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INFLUENCE OF DIETARY PROTEIN DEFICIENCY ON AMINO ACID AND FATTY ACID COMPOSITION IN TILAPIA, *OREOCHROMIS NILOTICUS*, FINGERLINGS

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Abstract

The influence of dietary protein deficiency on the amino acid and fatty acid compositions of tilapia (*Oreochromis niloticus*) fingerlings was studied. Two experimental diets (0.81% and 33.32% protein, dry matter) were prepared. The protein content of fish fed diet 1 (0.81% protein) decreased from 57.14% to 49.18% in eight weeks. Fish fed diet 2 (33.32% protein) had higher protein and amino acid contents. The lipid content of fish fed diet 1 was higher than that of fish fed diet 2, suggesting that carbohydrates transformed into lipids. The levels of fatty acids 16:0 and 18:2 n-6 in fish fed diet 1 remained nearly unchanged and did not reflect the diet, demonstrating that fatty acids in diet 1 may not have been incorporated into the triglycerides of the tissues. Possible impairment of lipid secretion from the liver, caused by depletion of protein in the blood lipoprotein, may have affected the transport of lipids to the muscles. A dietary protein deficiency results not only in a deficiency of essential amino acids in the body but also affects transport and storage of lipids within the fish.

Introduction

Protein is an important constituent of the fish diet. It is the source of building material for growth and is important in the production of enzymes (Steffens, 1989). Protein is the basic component of animal tissues and, therefore, an essential nutrient for maintenance and growth. Maintenance, according to Kaushik (1995), involves replacement of obligatory body protein losses (skin, digestive tract) and losses due to amino acid oxidation,

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utilization of amino acids for purposes other than synthesis and protein turnover.

Free amino acids in the body are supplied by dietary protein and by catabolism of tissue proteins. Free amino acids that are not used for protein synthesis and other essential functions are catabolized to ammonia and the corresponding α-ketoacids. The α-ketoacids are used as a source of energy or of carbon for fat synthesis or gluconeogenesis. Most α-ketoacids are oxidized to CO_2 and H_2O via the tricarboxylic acid cycle. Amino acid oxidation is principally influenced by the level of protein (or amino acids) and other energy sources in the diet (Kim et al., 1992).

In the circulatory fluid of animals including fish, most lipids are complexed with protein in the form of lipoproteins (Gotto et al., 1986). Lipoproteins of all animals have been identified as major carriers of lipids and other hydrophobic compounds (Ando and Mori, 1993). It is possible that a lack of adequate protein enhances the loss of serum lipoprotein and this can affect the transport and storage of lipids in *Oreochromis niloticus*. A deficiency of dietary protein may therefore impede physiological functions in fish, affecting yields. The optimal supply of the ideal protein has become an important factor in fish rearing for adequate fish growth.

Previous studies (Jauncey, 1982; Wang et al., 1985; El-Sayed and Teshima, 1992; Ogunji and Wirth, 2000) identified the protein requirements for different tilapia species. They demonstrated the effects of protein on growth rate, food conversion and body composition. The aim of this study was to investigate the influence of a dietary protein deficiency on the amino acid and fatty acid compositions of tilapia (*O. niloticus*) fingerlings.

Materials and Methods

Preparation of experimental diets. Two experimental feeds, with protein contents of 0.81% and 33.32% dry matter, were prepared. A dietary protein content of 33.32% dry matter (relative to fishmeal) was determined as optimal for *O. niloticus* (Ogunji and Wirth, 2000). Diet 1 was formulated from potato and wheat starch and contained no fishmeal whereas fishmeal was the sole protein source in diet 2 (Table 1). The dry diet components including a vitamin and mineral mixture were thoroughly mixed with sunflower oil. Water was added and the feed was pressed into pellets of 1 mm diameter. The formulated diets were dried at room temperature and stored in a refrigerator (5-7°C) throughout the experiment.

