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Effect of housefly maggot meal (magmeal) diets on catalase, and glutathione S-transferase in the liver and gills of carp *Cyprinus carpio* fingerling

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Abstract

Effect of housefly maggot meal (magmeal) diets on catalase, and glutathione S-transferase (GST) in the liver and gills of carp, *Cyprinus carpio* fingerling was studied. Eight iso-nitrogenous diets were formulated containing fishmeal and magmeal and in combination with soy meal to yield 41.0% crude protein dry matter. After 56 days feeding trial, weight gain and specific growth rate (SGR) of carp improved as fish meal was replaced in diets with magmeal up to an incorporation level of 45%. Results from GST indicate that experimental feed components did not contain unwanted chemicals, such as pharmaceutics or pesticides to critical concentrations that would adversely affect experimental fish performance. Inclusion of soy meal in diets did not confer any significant anti-oxidative and biotransformation stress effect on the fish but compromised the quantity of magmeal in carp diets able to bring about good performance when used without soy meal combination. Based on values of SGR, food conversion ratio (FCR) and enzyme activities, including magmeal above the level of 45% (diet A2) and below 67% (diet A3) in carp diets would improve optimal growth performance of carp. At such a level, magmeal is able to supply between 50% and 75% crude protein needed in the carp diet.

Keywords: *Cyprinus carpio*, Fishmeal, Fish meal replacement, Oxidative Stress, Housefly maggot meal

Introduction

A bulk (93%) of the total finfish production within developing countries in 2000 was contributed by omnivorous (and herbivorous) fish species, such as carp and tilapia (FAO 2006). As production of these fish species intensifies, higher feed inputs and nutritionally balanced diets are needed. Hardy (2000) estimated that the absolute amount of fish meal in feeds for carp will increase at a rate of 93% respectively till 2010. Given the huge production volume of farmed carp especially in developing countries (mainly Asia), significant increase in the usage of fish meal could be expected.

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Unfortunately, the cost of fishmeal and the pressure on the environment for the production of adequate quantity of fishmeal may slow down this anticipated growth in fish production (especially of carps) beyond 2010. This outlook has therefore given impetus to the research for alternatives to fishmeal. Magmeal is produced from the semi transparent larval stage of the housefly, *Musca domestica* and studies have shown that it is of high biological value. The percentage of crude protein ranges from 39 – 55% lipid 12.5 – 21% and crude fiber 5.8 – 8.2. Housefly maggot meal has been reported to have a balanced and rich amino acid profile (Spinelli et al. 1978; Ogunji et al. 2006) and contains higher methionine content than fishmeal (Ogunji et al. 2006, 2008a). Magmeal is also rich in phosphorous, trace elements and B complex vitamins (Teotia and Miller 1973). As a protein source in fish diets magmeal has been evaluated in tilapia and catfish species respectively (Adesulu and Mustapha 2000; Fasakin et al. 2003; Ajani et al. 2004; Ogunji et al. 2006, 2007a, 2008a). It was observed that magmeal can replace fishmeal at 100% dietary inclusion level in these species. One of the trials so far with carp (Ogunji et al. 2007b) indicates that the dietary inclusion of magmeal above the level of 45% would improve optimal growth performance of carp and provide outstanding economic advantage. In such a combination magmeal is able to supply above 50% crude protein needed in the carp diet. 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 including carp.

Emphasizing the effect of diets, Ogunji et al. (2007a) reported that 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. When fish are also fed diets containing substances or compounds capable of elevating biotransformation rate, oxidative stress can result. In any of these cases decreased growth and oxidative stress become impending. Rueda-Jasso et al. (2004), opine that a thorough knowledge of the fish's physiological condition and health is needed as a prerequisite to improving the nutritional profile of the diet.

Like all aerobic organisms, fish are susceptible to the attack of reactive oxygen species and have developed antioxidant defenses demonstrated by research primarily dating to the 1980s (Martínez-Álvarez et al. 2005). Specially adapted enzymes, such as catalase (CAT), superoxide dismutase (SOD), and enzymes dependent on glutathione (glutathione peroxidase, GPX, and glutathione reductase, GR) have been detected in most fish species investigated to date (Rudneva 1997). Together with these enzymes, lower-molecular- weight antioxidants, such as carotenoids, vitamins E, K and C, amino acids, and peptides (glutathione), have been detected in antioxidant defenses in fish.

This study assesses the influence of housefly maggot meal (magmeal) diets on both, growth and nutrient storage, and on the biotransformation and anti-oxidative response in the liver and gills of carp (*C. carpio*) fingerlings. The activities of catalase (CAT) and glutathione S-transferases (GST) respectively as the main anti-oxidative and phase II biotransformation enzyme were evaluated to further determine if magmeal may be harmful to fish. The growth parameters were evaluated to establish the nutritional quality of magmeal as a feed stuff for carps. Ichthyo-biochemical/physiology would be useful in the assessment of suitability of feeds and feed mixtures for fish nutrition (Ogunji et al. 2007a)

Materials and methods

Culture system

Experimental fish were reared in three recirculation systems, each comprising of nine tanks (individual volume: 50 × 30 × 30 cm) and a filtration unit with a sedimentation chamber for settlement of particulate matter and a biological filter. Mean and standard deviation (±) of water temperature, pH, O_2-content and conductivity (measured with WTW multi 340 I, Weilheim, Germany) during the experiment were similar in all recirculation systems: 25.95 °C (± 0.52); 7.63 (± 0.49); 8.51 mg/l (± 0.45 and 824 µsm/cm (± 2.31). The average concentration of total ammonia during the experiment was 0.25 mg l^{-1} and no critical values were detected for NO_2 and NO_3.

