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EFFECT OF DIETARY PROTEIN LEVEL AND VITAMIN C SUPPLEMENTATION ON PERFORMANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

AYYAT M.S.*, FAYZA S. ABBAS and G.O. EL-NAGGAR *****

* Department of Animal Production, Faculty of Agriculture, Zagazig University, Egypt

** Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad, Sharkia, Egypt

*** The World Fish Center, Africa and West Asia Regional Center, Abbassa, Abou-Hammad, Sharkia, Egypt

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SUMMARY

Effects of varying dietary protein and vitamin C levels on several nutritional parameters were calculated for young Oreochromis niloticus. Live body weight of Nile tilapia (O. niloticus) increased significantly (P<0.001) with increasing dietary protein level or vitamin C; supplementation in fish diet. Average daily gain weight increased with 37.31% in fish fed the high protein diet (31.1% crude protein) than those fed the normal protein diet (25.4%). Also, average daily weight gain increased with 5.41 and 14.86%, respectively, in fish fed diets supplemented with 50 and 100 mg vitamin C/ kg diet than those fed diets without supplementation. Increasing dietary protein level improved the feed conversion. Feed conversion improved by 18.85% in fish group fed high protein diet when compared with those fed the normal protein diet. Feed conversion improved by 1.37 and 7.88%, respectively, in fish groups fed diets supplemented with 50 and 100 mg vitamin C when compared with those fed diet without vitamin C supplementation. Serum total protein, albumin, creatinine and AST significantly (P<0.01) increased with dietary protein level, while ALT insignificantly affected. Also, vitamin C supplementation in fish diets significantly increased the concentrations of serum total protein, albumin and AST, while the concentration of ALT insignificantly decreased. Fish body composition did not affected significantly with dietary protein level or dietary vitamin C supplementation. The interaction between dietary protein level and vitamin C supplementation did not show any significant differences in body weight, daily

gain, daily feed intake, feed conversion, blood components or body composition.

Keywords: Dietary protein; vitamin C supplementation, growth rate; feed conversion; blood components; body composition.

INTRODUCTION

Protein is the most important component of the diet of fish because protein intake generally determines growth. As the protein is more expensive than carbohydrates and fat, the amount of protein in the diet should be just enough for growth and tissue repair (Lovell, 1980). The dietary protein requirements of several species of tilapia have been estimated to range between 20% and 56% (El-Sayed and Teshima, 1991). Al-Hafedh (1999) reported that for all sizes of tilapia, the growth performance showed progressive increase with increasing dietary protein level.

Although most land animals do not require a dietary source of vitamin C, most fish are extremely sensitive to vitamin C deficiency. It is true that vitamins are very important, but when the dosage is much higher than necessary, they are being abused. Vitamins are used as co-enzymes in the body. That is tiny bits of them are needed here and there to assist many body functions. Vitamins play a part in cellular oxidations, particularly the oxidation of the amino acid, tyrosine. It is necessary for the maintenance of normal connective tissue (Dwyer, 1979 and Marshall, 1985). Vitamin C helps to maintain the connective tissues, it helps in increasing absorption of iron and calcium from foods; it enhances red blood cell production and the production of the amino acids (Brody, 1981). Growth rate, feed conversion and protein efficiency ratio were improved with increasing dietary vitamin C supplementation (El-Naggar and lovell, 1991). When the marginal ascorbic acid, 20 mg/kg, was given, haematological measurements increased maximally with the low or high supplement of folic acid (0.4 or 4.0 mg/kg), but weight gain and red blood cell did not respond to folic acid supplementation. With dietary ascorbic acid 200 mg/kg, weight gain improved (Duncan and Lovell, 1994).

The objective of the present study was to investigate effects of dietary protein level and vitamin C supplementation on growth performance, feed efficiency, blood components and body composition of young Nile tilapia fish.

