fish showed

higher fat content than treated fish except fish in treatments (M2), (M3), (Y1), (Y2) and (Y3), which revealed no significant difference than control fish. Ash content showed significantly higher estimates in control fish than in all treated fish except in fish of treatment (M3) (Table 4). Thus, the addition of immunostimulants can improve fish body protein and decrease its fat.

It could be concluded that, the best treatments in this study were ranked as Echinacea 1% (E3) followed by Marjoram 1% (M1), Marjoram 3% (M3), Echinacea 0.5% (E2) and then by Echinacea 0.25% (E1) via compiling their results from tables of different used tools for assessment of immune response of experimented fish. Similar results were concluded by Maass *et al.* (2005) who stated that dietary administered *Echinacea purpurea* in form of cobs as feed additive might have a stimulating effect on the immune system, especially in situations with increased stress for the immune system. The effect of Echinacea on the immune system is probably caused by various substances such as alkamids, derivates of caffeic acid (cichoric acid), polysaccharides and glycoproteins (Bauer, 1997).

Although Yeast treatments gave the least results compared with Echinacea and Marjoram, Yeast 1‰ (Y2) was relatively the best rate among the 3 levels of Yeast used in this study. This dose was typical to that prescribed by the manufacturing company (1 kg/ton of fish feed). As a general perspective, most of the results indicated that the examined herbs and yeast induced their immunostimulant effects on the fish under study in the 1st and the 3rd months more than in the 2nd month, which showed the least figures in most of the measured parameters. Whereas, the fish in the control group demonstrated a regular and gradual increase in most measured parameters within prolonged application periods in the 1st, 2nd and 3rd months respectively.

Table 1. Average body weight and survival percent of fish treated with different levels of Echinacea, Marjoram and Yeast with feed of fish, 1, 2 and 3 months after start of experiment

Treatment	Average body weight of fish	Survival percent (%)
Mean ± standard error superscript Duncan Grouping after 1 month		
E1	4.800^{B}±0.187	94.6%
E2	4.900^{B}±0.163	96.7%
E3	4.925^{B}±0.225	90.4%
M1	4.633^{B}±0.338	92.8%
M2	5.275^{B}±0.165	94.6%
M3	4.650^{B}±0.272	91.7%
Y1	6.700^{A}±0.561	98.3%
Y2	5.275^{B}±0.085	95%
Y3	4.625^{B}±0.189	99.6%
Control	4.700^{B}±0.210	89.2%
Mean ± standard error superscript Duncan Grouping after 2 months		
E1	5.900^{B}±0.478	74.40%
E2	6.025^{B}±0.180	72.90%
E3	6.750^{B}±0.253	77.80%
M1	6.100^{B}±0.379	67%
M2	7.150^{B}±0.474	65.10%
M3	5.875^{B}±0.470	71.80%
Y1	11.875^{A}±2.148 (Discarded)	72.90%
Y2	7.900^{B}±0.478	59.70%
Y3	6.575^{B}±0.591	59.80%
Control	7.325^{B}±0.392	62.60%
Mean ± standard error superscript Duncan Grouping after 3 months		
E1	7.3000^{B}±0.432	86.90%
E2	7.525^{B}±0.502	88.10%
E3	8.5255^{B}±0.712	84.50%
M1	7.867^{B}±0.801	86.70%
M2	8.800^{B}±0.780	83.10%
M3	7.150^{B}±0.771	86.10%
Y1	16.7775^{A}±5.281 (Discarded)	86.10%
Y2	13.500^{AB}±3.616 (Discarded)	85.30%
Y3	8.400^{B}±0.975	81.70%
Control	8.975^{B}±0.851	79.10%

Table 2. Results of challenge test using virulent isolate of *Aeromonas hydrophila* in fish treated with different levels of Echinacea, Marjoram and Yeast with feed of fish

Treatment	Mortalities during the 1st seven days after injection							No. of dead fish	Mortality %
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7		
1 month after start of the experiment									
E1	0	6	1	0	0	0	0	7	70
E2	0	9	1	0	0	0	0	10	100
E3	1	0	0	0	0	0	0	1	10
M1	0	0	0	0	0	0	0	0	0
M2	0	0	0	0	0	0	1	1	10
M3	0	0	0	0	0	0	0	0	0
Y1	0	0	0	0	0	0	0	0	0
Y2	0	1	3	0	0	0	0	4	40
Y3	0	2	0	0	0	0	0	2	20
Control	0	0	0	0	0	0	10	10	100
2 months after start of the experiment									
E1	0	5	0	0	0	0	0	5	50
E2	0	4	1	0	1	0	0	6	60
E3	0	8	0	1	0	0	0	9	90
M1	0	7	0	1	1	0	0	9	90
M2	0	9	1	0	0	0	0	10	100
M3	0	7	0	0	0	0	0	7	70
Y1	0	10	0	0	0	0	0	10	100
Y2	0	10	0	0	0	0	0	10	100
Y3	0	6	0	1	1	0	0	8	80
Control	0	9	0	0	0	0	0	9	90
3 months after start of the experiment									
E1	0	2	0	0	0	0	0	2	20
E2	0	0	0	0	0	0	0	0	0
E3	1	0	0	0	0	0	0	1	10
M1	1	0	0	0	0	0	0	1	10
M2	1	0	0	0	0	0	0	1	10
M3	1	0	0	0	0	0	0	1	10
Y1	0	1	0	0	0	0	0	1	10
Y2	0	0	0	0	0	0	0	0	0
Y3	1	0	0	0	0	0	0	1	10
Control	3	2	0	0	1	1	0	7	70