Feedstuff and formulation of experimental diets

Magmeal used for this study was got from Nigeria and produced using the semi-transparent larval stage of housefly, *Musca domestica* that grew on poultry droppings. The droppings were kept in a moist state to attract houseflies that laid eggs. Maggots emerged within 8 h to 3 days (Adesulu and Mustapha 2000; Ajani et al. 2004). The best conditions for the larval development are at a temperature of 27 ºC and moisture of 600 –750 g/kg like it is found in

fresh animal droppings (Kling and Wöhlbier 1974). Fishmeal and magmeal formed the major dietary protein sources.

Formulation and chemical composition of experimental diets and some feed ingredients are shown in Table 1 and 2. Eight iso-nitrogenous diets (C; A1 – A4; B1 – B3) were formulated to yield a content of around 41% crude protein dry matter (dm) using fishmeal and magmeal as major dietary protein sources. Diets B1 – B3 however, contained a non-varying amount of soy meal. Fishmeal concentration in the diets decreased with increasing concentration of magmeal. Diet C, formulated with the highest inclusion level of fish meal and without magmeal served as the control. Silicate gel was used to balance the nutrient content of the diets. All dry diet components, including vitamin and mineral mixtures, were thoroughly mixed with oil. Water was added and the feed extruded into sizes 1 mm diameter. The wet pellets were dried for 3 days at room temperature and afterwards stored in a cold chamber at -2 °C until used.

Table 1. Formulation and proximate nutritional composition (% dry matter) of experimental diet

	Diets							
Component	C[1]	A1	A2	A3	A4	B1	B2	B3
Fishmeal	62.0	52.0	30.0	15.0	-	-	30.0	15.0
Magmeal	-	15.0	45.0	67.0	86.0	76.0	36.0	57.0
Soy meal	-	-	-	-	-	13.0	12.0	12.0
Wheat flour	16.0	15.0	13.0	1.00	10.0	8.0	9.0	6.0
Fish oil	7.0	6.0	3.0	-	-	-	2.5	3.5
Canola oil	8.0	6.0	3.0	2.0	1.0	-	3.0	3.5
Vit./Min Mix[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Silicate gel	5.0	4.0	4.0	4.0	1.0	1.0	5.5	1.0
	Proximate composition							
Dry matter (Dm)	95.3	95.1	95.4	94.5	92.7	92.8	92.6	93.2
Crude protein	41.7	41.8	41.0	41.1	40.1	40.4	42.7	41.3
Crude lipid	20.5	21.5	21.7	21.9	25.5	24.1	20.4	26.4
Crude ash	18.1	17.0	16.3	16.0	12.6	12.3	17.4	12.4
NFE [3]	19.8	19.7	21.0	21.0	21.8	23.2	19.5	19.9
Gross energy (kJ /g)[4]	21.61	22.01	22.13	22.24	23.57	23.33	21.76	23.88
P/E ratio[5]	19.30	18.99	18.53	18.48	17.01	17.32	19.62	17. 29
Amino Acids								
Alanine	4.7.1	4.70	4.58	3.78	3.82	3.78	3.06	4.17
Arginine*	2.5.2	2.92	2.61	2.58	2.82	2.78	3.52	3.44
Aspartic acid	3.4.2	6.35	6.51	5.81	6.21	6.09	6.71	6.43
Glutamic acid	12.2.1	12.30	11.97	9.84	10.32	11.10	11.91	11.02
Histidine*	2.33	2.10	3.02	2.52	3.08	2.86	2.58	2.96
Isoleucine*	3.3.1	3.27	3.34	2.83	2.95	2.86	2.58	2.96
Leucine*	1.6.9	1.85	1.68	1.41	1.44	1.50	1.61	1.53
Lysine *	2.5.7	2.68	2.64	2.20	2.34	2.35	2.50	2.39
Phenylalanine*	3.6.1	3.45	4.07	3.99	4.47	2.53	2.24	2.47
Serine	2.6.4	2.68	2.62	2.14	2.18	2.47	2.61	2.50
Taurine	1.2.8	1.34	1.09	1.37	1.06	0.57	0.30	0.47
Threonine*	1.8.0	1.90	1.93	1.33	1.40	1.87	1.94	1.72
Tryptophan*	0.22	0.29	0.19	0.30	0.16	2.06	1.82	1.75
Tyrosine	0.44	0.24	0.35	0.36	0.45	0.98	0.74	1.07
Valine*	2.83	2.81	2.99	2.5.9	2.77	2.81	2.84	2.79
Total	45.58	48.88	49.59	43.05	45.47	46.61	46.96	47.67

*Essential Amino Acids [1]C = Control. [2]Vitamin and Mineral mix (Spezialfutter Neuruppin - VM BM 55/13 Nr. 7318) supplied per 100g of dry feed: Vitamin A 12000 I.E; Vitamin D3 1600 I.E; Vitamin E 160mg; Vitamin K3 6.4mg; Vitamin B1 12mg; Vitamin B2 16mg; Vitamin B6 12mg Vitamin B12 26.4µg; Nicotinic acid 120mg; Biotin 800µg; Folic acid 4.8mg; Pantothenic acid 40mg, Inositol 240mg; Vitamin C 160mg; Antioxidants (BHT) 120mg; Iron 100mg; Zink 24mg; Manganese 16mg; Cobalt 0.8mg; Iodine 1.6mg; Selenium 0.08mg. [3]Nitrogen free extract + fibre, (NFE) = 100 - (% protein + % fat + % ash). [4]Calculated by: Crude protein = 23.9 kJ k/g Crude lipids = 39.8 kJ k/g, NFE = kJ /g (Schulz et al. 2005); [5]P/E = Protein to energy ratio in g protein $(kJ)^{-1}$ gross energy.