MATERIALS AND METHODS

The study was conducted at the Department of Animal Production, Agriculture Faculty, Zagazig University. Young Nile tilapia averaged about 6.5 g were used in this study. The fish were stocked in eighteen glass aquaria (70 X 40 X 60 cm) supplied with fresh, aerated tap water. Fish were divided into six groups, each group of fish was stocked into 3 aquaria and each contains 40 fish.

The fish of the first three groups were fed a normal protein diet (25.4%), other three groups were fed on high protein diet (31.1%; Table 1). Fish were fed one time daily at a feeding rate of 3% of body weight per day. The total duration of the experimental feeding trial was 4 months (from April to August, 2001). Within each dietary protein level, three fish groups were fed diets supplemented with vitamin C to supply 0, 50 and 100 mg per kg diet. Fish wastes were siphoned out and 30% of the water in each aquarium was removed daily and replaced with fresh new water. Fish were individually weighed to the nearest 0.1 gm at the beginning of the experiment and biweekly intervals throughout the experimental period. Feed conversion was calculated as the quantity of feed required to obtain one unit growth during the experimental period, according to Berger and Halver (1987). Blood samples were taken from the caudal vein from randomly selected three fish in each group. The blood samples were centrifuged at 3000 RPM for 20 min. to separate the serum. Total protein, albumin, urea-N, creatinine and serum transaminase enzymes (AST; aspartate amino transferase and ALT; alanine amino transferase) were measured in blood serum by colormetric methods using commercial kits. Proximate chemical compositions of experimental diets and fish body were determined according to AOAC (1980).

The obtained data were statistically analyzed by 2 X 3 factorial experiment (Sendecor and Cochran, 1982) as the following model:

$$Y_{ijk} = \mu + P_i + V_j + PV_{ij} + E_{ijk}$$

Where, μ is the overall mean, Pi is the fixed effect of ith dietary protein level, Vjj is the fixed effect of jth dietary vitamin C supplementation level, PVij is the interaction effect of ith dietary protein level and jth dietary vitamin C level and Eijk is the random error. Differences between treatments were statistical tested by Duncan's multiple range test (Duncan, 1955).

RESULTS

Growth performance:

Live body weight of Nile tilapia fish increased significantly ($P<0.001$) with increasing dietary protein level (Table 2). Average daily weight gain increased with 37.31% in fish fed the high protein diet (30% crude protein) than those fed the normal protein diet (25%).

Live body weight of Nile tilapia increased significantly ($P<0.001$) as affected with vitamin C supplementation in the diets (Table 2). Average daily gain weight increased with 5.41 and 14.86%, respectively, in fish fed diets supplemented with 50 and 100 mg vitamin C/ kg diet than those fed diets without supplementation.

The interaction between dietary protein level and vitamin C supplementation did not show any significant differences in live body weight or daily body gain (Table 2). Fish group fed high protein

Table (1): Ingredients and chemical composition of the experimental diets.

Ingredients %	Dietary protein levels	
	Normal	High
Fish meal	8.0	12.0
Soybean meal	28.0	40.0
Corn	14.0	8.0
Wheat bran	35.0	30.0
Alfalfa hay	12.0	7.0
minerals mix.*	0.5	0.5
Vitamin mix.**	1.5	1.5
Carboxymethyl cellulose	1.0	1.0
Chemical composition%		
Crude protein	25.4	31.1
Ether extract	28	2.7
Crude fiber	8.5	8.3
Gross energy (kcal/Kg)[1]	3348.0	3577.0

* Each one Kg of mineral mixture contained: Zinc 1.23g, manganese 930 mg, Iron 630 mg, Copper 105 mg and selnium 2.1mg.

** Each one Kg of vitamin mixture contained: Vit. A 72000IU, Vit. B16 mg, Vit. B3 12000 Iu, Vit. B6 9 mg, B12 0.06 mg, Vit E 60 mg, Vit. K 12mg, Pantothonic acid 60 mg, Vit. K 12 mg, Pantothonic acid 60 mg, Nicotinic acid 120 mg, Folic acid 6 mg, Biotin 0.3 mg and Choline chlorids 3 mg.