Table 3. Total and fractionation protein estimates (g/dl) of fish treated with different levels of Echinacea, Marjoram and Yeast with feed of fish, after 1, 2 and 3 months

Treatment	Protein %	Fat %	Ash %	Moisture %
Mean ± standard error superscript Duncan Grouping after 3 months				
E1	$58.450^{BC}\pm0.115$	$29.033^{CD}\pm0.498$	$11.850^{CD}\pm0.276$	$75.310^{AB}\pm0.497$
E2	$59.913^{B}\pm0.365$	$28.470^{D}\pm0.265$	$11.417^{D}\pm0.589$	$72.477^{D}\pm0.702$
E3	$65.837^{A}\pm0.456$	$20.703^{E}\pm0.259$	$12.400^{CD}\pm0.470$	$73.960^{BCD}\pm0.342$
M1	$65.337^{A}\pm0.254$	$20.823^{E}\pm0.458$	$13.507^{B}\pm0.271$	$76.500^{A}\pm0.100$
M2	$58.353^{BC}\pm0.034$	$29.267^{BCD}\pm0.273$	$11.470^{D}\pm0.061$	$73.333^{BCD}\pm1.037$
M3	$56.123^{DE}\pm1.382$	$29.393^{BCD}\pm0.652$	$15.233^{A}\pm0.260$	$74.850^{ABC}\pm0.269$
Y1	$57.377^{CD}\pm0.285$	$29.547^{BCD}\pm0.387$	$12.643^{BC}\pm0.075$	$73.260^{BCD}\pm0.622$
Y2	$56.050^{DE}\pm0.159$	$30.153^{BC}\pm0.396$	$12.063^{CD}\pm0.515$	$73.000^{CD}\pm1.316$
Y3	$57.267^{CD}\pm0.466$	$31.537^{A}\pm0.331$	$11.763^{CD}\pm0.182$	$71.973^{D}\pm0.451$
Control	$55.477^{E}\pm0.436$	$30.457^{AB}\pm0.193$	$15.400^{A}\pm0.316$	$72.587^{D}\pm0.316$

Table 4. Proximate analysis for fish meat treated with different of Echinacea, Marjoram and Yeast with feed of fish, 3 months after start of the experiment

