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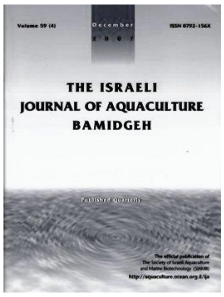

Effect of Total Fish Meal Replacement with Vegetal Protein Alone or Combined with Rendered Animal Protein on Growth Performance and Tissue Composition of European Catfish *(Silurus glanis L.)*

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Abstract

A feeding trial was conducted for six weeks to determine the effect of the replacement of fishmeal (FM) either with vegetal protein (mainly soybean) and rendered animal protein combined or vegetal protein alone on growth and carcass composition of European catfish, *Silurus glanis*. One hundred and thirty five fish (average weight 70.90±5.10g ±SD) were randomly distributed into three treatments in triplicate. Fish were fed with either of three isonitrogenous (35% crude protein) and isocaloric (16.70 MJ/kg DE) experimental diets. The diets were: fish meal (FM) based diet, fish meal protein replaced with a combination of vegetal protein and rendered animal protein (AP), and fish meal replaced with vegetal protein only (SM). Weight gain (%), specific growth rate, protein efficiency ratio, and apparent net protein utilization of FM diet fed groups were similar ($P>0.05$) with AP diet fed group being significantly higher ($P < 0.05$) than the SM group. Feed conversion ratio with FM diet was similar ($P < 0.05$) to that of the AP diet, and significantly lower than SM diet. The present experiment indicated that vegetal protein alone cannot replace total FM protein in the diet of *Silurus glanis*, however, vegetal protein and rendered animal protein combined, can replace total fish meal protein, with no detrimental effect on growth performance, feed utilization, and body composition.

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Introduction

European catfish *Silurus glanis*, is a favoured farmed fish species in Europe because of its large size, rapid growth, delicious meat, and high market price. It has been cultivated extensively in ponds in Central and Eastern Europe for over 100 years, but development of new diets and feeding regimes for cost effective production and satisfactory quality of the final edible product is crucial in order to establish viable European catfish farming.

To date, diets for European catfish have been based on high quality fish meal (FM). Fish meal is the most important and widely used protein ingredient in aquaculture, especially for the production of piscivorous fish species. However, with the expansion of aquaculture, increasing demand, uncertain availability, and high price has necessitated the search for alternate protein sources. Vegetal proteins, the most common of which is soybean meal (SM) (Kisssil and Lupatsch, 2004), which has high protein content and contains most of the essential amino acids required by fish, currently represent the only economic and sustainable protein alternative to fishmeal and are increasingly being used in commercial fish feed. However, soybeans are also used for human food, so there is a need to identify other protein-rich resources that could be used in aquatic diets. Finding other protein sources will reduce price volatility and European Union dependency on imports. Rendered by-products of terrestrial animals may also be a suitable alternative for replacement of fishmeal as a protein source (Altan et al., 2010). Meat and bone meal (MBM), one of the economically rendered animal by-products, has been widely used in poultry feed (Parsons et al., 1997; Wang and Parsons, 1998). MBM has relatively higher protein content, and better growth promotion effect in comparison with alternative plant proteins. Recently, MBM was introduced to replace FM in fish diets. Some studies have shown that MBM could partially replace dietary FM without affecting growth and feed efficiency of experimental fish (Davies et al., 1991; El-Sayed, 1998; Bureau et al., 2000; Webster et al., 2000). 80% of the fish meal can be successfully replaced with meat and bone meal (4:1) in grouper (*Epinephelus coioides*) feeds (Millamena, 2002).

Previous research has indicated that neither vegetal protein nor animal protein alone can replace total fish meal protein in the diet of carnivorous fish. The present experiment aims to study the effect on growth performance and body composition of total FM replacement with only vegetal protein or in combination with animal protein in the diet of European catfish (*Silurus glanis* L.)

Materials and Methods

Diets. Three isonitrogenous (35% crude protein) and isocaloric (16.70 MJ/kg DE) experimental diets were formulated: Fish meal (FM) based diet, fish meal protein replaced with a combination of vegetal protein and rendered animal protein (AP), and fish meal replaced with vegetal protein only (SM). All ingredients were ground before final mixing with a hammer mill and then blended with the oils. Ingredients were steam pelleted and dried in a forced air oven at 50^0C. The dry pellets were placed in covered plastic containers and stored at 4^0C. All diets were analysed in duplicate to establish their proximate composition.

Experimental animals and layout. Juvenile European catfish (*Silurus glanis*) were brought from a commercial farm in Hungary. Prior to the start of the six week experiment the juveniles were reared in a recirculation tank system and fed with fish meal for 2 weeks to acclimate them to the experimental conditions.