1 Calculated according to NRC (1993).

diet and supplemented with 100 mg vitamin C recorded higher growth rate than the other experimental groups.

Feed efficiency:

Daily feed intake increased as affected with increasing body weight. Increasing dietary protein level improved the feed conversion during the whole experimental periods (Table 3). Fish fed the high protein diet recorded the best feed conversion ratio during the whole experimental period (0 – 4 months). Feed conversion improved by 18.85% in fish group fed high protein diet when compared with those fed the normal protein diet.

Vitamin C supplementation in Nile tilapia fish diets improved feed conversion during the whole experimental period (0-4 months). Fish fed the diets supplemented with 100 mg vitamin C recorded the best feed conversion during the whole experimental periods. Feed conversion improved by 1.37 and 7.88%, respectively, in fish groups fed diets supplemented with 50 and 100 mg vitamin C when compared with those fed diet without vitamin C supplementation (Table 3).

The interaction between dietary protein level and vitamin C supplementation did not show any significant differences in daily feed intake or feed

Table (2): Live body weight (g) daily body gain (g/day) of *O. niloticus* fish as affected by dietary protein level, vitamin C supplementation and their interaction.

Items	M0	Body weight at M2	M4	0-2 M	Daily gain at 2-4 M	0-4 M
Dietary protein level (%):						
25%	6.63 ± 0.03	10.63 ± 0.16	14.62 ± 0.16	0.067 ± 0.003	0.067 ± 0.003	0.067 ± 0.001
30%	6.63 ± 0.02	12.28 ± 0.14	17.6 ± 0.32	0.094 ± 0.002	0.089 ± 0.003	0.092 ± 0.003
Significant	NS	***	***	***	***	***
0	6.61 ± 0.03	11.02 ± 0.41a	15.48 ± 0.57a	0.073 ± 0.007a	0.074 ± 0.003	0.074 ± 0.005a
50	6.62 ± 0.03	11.42 ± 0.34b	15.98 ± 0.70a	0.080 ± 0.006b	0.076 ± 0.007	0.078 ± 0.006b
100	6.63 ± 0.04	11.92 ± 0.39c	16.88 ± 0.79b	0.088 ± 0.006c	0.083 ± 0.007	0.085 ± 0.007c
Significant	NS	***	***	***	NS	
Interaction between dietary protein and vitamin C levels: 25% dietary protein level						
0	6.65 ± 0.05	10.11 ± 0.11	14.27 ± 0.15	0.058 ± 0.002	0.069 ± 0.004	0.064 ± 0.001
50	6.63 ± 0.04	10.71 ± 0.26	14.47 ± 0.26	0.068 ± 0.004	0.063 ± 0.008	0.065 ± 0.002
100	6.63 ± 0.06	11.08 ± 0.06	15.12 ± 0.08	0.074 ± 0.001	0.068 ± 0.003	0.071 ± 0.007
30% dietary protein level						
0	6.64 ± 0.04	11.93 ± 0.07	16.8 ± 0.34	0.088 ± 0.009	0.079 ± 0.005	0.084 ± 0.003
50	6.62 ± 0.04	12.13 ± 0.12	17.48 ± 0.27	0.092 ± 0.002	0.089 ± 0.003	0.091 ± 0.002
100	6.64 ± 0.05	12.76 ± 0.18	18.64 ± 0.21	0.102 ± 0.003	0.098 ± 0.002	0.100 ± 0.002
Significant	NS	NS	NS	NS	NS	NS

M= Month

NS= Not significantly and *** $P < 0.001$.

Means in the same column within each classification with different letters differ significantly ($P < 0.05$).

Table (3): Daily feed intake (g/day) and feed conversion ratio (g feed/1 g gain) of ***O. niloticus*** fish as affected by dietary protein level, vitamin C supplementation and their interaction.