Treatment	Albumin	α-globulin	B-globulin	λ-globulin	Total protein
	Mean ± standard error superscript Duncan Grouping after 1 month				
E1	$3.140^{A}\pm0.254$	$1.073^{C}\pm0.027$	$1.183^{AB}\pm0.096$	$1.937^{A}\pm0.233$	$7.330^{A}\pm0.420$
E2	$3.717^{A}\pm0.200$	$1.210^{C}\pm0.044$	$1.193^{AB}\pm0.065$	$1.490^{BC}\pm0.122$	$7.613^{A}\pm0.113$
E3	$3.493^{A}\pm0.155$	$1.173^{C}\pm0.076$	$1.203^{AB}\pm0.078$	$1.773^{AB}\pm0.032$	$7.640^{A}\pm0.266$
M1	$3.223^{A}\pm0.083$	$2.503^{B}\pm0.696$	$1.107^{B}\pm0.087$	$1.487^{BC}\pm0.007$	$6.923^{A}\pm0.248$
M2	$3.633^{A}\pm0.125$	$3.633^{A}\pm0.125$	$1.180^{AB}\pm0.076$	$1.557^{ABC}\pm0.104$	$7.660^{A}\pm0.361$
M3	$3.380^{A}\pm0.205$	$3.380^{A}\pm0.205$	$1.293^{AB}\pm0.065$	$1.660^{ABC}\pm0.040$	$7.517^{A}\pm0.263$
Y1	$3.213^{A}\pm0.174$	$1.057^{C}\pm0.054$	$1.147^{AB}\pm0.003$	$1.533^{BC}\pm0.090$	$6.950^{A}\pm0.125$
Y2	$3.560^{A}\pm0.145$	$1.023^{C}\pm0.098$	$1.197^{AB}\pm0.081$	$1.473^{BC}\pm0.122$	$7.257^{A}\pm0.303$
Y3	$3.163^{A}\pm0.228$	$1.193^{C}\pm0.065$	$1.247^{AB}\pm0.003$	$1.740^{ABC}\pm0.177$	$7.347^{A}\pm0.352$
Control	$3.517^{A}\pm0.349$	$1.240^{C}\pm0.155$	$1.393^{A}\pm0.135$	$1.357^{C}\pm0.111$	$7.507^{A}\pm0.206$
	Mean ± standard error superscript Duncan Grouping after 2 months				
E1	$3.687^{AB}\pm0.114$	$1.133^{A}\pm0.210$	$1.190^{A}\pm0.072$	$1.700^{A}\pm0.070$	$7.710^{AB}\pm0.395$
E2	$3.293^{B}\pm0.063$	$1.427^{A}\pm0.107$	$1.230^{A}\pm0.035$	$1.670^{A}\pm0.085$	$7.620^{AB}\pm0.227$
E3	$3.427^{AB}\pm0.306$	$1.143^{A}\pm0.092$	$1.290^{A}\pm0.065$	$1.767^{A}\pm0.071$	$7.630^{AB}\pm0.311$
M1	$3.403^{AB}\pm0.122$	$1.200^{A}\pm0.074$	$1.400^{A}\pm0.027$	$1.667^{A}\pm0.212$	$7.667^{AB}\pm0.202$
M2	$3.150^{B}\pm0.072$	$1.280^{A}\pm0.141$	$1.263^{A}\pm0.075$	$1.573^{A}\pm0.230$	$7.267^{B}\pm0.101$
M3	$3.487^{AB}\pm0.258$	$1.253^{A}\pm0.046$	$1.280^{A}\pm0.061$	$1.793^{A}\pm0.204$	$7.813^{AB}\pm0.270$
Y1	$3.110^{B}\pm0.053$	$1.247^{A}\pm0.046$	$1.237^{A}\pm0.075$	$1.650^{A}\pm0.072$	$7.243^{B}\pm0.070$
Y2	$3.377^{B}\pm0.088$	$1.243^{A}\pm0.069$	$1.207^{A}\pm0.052$	$1.683^{A}\pm0.215$	$7.843^{AB}\pm0.236$
Y3	$3.957^{A}\pm0.221$	$1.063^{A}\pm0.075$	$1.417^{A}\pm0.282$	$1.667^{A}\pm0.071$	$8.097^{A}\pm0.117$
Control	$3.197^{B}\pm0.211$	$1.377^{A}\pm0.318$	$1.217^{A}\pm0.075$	$1.590^{A}\pm0.051$	$7.380^{AB}\pm0.284$
	Mean ± standard error superscript Duncan Grouping after 3 months				
E1	$3.717^{AB}\pm0.104$	$1.423^{A}\pm0.090$	$1.227^{A}\pm0.078$	$1.673^{BC}\pm0.120$	$8.013^{A}\pm0.171$
E2	$3.747^{A}\pm0.061$	$1.397^{A}\pm0.056$	$1.210^{A}\pm0.047$	$1.630^{C}\pm0.114$	$7.980^{A}\pm0.144$
E3	$3.700^{AB}\pm0.142$	$1.367^{A}\pm0.063$	$1.180^{A}\pm0.057$	$1.783^{ABC}\pm0.111$	$8.030^{A}\pm0.093$
M1	$3.453^{ABC}\pm0.130$	$1.463^{A}\pm0.035$	$1.267^{A}\pm0.029$	$1.927^{AB}\pm0.121$	$8.113^{A}\pm0.116$
M2	$3.520^{ABC}\pm0.113$	$1.530^{A}\pm0.015$	$1.320^{A}\pm0.015$	$1.790^{ABC}\pm0.050$	$8.157^{A}\pm0.158$
M3	$3.720^{AB}\pm0.123$	$1.423^{A}\pm0.024$	$1.290^{A}\pm0.047$	$1.747^{ABC}\pm0.088$	$8.180^{A}\pm0.055$
Y1	$3.317^{C}\pm0.096$	$1.377^{A}\pm0.041$	$1.187^{A}\pm0.035$	$1.957^{A}\pm0.032$	$7.837^{A}\pm0.190$
Y2	$3.473^{ABC}\pm0.122$	$1.440^{A}\pm0.050$	$1.243^{A}\pm0.042$	$1.717^{ABC}\pm0.037$	$7.873^{A}\pm0.182$
Y3	$3.543^{ABC}\pm0.079$	$1.440^{A}\pm0.035$	$1.247^{A}\pm0.032$	$1.947^{AB}\pm0.020$	$8.173^{A}\pm0.113$
Control	$3.370^{BC}\pm0.089$	$1.407^{A}\pm0.038$	$1.363^{A}\pm0.119$	$1.813^{ABC}\pm0.042$	$7.947^{A}\pm0.217$

