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EFFECT OF PROTEIN SOURCES ON DISEASE RESISTANCE FACTORS AND GROWTH PERFORMANCE OF NILE TILAPIA (OREOCHROMIS NILOTICUS)

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INTRODUCTION

Nowadays fish culture is one of the best methods by which much protein can be produced. Many countries now practice fish culture very successfully not only as food industry but as major source of income.

Nile tilapia *(O. niloticus)* was selected as the species for investigation in the present study as it is probably the most extensively cultured tilapia in the world (Bardach et al. 1972). In Egypt, the importance of aquaculture take wide step especially with Nile tilapia that are very palatable for Egyptian people. Therefore, it is essential to develop suitable feed to be used either as a supplementry diet in ponds or as a complete diet in tanks reared with Nile tilapia.For economic and practical reasons these diets must be locally from available protein sources, preferably those unsuitable for direct human consumption.

The effect of diets on both specific immune response and nonspecific disease resistance factors have been well studied in homeothermic animals. However, relatively little is known about the relationship between diet and disease resistance in fish. Selected vitamins (particularly C and E) and minerals have been shown to affect immunity and disease resistance in salmonids (Blazer and Wolke, 1984 and Paterson et al., 1985) as well as channel catfish (Durve and Lovell, 1982 and

Li and Lovell, 1985). Indeed, quantity and quality of macronutrients, as well as essential vitamins and minerals, are known to affect both the incidence and severity of certain infectious diseases of fish(Wedemeyer and Ross, 1973 and Taniguichi, 1983). In addition, commercial feeds used to maintain laboratory fish for basic immunological, disease susceptibility and toxicological research may be an important source of variation in these studies, even when the fish appear normal and healthy. Therefore, this research was conducted to evaluate the effect of protein sources on the growth performance and disease resistance factors of Nile tilapia (*O. niloticus*).

MATERIAL AND METHODS

Fish:

One hundred and twenty fingerling Nile tilapia weighted 10.5 g were used. . The fish were devided into 4 groups each contain 30 fish. The experiment was conducted in glass aquaria (80x50x40 cm) supplied with dechlorinated tap water. The water temperature was maintained at 27 C throughout the experiment by automatic heater. The experimental period was extended up to 155 days.

Diet and feeding regime

The experiment was undertaken at Fish Research Center, Suez Canal University. Four diets were formulated isonitrogenous and as far as possible isoenergetic for metabolizable energy (Table 1). The fish were fed at rate of 3 % body weight/day. The daily amount of diet was offered on 2 occasions at 9.00 am and 3.00 pm. The diets were offered 6 days/week. The fish were weighted initially and every 10 days of the experiment. Weight gain (WG) percent, specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) were calculated according to the equation:

$$WG\ \% = \frac{\text{Mean final wt-mean initial wt}}{\text{Mean initial wt}} \times 100$$

$$SGR = 100 (\text{Log } W_2 - \text{Lof } W_1 / t)$$

Where W_1 is the initial weight (g), W_2 the final weight (g) and t the time in days between weightings.

FCR = Food fed (g) / wet weight gain (g).

PER = Wet weight gain (g) / protein intake (g).

Statistical Analysis

Statistical analysis were carried out by one way analysis of variance (Snedercor and Cochran. 1980).

Vaccine preparation

Formalin-killed *A. hydrophila* bacterin was prepared according to Baba et al. (1988). The organism used in the bacterin preparation was isolated from diseased Nile tilapia by Badran and Danasoury (1990). Sterility and safety of bacterin to fish was tested according to Ward (1982). After 120 days of feeding, 10 fish from each group were taken for intraperitonial (I/P) immunization with *A. hydrophila* bacterin. The fish were anasthetized with MS_{222} (Tricain Methan Sulfonate) and injected with 0.1 ml of formalin-killed bacterial cells diluted withthe same volume of sterial saline solution for each fish.

Antibody response:

Weekly post-vaccination, 2 fish from each group were used for blood collection. Specific antibody titers in collected sera to *A. hydrophila* were determined using bacterial agglutination test. For agglutination formalin-killed bacterial cells were washed twice with sterile saline solution and prepared to a concentration for 3 mg wet weight/ml saline (Baba et al. 1988).

