PRELIMINARY ASSESSMENT OF THE POTENTIAL TO PARTIALLY SUBSTITUTE FISH MEAL IN THE DIET OF CATFISH (*SILURUS GLANIS* LINNAEUS, 1758)

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Abstract

In the context of demographic growth, aquaculture provides high-quality proteins for human consumption. The main costs in fish farming are related to feed expenses, which depend on the fish meal content. This study aims to explore solutions for partially replacing fish meal (30%) in existing diet formulas for European catfish, using insect meal (V1) or soybean meal (V2). The experiment lasted 45 days. Biometry and gravimetry analyses were performed, along with blood samples to assess blood biochemistry, hemoglobin, and hematocrit. Erythrocyte counts were also conducted under a microscope. The weight gain in the control group was Vc=368±3.84g, in the insect meal-fed group V1 was 387±11.81g, and in the soybean meal-fed group V2 was 245±7.51g. Results indicate that insect meal can successfully reduce the amount of fish meal in the diet of Silurus glanis.

Key words: *catfish feeding, insect meal, soybean meal*

INTRODUCTION

One significant challenge in global demographic growth is finding new, quality protein sources for livestock and human consumption. Worldwide aquaculture has experienced an upward trend in recent decades, although fish feed costs account for 56.45-58.49% of production costs [1].

Continuous efforts are underway to develop solutions involving the partial substitution of fishmeal in feed formulations to enhance the profitability of aquaculture operations. Fishmeal, characterized by its favorable palatability, digestibility, and absorption, with a high crude protein content of 60-72% [2]. It provides essential amino acids and unsaturated fatty acids, with lipid levels ranging from 5% to 10%. However, it also incurs high and variable costs [3]. Human activities such as coastal development, water pollution, overexploitation of marine resources, and climate change have led to a persistent decline in wild fish populations [4]. In recent decades, research has focused on the partial replacement of fish protein with soybean meal [5], yielding notable results in the nutrition of omnivorous species such as common carp (*Cyprinus carpio* Linnaeus, 1758), as well as carnivorous species such as rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792).

Soybean cultivation necessitates extensive land area, substantial water consumption, and the use of fertilizers and pesticides that leave residual effects within the soil and groundwater. This practice ultimately results in a decline in plant biodiversity over time. In recent years, climate change and increasingly dry seasons have decreased agricultural yield profitability [7]. Although soybean meal is extensively utilized in formulating fish feed recipes due to its high plant protein content

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[8], it contains antinutritional factors such as phytates and tannins, and is deficient in essential amino acids. An alternative solution to meet the demand for high-quality proteins for animal and human nutrition is insect farming. Insects present a feasible option for partially substituting fishmeal in aquaculture feeds [9]. Among farmed insect species, the black soldier fly (*Hermetia illucens* Linnaeus, 1758) demonstrates superior performance owing to its high protein content [10] and the fact that it does not serve as a carrier of pathogens. Given that adult flies lack mouthparts and do not feed, they are not considered vectors for zoonotic disease transmission. The larvae can convert organic waste and animal manure into high-quality protein, contributing to a circular economy. Furthermore, before transforming into prepupae, the larvae expel their intestinal contents-which reduces the risk of contamination by pathogenic microorganisms-and retreat to a dry substrate, naturally separating themselves. This process eliminates additional labor costs associated with this technological stage.

From a pricing perspective, fish meal represents the most costly component within a fish feed formulation, followed by insect meal. The most economically advantageous alternative is soybean meal; however, it exhibits significant deficiencies concerning essential amino acids and contains antinutritional factors. This experiment investigates the comparative effects of partially substituting fish meal with soybean meal and full-fat black soldier fly meal (*Hermetia illucens*).

MATERIAL AND METHOD

The experiment was conducted over 45 days in duplicate, utilizing 60-liter aquariums equipped with aeration and water filtration systems. Each aquarium contained 16 catfish (Silurus glanis Linnaeus, 1758). The experimental variants involved feed formulations comprising around 70% base feed and 30% full-fat black soldier fly (*Hermetia illucens*) larva meal - V1, or with soy flour - V2. Both experimental variants and the control (Vc) maintained a total crude protein content (C.P.) of 40%. For V1, the feed consisted of 71.9 g of base feed and 30 g of BSFL meal, whereas for V2, it consisted of 72.6 g of base feed, 30 g of soy flour, and 1% methionine. An attractant derived from *Chironomidae* larvae was utilized at 14 g larvae per hundred grams of prepared feed to ensure consistent palatability. The nutrient content of the feed variants containing fish meal (support feed) for the control variant, feed with 30% substitution of the support feed with BSFL larvae meal, and feed with 30% substitution of soybean meal with added methionine are presented in Table 1.

