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Evaluation of the influence of housefly maggot meal (magmeal) diets on catalase, glutathione S-transferase and glycogen concentration in the liver of *Oreochromis niloticus* fingerling

Johnny O. Ogunji [a,b,*], Jorge Nimptsch [a], Claudia Wiegand [a], Carsten Schulz [c]

[a] *Institute of Freshwater Ecology and Inland Fisheries Berlin, Germany*
[b] *Ebonyi State University, Department of Animal Production and Fisheries Management, P.M.B. 053 Abakaliki, Nigeria*
[c] *Institute of Animal Science, Faculty of Agriculture and Horticulture, Humboldt University Berlin, Germany*

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Abstract

Influence of housefly maggot meal (magmeal) diets on the activities of catalase (CAT), glutathione S-transferase (GST) and glycogen concentration in liver of Tilapia *Oreochromis niloticus* fingerling was evaluated. Triplicate groups of fifteen fish (initial average weight 2.0±0.1 g) were fed eight weeks with seven test diets (in average 36% crude protein, dry matter) formulated by replacing fish meal with magmeal. Percentage body weight gain (591–724.46%), food conversion ratio (1.05–1.22) and standard growth rate (3.45–3.76) in all feeding groups were not significantly different ($P<0.05$). No significant difference ($P<0.05$) was observed in liver glycogen reserve (175.27–236.88 μmol g^{-1}) among the fish groups. Hepatic catalase activity also did not differ significantly. However, elevated glutathione S-transferases activities were observed when fish received higher dietary magmeal concentration. This might have been temporary with no real physiological implication when appraised by the growth responses. These results indicate that magmeal was well utilized by the fish and its incorporation into tilapia diets seems to have no oxidative stress generating effect on fish metabolism and may not be containing any compound that stimulates the generation of reactive oxygen species. Magmeal can effectively be used as an alternative protein source in tilapia fingerling production.

Keywords: Fish nutrition; Tilapia *Oreochromis niloticus*; Housefly maggot meal; Catalase; Glutathione S-transferases; Liver glycogen; Oxidative stress

1. Introduction

Due to the scarcity and cost of fish meal in the world market, there is a tendency to substitute fish meal in artificial diets of culturable fish species. Several feed ingredients have been investigated and they include both animal protein and plant protein sources. It has been reported that housefly maggot meal (magmeal) which is of animal origin possesses a great potential to replace fish meal in tilapia (*Oreochromis niloticus*) diets (Adesulu and Mustapha, 2000; Fasakin et al., 2003; Ajani et al., 2004). Based on cost effectiveness, availability and crude protein content, the housefly larvae grown on animal waste seem to have an immense potential as a good protein source for fish.

However, when fish is fed with diets unaccustomed to them two extreme situations may arise. The diet may be rejected and the situation of starvation or lack of nutrients in the fish may result. On the other hand the diet may not be qualitative enough to supply the nutrient requirements of the fish hence, a situation of decreased feeding may arise. In any of these cases decreased growth and oxidative stress become impending. Hidalgo et al. (2002) observed oxidative stress conditions in rainbow trout as a consequence of deficient Zn supplement. When fish are fed diets containing substances or compounds capable of elevating biotransformation rate, oxidative stress can also result.

In the process leading to oxidative stress depletion of organ antioxidant stores and increases in the generation of oxygen free radicals particularly in liver (Robinson et al., 1997) arises. As a

* Corresponding author. Department of Animal Production and Fisheries Management, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria. Tel.: +234 8067558863.
E-mail address: Ogunjijo@yahoo.com (J.O. Ogunji).

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defence mechanism, antioxidative enzymes, in addition to small antioxidant molecules, are active in the cell. Main antioxidative enzymes are the super-oxide dismutase, which hastens the dismutation of O_2^- to H_2O_2, the catalase (CAT) and glutathione peroxidase which are in charge of reducing H_2O_2 to H_2O, and are considered the most important antioxidant enzymes found in vertebrates. Their activities differ among the organs and tissues of freshwater and marine fish (Wdzieczak et al., 1982), depending upon feeding behaviour (Radi and Matkovics, 1988), environmental factors and other ecological conditions (Winston and Di Giulio, 1991; Roche and Bogé, 1996). When the antioxidant defences are insufficient to combat the action of the reactive oxygen species (ROS) the result is oxidative stress, leading to oxidation of lipids, proteins and DNA as consequence.

To prove, if the fish diets may contain compounds elevating the biotransformation rate, the activities of glutathione S-transferases (GST) were determined as one phase II biotransformation enzyme. In the detoxification of organic environmental pollutants, GSTs play a crucial role, catalyzing the conjugation of electrophilic substrates to the co-substrate glutathione (GSH), thereby enhancing water solubility of the compound and aiding excretion processes (George et al., 1990). Beside their role in biotransformation, GSTs display many other functions like peroxidase-, isomerase-activities, inhibition of Jun N-terminal kinase, protection against H_2O_2 induced apoptosis (Sheehan et al., 2001) and conjugating subproducts of the lipid peroxidation (e.g. 4 hydroxynonenal) (Awashti et al., 2004).

