Deutscher Tropentag 2006
Stuttgart-Hohenheim, October 11-13, 2006
Conference on International Agricultural Research for Development

Housefly Maggot Meal (Magmeal): An Emerging Substitute of Fishmeal in Tilapia Diets

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Abstract
Several feed ingredients have been investigated in an attempt to substitute fishmeal in the diets of fish and livestock. These include both animal protein and plant protein sources. Unfortunately attempts to use these ingredients for complete replacement of the fishmeal component in tilapia diets have not entirely been successful. A major reason is the suboptimal content of essential amino acids in the diets especially methionine. Based on cost effectiveness, availability, crude protein content and amino acid profile, magmeal seem to be a candidate for replacement of fishmeal in fish diets. In this study, seven test diets (36% crude protein, dry matter) formulated by replacing fishmeal with magmeal were fed to triplicate groups of fifteen fingerlings (initial average weight 2.0±0.1g) for 56 days. Results obtained from growth parameters, protein utilisation, stress indicators and haematological parameters revealed no significant difference among all feeding groups. The observation suggests that magmeal can completely replace fishmeal in the diet of Tilapia *Oreochromis niloticus* fingerling and can meet the nutrient requirements of this species.

Introduction
Approximately 30% of the total fish catch are converted to fish meal and fish oil for the use in animal and fish feed actually. The percentage of the fish meal used for aquaculture feeds has increased from 10% in 1988 to around 45% in 2002 and the actual amount of fish harvested to produce fish meal has remained constant at 30 million/year (FAO 1999). Aquaculture is likely to grow over the next 20 years and some experts are concerned that rising demand for fish meal and fish oil could place heavier fishing pressure on already threatened stocks of fish used for feed (Delgado et al. 2003). It will also increase the already high cost of fishmeal in the world market.

Several attempts have therefore been made to find adequate substitutes for fish meal in fish diets. Unfortunately attempts to use protein sources of plant or animal origin as complete replacements for the fish meal component in fish diets showed varying success. Ogunji (2004) summarised some of the factors which may have contributed to the variation in the results obtained like protein composition and amino acid profile of alternative feeds; apparent digestibility of feeds; phosphorus content of alternative feeds; anti-nutritional factors in alternative feeds (especially in protein sources of plant origin) and palatability/acceptability of alternative feeds.

A relatively new approach is the use of insects as a source of animal protein in fish nutrition. Bondari and Sheppard (1981) stressed that insects in various developmental stages have been used to feed fish and farm animals. Hickling (1962) and Kling and Wöhlbier (1974) noted that silkworm pupae have been an important component of carp diet in Japan and China. Newton et al. (1977) used dried ground soldier fly larvae as a dietary supplement for young pigs with success. Interestingly study of the use of housefly maggot meal (magmeal) as substitute for fish meal in fish diets have increased in recent times (Adesulu and Mustapha, 2000; Fasakin et al., 2003; Ajani et al., 2004).

Maggots are produced from the semi transparent larval stage of the housefly *Musca domestica* and are used to process magmeal. Studies have shown that magmeal is of high biological value. The percentage of crude protein ranges from 39–61.4%, lipid 12.5–21%, and crude fiber 5.8–8.2%. Magmeal is also rich in phosphorus, trace elements and B complex vitamins (Teotia and Miller, 1973). Examination of the comparative amino acid profiles of fish and fly larvae protein showed that no essential amino acid was limiting. Spinelli et al. (1979) used magmeal protein in the diets of rainbow trout. The protein provided growth and feed conversion levels equivalent to fish meal at substitution levels ranging from 25-100%. Ajani et al. (2004) and Fashina-Bombata and Balogun (1997) reported that magmeal can replace up to 100 percent of fish meal in the diets of Nile tilapia *(O. niloticus)*. The authors concluded that the biological value of magmeal was equivalent to that of whole fish meal and that the larvae contained no anti-nutritional or toxic factors sometimes found in alternative protein sources of vegetable origin.