Infection method:

Fish remained in each group (20 fish) were exposed to artificial infection by immersion method at day 120

from beginning of feeding. *A. hydrophila* was inoculated in brain heart infusion broth and incubated at 28C for 24 hrs. The infection was performed by immersing the fish in the broth culture diluted by 1:5 in 0.5 % Nacl solution to give a concentration of 1.0 gm/L for 10 minutes. Before immersion in the broth culture, the fish were preimmersed in 3 % Nacl solution for 5 minutes as stress factor (Muroga and Nakajima, 1981). Challenged fish were placed under observation for 2 weeks and dead ones were collected for re-isolation of *A. hydrophila* organism.

RESULTS

Growth performance:

Table (2) shows a summary of the results obtained in this study. Group of fish fed diet 1 (fish meal) had a significantly ($P < 0.05$) higher WG %, SGR, FCR, PER and protein retention followed by those fed diet 2 (meat and bone meal), diet 3 (cottonseed meal), and diet 4 (sunflower meal) respectively.

Antibody response:

The results demonestrated in Table (3) revealed that, fish fed fish meal diet throughout 120 days responded to immunization with *A. hydrophila* bacterin with production of specific antibody in high levels followed by fish fed bone and meat meal, cottonseed meal and sunflower meal respectively.

Artifical infection:

The results demonestrated in Table (4) revealed that, the fish fed protein of animal sources (fish meal and meat & bone meal) were suffered from 20 and 25 % mortalities respectively while the fish fed protein of plant sources (cottonseed meal and sunflower meal) were suffered from 35 % mortalities for each group.

Table (1). Composition of experimental diets.

Component	Diet No. (1) Control%	(2)%	(3)%	(4)%
Fish meal	48.00	-	-	-
Meat and bone meal	-	56.81	-	-
Cottonseed meal	-	-	53.20	-
Sunflower meal	-	-	-	67.40
Starch	25.00	23.00	25.00	17.00
Corn oil	9.00	2.00	8.00	9.00
Vitamin Mix	1.00	1.00	1.00	1.00
Mineral Mix	2.00	2.00	2.00	2.00
CMC *	1.00	1.00	1.00	1.00
Cellulose	14.00	14.19	9.80	2.60
Total	100.00	100.00	100.00	100.00
Proximate Analysis				
Moisture	8.10	9.10	8.10	8.20
Protein	25.00	25.00	25.00	25.00
Ether extract	15.30	16.30	10.10	10.10
Ash	15.10	15.10	9.20	9.20
NFE **	36.50	34.50	47.60	47.50
ME ‡	360.10	356.34	347.90	354.10

* Carbxy-methyl cellulose.

** Nitrogen Free Extract.

‡ Metabolizable energy content, based on physiological fuel values 4, 4 and 9 K cal ME/g for carbohydrate, protein and lipid respectively.

Table (2). Protein evaluation data.

Parameters	Diet number 1	2	3	4
Fish number	30	30	30	30
Initial weight (g)	10.50	10.50	10.50	10.50
Final weight (g)	30.30	26.80	23.80	20.10
Gain in weight (g)	19.80	16.30	13.30	9.60
Gain in weight (%)	188.57	155.23	126.66	91.42
SGR (%/day)	0.38	0.34	0.30	0.23
SGR as % of diet 1	100.0	89.47	78.94	60.52
FCR	1.90	2.50	2.50	3.10
PER	1.75	1.33	1.17	1.08
Protein retention +	0.06	0.02	0.004	0.005

$$+ = \frac{\text{Final protein - initial protein}}{(\text{Final + initial weight})/2 \text{ X time (days)}} \text{ X } 100$$

Figures with common superscript in each horizontal row are significantly different ($P < 0.05$).

Table (3). Results of agglutination test on sera+ from immunized fish fed on protein of different sources.

Fish group	Protein source	Antibody titer++(Log2) weekly post-vaccination 1	2	3	4	5
1	Fish meal	3	5	7	9	11
2	Bone & meat meal	3	5	7	8	10
3	Cottonseed meal	3	4	6	7	8
4	Sunflower meal	3	4	6	7	8

+ Two fish per serum pool.

++ Reciprocal of last dilution with postive agglutination.

Table (4). Results of artificial infection to fish fed protein of different sources.