Experimental fishes and feeding trial

Experimental fish were obtained from Warmwasserfischzucht Kraftwerk Jäntschwalde, Brandenburg, Germany, three days after hatching. They were brought to the facilities of Institute of Freshwater Ecology and Inland Fisheries Berlin, Germany, where the carps were reared and acclimatized for six weeks. Prior to the commencement of growth trial, ten carps each with an initial average body weight of 0.74 ± 0.01 g were randomly distributed in 24 tanks. Experimental diets were assigned to the carps in triplicate tanks respectively in a way that all feeding groups were represented in the three recirculation systems evenly. The fish were given restricted ration at a level of 15% body weight in 6 portions at 8.00, 10.00, 12.00, 14.00, 16.00, 17.30 hours, respectively per day. Bryant and Matty (1981) suggested that carp fry (100 mg to 3g) required 10 – 15% body weight per day for optimum growth at water temperatures of 24 °C. This feeding level was reduced to 10% body weight after three weeks when fish failed to consume their total ration. The fish were weighed every two weeks and quantity of food adjusted accordingly. Experimental tanks were cleaned regularly, and the trial lasted for 43 days (Molnar et al. 2006).

Enzyme preparation and measurement of activity

At the end of the experiment, all fish were killed and weighed individually. Liver and gills of four randomly taken fish from each tank was excised, shock frozen in liquid N_2 and stored at -80 °C. Enzyme extraction was done according to Wiegand et al. (2000). Samples were homogenized adding sodium-phosphate buffer (0.1 M, pH 6.5), containing 1.4 mM dithioerythritol (DTE), 20% glycerol and 1 mM ethylenediaminetetraacetic acid (EDTA). Membranes were discarded by centrifugation and cytosolic proteins concentrated by ammonium sulphate precipitation, centrifugation and re-suspension of the pellet 1 mL of 20 mM sodium-phosphate buffer (pH 7.0) and desalting via sephadex columns. Protein extracts were stored at -80 °C until enzyme activity assays. The activity of glutathione S-transferase (GST: EC 2.5.1.18) was measured in the soluble (cytosolic) fraction according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The catalase (CAT: EC 1.11.1.6) activity was measured according to Claiborne (1985). Enzyme activity is related to the protein content of the extract, which was assessed using bovine serum albumin as standard.

Proximate analysis

The freeze-dried samples of experimental diets were analyzed for proximate composition. Every analysis was carried out in duplicate and fish samples per tank. Protein (N × 6.25) was analysed using a Kjeltec System (Tecator) and crude fat using a Soxtec System HT (Tecator) with petroleum ether as the solvent. Ash was determined by burning in a muffle furnace at 550 °C for 10 hours. Gross energy was calculated using the following values: crude protein = 23.9 kJ/g, crude lipids = 39.8 kJ/g and NFE = 17.6 kJ/g (Schulz et al. 2005). Acid Detergent Fibre (ADF) content of magmeal was determined following the method of Van Soest (1963). To determine the amino acid concentrations of the experimental diets, 5 mg of freeze-dried samples were hydrolyzed with 6N HCl at 110 °C for 24 hours. No protecting reagents were added to avoid destruction of sulphur amino acids and methionine values determined were very insignificant and are not reported here. Other analytical procedures for amino acids followed the description of Ogunji and Wirth (2001).

Growth parameter analyses

From the experimental data obtained specific growth rate (SGR), food conversion ratio (FCR), percentage body weight gain (BWG), protein efficiency ratio (PER) and survival (%) were calculated as follows:

FCR = food fed/live weight gain;

$SGR = (\ln W_2 - \ln W_1 \times T_2\text{-}T_1^{-1}) \times 100$;

$\text{Body weight gain (BWG) (\%)} = [(W_2 - W_1) \times W_1^{-1}] \times 100$;

Protein efficiency ratio (PER) = live weight gain (g)/protein fed (g);

Where: W2 = final weight of fish, W1 = initial weight of fish and T1 and T2 = time (day);

$\text{Survival (\%)} = F_2 \times F_1^{-1} \times 100$

Where: F_1 = number of fish at the end of experiment, F_2 = number of fish at the beginning of experiment. All calculations were based on each of the triplicate tank per treatment.

Statistical analyses

All growth data were subjected to one way analysis of variance (ANOVA). The significance of difference between means was determined by Duncan's multiple range test ($P < 0.05$) using SPSS for Windows (Version 12). Values

are expressed as means ± SEM. One-way ANOVA, followed by Tukey's test ($P < 0.05$) using Statistica software was performed with the enzyme data. According to feeding groups the following comparisons were conducted: all feeding groups to control (diet C), A and B groups within each other, A2 to B2, A3 to B3. Pearson Correlation Coefficient was also used to compare the enzyme data.