Until now the use of magmeal in fish nutrition is not connected with any economic advantage. According to Fashina-Bombata and Balogun (1997) the cost of harvesting and processing one kg of magmeal is less than 20% of cost of 1 kg of fish meal. Based on cost, availability, biological value and feed conversion ratio, magmeal is said to be a viable alternative to all fish meal in the diet of fish. It seems that the utilization of magmeal offers a good opportunity for the development of low cost fish feeds, especially in the developing countries where fish meal is imported very expensively and therefore not readily available. In this study multi-dimensional biological approach was used to evaluate the suitability of housefly maggot meal (magmeal) as an alternative protein sources for Tilapia (*Oreochromis niloticus* L.) fingerling. Growth parameters, protein utilisation, carcass composition, stress indicators and haematological parameters were examined.

Methods

House fly maggots produced in Nigeria on poultry droppings were used to process magmeal as described by Ajani et al. (2004) and Adesulu and Mustapha (2000). Seven test feeds with nominal 36% protein content were formulated with fishmeal and magmeal as the main protein sources (Table 1). Fifteen tilapia fingerlings (initial mean weight 2.0±0.1g) were stocked into tanks (28 x 28 x 51.5 cm) within a recirculation system with triplicate tanks for each feed treatment. The fish were manually fed 5% of their body weight in two portions per day at 9.00 and 15.00 for 56 days (Ogunji and Wirth 2000). Fish were weighed every two weeks and the quantity of food adjusted accordingly. Oxygen saturation was kept above 60% and temperature was maintained at 26 ± 1°C.

Table 1. Formulation and nutrient 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[1]	4	4	4	4	4	4	4
Potato Starch	36	30	26	22	18	13.5	7
Dry Matter	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	13.57	14.78	15.89	16.88	18.44	20.27
NFE[2]	39.9	36.93	35.12	33.91	30.52	29.36	28.33
Gross energy (kJ/g)[3]	20.11	20.29	20.24	20.22	20.65	20.38	20.04

[1]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

At the start of the experiment, 20 fish were sacrificed homogenised and kept frozen until analysed for whole- body composition. At the end of the experiment all fish were killed and individually weighed, then 21 from each feeding group (seven per replicate) were randomly selected and homogenised. Homogenised fish as well as diets and ingredients samples were freeze dried at a temperature of – 54 °C. All samples were analysed for proximate composition in duplicate. Protein (N x 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 factors: crude protein = 23.9 kJg^{-1}, crude lipids = 39.8 kJg^{-1} and NFE = 17.6 kJg^{-1} (Schulz et al. 2005). Amino acids analysed as described by Buchholz (1997) and Ogunji and Wirth (2001) with minor modifications that allowed for effective measurement. Fatty acid composition of fish samples and feed ingredients were analyzed using gas-liquid chromatography as described by Schulz et al. (2005) using triclosan acid as the internal standard.

At the end of the experiment three fish from each tank were randomly taken for blood sampling. Blood was collected from the vertebral blood vessels towards the caudal peduncle of each fish using separate heparinized syringes. The blood and plasma produced after centrifugation were analysed for haematocrit (PCV), whole blood haemoglobin (Hb), plasma glucose and cortisol. Hb concentration estimates were determined with cyanmetahemoglobin method that makes use of a spectrophotometer (wavelength = 540nm). PCV was determined by the microhaematrocrit method in which the blood samples were centrifuged for 5 minutes at 10,000rpm as described by Wedemeyer and Yasutake (1977). Cortisol was extracted from 50µl blood plasma using 1ml volume of 99.8% ethanol. The concentration was measured using a radioimmunoassay (RIA) technique as described by Kloas *et al*. (1994). The plasma glucose concentration was determined spectrophotometrically using the Glucose Liquicolor PAP kit (Rolf Greiner Biochemica GmbH, Flacht, Germany).