The total amino acid content of the BSFL larvae meal used in the experiment is shown in Table 2.

The water resistance of the formed pellets was enhanced by incorporating 2.5% gelatin dissolved in water. In experimental groups, the diets characterized by identical protein content were utilized; this feed also included vitamin-mineral premixes to prevent nutritional deficiencies in catfish. Adding methionine to the soybean meal-based feed formulation aimed to compensate for the amino acid-deficient composition, recognizing the essential role of methionine in growth processes.

The feed formulations were designed to be isoproteic. At the beginning of the experiment, the V1 group had an average mass of 7.35±2.10 g, the V2 group 7.26±1.48 g, and the control group 7.02±1.38 g. Biometric measurements, body mass assessments of the catfish specimens, and venous blood sampling were conducted following narcosis with 2-phenoxyethanol. Growth parameters were evaluated through the calculation of the Relative Growth Rate (RGR%); $RGR = 100 \times (W_f - W_i) / W_i$, and the Specific Growth Rate (SGR%); $SGR = 100 \times (\ln W_f - \ln W_i)/t$. Feed utilization parameters were compared using the Feed Conversion Ratio (FCR); FCR = offered feed (g) / (final fish weight (g) - initial fish weight (g)), and the Protein Efficiency Ratio (PER g/g); PER = weight gain (g) / protein intake (g). The specimens' survival rate (S%) was 100%.

Table 1: The composition of the feed recipes used in the experiment

Nutrition facts (%)	Support fooder	*Hermetia illucens* larvae meal	Soybean meal
Crude protein	40	37.5	36.5
Crude fat	10	19.82	19.9
Crude fibre	2.2	11.56	9.3
Crude ash	10.2	8.8	4.5
Phosphorus	1.37	6.36	0,65
Calcium	1.2	12.51	0.4
Sodium	0.3	0.79	0.01

Table 2: Amino acid content of BSFL larvae meal

Aminoacid	Proportion (%)
Alanine	2.06
Aspartic acid	3.5
Arginine	1.7
Glutamic Acid	4.74
Glycine	1.55
Histidine	0.82
Isoleucine	1.33
Leucine	2.56
Lysine	1.33
Phenylalanine	1.39
Proline	2.09
Serine	1.35
Tyrosine	1.45
Valine	1.77
Threonine	1.17
Cistine+Cysteine	0.131

The water temperature and dissolved oxygen levels were continuously monitored throughout the experiment using the HQ 40 D multiparameter device. For hemoglobin analysis, a Spekol Analytik Jena 1300 spectrophotometer was utilized, along with a Dlab DM 1224 centrifuge for hematocrit, and erythrocytes were counted on a hemocytometer under a Euromex microscope equipped with a 3 MP video camera. The red blood cell count (RBCC, x $10^6/mm^3$) was determined by counting erythrocytes in five small squares of Neubauer hemocytometers, using Vulpian dilution solution. Hematocrit (PCV, %) was measured in duplicate using capillaries centrifuged for five minutes at 12000 rpm in a hematocrit centrifuge. Hemoglobin concentration (Hb, g/100 ml) was estimated using the Sahli method. To evaluate the influence of differential feeding on chatfish, hemoglobin, hematocrit, erythrocyte count, and various biochemical blood parameters were analyzed. The levels of ALB, TP, ALT, ALP, Creat, URE, TC, TG, TCo2, Ca, PHO, LAC, Mg, K, Na, Cl, and pH in the blood samples collected from catfish specimens in the two experimental groups, compared to the control group, were recorded and shown in Fig. 5 and 6.

Significance tests, ANOVA, and post-hoc Tukey's were performed for statistical analysis. Data were analyzed using Microsoft Excel software.

RESULTS

During the experiment, the lowest recorded temperature was 24.5°C, and the highest was 27.9°C. The dissolved oxygen and oxygen saturation values recorded a minimum of 3.98 mg/l with 50.5% for V1, 3.81 mg/l with 50.5% for V2, and 6.52 mg/l with 82.9% for Vm. The maximum recorded values for dissolved oxygen and water oxygen saturation were 7.6 mg/l with 93.4% for V1, 7.75 mg/l with 94.1% for V2, and 7.73 mg/l with 93.4% for Vm. The final results from the experiment are presented in Table 3.

The results showed the highest growth rate in the insect flour feeding variant V1, 387±11.81g, compared to the control variant with fish flour, Vc, 368±3.84 g, and the soybean flour variant, V2, 245±7.51g. The final results regarding the weight gain in the three experimental variants are presented in Fig. 1.