At onset of the experiment, the fish were fasted for 24 h and weighed. 135 juveniles (average individual weight 70.90 ± 5.10 g) were randomly distributed into three treatment groups with three replicates each in 9 glass aquaria (130x50x60 cm) equipped with a recirculation system. The water flow rate through the aquaria was about 2 L/min and water exchange was 10%/day. Continuous aeration was provided to all from a compressed air pump. During the 6 week experimental period the groups were fed with the three experimental diets respectively. Juveniles in all groups were fed twice a day with 2.5% of actual stock body weight. All water quality parameters were analysed on alternate days and found to be within the normal range for rearing of *Silurus glanis* (Copp et al., 2009).

Growth study. Fish in each aquarium were measured individually at the end of the experiment to assess weight gain. Growth performance of fish such as weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated based on the following standard formulae:

WG (%) = [(Final weight –Initial weight) / Initial weight] x 100

SGR = [ln Final weight – ln Initial weight/ Number of days] x 100

FCR = Total dry feed intake (g)/ wet weight gain (g)

PER = Net weight gain (wet weight) / Protein fed (dry weight)

ANPU = [(Final carcass protein - Initial carcass protein) / Protein fed] X 100

Sampling. At the end of experiment, five juveniles from each aquarium i.e fifteen juveniles from each treatment group, were randomly collected for proximate analysis. Another three juveniles from each aquarium of each treatment were anesthetized with clove oil (50 µl/L), sacrificed with a blow to the head, and dissected to collect the liver and visceral fat for determination of weight of liver and visceral fat (g/100 g fish).

Analysis of samples. Proximate composition of the feed and fish was analyzed by standard methods of AOAC (1990). Moisture was determined by drying the sample at 105^0C to a constant weight. Nitrogen content of the sample was analyzed using Kjeltec system (FOSS, Denmark), and crude protein (CP) was calculated by multiplying nitrogen percentage by 6.25. Lipid was determined using Soxtec system (FOSS, Denmark) using diethyl ether (boiling point, 40^0 - 60^0C) as a solvent, and ash content was estimated by incinerating the sample in a muffle furnace at 600^0C for 6 h. Crude fiber content was analyzed by alkali and acid digestion. Nitrogen free extract (NFE) was calculated by differences as, 100–(%CP + %EE + %ash + %fiber). The content of amino acids in different diets was determined by low pressure ion exchange chromatography according to European standards. The dietary energy value of experimental diets was calculated as described in Kumar et al., (2013).

Statistical analysis. Mean value of all parameters were subjected to one-way analysis of variance (ANOVA) to study the treatment effect, and Tukey and Tamhane's post hoc tests were used to determine the significant differences between the mean value. Comparisons were made at 5% probability level. All the data were analysed using statistical packages SPSS (Version 16; spss Inc., Chicago, IL, USA).

Results

For the proximate composition of the different experimental diets (% DM)see Table 1.

Table 1. Formulation and proximate composition of different experimental diets (% DM basis)

Ingredients (%)	*Experimental diets*		
	FM	*AP*	*SM*
Fishmeal[1]	19.00	0.00	0.00
Rendered animal protein[2]	0.00	17.00	0.00
Soybean meal, solvent extracted[3]	30.30	25.05	40.00
Corn[3]	26.50	27.14	16.48
Corn gluten[3]	12.20	18.80	19.10
Wheat[3]	10.00	10.00	10.00
Canola meal, solvent extracted[3]	0.00	0.00	10.00
Mono-calcium phosphate[3]	0.00	0.00	1.30
DL- Methionine[3]	0.00	0.10	0.00
L-lysine[3]	0.00	0.54	0.42
Sunflower oil[3]	1.50	0.87	2.20
Vitamin and Mineral Premix[3*]	0.50	0.50	0.50
Proximate composition (% DM basis)			
Dry matter	89.40	90.70	88.90
Moisture	10.60	9.30	11.10
Crude protein	34.55	35.6	34.65
Crude lipid	5.05	5.15	4.39
Crude fibre	0.44	1.52	3.62
Nitrogen free extract	52.86	51.83	51.74
Ash	7.10	5.90	5.60
Dietary energy (MJ/kg)	16.61	16.83	16.72

[1]HALTAP, Szarvas, Hungary.
[2]Animal feed production company (ATEV), Budapest, Hungary.
[3]UBM, Feed Kft, 2085 Pilisvörö svár, Fóv. 130.
*Vitamin and Mineral Premix (per kg): Vitamin A: 400 000 IU, Vitamin D_3: 200 000 IU, Vitamin E: 6000 mg, Vitamin K_3: 918 mg, Vitamin B_1: 500 mg, Vitamin B_2: 1200 mg, Vitamin B_6: 1000 mg, pantothenic acid: 3000 mg, folic acid: 500 mg, Vitamin C: 10 000 mg, Ca: 228 g, Fe: 6000 mg, Zn: 40 324 mg, Mn: 5022 mg, Cu: 1000 mg, Se: 22.5 mg, I: 496 mg, antioxidant: 2000 mg.