The experimental feed given to fish could affect the fish glycogen levels in the muscle and liver tissues. Fish do not accept or utilize experimental feeds in the same way due to several factors like palatability, nutrient composition and digestibility. As such the supply of energy needs of fish from the feed source may be hampered. Carbohydrates are stored as glycogen in fish tissue and organs like the muscle and liver in order to supply the energy needs when there are hypoxic conditions, intensive stocking and a lack of food (Wendelaar-Bonga, 1997). It has been demonstrated that liver glycogen levels decreased in *Oncorhynchus mykiss* as a result of the activation of glycolytic enzymes via catecholamines under lack of food and hypoxic conditions (Vijayan and Moon, 1992).

This study assesses the influence of housefly maggot meal (magmeal) diets on both, growth and nutrient storage, and on the biotransformation and antioxidative response in the liver of *O. niloticus* fingerlings. The activities of catalase (CAT) and glutathione S-transferases (GST) respectively as the main antioxidative and phase II biotransformation enzyme, were evaluated to further determine if magmeal may be harmful to fish. The growth parameter and biochemistry of nutrient storage (glycogen concentration) were evaluated to establish the nutritional quality of magmeal as a feed stuff. Though very few similar studies have been undertaken (Hidalgo et al., 2002), ichthyo-biochemical/physiology would be useful in the assessment of suitability of feeds and feed mixtures for fish nutrition.

2. Materials and methods

2.1. Experimental fish and diets

In this study, tilapia *O. niloticus* fingerlings bred at the facilities of Institute of Freshwater Ecology and Inland Fisheries Berlin, Germany and reared in a recirculation system were used as experimental fish. Fifteen of the fingerlings (initial average weight 2.0±0.1 g) were stocked in each of the 21 experimental tanks (28×28×51.5 cm) used. Seven test diets were formulated to yield a protein content of 34.0–38.1% (±36%) crude protein dry mater (dm) using fish meal and magmeal as major dietary protein sources. Fish meal concentration in the test diets decreased with increasing concentration of magmeal. Diet 1 which was formulated with the highest inclusion level of fish meal and without magmeal served as the control (Table 1).

2.2. Experimental conditions

Feeding of the experimental diets was carried out in triplicates. The fish were manually fed 5% of their body weight in two portions per day at 9.00 and 15.00 for 56 days. The ration was completely consumed and has been established in previous experiments, not to limit fish growth (Ogunji and Wirth, 2000). Fish were weighed every two weeks and the quantity of food adjusted accordingly. Experimental tanks were cleaned regularly. Conductivity, pH, oxygen concentration and water temperature were measured regularly. The water was well aerated and oxygen concentration was kept above 7.6 mg/l. Temperature was maintained at 26±1 °C throughout the experiment.

Table 1
Ingredients and proximate nutritional composition of experimental diets (%)

Ingredients	Experimental diets						
	1 (control)	2	3	4	5	6	7
Fish meal (FM)	43	34	28	22	16	10.5	–
Magmeal	–	15	25	35	45	55	68
Soy meal (SM)	12	12	12	12	12	12	16
Sunflower oil	5	5	5	5	5	5	5
Vita/min mix [a]	4	4	4	4	4	4	4
Potato starch	36	30	26	22	18	13.5	7
Dry matter (dm)	92.4	93.4	93.6	94.2	94.5	94.8	95.6
Crude protein	38.1	37.2	37.0	36.0	35.6	35.0	34.0
Crude fat	10.0	12.3	13.1	14.2	17.0	17.2	17.4
Ash	12.03	13.6	14.8	15.9	16.9	18.4	20.3
NFE [b]	39.9	36.9	35.1	33.9	30.5	29.4	28.3
Gross energy (kJ g^{-1}) [c]	20.11	20.29	20.24	20.22	20.65	20.38	20.04
P/E ratio [d]	18.95	18.34	18.28	17.80	17.24	17.18	16.97

[a] Vitamin and mineral mix (Spezialfutter Neuruppin — VM BM 55/13 Nr. 7318) supplied per 100 mg of dry feed: Vitamin A 12,000 I.E; Vitamin D3 1600 I.E; Vitamin E 160 mg; Vitamin K3 6.4 mg; Vitamin B1 12 mg; Vitamin B2 16 mg; Vitamin B6 12 mg; Vitamin B12 26.4 µg; Nicotinic acid 120 mg; Biotin 800 µg; Folic acid 4.8 mg; Pantothenic acid 40 mg; Inositol 240 mg; Vitamin C 160 mg; Antioxidants (BHT) 120 mg; Iron 100 mg; Zinc 24 mg; Manganese 16 mg; Cobalt 0.8 mg; Iodine 1.6 mg; Selenium 0.08 mg.

[b] Nitrogen free extract+fibre, (NFE)=100−(% protein+% fat+% ash).

[c] Calculated by: Crude protein=23.9 kJ g^{-1}; Crude fat=39.8 kJ g^{-1}; NFE=17.6 kJ g^{-1} (Schulz et al., 2005).

[d] P/E=Protein to energy ratio in mg protein/kJ energy.