Results

Growth performance, feed quality and utilization

Formulation and nutrient composition of experimental diets are shown in Table 1. Proximate composition of fish meal and magmeal used in this study is presented in Table 2. All diets were iso-nitrogenous, iso-energetic and have similar amino acid profile. The inclusion of soybean in diets B1 – B3 did not markedly affect the proximate composition of the diets.

Table 2. Proximate composition (% dry matter) of fish meal and magmeal[1] used in diet formulation

Proximate Composition	Fish Meal	Magmeal
Dry matter	80.52	92.43
Crude protein	66.40	46.56
Crude lipid	11.50	25.82
Ash	17.30	11.10
NFE[2]	4.80	16.52
ADF	-	13.78
Gross energy (kJ/g)[3]	21.29	23.36

[1]Values are mean of duplicate determinations. [2]Nitrogen free extract + fibre, (NFE) = 100 - (% protein + % fat + % ash). [3]Calculated by: Crude protein = 23.9 kJ /g Crude lipids = 39.8 kJ /g, NFE = 17.6 kJ /g (Schulz et al. 2005).

The results of growth performance in terms of BWG, SGR, FCR and PER of fingerlings carp fed experimental diets are shown in Table 3. During the experiment no mortality was recorded. Fish fed by diets C, A1 and A2 showed the significantly best BWG, SGR, FCR and PER. Fish fed diet A4 realized a significantly reduced performance. Reduction of fishmeal from 62% in the control diet to 53% (supplying 35.19% crude protein) and 30% (supplying 19.92% crude protein) respectively, by substituting with 15% magmeal (supplying 6.98% crude protein) in diet A1 and 45% magmeal (supplying 20.95% crude protein) in diet A2 improved final fish growth and performance. Diets high in magmeal impacted negatively on the growth of fish (particularly diets A4 and B1).

Table 3. Mean (± SEM) of growth performance parameter of *Cyprinus carpio* fed experimental diets*

Diet	Final weight (g) (per fish)	BWG (%)[1]	SGR[2]	FCR[3]	PER[4]	Survival (%)
C	7.90 ± 0.10^{a}	977.9 ± 12.1^{a}	5.53 ± 0.26^{a}	1.52 ± 0.02^{a}	1.72 ± 0.22^{a}	100
A1	9.44 ± 0.55^{a}	1163.9 ± 6.9^{a}	5.89 ± 0.13^{a}	1.38 ± 0.04^{a}	2.08 ± 0.13^{a}	100
A2	8.59 ± 0.66^{a}	1071.4 ± 2.0^{a}	5.71 ± 0.16^{a}	1.41 ± 0.05^{a}	1.91 ± 0.16^{a}	100
A3	6.35 ± 0.53^{b}	752.2 ± 76.5^{b}	4.97 ± 0.20^{b}	1.69 ± 0.09^{a}	1.37 ± 0.15^{b}	100
A4	4.38 ± 0.28^{c}	485.6 ± 47.6^{c}	4.09 ± 0.20^{c}	2.19 ± 0.21^{b}	0.91 ± 0.09^{c}	100
B1	4.48 ± 0.42^{c}	502.4 ± 57.0^{c}	4.15 ± 0.23^{b}	2.12 ± 0.22^{b}	0.92 ± 0.17^{c}	100
B2	6.61 ± 0.27^{b}	804.8 ± 37.2^{b}	5.12 ± 0.10^{a}	$1.66 \pm .04^{ab}$	1.38 ± 0.06^{b}	100
B3	4.88 ± 0.36^{c}	564.7 ± 58.9^{c}	4.39 ± 0.21^{b}	2.09 ± 0.17^{b}	1.00 ± 0.09^{c}	100

*Values are mean of triplicate feeding groups; within each column figures with different superscript letter differ significantly ($P < 0.05$); [1]Body weight gain (BWG) (%) = $[(W_2 - W_1)/ W_1] \times 100$; [2]Specific growth rate (% day^{-1}) = $(\ln W_2 - \ln W_1/ T_2 - T_1) \times 100$ [3]Food conversion ratio = total diet fed (g)/live weight gain (g); [4]Protein efficiency ratio (PER) = live weight gain (g)/protein fed (g).

The inclusion of soybean in diets B1 – B3 did not significantly improve values of BWG, SGR, FCR and PER compared to control diet. However, fish fed Diet B2 had a comparable FCR and SGR with those fed control diet. Those fed diet B1 and B3 were comparable to fish fed diet A4 that showed performance poorer than diets A1 – A3. Diets A4, B1 and B3 recorded high gross energy but reduced PE ratio.

Anti-oxidative and biotransformation response in the liver and gill

In the liver, CAT activities were highest in the feeding group A1 (Fig. 1). Then the activities decreased with increasing magmeal in the diets, but A2 was still higher than the control and the corresponding B2 group having comparable magmeal but addition of soy meal. A3 did not differ from B3. Within the B feeding groups, only B2 was lower than the control and the corresponding A2 group.