Experimental conditions. Tilapia fingerlings, bred and reared in recirculating tanks at the facilities of the Institute of Freshwater Ecology and Inland Fisheries (Berlin), were used for the study. Twenty fingerlings (initial weight 3-4.1g) were stocked into each of three glass tanks (28 x 28 x 51.5 cm). Two trials were conducted, one for each diet. The two trials were conducted one after the other. Each experiment started after an adaptation period of two weeks and lasted eight weeks. The fish were fed at the rate of 5% (wet weight basis) of their total biomass per day, divided into three portions. They were weighed every two weeks and the feed quantity was adjusted accordingly. Tanks were cleaned twice a week. Conductivity, pH, oxygen concentration and water temperature were measured three times a week. The water was aerated and the oxygen content exceeded 60%. The temperature was maintained at 27±1°C.

Analyses of feed and fish samples. Prior to the start of the experiment, twenty-five fish were randomly selected and homogenized after removal of the intestines. At the end of the experiment, all the fish in each tank were weighed. Fish from similar treatments were combined (March et al., 1985; Kim and Lall, 2001), and twenty were randomly chosen. Their intestine was removed and the carcass homogenized (Kim et al., 1992). Freeze-dried samples of fish at the beginning and at the end of the experiment as well as samples of the test diets were analyzed in triplicate for proximate, fatty acid and amino acid composition. Protein (N × 6.25) was determined by the Kjeltec System (Tecator) and crude fat by the Soxtec System HT (Tecator) using petroleum ether (Sadiku and Jauncey, 1995). Ash was determined by burning in a muffle fur-

Table 1. Ingredients and proximate composition of experimental diets for *Oreochromis niloticus* fingerlings.

	Diet 1	*Diet 2*
Ingredients (%)		
Fishmeal	-	45
Sunflower oil	10	10
Potato starch	35	40
Wheat starch	50	-
Vitamin and mineral mix[1]	5	5
Proximate composition		
Dry matter (%)	92.39	91.47
Crude protein (% of dry matter)	0.81	33.32
Crude fat (% of dry matter)	12.61	16.02
NFE and fiber[2] (% of dry matter)	83.18	40.38
Ash (% of dry matter)	3.40	10.28
Gross energy (kJ/g)	19.23±0.04	20.49±0.33

[1] Vitamin and mineral mix (Spezialfutter Neurupin,VM BM 55/13) supplied per 100 g of dry feed: Vitamin A 15,000 IU, D3 2500 IU, E 500 mg, K3 23 mg, B1 42 mg, B2 18 mg, B6 21 mg, B12 59 µg, C 66.7 mg, nicotinic acid 100 mg, biotin 544.7 µg, folic acid 13 mg, pantothenic acid 123 mg, inositol 1230 mg, antioxidants (BHT) 121.9 mg, calcium 20.2%.

[2] Nitrogen free extract and fiber = 100-(%protein + %fat +%ash)

nace at 750°C for four hours. An oxygen bomb calorimeter (Framo-MK 200) was used to determine energy content (Zeitler et al., 1984). Fatty acid composition of the samples was analyzed using gas-liquid chromatography as described by Wirth and Steffens (1998). The lipids were separated by thin layer chromatography on silica gel G 60 (Merck, Darmstadt), using n-hexane/ethylether/acetic acid (73/25/2/v/v/v) as the developing solvent. Fatty acids of phospholipids and triglycerides were transformed with sodium methylate into methylesters. High Performance Liquid Chromatography (HPLC) was used to estimate the amino acid concentrations of the samples. Five mg of the freeze-dried samples was hydrolyzed with 6N HCl at 110°C for 24 hours. No protecting reagents were added to avoid destruction of sulphur amino acids. Other procedures for this analysis have been reported (Ogunji and Wirth, 2001).

Statistical analyses and calculation. The *t*-test was performed to compare means (SPSS for windows, Version 9). Weight gains, specific growth rate (SGR) and feed conversion ratio (FCR) were calculated. SGR = $(\text{Ln}W_2 - \text{Ln}W_1)/(T_2-T_1)100$ where W_1 and W_2 = initial and final weight of fish and T_1 and T_2 = time in days.

Results

Feed performance and growth. Both experimental diets were accepted by the fish. All

but one of the fish fed the protein-deficient diet 1 (0.81% protein) survived the 8-week experiment but no weight gain was recorded in this group. The mean weight gains, growth rates, protein to energy ratios and feed conversion ratios are presented in Table 2. Fish fed diet 2 (33.32% protein) gained 23.28 g and had a low FCR (1.19). The FCR among fish fed diet 1 was extraordinarily high at 9.57.