Specific growth rate (SGR) and food conversion ratio (FCR) were calculated as follows:
FCR = food fed/live weight gain; SGR = (ln W2 - ln W1/T2-T1) x 100.
Where: W2 = final weight of fish, W1 = initial weight of fish and T1 and T2 = time (day);
Survival % = $F_2/F_1 \times 100$; 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. 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 ± SE.

Results and Discussion

The amino acid and nutrient composition of fish meal and magmeal used in this study is compared in Table 2. Magmeal had low crude protein content (37.5% dry matter; dm). This is lower than the values reported previously (range 40 to 61.4% dm) (Teotia & Miller, 1973; Spinelli et al. 1979; Ajani et al., 2004). The differences may be due to processing, drying or storage methods. For example, Fasakin et al. (2003) suggested that variations in crude protein of magmeal may be related to the quality of poultry droppings given to maggots used to produce the magmeal. More work will be needed to standardise methods for magmeal production. The crude fat content of experimental feeds was influenced by the high fat content of magmeal (19.8% dm). It however, did not affect the fish growth but the fatty acid composition of fish body (not shown here). The dietary fat contents (10.0 – 17.4 %) were within the levels tolerable to *O. niloticus*. De Silva et al. (1989) used a similar level (12.00 – 15.20% lipid and 19.40 – 20.60 kJg^{-1} energy) for same species. According to NRC (1993) no definite percentage of dietary lipids can be given for fish diets without considering the type of lipid as well as the protein and energy content of the diet.

No significant differences were observed between different feeding groups in terms of fish final weight (13.16 – 17.17 g), SGR (3.45 – 3.76 % /day), and FCR (1.05 – 1.22; Table 3). This Indicates that magmeal was well utilized by the fish thus resulting in good fish performance. One point, in favour of magmeal over many other alternative protein sources especially plant protein may be its balanced amino acid (Table 2) particularly for tilapia. There seem to be no evidence that any amino acids are severely limiting

(Spinelli et al. 1979). Several other ingredients of animal origin (e.g. feather meal, poultry by-product meal and meat with bone meal) may not have been completely successful as fish meal substitutes due to their inferior amino acid profile compared to fishmeal (Abdelghany, 2003). Magmeal is also rich in phosphorus, trace elements and B complex vitamins (Teotia & Miller, 1973).

Table 2. Comparison of amino acid and nutrient composition (% dry matter) of fish meal and magmeal used in this study[1].

Components	Fish Meal	Magmeal
Dry Matter	91.0	96.4
Crude Protein	70.7	37.5
Crude Fat	7.8	19.8
Ash	18.3	23.1
NFE[2]	3.21	19.6
Gross energy (kJ g^{-1})[3]	20.6	20.3
Amino Acids		
Aspartic acid	3.74	1.69
Glutamic acid	2.69	2.53
Serine	1.92	1.47
Histidine*	1.76	1.90
Glycine	0.94	0.35
Threonine*	2.53	2.83
Arginine*	3.34	1.74
Alanine	3.60	1.64
Tyrosine	0.71	0.95
Tryptophan*	1.91	0.58
Methionine*	1.29	1.66
Valine*	0.95	0.50
Phenylalanine*	2.90	3.83
Isoleucine*	0.99	0.63
Leucine*	2.74	2.11
Lysine*	3.96	1.66

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

Table 3. Growth data and feed conversion ratio of *O. niloticus* fingerlings fed experimental diets*