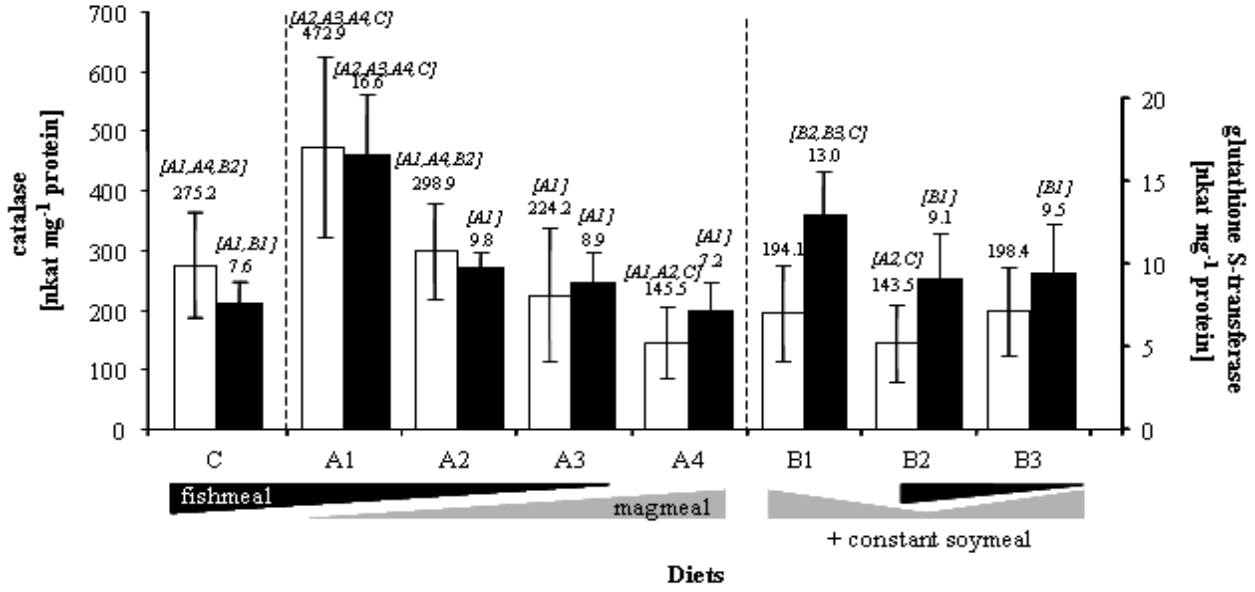

Fig. 1. Activities of antioxidant enzyme catalase (white bars, left y-axis) and biotransformation enzyme glutathione S-transferase (black bars, right y-axis) in liver of carp of the feeding groups.

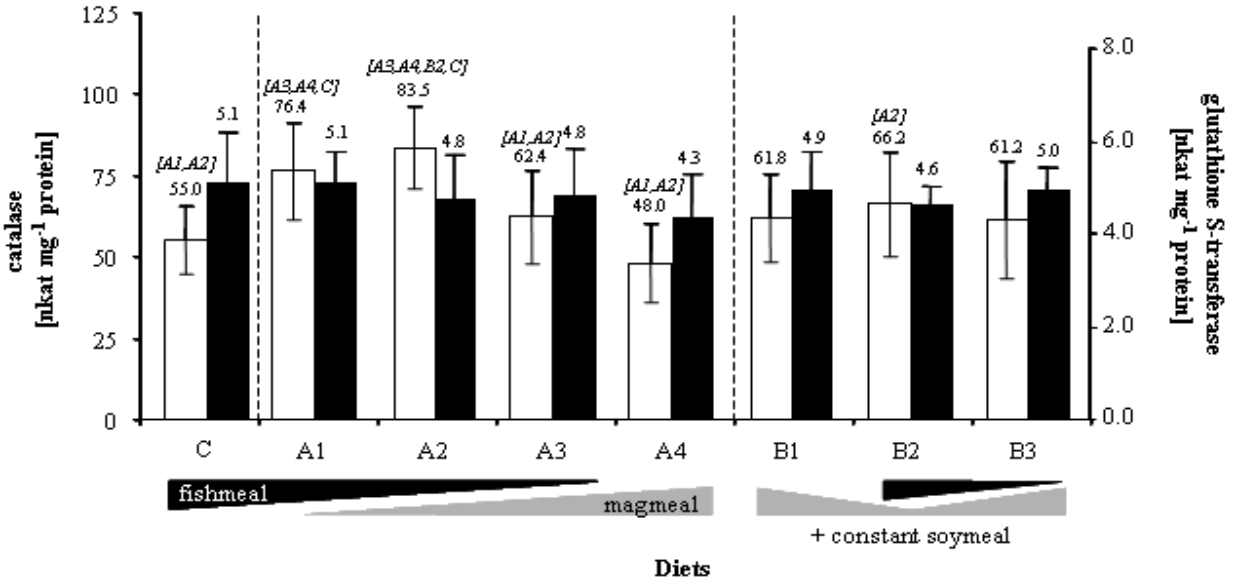

Fig. 2. Activities of antioxidant enzyme catalase (white bars, left y-axis) and biotransformation enzyme glutathione S-transferase (black bars, right y-axis) in gills of carp of the feeding groups.

Similar to CAT, also GST activity of A1 was higher than of the control group and the other A groups. GST activities of A2-A4 decreased with increasing magmeal, but were not different from control. Within the B group, the pattern was alike, only B1 significantly higher than control. No differences occurred between the diets having comparable magmeal but some replacement of fishmeal by soy meal (A2-B2; A3-B3). Therewith, highest enzyme activities were measured in carps fed with 52% fishmeal and 15% magmeal as protein source (diet A1).