Fig. 2 to 4 display the relationship between final body mass and length for each experimental group of catfish: the control group, the insect meal-feeding group, and the soybean meal-feeding group.

Table 3: The values for assessment of growth and feeding in experimental groups

Growth parameter	Groups		
	V1	V2	VC
WG (g)	387±11.81	245±7.51	368±3.84
RGR (%)	329.42±62.31	211.03±128.56	327.78±45.44
SGR (%)	3.19±0.31	2.46±0.96	3.15±0.24
FCR (g feed/g gain)	1.29	2.04	1.36
PER (g gain/g prot. intake)	1.935	1.225	1.84

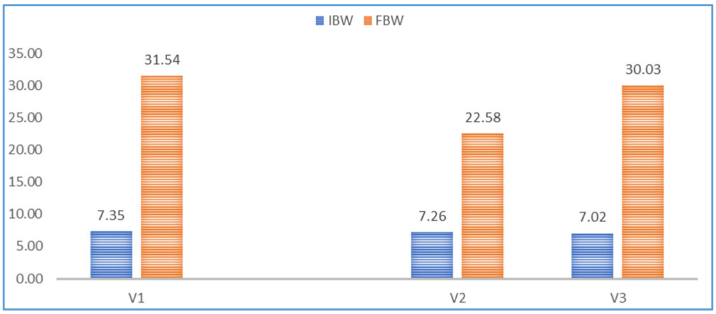

Fig. 1 - The weight gain in the experimental groups

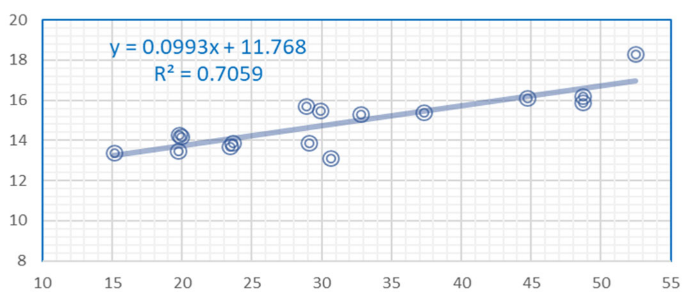

Fig. 2 - Relation Weight-Length after 45 days in VC

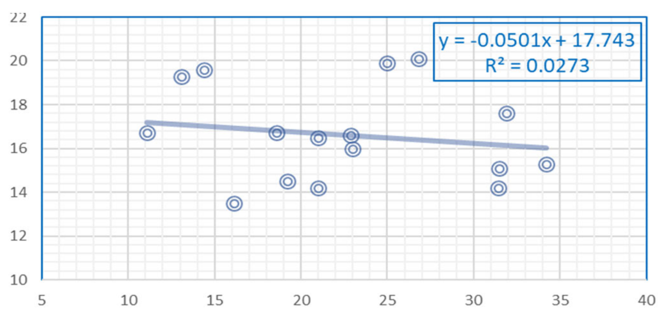

Fig. 3 - Relation Weight-Length after 45 days in V1

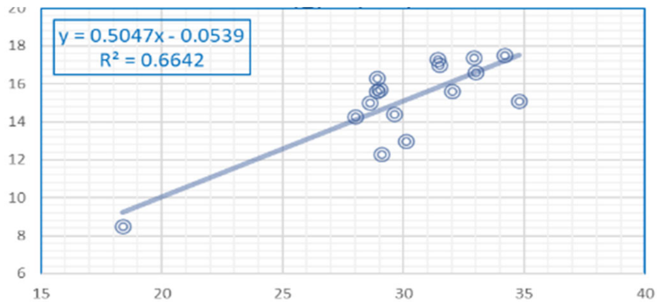

Fig. 4 - Relation Weight-Length after 45 days in V1

RGR and SGR recorded the highest values in the V1 variant of partial substitution, with 30% of the feed with fish meal replaced by black soldier fly meal, followed by the control variant V and the V2 feeding variant with 30% of the fish meal replaced by soybean meal. The experiment results are comparable to those obtained for African catfish (*Clarias gariepinus*) and tilapia (*Oreochromis mossambicus*), where it was demonstrated that both species can utilize an insect-based diet up to 50% inclusion levels without adverse effects on growth performance and nutrient utilization [11]. The conversion factor and protein efficiency ratio are similar to those obtained with a 65% substitution of fish meal with BSFL larvae meal in the diet of common carp (*Cyprinus carpio*) [12].