The protein content in all three diets averaged 35%. The amino acid compositions of the different experimental diets are presented in Table 2. The methionine and lysine content in all experimental diets were similar. At the end of experiment, the carcass compositions of *Silurus glanis* whole body were significant among the treatments (Table 3).

Table 2. Amino acid profile (% DM basis) of different experimental diets

Amino acid	*Diet*		
	FM	*AP*	*SM*
Cysteine	0.49	0.51	0.58
Asparagine	2.98	2.53	2.82
Methionine	0.76	0.79	0.80
Threonine	1.30	1.17	1.24
Serine	1.50	1.55	1.60
Glutamine	5.30	5.31	5.81
Proline	1.89	2.56	2.07
Glycine	1.73	2.27	1.28
Alanine	1.95	2.13	1.80
Valine	1.53	1.47	1.54
Isoleucine	1.38	1.24	1.40
Leucine	2.78	2.96	2.98
Tyrosine	1.23	1.22	1.35
Phenylalanine	1.52	1.49	1.62
Histidine	0.86	0.79	0.86
Lysine	1.86	1.81	1.89
Arginine	1.93	1.98	1.79

Table 3. Carcass composition (%) of *Silurus glanis* juveniles fed with different experimental diets (wet weight basis; mean ± standard deviation)

Carcass composition	*Diets*			
	FM	*AP*	*SM*	*Initial**
Dry matter	21.60	21.70	21.20	22.90
Moisture	78.40	78.30	78.80	77.10
Crude protein	14.46	14.57	13.96	13.84
Lipid	4.81	5.22	4.79	6.81
Ash	2.52	2.13	2.27	2.37

* Fish sample taken before start-up of the experiment

No mortality was registered in any of the groups throughout feeding experiment. The growth performance (weight gain and SGR) and feed efficiency (FCR, PER and ANPU) were significantly different ($P < 0.05$) among the treatment groups (Table 4).

Table 4. Effect of different treatments on the Growth parameters of *Silurus glanis* (mean ± standard deviation)

Parameters	*Diets*		
	FM	*AP*	*SM*
Initial weight (g)	70.3 ± 9.0	71.2 ± 10.0	71.2 ± 9.3
Final weight (g)	131.0 ± 23.3^a	121.4 ± 21.2^a	97.7 ± 13.0^b
Weight gain (%)	86.2 ± 8.6^a	70.5 ± 6.2^a	37.2 ± 6.6^b
SGR	1.26 ± 0.09^a	1.10 ± 0.12^a	0.70 ± 0.06^b
FCR	1.40 ± 0.08^b	1.64 ± 0.10^b	2.63 ± 0.18^a
PER	2.13 ± 0.17^a	1.84 ± 0.10^a	1.05 ± 0.11^b
ANPU	31.38 ± 2.60^a	26.73 ± 2.17^a	15.67 ± 1.36^b

Values within same row with different superscripts are statistically different ($P<0.05$)

The weight gain (%), SGR, PER, and ANPU, of fish fed FM or AP diets were similar ($P > 0.05$) but significantly higher than those of fish fed diet SM. Feed conversion ratio (FCR) of FM diet fed group were similar ($P > 0.05$) to the AP group, and lower ($P < 0.05$) than in the SM group. Visceral fat (%) and liver weight (%) of *Silurus glanis* did not differ (Figure 1).

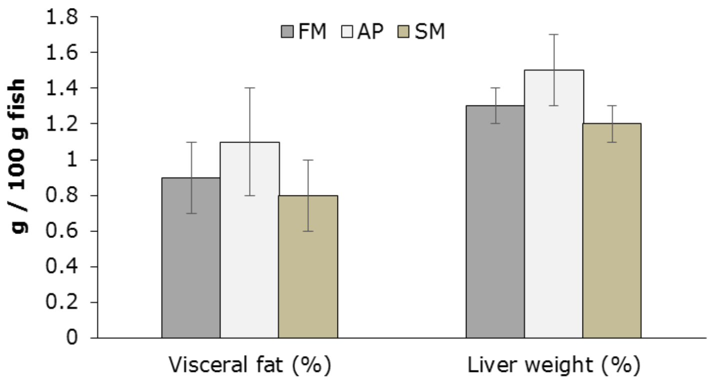

Figure 1. Effect of different treatments on visceral fat (%) and liver weight (%) of *Silurus glanis*.