In the gills, similar to the liver, activities of CAT were higher in A1 and A2 feeding group compared to control and the other A groups (Fig. 2). CAT activity of A2 was furthermore higher than in the B2 group, and, as in the liver, A3 and B3 did not differ to control or between each other. CAT activity of the B group did not differ from control or within each other; just B2 had lower activities than A2. GST activity in gills did not vary significantly throughout the experiment between diets of type A or B or compared to the controls.

Discussion

In this study it is shown that magmeal can be used as a good alternative protein source in carp diets. Replacement of up to 50% crude protein need of carp by magmeal or a magmeal dietary inclusion of 45% resulted in better growth compared to the fishmeal only diet. Further increase of magmeal above 67% dietary inclusion level resulted in diminished growth performance of carp. In contrast 100% replacement has been reported for tilapia, *Oreochromis niloticus* without any growth inhibition (Ajani et al. 2004; Fashina-Bombata and Balogun 1997; Ogunji et al. 2008a).

The reduced growth performance when magmeal completely substitute fishmeal may be due to inefficient utilisation of magmeal protein by carp resulting from low digestibility. Magmeal used for diet formulation contained an acid detergent fibre (ADF) concentration of 13.78%. Most fish can tolerate up to 8% fibre in their diets, but higher concentrations (8 to 30%) depress growth (Edwards et al. 1977; Hilton et al. 1983; Poston 1986). According to Schwarz and Kirchgeßner (1982) carp is not able to digest fibre. High levels of fibre in fish diets are able to impair calcium (Ca), lipid and fat-soluble nutrient absorption, between others (Lall and Lewis-McCrea 2007). Fasakin et al. (2003) reported that the reduction in growth performance of African catfish fed full-fat maggot meal may be, among other reasons, due to low protein digestibility of magmeal. Consequently, a study on digestibility of magmeal in diets of young carps would be needed.

The weight gain and SGR of carp improved as fish meal was replaced in experimental diets with magmeal up to an incorporation level of 45% (diet A2). There seems to be a form of nutrient fortification when fishmeal is combined with magmeal in the diet of carp up to a certain level. It is, however, unclear which particular nutrients that were improved. Adesulu and Mustapha (2000) and Ogunji et al. (2006, 2008a) reported that levels of some essential amino acids in magmeal were higher than in fish meal and soybean meal.

FCR for carp fed diets A1 – A3 containing various percentages of magmeal were not significantly different, ranging from 1.38 (diet A1) to 1.69 (diet A3). These values were comparable to the results of Khan et al. (2003) and Lenka et al. (2005) for fingerling carps. De Silva and Anderson (1995) suggested that low FCR values indicate an improved feed outcome. Incorporation of magmeal in carp diets, above the level 45% (diet A2: FCR 1.41) and below 67% (diet A3: FCR 1.69) would seem to enhance optimal growth performance of carp and provide outstanding economic advantage.

The inclusion of soybean in diets B1 – B3 affected significantly the values of BWG, SGR, FCR and PER compared to control diet. The fish groups fed Diet B2 had a comparable FCR and SGR with those fed control diet (Table 4). This indicates that dietary soy meal inclusion compromised the quantity of magmeal in carp diets able to bring about good performance when used without soy meal combination. Soy meal is characterised by an insufficient methionine and lysine content (Viola et al. 1981). Carp nevertheless might be able to meet partially the requirements for sulphur amino acids by cystine and phenylalanine by tyrosine (Nose 1978). However, the effect of amino acid content of the diets may not play any major role in this case since the amino acid profile of diets B1 – B3 was similar to those of diet C and A1 – A3 that performed better.

Even though carp seems to be more tolerant to anti-nutritional factors in soybean than other species (Viola et al. 1983), it is worth mentioning that the soy meal used for diet formulation was industrially proceeded and treated for anti-nutritional factors. The reduced protein to energy ratio of diets B1, B3 as well as A4 may be the reason for the poor performance (Table 1). This was due to slight increase in crude lipid content and consequently gross energy content resulting from the high fat content of magmeal and soy meal. Ogunji et al. (2008c) observed a decreased

weight gain, FCR and SGR when the dietary protein to energy ratio (P/E ratio) of tilapia diet decreased from 17.6 in diet 1 to 15.6. They stressed the importance of maintaining a proper ratio of protein to energy in the diet, noting that excessive energy can cause reduced feed intake and will result in decreased growth rates.

Since the liver is the first organ that receives absorbed nutrients from the digestive tract, activities of the biotransformation enzyme GST and the antioxidant enzyme CAT were much higher in all treatment groups compared to activities in gills. The lipid content of experimental diet may have also contributed to this. Rueda-Jasso et al. (2004) reported that activity levels of anti-oxidant enzymes CAT and SOD were higher in livers of fish fed diets with high lipid level. Growth performance was best when fish were fed diet A1 having 52% fishmeal and 15% magmeal as protein sources, and this was paralleled by highest activities of both CAT and GST. On the other hand, total replacement of fishmeal by magmeal (86%, diet 4) resulted in lowest growth performance and also in lowest enzyme activities of CAT and GST.

Liver CAT activities are in good correlation to the SGR ($r^2 = 0.58$) and the PER ($r^2 = 0.69$), suggesting higher enzyme activities at higher growth rates and at increased protein efficiency ratios. This is only partly mirrored by decreasing fishmeal and increasing magmeal content, as growth performance and CAT activity in the group receiving the fishmeal only diet resulted was 3rd best respectively 3rd highest. Consequently, in this study, it seems that enhanced CAT activities in the carp liver are ruled mainly by higher metabolic rates due to higher growth rates. Presumably the oxidative stress to which CAT is responding originates from high oxidative catabolism. In gills, alterations of CAT activities were not as pronounced as in liver, but CAT still reflected similar tendency in activities correlating to growth performance.