Items	M0	Daily feed intake (g) at M2	Daily feed intake (g) at M4	Feed conversion (g feed/g gain) at 0-2 M	Feed conversion (g feed/g gain) at 2-4 M	Feed conversion (g feed/g gain) at 0-4 M
Dietary protein level (%):						
25%	0.17 ± 0.000	0.25 ± 0.003	0.21 ± 0.002	2.59 ± 0.115	3.82 ± 0.178	4.13 ± 0.047
30%	0.18 ± 0.001	0.29 ± 0.003	0.23 ± 0.002	1.91 ± 0.041	3.30 ± 0.107	2.54 ± 0.053
Significant	***	***	***	***	*	***
Dietary vitamin C level (mg/kg diet):						
0	0.17 ± 0.002	0.26 ± 0.011a	0.21 ± 0.006a	2.48 ± 0.222a	3.57 ± 0.128	2.92 ± 0.105a
50	0.18 ± 0.002	0.27 ± 0.008a	0.22 ± 0.004b	2.24 ± 0.145b	3.67 ± 0.300	2.88 ± 0.159a
100	0.18 ± 0.011	0.28 ± 0.009b	0.23 ± 0.006b	2.03 ± 0.121c	3.45 ± 0.181	2.69 ± 0.149
Significant	NS	***	***	***	NS	**
Interaction between dietary protein and vitamin C levels: 25% dietary protein level						
0	0.17 ± 0.000	0.24 ± 0.000	0.20 ± 0.000	2.96 ± 0.125	3.49 ± 0.208	3.14 ± 0.067
50	0.19 ± 0.000	0.25 ± 0.003	0.21 ± 0.000	2.52 ± 0.157	4.14 ± 0.481	3.22 ± 0.088
100	0.17 ± 0.000	0.26 ± 0.000	0.21 ± 0.003	2.29 ± 0.044	3.84 ± 0.019	3.02 ± 0.066
30% dietary protein level						
0	0.18 ± 0.003	0.029 ± 0.003	0.23 ± 0.003	2.00 ± 0.026	3.65 ± 0.179	0.70 ± 0.043
50	0.18 ± 0.000	0.29 ± 0.003	0.23 ± 0.000	1.96 ± 0.33	3.21 ± 0.065	2.54 ± 0.057
100	0.18 ± 0.000	0.30 ± 0.006	0.24 ± 0.003	1.76 ± 0048	3.53 ± 0.088	2.37 ± 0.007
Significant	NS	NS	NS	NS	NS	NS

M= Month

NS= Not significantly and * $P < 0.05$, ** $P<0.01$ and *** $P < 0.001$.

Means in the same column within each classification with different letters differ significantly ($P < 0.05$).

Table (4):Some blood components of ***O. niloticus*** fish as affected by dietary protein level, vitamine C supplementaton and their interaction.

Items	Total protein (g/dl)	Albumin (g/dl)	Urea-N (mg/dl	Creatinine (mg/dl	AST (U/l)	ALT (U/l)
Dietary protein level (%):						
25%	4.79 ± 0.12	2.73 ± 0.07	3.75 ± 0.08	0.906 ± 0.023	27.61 ± 1.06	16.87 ± 0.86
30%	5.37 ± 0.14	3.21 ± 0.11	3.91 ± 0.07	1.009 ± 0.029	36.77 ± 0.96	13.74 ± 0.48
Significant	***	***	NS	**	***	**
Dietary vitamin C level (mg/kg diet):						
0	4.71 ± 0.14a	2.71 ± 0.09a	3.65 ± 0.12	0.898 ± 0.038	28.95 ± 2.10a	16.62 ± 1.28
50	5.10 ± 0.17b	2.93 ± 0.11b	3.87 ± 0.06	0.970 ± 0.032	32.68 ± 2.40b	14.95 ± 0.98
100	5.43 ± 0.17c	3.27 ± 0.16c	3.97 ± 0.06	1.006 ± 0.036	34.93 ± 1.92c	14.35 ± 0.80
Significant	***	***	NS	NS	***	NS
Interaction between dietary protein and vitamin C levels: 25% dietary protein level						
0	4.47 ± 0.17	2.52 ± 0.06	3.60 ± 0.24	0.852 ± 0.041	24.59 ± 1.15	19.20 ± 0.82
50	4.83 ± 0.19	2.73 ± 0.04	3.78 ± 0.09	0.913 ± 023	27.53 ± 1.25	16.17 ± 1.70
100	5.07 ± 0.12	2.93 ± 0.09	3.870.04	0.953 ± 0.039	30.80 ± 0.850	15.23 ± 0.96
30% dietary protein level						
0	4.95 ± 0.08	2.90 ± 0.07	3.70 ± 0.13	0.943 ± 0.058	33.40 ± 0.02	14.03 ± 0.90
50	5.37 ± 0.18	3.14 ± 0.11	3.95 ± 0.05	1.027 ± 0.035	37.83 ± 0.88	13.73 ± 0.62
100	5.80 ± 0.06s	3.60 ± 0.08	4.08 ± 0.05	1.058 ± 0.047	39.07 ± 0.81	13.47 ± 1.22
Significant	NS	NS	NS	NS	NS	NS