Discussion*.*

Research has been conducted to examine the replacement of FM by different protein sources fed to different fish species, but very few studies have been conducted on European catfish, *Silurus glanis* (Bekcan et al., 2006). This is due to limited knowledge of the nutrient requirements of *Silurus glanis*. In the present study different experimental feeds were formulated according to the requirements of a related species, *Ictalurus punctatus* (Li et al., 2004). Soybean meal and rendered animal protein, similar to meat and bone meal have been shown to be nutritionally adequate protein sources for many species (Altan, 2010;; Robaina et al., 1997; Higgs et al., 1995). Essential amino acid deficiency is one of the factors limiting the utilization of less expensive protein sources as FM substitutes (Glencross et al., 2007). Compared to FM, SM proteins are lower in lysine content whereas rendered animal proteins are lower in methionine and lysine contents (Kissil and Lupatsch, 2004; Hertrampf and Piedad-Pascual, 2000). In the present study, test diets were formulated to be isonitrogenous and similar in methionine and lysine contents.

Whole body carcass composition of *Silurus glanis* was not affected by the replacement of FM either with soybean meal protein (SM diet) only, or SM protein combined with rendered animal protein (AP diet). This is in agreement with Takagi et al (2000) who reported no significant difference in whole body composition of red sea bream fed different level of poultry by-product meal. Similar results were also reported in grouper (Shapawai et al., 2007 and Gunben et al., 2014), in rainbow trout (Bureau et al., 2000) and in gilthead seabream (Robaina et al., 1997). However, significantly similar ($p > 0.05$) but higher carcass lipid content was found in the treatment group fed AP diet compared to FM and SM diet. This may be due to slightly higher lipid content in AP diet. This may also be caused by higher but significantly similar ($p > 0.05$) visceral fat content in AP treatment group. Higher liver weight of fish fed the AP diet were similar ($P > 0.05$) to other groups. This indicates that the inclusion of animal protein has little influence on body indices of *Silurus glanis* (Fig. 1).

The present study showed that the total replacement of FM with SM in the diet of *Silurus glanis* significantly decreased ($P < 0.05$) the growth performance, whereas no effect ($P > 0.05$) was seen in the treatment group fed a diet where FM was substituted with SM along with rendered AP. The limitation of use of alternative protein sources may be due to three factors: (i) lower feeding rate with replacement of FM by alternate protein (Robaina et al., 1997); (ii) lower digestibility of the alternate proteins (Bureau, et al., 1999); (iii) imbalance of the essential amino acids (Ai and Xie, 2005). The amino acid composition of different experimental feeds in the present study is quite similar, therefore lower feeding rate, lower digestibility, or both may be the cause of lower growth performance in the SM fed diet group. The reduced digestibility of SM diet may be due to high fibre content. Excretory waste of energy in *Silurus meridionalis* increased as the portion of soybean meal in the feed increased which subsequently provided less available energy for growth (Ai and Xie, 2005). Very promising results were obtained in the present study that show that the replacement of FM protein with a combination of plant protein and rendered animal protein in the diet did not affect the growth performance of *Silurus glanis*. Fish meal was successfully replaced with meat and bone meal up to 25% digestible protein in the diet of rainbow trout without compromising feed utilization and growth performance (Bureau et al., 2000). In the diet of grouper (*Epinephelus coioides*) 80% fishmeal was replaced with meat and bone meal with no effect on growth performance (Millamena, 2002).

Increased FCR correlated with increased vegetal protein inclusion in the fish diet and can be explained as a compensatory intake to meet the demand for protein to maintain maximum growth (Refstie et al., 2006). Reduced palatability of the SM diet may be another reason for high FCR. Loss of palatability has been responsible for reduced feed intake and growth of fish fed diets when high levels of dietary fish meal were replaced with cheaper plant or animal ingredients (Davis et al., 1995; Xue and Cui, 2001). This indicates that AP diets are more palatable when compared to SM diet.

Lower protein utilization of SM diet than FM may be due to the presence of anti-nutritional factors such as trypsin. These are in the range of 2-6 mg/g, averaging 4 mg/g (Francis et al., 2001) causing a decrease in mucosal enzymes that coincide with lower protein utilization and feed conversion (Krogdahl et al., 2003). Soybean meal also contains undigested fibre, which reduces the digestible energy causing amino acid oxidation and/or conversion of amino acids to glucose and/or fat. Lower fibre content in the AP diet compares to that of the SM diet. This suggests a higher level of digestible energy in the AP diet, and better utilization of amino acids leading to an increase of the PER and ANPU.

Our results showed that vegetal protein alone has a negative impact on growth performance and protein utilization efficiency. These results still need to be optimised for *Silurus glanis*. Supplemented rendered animal protein for fish, which is authorized in EU countries (*Commission Regulation EU No 56/2013*), may be a suitable alternative protein source to fishmeal in the diet of *Silurus glanis*.

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