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دراسة حقلية لإستخدام الإكينيسيا والبردقوش والخميرة كإضافات أعلاف لأسماك البلطى النيل"

أحمد سعيد دياب ، ياسر محمد عبد الهادى ، محمد حسن أحمد ، صالح فتحى صقر ، محمد السيد أبو العطا

المعمل المركزى لبحوث الثروة السمكية بالعباسة ، أبوحماد ، الشرقية ، مصر

تم إضافة مسحوق الإكينيسيا والبردقوش والخميرة على عليقة إصبعيات البلطى النيلى بنسب مختلفة ماعدا المجموعة الضابطة التى تحتوى على أسماك تم تغذيتها على عليقة أساسية (٢٥% بروتين) دون أى إضافات. وقد استخدم عدد "٢٤٠٠" سمكة لإجراء التجربة وزعت بواقع "٦٠" إصبعية لكل هابة من عدد "أربعون هابة" خصص منها أربع هابات لكل معاملة كمكررات. أى أن عدد المعاملات عشرة وهى كالتالى: المعاملة الأولى (E1) عليقة تحتوى على مادة الإكينيسيا بنسبة ٠,٢٥% والثانية (E2) عليقة تحتوى على الإكينيسيا بنسبة ٠,٥% والثالثة (E3) عليقة تحتوى على الإكينيسيا بنسبة ١% والرابعة (M1) عليقة تحتوى على البردقوش بنسبة ١% والخامسة (M2) عليقة تحتوى على مسحوق البردقوش بنسبة ٢% والسادسة (M3) عليقة تحتوى على البردقوش بنسبة ٣% والسابعة (Y1) عليقة تحتوى على مسحوق الخميرة (البيوبادز) بنسبة ٠,٥‰ والثامنة (Y2) عليقة تحتوى على الخميرة بنسبة ١‰ والتاسعة (Y3) عليقة تحتوى على الخميرة بنسبة ٢‰ أما المعاملة العاشرة فهى المجموعة الضابطة (Control) حيث تم تغذية الأسماك بها على عليقة أساسية تحتوى على ٢٥% بروتين. واستمرت التجربة لمدة ثلاثة أشهر حيث قسمت التجربة إلى ثلاثة مراحل مدة كل مرحلة شهر واحد. وقد غذيت الأسماك بمعدل ١٠% من وزن الجسم فى الشهر الأول ثم ٥% فى الشهر الثانى وأخيرا بمعدل ٣% فى الشهر الثالث. ثم تم وزن الأسماك وعدها فى كل هابة على حدة بعد تجميعها من الهابات ثم تم أخذ عدد خمس سمكات من كل هابة بإجمالى عشرون سمكة من كل معاملة شهريا حيث نقلت إلى أحواض زجاجية لأخذ عينات الدم والكبد اللازمة لإجراء تحاليل البروتين الكلى ومشتقات البروتين. وقد تم إجراء عدوى تجريبية للأسماك (challenge test) بحقن عشر سمكات من كل معاملة بعترة ضارية لميكروب الإيروموناس هيدروفيلا *(Aeromonas hydrophila)*. وكذلك أخذت عينة من الأسماك من كل معاملة قبل بدء التجربة وبعد نهاية التجربة لعمل تحليل محتوى الجسم الكلى (Proximate analysis). وقد أوضحت النتائج أن أفضل المعاملات فى هذه الدراسة هى على الترتيب الإكينيسيا بنسبة ١% على العليقة (E3) ثم البردقوش بنسبة ١% (M1) ثم البردقوش بنسبة ٣% (M3) ثم الإكينيسيا بنسبة ٠,٥% (E2) ثم الإكينيسيا بنسبة ٠,٢٥% (E1) وذلك استنادا على نتائج المستخلصة من المقاييس المناعية المختلفة التى تم استخدامها وهى نسبة الإعاشة الطبيعية ومتوسط وزن الأسماك ونسبة النفوق فى اختبار التحدى وفى محتوى جسم الأسماك من بروتين ودهون ورماد ورطوبة

وكذلك محتوى البروتين الكلى ومشتقاته من الألبيومين والجلوبيولين بأنواعه (ألفا وبيتا وجاما). ورغم أن معاملات الخميرة بنسبها الثلاث قد أعطت أقل النتائج نسبيا بالمقارنة بمادتى الإكينيسيا والبردقوش إلا أن الخميرة بنسبة ١‰ (Y2) قد أعطت أفضل النتائج من بين نسب الخميرة الثلاث التى جربت فى هذه الدراسة.