NS= Not significantly and * * $P < 0.05$ and *** $P < 0.001$.

Means in the same column within each classification with different letters differ significantly ($P < 0.05$).

Table (5): The hole body of *O. niloticus* fish as affected by dietary protein level, vitamine C supplementaton and their interaction.

Items	Moisture	Protein[1]	Ether extract[1]	Ash[1]
Dietary protein level (%):				
25%	74.95 ± 0.37	58.71 ±1.17	6.65 ± 0.26	23.93 ± 0.41
30%	74.13 ± 0.42	59.81 ±0.51	6.59 ± 0.32	24.46 ± 0.30
Significant	NS	NS	NS	NS
Dietary vitamin C level (mg/kg diet):				
0	74.32 ± 0.56	59.54 ± 0.63	7.20 ± 0.34	24.22 ± 0.27
50	74.58 ± 0.47	58.23 ± 1.58	6.32 ± 0.35	24.37 ± 0.47
100	74.72 ± 0.54	60.01 ± 0.94	6.35 ± 0.26	24.01 ± 0.61
Significant	NS	NS	NS	NS
Interaction between dietary protein and vitamin C levels: 25% dietary protein level				
0	74.90 ± 0.99	59.30 ± 1.06	7.22 ± 0.27	23.80 ± 0.34
50	74.93 ± 0.33	56.99 ± 3.09	6.21 ± 0.51	24.56 ± 0.62
100	75.01 ± 0.72	59.85 ± 1.89	6.51 ± 0.43	23.44 ± 1.10
30% dietary protein level				
0	73.74 ± 0.50	59.78 ± 0.88	7.17 ± 0.70	24.63 ± 0.27
50	74.23 ± 0.93	59.48 ± 1.16	6.42 ± 0.59	24.19 ± 0.83
100	74.42 ± 0.91	60.17 ± 0.90	6.18 ± 0.36	24.58 ± 0.54
Significant	NS	NS	NS	NS

[1] On dry matter basis.
NS= Not significantly

conversion (Table 3). Fish group fed high protein diet and supplemented with 100 mg vitamin C recorded the best feed conversion than the other experimental groups.

Blood components:

Serum total protein, albumin, creatinine (as indicator of kidney function) and AST (as indicator of liver function) significantly ($P<0.01$) increased with dietary protein level, while ALT insignificantly affected (Table 4).

Serum total protein, albumin and AST significantly ($P<0.001$) affected with supplementation of vitamin C in fish diet, while urea-N, creatinine and ALT insignificantly affected (Table 4). Vitamin C supplementation significantly increased the concentrations of serum total protein, albumin and AST, while the concentration of ALT insignificantly decreased.

Studied serum parameters insignificantly affected with the interaction between the dietary protein level and vitamin C supplementation.