Fish group	Protein source	Fish No.	Dead fish	Mortality %
1	Fish meal	20	4	20
2	Bone & meat meal	20	5	25
3	Cottonseed meal	20	7	35
4	Sunflower meal	20	7	35

DISCUSSION

In the present study, the highest growth rate of Nile tilapia *O. niloticus* was obtained with group of fish fed fish meal diet followed by those fed meat and bone meal, cottonseed meal and sunflower meal diets respectively. From this results it could be concluded that the higher WG %, SGR, FCR, PER and protein retention of Nile tilapia is proportional to the percent of animal protein (fish meal and meat & bone meal) which were contained higher percentage of essential amino acid profile in the diet. The factors which are likely to produce the decreased growth rates observed at higher plant protein inclusion levels are (1) lower digestibility of plant protein and carbohydrates as reported by Hastings (1969); (2) limiting levels of essential amino acids, particulary methionine and sine (Olukunle, 1982; Jackson et al. and Kamara 1982). Similar results were obtained by other author Nose '1970), Koops et al. '1976) and Atack et al. (1979). Jackson (1982) reported that the diets could be improved by supplementation with essential amino acid.Tilapia species are able to utilize pure amino acids presented to them in this way.

The results of the present study also revealed that the protein sources in fish diets has an important influence on the host defence mechanism. Immunized fish fed protein of animal source (fish meal and meat & bone meal) produced high level of specific antibodies and the non-immunized fish were protected against artificial infection more than the fish fed protein of plant sources (cottonseed meal and sunflower meal). This may be attributed to the higher percent of essential amino acids in the protein of animal sources which takes place in the antibody formation and enhancing the function of non-specific immune response (macrophage activity, promoting phagocytosis and cytotoxic activity). This is important to fish culturists as well as to researchers involved in evaluating normal disease

resistance mechanisms of fish. Since many of the diseases of cultured fresh water fish are caused by organisms commonly found in water systems (Frerich and Hendrie, 1985), any impairment of disease resistance can lead to infection and mortality. Unfortunately, no avaliable reports dealt with the effect of protein sources on the host defence mechanisms, while several reports (Johansson-Sjabeck et al. , 1975; Mahajan and Dheer, 1983 and Henken et al. ,1987) were explained the effect of feeding level on the antibody response and number of peripheral white blood cells in fish. Long-term starvation or even continuously feeding at maintenance level reducing the antibody response and decreasing the leucocyte number. Other reports (Durve and Lovell, 1982; Li and Lovell, 1985 and Blazer et al. 1989) explained that catfish fed diets with low level or no ascorbic acid were more susceptible to *E. tarda* and *E. intaluri* infection and has significantly lower circulating antibody concentration to *E. ictaluri* antigen and a lower phagocytic index when when compared to fish fed levels ranging from 30-3000 mg/kg feed. Moreover, the highly incidence and severity of bacterial kidney disease in fish maintained on commercial feeds was attributed to deficiencies of traceminerals and/or deficiences of vitamines C or A (Paterson et al. 1981).

From this work it could be concluded that:

1) The above-mentioned plant protein sources cannot be used as replacement for animal protein sources at 100 % of the dietary protein without sacrificing growth and feeding efficiency.
2) The diets of animal protein sources enhances the host defence mechanisms consequently increasing the host protection against infection more than the diets of plant sources.

SUMMARY

Four groups of Nile tilapia (Oreochromis niloticus) each contain 30 fish were fed four approximately . .

isonitrogenous and isoenergetic diets formulated with different protein sources. The growth performance were studied during the period of 120 days. After this time, 10 fishes from each group were immunized with *Aeromonas hydrophila* bacterin and the remainder fish (20 fish) were exposed to artificial infection with *A. hydrophila* by immersion method. The antibody titres were detected weekly by agglutination test while the mortalities percent were recorded throughout 15 days post challenge.

The growth performance including weight gain (WG) percent, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and protein retention were higher in fish fed the diet containing fish meal than bone and meat meal, cottonseed meal and sunflower meal. There was a significant different ($P < 0.05$) between fish meal diet and all experimental diets in the most studied criteria.

Fish fed diets contain protein of animal sources (fish meal and meat & bone meal) were responded to immunization with production of specific antibodies more than the fish fed diets contain protein of plant sources (cottonseed meal and sunflower meal).

The mortalities percent resulted from artificial infection with *A. hydrophila* were 20, 25, 35 and 35 in groups fed diets contain fish meal, bone and meat meal, and sunflower meal respectively.

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