Body composition. The protein content of fish fed diet 2 increased from 58.4% to 62.83% while that of fish fed diet 1 decreased from 57.14% to 49.18% (Table 3). The fat content in both groups decreased, however, the decrease was greater in fish fed diet 2 (21.48%) than in fish fed diet 1 (24.53%). In fish fed diet 1, all essential amino acid levels decreased except for that of histidine (we have no explanation for the high content of histidine) and most of the nonessential amino acids slightly increased. On the other hand, fish fed diet 2 accumulated higher values of both essential and nonessential amino acids.

Fatty acid composition of samples. Fatty acid compositions of triglycerides and phospholipids from the test diets and fish samples are presented in Tables 4 and 5. Both diets were rich in linoleic acid (18:2 n-6), however, diet 2 contained more n-3 fatty acids than diet 1. During the experiment, linoleic acid in fish fed diet 2 rose from 5.1% to 36.5% in triglycerides and from 2.6% to 21.7% in phospholipids. Among fish fed diet 1, linoleic acid slightly increased in triglycerides (from 5.1% to 6.1%) and decreased in phospholipids (from 2.8% to 2.3%), significantly different ($p<0.05$) from the changes in fish fed diet 2. Long chain polyunsaturated fatty acids such as eicosapentaenoic (20:5 n-3) and docosahexanoic acid (22:6 n-3) decreased in the triglycerides as well as in the phospholipids in fish of both groups. Concentrations of fatty acids 18:3, 20:4, 20:5 and 22:6 were low in both diets and did not affect body composition of the experimental fish.

Table 2. Growth, protein/energy ratio and food conversion ratio (FCR) of *Oreochromis niloticus* fingerlings fed diet 1 and 2 (n=3; mean±SD).

	Diets 1 *(0.81% protein)*	*Diet 2* *(33.32% protein)*
Initial weight (g)	3.2±0.0	4.10±0.1
Final weight (g)	3.21±0.07[b]	27.38 ±2.1[a]
Weight gain (g)	0.01±2.0[b]	23.28±0.07[a]
SGR[1]	0.005±0.04	3.39±0.1
Food fed (g/fish)	8.76	27.63
Protein/energy ratio[2]	0.42	16.26
FCR[3]	9.57[b]	1.19[a]

Figures in the same row with different superscript letters are significantly different ($p<0.05$) from each other.

[1] Specific growth rate (%/d) = $(LnW_2 - LnW_1/T_2 - T_1)100$

[2] Protein to energy ratio (P/E) = mg protein/kJ energy

[3] Food conversion ratio = food fed (g)/live weight gain (g)

Table 3. Change in proximate and selected amino acid compositions of fish fed Diet 1 and 2 (n = 3; mean ± standard deviation).

	Diet 1 (0.81% protein)		*Diet 2 (33.32% protein)*	
	Initial	*Final*	*Initial*	*Final*
Nutrient content				
Dry matter (%)	27.32	27.14	25.70	28.16
Protein (% of dry matter)	57.14	49.18	58.40	62.83
Lipid (% of dry matter)	27.16	24.53	27.28	21.48
Amino acid composition (% of DM)				
Aspartic acid	3.63±0.32	3.77±0.13	3.84±0.05	4.68±0.13
Glutamic acid	5.41±0.55	5.50±0.13	5.60±0.29	6.48±0.41
Serine	1.51±0.17	1.51±0.06	1.66±0.03	1.91±0.01
Histidine*	0.47±0.05	0.77±0.02	0.86±0.04	1.15±0.03
Glycine	2.59±0.29	2.78±0.11	2.79±0.14	2.87±0.15
Threonine*	1.79±0.22	1.70±0.06	1.76±0.006	2.08±0.02
Arginine*	2.63±0.33	2.51±0.10	2.83±0.05	3.38±0.06
Carnosine	0.15±0.02	0.13±0.00	0.18±0.03	0.24±0.006
Alanine	2.67±0.33	2.83±0.12	2.94±0.05	3.31±0.01
Tyrosine	0.15±0.02	0.15±0.006	0.25±0.00	0.35±0.006
Valine*	1.68±0.22	1.66±0.08	1.76±0.05	2.07±0.006
Phenylalanine*	1.63±0.20	1.58±0.07	1.66±0.03	1.94±0.02
Isoleucine*	1.53±0.19	1.49±0.08	1.58±0.08	1.91±0.00
Leucine*	2.92±0.34	2.91±0.14	3.08±0.06	3.68±0.24
Lysine*	3.06±0.34	2.89±0.17	3.00±0.06	3.80±0.17