Parameters	Experimental diets						
	1 (Control)	2	3	4	5	6	7
Initial weight (g)	2.08±0.1[a]	2.09±0.1[a]	2.09±0.1[a]	2.01±0.17[a]	1.93±0.2[a]	1.94±0.11[a]	1.91±0.10[a]
Final weight (g)	14.46±1.3[a]	15.39±2.3[a]	17.17±0.5[a]	15.97±2.1[a]	16.00±2.0[a]	14.37±0.1[a]	13.16±0.1[a]
Tot. feed intake (g)	13.95±0.8[a]	14.53±1.6[a]	15.83±0.8[a]	15.23±1.7[a]	14.90±1.6[a]	14.24±0.6[a]	13.73±0.5[a]
SGR	3.45±0.7[a]	3.53±0.2[a]	3.76±0.1[a]	3.68±0.1[a]	3.76±0.1[a]	3.58±0.1[a]	3.45±0.1[a]
FCR	1.14±0.1[a]	1.12±0.1[a]	1.05±0.0[a]	1.10±0.0[a]	1.06±0.0[a]	1.15±0.1[a]	1.22±0.1[a]
Survival %	95.6±4.4[a]	100±0.0[a]	93.3±6.7[a]	91.1±4.4[a]	76.8±3.3[b]	91.1±2.2[a]	73.3±6.6[b]

*Values represent mean ± SE of each replicate per treatment. Values in the same row with different superscript letters are significantly different ($P < 0.05$) from each other.

The mean values for haematocrit, plasma cortisol and glucose were not significantly different ($P<0.05$) among the feeding groups (Table 4). This shows that no physiological stressful condition was introduced in the fish by feeding magmeal diets. Stress is defined as a condition in which the dynamic equilibrium of animal organisms called homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors (Chrousos and Gold, 1992). It has been shown that nutritional deficiency is one of the main causes of stress in cultured fishes (Martins *et al*. 2002). The fish diet can certainly be one of these challenges depending on the feed nutrient composition. Fish react to

stress with a series of physiological changes called general adaptation syndrome, which was first described in mammals. Increased packed cell volume (PCV) values, increased whole blood glucose (hyperglycaemia) and plasma cortisol concentrations and decreased plasma chloride concentrations (hypochloremia) have all been associated with stressful conditions in mammals (Selye, 1950). Similar severe effects have also been reported in fishes (Wendelaar, 1997). No such physiological changes were observed in this study.

The observations suggest that magmeal is well utilized by *O. niloticus* thus enhancing good growth. Feeding tilapia fingerlings with magmeal diets did not result in any form of physiological stress at all inclusion levels. Hence magmeal can therefore completely replace fishmeal in the diet of Tilapia *Oreochromis niloticus* fingerling since it is able to meet the nutrient requirements of this species.

Table 4: **Haematocrit, Haemoglobin, Plasma Cortisol and Glucose concentrations of *Oreochromis niloticus* larvae fed magmeal diets at different dietary inclusion rates.***

Experimental Diets	Haemoglobin (Hb) (g dl^{-1})	Haematocrit (%)	Cortisol (ng ml^{-1})[1]	Glucose (mg dl^{-1})
1	5.96 ± 0.2^{a}	32.67 ± 1.0^{a}	9.16±3.6	43.01 ± 1.6^{a}
2	6.49 ± 0.2^{ab}	35.00 ± 2.4^{a}	30.71±24.1	40.96 ± 3.1^{a}
3	6.72 ± 0.2^{b}	32.67 ± 1.5^{a}	32.75±8.7	43.73 ± 2.7^{a}
4	6.95 ± 0.3^{b}	35.22 ± 2.1^{a}	31.83±7.3	40.25 ± 1.4^{a}
5	6.91 ± 0.3^{b}	37.44 ± 1.3^{a}	34.25±9.5	41.96 ± 3.7^{a}
6	6.97 ± 0.3^{b}	33.11 ± 1.2^{a}	25.67±9.7	38.44 ± 2.0^{a}
7	6.76 ± 0.2^{b}	36.57 ± 1.7^{a}	14.12±5.1	37.07 ± 2.1^{a}

*Figures on the same column with the same superscripts are not significantly different ($p<0.05$) Values represent mean ± SE of 9 fish per treatment. [1]Due to initial analytical problems that resulted in loss of some samples, not less than 3 cortisol values in any feeding group were used for calculation.

Acknowledgement
The first author is grateful to Alexander von Humboldt Foundation (AvH) Germany, for the award of a Post Doctoral Research Fellowship under which this work was performed.

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