Liver GST however, did not show similar correlation, apart from the highest and lowest activities, corresponding to highest and lowest growth performance. In gills GST did not show any significant changes. From GST activities we can conclude, that the components used for feed preparation seemed not to contain unwanted chemicals, such as pharmaceutics or pesticides to critical concentrations that would cause adverse effects on the fish. Ogunji et al. (2007a) reported that the activities of glutathione S-transferases (GST) can be used to prove, if fish diets may contain compounds elevating the biotransformation rate. On the other hand, in the detoxification of organic environmental pollutants, GSTs also 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). Similarly it has been reported that magmeal is well utilized by the *Oreochromis niloticus* and its incorporation into tilapia diets seems to have no oxidative stress generating effect on fish metabolism and did not contain any compound that stimulates the generation of reactive oxygen species (Ogunji et al. 2007a). Magmeal also does not cause any form of physiological stress when fed to tilapia fingerlings (Ogunji et al. 2008b).

The data obtained during this experiment suggest that the replacement of fishmeal by magmeal in artificial diets of carp fingerlings when looking at anti-oxidative and biotransformation stress responses produced minor effects on CAT and GST activities in both liver and gills. These responses may have originated from high oxidative catabolism and did not impinge on fish performance. Inclusion of soy meal in Diets B1 – B3 did not confer any significant anti-oxidative and biotransformation stress effect on the fish.

Based on values of SGR, FCR, and enzyme activities, including magmeal in the diet of carp, *Cyprinus carpio* containing 41% Crude protein above the level of 45% (Diet A2) and below 67% (Diet A3) would improve optimal growth performance of carp and provide outstanding economic advantage. At such a level, magmeal is able to supply between 50% and 75% crude protein needed in the carp diet.

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References

Adesulu EA, Mustapha AK. 2000. Use of housefly maggots as a fishmeal replacer in tilapia culture: a recent vogue in Nigeria. In: 5th Inter. Symp. On tilapia Aquaculture (Fitzsimmons, K. & Filho, J.C. eds.) Vol. 1, pp. 138. Rio De Janeiro, Brasil.

Ajani EK, Nwanna LC, Musa BO. 2004. Replacement of fishmeal with maggot meal in the diets of Nile tilapia, *Oreochromis niloticus*. World Aqua 35: 52-54.

Bryant PL, Matty AJ. 1981. Adaptation of carp (*Cyprinus carpio*) larvae to artificial diets: 1. Optimum feeding rate and adaptation age for a commercial diet. Aquaculture 23: 275-286.

Edwards DJ, Austreng E, Risa S, Gjedrem T. 1977. Carbohydrate in rainbow trout diet. 1. Growth of fish of different families fed diets containing different proportions of carbohydrate. Aquaculture 111: 31-38.

FAO 2006. The state of world fisheries and aquaculture. 2006. FAO Fisheries Department, Rome, 165pp. ISBN 978-92-5-105568-7.

Fasakin EA, Balogun AM, Ajayi OO. 2003. Evaluation of full-fat and defatted maggot meals in the feeding of Clariid catfish *Clarias gariepinus* fingerlings. Aqua Res 34: 733-738.

Fashina-Bombata HA, Balogun O. 1997. The effect of partial or total replacement of fish meal with maggot meal in the diet of tilapia (*Oreochromis niloticus*) fry. J Prospects Sci 1: 178-181.

Felton GW. 1995. Oxidative stress of vertebrates and invertebrates. In: Ahmad, S. (Ed.), Oxidative Stress and Antioxidant Defenses in Biology. Chapman and Hall, New York, pp. 356-434.

George S, Young P, Leaver M, Clarke D. 1990. Activities of pollutant metabolising and detoxication systems in the liver of the plaice, *Pleuronectes platessa*: Sex and seasonal variations in non-induced fish. Comp Biochem Physiol 96C: 185-192.

Habig W, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferase: the first step in mercapturic acid formation. J Biol Chem 249: 1730-1739.

Halliwell B, Gutteridge JMC. 1996. Lipid peroxidation: a radical chain reaction. In: Free Radicals in Biology and Medicine (Halliwell, B. & Gutteridge, J.M.C. eds), pp. 188-266. Clarendon Press, Oxford.

Hardy RW. 2000. New developments in aquatic feed ingredients, and potential of enzyme supplements. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MA, Civera-Cerecedo R, (Eds.). Avances en Nutrición Acuicola V. Memorias del V Symposium Internacional de Nutrición Acuicola. 19-22 Noviembre, 2000. Mérida, Yucatán, Mexico: 216-226.

Hilton JW, Atkinson JL, Slinger SJ. 1983. Effect of dietary fibre on the growth of rainbow trout (*Salmo gairdneri*). Can J Fish Aquat Sci 40: 81-85.

Khan MN, Parveen M, Naqvi SMHM. 2003. Effect of replacement of fish meal by soybean and sunflower meal in the diet of *Cyprinus carpio* Fingerlings. Pakistan J Biol Sci 6: 601-604.