Body composition:

Fish body composition did not affected significantly with dietary protein level, dietary vitamin C supplementation or the interaction between them (Table 5).

Protein content in fish body insignificantly increased with increasing dietary protein level or vitamin C supplementation, while the fat content in fish body decreased with vitamin C supplementation.

DISCUSSION

Fish fed diet with 30% crude protein recorded higher body weight and daily gain than those fed diets with 25% crude protein. Similar results were reported by Ayyat et al. (2000). Lochman and Phillips (1994) found that weight gain was the best in fish fed on diets containing 28.9% protein than the diets contained 21.2, 25.3, 31.1, or 34.5% crude protein levels. However, Tidwell et al. (1992) reported that fish fed diet with 37% protein had significantly ($P<0.05$) higher body gain and specific growth rate than fish fed on diets with 26 and 31% protein levels. On the other hand, Watanabe et al. (1990) found that final body weight and body gain were higher for tilapia fish given diets with 28% crude protein than these fed diets with 32%.

Body weight and daily body gain weight increased with increasing dietary protein level and vitamin C supplementation in fish diets. Fish fed diet supplemented with 100 mg vitamin C/kg diet recorded higher body weight and daily gain than those fed diets supplemented with 50 or 0 mg vitamin C. The obtained results indicated that fish fed 30% protein diet and supplemented with 100 mg vitamin C recorded higher daily body gain. Similar results were reported by Abdelghany

(1996 and 1998) and Frischknecht et al. (1994). Abdelghany (1998) indicated that diets supplemented with 5 or 50 mg ascorbic acid per kg diet promoting growth, improving food efficiency, increasing nutrient retention and preventing scurvy in Nile tilapia.

Abdelghany (1996) reported that the best growth, food conversion ratio, survival rate, and ascorbic acid content in liver were obtained with the diet containing 50 mg ascorbate equivalent / kg diet from L-ascorbyl-2-polyphosphate followed by the diet containing 50 mg ascorbate equivalent / kg diet from L-ascorbyl-2-sulfate. Fish fed diets with 50 mg ascorbic acid/kg diet from L- ascorbyl -2- sulfate and L-ascorbyl -2-polyphosphate gained 27.9% and 36.2% more weight, respectively, than fish fed diets with 50 mg ascorbic acid/kg diet from L-ascorbic acid.

Also, Frischknecht et al. (1994) reported that the young rainbow trout fish fed a diet deficient in vitamin C and E exhibited a high mortality and were anemic after 8-12 weeks. Fish fed a diet deficient in vitamin C but high in vitamin E developed the typical signs of vitamin C deficiency after 16-20 weeks, including reduced growth rate, haemorrhages and gill alterations as well as severe deformations and fractures of the vertebral column.

Fish fed high protein diet (30%) recorded the best feed conversion than the normal dietary protein level (25%). Feed conversion improved with vitamin C supplementation in fish diets. The best-feed conversion was obtained in fish fed diets supplemented with 100 mg vitamin C. The obtained results are in agreement with those obtained with Ayyat et al. (2000).

The results obtained from the blood components indicated that the kidney function (urea-N and creatinine) increased as affected the increasing dietary protein, and the obtained results may indicate that the protein synthesis increased in fish fed high protein diet. Vitamin C supplementation in fish diets significant ($P<0.01$) increased on serum total protein, albumin and AST and insignificant increased urea-N and creatinine and decreased in ALT concentration. Increasing the level of AST (within the normal level) in the serum of fish fed the high protein diet or diet supplemented with vitamin C may be related with the increasing of protein synthesis in the liver.

Carcass protein content increased insignificantly with increasing dietary protein level or vitamin C supplementation in fish diets, while the fat content decreased insignificantly. El-Dahhar and Lovell (1995) and Lochmann and Phillips (1994) found that dietary protein content in the diet had no effect on the body composition. On the other hand, Mohantly and Samantaray (1996) reported that there was a significant increase in carcass protein and a significant decrease in ash content with progressive dietary protein content.

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