*Essential amino acids

Discussion

The proportion of sunflower oil in both diets was 10%. Sunflower oil, like other vegetable oils, is rich in linoleic acid (18:2, n-6; Takeuchi et al., 1983). It was used in the diets because of the need of *O. niloticus* for n-6 fatty acids (Kanazawa et al., 1980). The fishmeal affected the crude fat content of diet 2, making it 16.02% as compared to 12.61% in diet 1, but this did not seem to influence fish performance. The dietary fat and energy contents in our study are similar to those of De Silva et al. (1989), i.e., 12.0-15.2% lipid and 19.4-20.6 kJ/g energy, for the same species. In his study, Hanley (1991) noticed that increasing the dietary lipid level in the feed of Nile tilapia (*O. niloticus*) from 5% to 12% produced no significant effects on growth rate, food conversion ratio or protein gain.

Table 4. Selected fatty acid composition (%) of triglycerides from feed and fish at the beginning and at the end of the feeding experiment (n = 3; mean±standard deviation).

	Diet 1 (0.81% protein)			*Diet 2 (33.32% protein)*		
	Feed	*Initial body*	*Final body*	*Feed*	*Initial body*	*Final body*
16:0	6.2±0.1	20.2±0.5	21.2±0.06	8.5±0.06	15.9±0.06	11.6±0.06
18:1 (n-9)	22.8±0.2	21.5±0.06	24.5±0.15	21.4±0.06	20.2±0.06	26.8±0.1
18:2 (n-6)	62.1±0.1	5.1±0.06	6.1±0.06	52.7±0.06	5.1±0.3	36.5±0.1
18:3 (n-3)	0.8±0.06	1.0±0.06	0.5±0.1	0.5±0.06	1.7±0.06	0.2±0.00
20:4 (n-6)	0.8±0.06	0.3±0.00	0.3±0.06	0.8±0.06	0.6±0.00	0.5±0.06
20:5 (n-3)	trace	2.5±0.06	0.5±0.06	0.5±0.00	2.1±0.06	0.4±0.06
22:6 (n-3)	0.1±0.00	3.7±0.06	2.0±0.1	2.1±0.06	11.7±0.3	2.7±0.06

Table 5. Selected fatty acid composition (%) of phospholipids from feed and fish at the beginning and at the end of the feeding experiment (n = 3; mean±standard deviation).

	Diet 1 (0.81% protein)			*Diet 2 (33.32% protein)*		
	Feed	*Initial body*	*Final body*	*Feed*	*Initial body*	*Final body*
16:0	16.2±0.06	26.2±0.06	26.8±0.1	20.2±0.25	24.1±0.4	19.2±0.1
18:1 (n-9)	16.3±0.06	20.5±0.15	24.6±0.1	16.1±0.06	17.4±0.1	25.3±0.2
18:2 (n-6)	41.9±0.1	2.8±0.00	2.3±0.06	6.0±0.06	2.6±0.06	21.7±0.15
18:3 (n-3)	1.6±0.06	0.7±0.06	0.4±0.00	0.8±0.06	0.8±0.1	0.3±0.06
20:4 (n-6)	0.6±0.06	0.6±0.00	0.4±0.06	1.0±0.1	1.7±0.15	1.2±0.06
20:5 (n-3)	0.8±0.06	1.4±0.2	0.1±0.00	5.0±0.2	2.1±0.06	0.3±0.06
22:6 (n-3)	1.7±0.06	5.3±0.06	1.5±0.06	14.3±0.2	17.1±0.5	4.3±0.3

In spite of the high content of carbohydrate in their diet (NFE 83.63%), the protein content decreased in fish fed diet 1. There was no significant weight gain in this group at the end of the experiment. Carbohydrates are poorly utilized by fish and the main sources of energy in fish appear to be protein and lipids, in contrast to mammals in which carbohydrates and lipids are more important (Walton, 1985). It appears that the inability of fish to produce sufficient maintenance energy resulted in the use of body protein, thereby depleting the body protein and essential amino acids. When food is insufficient for both maintenance and growth, growth will be inhibited or will cease entirely (Hepher, 1988).