Kling M, Wöhlbier W. 1974. Handelsfuttermittel 1: Gesamtschau, Futtermittel tierischer Herkunft, Fette und Öle, Zusatzstoffe. Eugen Ulmer, GmbH, Stuttgart, 526-535.

Lall SP, Lewis-McCrea LM. 2007. Role of nutrients in skeletal metabolism and pathology in fish — An overview. Aquaculture 267: 3-19.

Lenka S, Giri SS, Hota PK. 2005. Performance of *Cyprinus carpio* (var. communis) fingerlings fed on diets containing water washed neem (*Azadirachta indica*) seed cake. Deutsher Tropentag 2005 Stuttgart-Hohenheim, October 11-13, 2005, Conference on International Agriculture Research for Development.

Martínez-Alvarez RM, Morales AE, Sanz A. 2005. Antioxidant defenses in fish: Biotic and abiotic factors. Rev Fish Biol Fisheries 15: 75-88.

Molnar A, Szabo A, Sazbo G, Sazbo C, Hancz C. 2006. Effect of different fat content and fat type on the growth and body composition of intensively reared pikeperch *Sander lucioperca* (L.). Aqua Nutr 12: 173-182.

Nose T. 1978. Summary report on the requirements of essential amino acids for carp. Proc. World Symp. On Finfish Nutrition and Fish feed Technology, Hamburg 20 - 23 June,1978. Vol. I, Heenemann GmbH, Berlin, 145-156.

Ogunji JO, Wirth M. 2001. Alternative protein sources as substitutes for fish meal in the diet of young Tilapia *Oreochromis niloticus* (Linn.). Israeli J Aqua – Bamidgeh 53: 34-43.

Ogunji JO, Kloas W, Wirth M, Schulz C, Rennert B. 2006. Housefly maggot meal (Magmeal): An emerging substitute of fishmeal in tilapia diets. http://www.tropentag.de/2006/abstracts/full/76.pdf.

Ogunji JO, Nimptsch J, Wiegand C, Schulz C. 2007a. 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. Comp Biochem Physiol 147A: 942-947.

Ogunji JO, Sutter D, Rennert B, Kloas W, Schulz C. 2007b. Growth performance and body composition of carp (*Cyprinus carpio*) fed diets containing housefly maggot meal (magmeal). Berichte des IGB, 140-148.

Ogunji, JO, Kloas W, Wirth M, Schulz C, Rennert B. 2008a. Housefly maggot meal (magmeal) as a protein source for *Oreochromis niloticus* (Linn.). Asian Fisheries Sci 21: 319-331.

Ogunji, JO, Kloas W, Wirth M, Neumann N, Pietsch, C. 2008b. Effect of Housefly Maggot Meal (Magmeal) diets on the Concentration of Plasma Glucose, Cortisol and Blood characteristics of *Oreochromis niloticus* fingerlings. J Anim Physiol Anim Nutr 92: 511-518.

Ogunji JO, Rahat-ul-Ain S, Schulz C, Kloas W. 2008c. Growth performance, nutrient utilization of Nile Tilapia *Oreochromis niloticus* fed housefly maggot meal (Magmeal) Diets. Turk J Fisheries Aquat Sci 8: 141-147.

Poston HA. 1986. Response of lake trout and rainbow trout to dietary cellulose. Fish and Wildlife Technical Report No. 5, pp. 1. Washington, D.C.: U.S. Fish and Wildlife Service.

Rudneva II. 1997. Blood antioxidant system of Black Sea elasmobranch and teleost. Comp Biochem Physiol 118C: 255-260.

Rueda-Jasso R, Conceica˜o LEC, Dias J, De Coen, W, Gomes E, Rees JF, Soares F, Dinis MT, Sorgeloos P. 2004. Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese sole (*Solea senegalensis*) juveniles Aquaculture 231: 417-433.

Schulz C, Knaus U, Wirth M, Rennert B. 2005. Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (*Sander lucioperca*). Aqua Nutr 11: 1-11.

Schwarz FJ, Kirchgeßner M. 1982. Zur Bestimmung der Nährstoffverdaulichkeit beim Karpfen (*Cyprinus carpio L.*). 1. Mitt. Aquarienaufbau und Versuchsmethodik. Bayer Landwirtsch Jahrb 59: 79-84.

Spinelli J, Mahnken C, Steinberg M. 1979. Alternative sources of protein for fish meal in Salmonid diets. In: Finfish Nutrition and Fish feed Technology (Halver, J.E. & Tiews, K. eds.), 2: 132-143.

Teotia JS, Miller BF. 1973. Fly pupae as a dietary ingredient for starting chicks. Poultry Sci 53: 1830-1835.

Van Soest PJ. 1963. Use of Detergents in the Analysis of Fibtous Feeds. II. A Rapid method for the determination of fibre and lignin. J Analyt Chem 46: 829-835.

Viola S, Arieli Y, Rappaport U, Mokady S. 1981. Experiments in the nutrition of carp, Replacement of fishmeal by soyabean meal. Israeli J Aqua-Bamidgeh 33: 35-49.

Viola S, Arieli Y, Mokady S. 1983. Effects of soybean processing methods on the growth of carp (*Cyprinus carpio*). Israeli J Aqua- Bamidgeh 32: 27-38.

Wiegand C, Pflugmacher S, Oberemm A, Steinberg C. 2000. Activity development of selected detoxication enzymes during the ontogenesis of the zebra fish (*Danio rerio*). Int Rev Hydrobiol 85(4): 413-422.