Amino acid composition. As observed above, essential amino acids (except histidine) were depleted while nonessential amino acids (except carnosine) increased among fish fed diet 1. The increase may be attributed to the ability of the fish to synthesize nonessential amino acids. The decrease of essential amino acids may emphasis their essentiality for *O. niloticus*. Steffens (1989) points out that a number of amino acids cannot be synthesized by animals because their carbon skeleton is particularly complex; they must therefore be supplied as part of the diet and are referred to as essential amino acids. The insufficient protein and essential amino acids in diet 1 may have resulted in retarded growth and depletion of body protein and amino acids in fish fed this diet.

Fatty acid composition. The high level of linoleic acid (18:2 n-6) in both diets is derived from the lipid source (sunflower oil). The higher n-3 fatty acids in diet 2 originated from the fishmeal. The fatty acid pattern of triglycerides in a body depends on the fatty acid composition of the diet (e.g., Watanabe, 1982; Steffens et al., 1995). The level of linoleic acid in fish fed diet 2 increased from 5.1% to 36.5% while that of palmitic acid (16:0) decreased from 15.9% to 11.6%. Among fish fed diet 1, the level of linoleic acid remained nearly unchanged and the palmitic acid slightly increased. This, however, does not correspond to the fatty acid composition of the diet. Results indicate that fatty acids from diet 1 were not incorporated into the triglycerides of the fish body. This may be due to impaired lipid secretion from the liver caused by depletion of the protein content of the blood lipoprotein which affected the transportation and storage of lipids in the muscles.

Very low-density lipoproteins (VLDL) are the transport vehicle of tryglycerides in the bloodstream (De Silva and Anderson, 1995). It has been reported that VLDL are synthesized from triglycerides and apolipoproteins (lipoprotein protein component) in the liver, and secreted as triglycerides-rich lipoprotein. Ando and Mori (1993) observed that the level of VLDL in the bloodstream seemed to be the determining factor by which lipids were stored in the muscle or liver. Apolipoprotein deficiency results in impaired secretion of lipid from the liver, causing lipid accumulation in the liver. In our study, the concentration of lipids in the liver was not measured, however, the dietary protein deficiency in diet 1 may have caused an apolipoprotein deficiency in the fish fed this diet.

The lipid content in the body of the fish fed diet 1 was higher than that of the fish fed diet 2. A slight increase in the concentration of 16:0 triglycerides in fish fed diet 1 was also observed, while in fish fed diet 2 (with the optimal protein content) the concentration decreased. The higher body lipid content obtained after feeding the protein deficient diet may be due to synthesis of fatty acids from carbohydrates. Anderson et al. (1984) pointed out that addition of carbohydrates to the diet of *O. niloticus* resulted in increased fat contents in the fish. Henderson (1996) reported that fish can transform carbohydrates into saturated and monounsaturated fatty acids. According to Lovell (1989), fatty acids are synthesized through acetyl-CoA from 2-carbon residues that come primarily from glucose and deaminated aminoacids. The 2-carbon units (acetate) are synthesized primarily into palmitic acid, but also into stearic (18:0) and myristic (14:0).

In diet 1, although the dietary level of 18:2 in the phospholipids was high, it was not incorporated into the membrane lipids. In fish fed diet 2, with a lower level of 18:2, this

polyunsaturated fatty acid rose (from 2.6% to 21.7%). This observation corroborates the results obtained with triglycerides, supporting the conclusion that a deficiency of protein in the fish diet results not only in a deficiency of essential amino acids but also affects the transport and storage of lipids by the fish. This study has shown that feeding tilapia fingerlings with a protein deficient diet influences the gross composition, amino acid and fatty acid composition of the fish body. Only one fish died among the fish fed diet 1 during the 8-week experimental period. It is possible that more would have died with time, when the body protein reserves could no longer supply the metabolic needs of the fish.

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