Blood tests show no significant differences among the experimental variants in ALB, PT, and GLOB levels. Additionally, significant differences are observed for TB, ALT, ALP, TBA, LPS, LDH, CREA, UREA, GLU, TC, PHOS, and TG, which are presented in Fig. 5 and 6. The obtained data indicate a noticeable decrease in total blood protein for the variant fed with insect meal, possibly due to the increased chitin content that reduces absorption [13]. Alanine aminotransferase (ALT) exhibits elevated values in all variants, peaking in the control group. This suggests possible liver dysfunction, likely due to the antiparasitic treatments administered during the experiment. LPS levels exceeded permissible limits in all three variants, reaching a value nearly three times that of the control in the soybean feeding variant. This indicates possible intestinal inflammation, likely caused by antinutritional factors in plant meals. LDH levels are nearly double in the soybean-fed group compared to the control. This increase may serve as a specific indicator for growth or could be associated with dysfunctions in the hepatobiliary, kidney, or pancreas systems.

Creatinine levels are elevated three times above the maximum limit in the soybean feeding variant, while the other experimental variants remain within normal ranges. This may suggest the possibility of renal dysfunction. In conclusion, although the feed formulas for the three experimental variants are isoproteic, the differences observed are likely due to variations in digestibility and the degree of feed absorption. The low level of urea observed in the group fed with insect meal, along with its lower total protein content compared to the other two groups, may suggest that either the feed has a low protein level or there are absorption issues related to the chitin content in the insect meal. The slightly low PT values observed in VC and V1 are linked to intestinal malabsorption and malnutrition. In contrast, V2, which is fed a soy-based diet, shows values that slightly exceed the standard limit due to the higher carbohydrate content in the feed. The use of plant-based meals has also been effectively applied to other carnivorous fish in the *Salmonidae* family. The results of this experiment indicate that the blood carbohydrate levels in the soy-fed variant are comparable to those found in Atlantic salmon and do not negatively impact its development. In terms of triglycerides (TG) and albumin (ALB), the values observed are similar to those recorded in grass carp (*Ctenopharyngodon idellus*) when fed an optimal inclusion of 50% black soldier fly (BSFL) larvae meal.

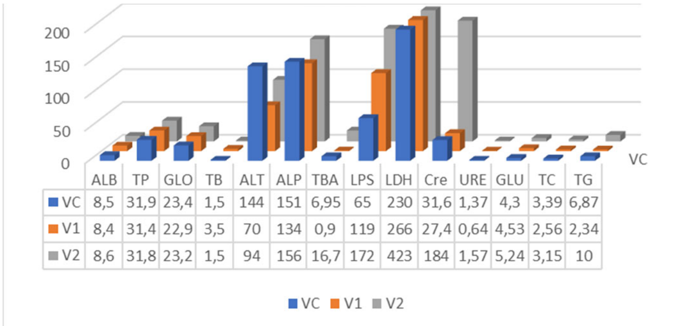

Fig. 5 - Blood analysis in *Silurus glanis*

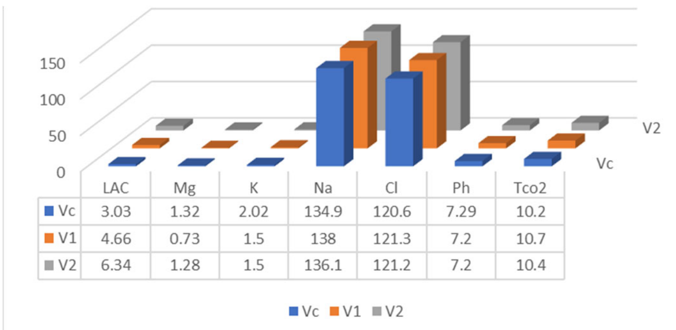

Fig. 6 - Blood electrolyte analyses in *Silurus glanis*

Table 4 - The values of hematocrit, hemoglobin, and erythrocytes

	Ht (%)	**Apb**	**Astd**	**Cst (%)**	**Hb (g/dl)**	**RBCC (million/µL)**
V1	19.40	0.288	0.1	18	5.18	1.38±2.3
V2	18.06	0.396			7.12	1.49±1.8
VC	18.92	0.354			6.37	1.34±0.8

Table 4 presents the analyses of hemoglobin content in the blood, hematocrit, and erythrocytes performed for the three experimental variants. Ve was 13 in the V1 and V2 groups and 14 in Vc, and Vt was 67 in V1, 72 in V2, and 74 in Vc.

The evaluation of hematological parameters revealed results that, while slightly lower, are comparable to those observed in Nile tilapia fed with black soldier fly larvae (BSFL) meal at optimal inclusion levels of 25% and 50% [16]. Statistical analysis indicated significant differences between groups V1 and V2, as well as between groups V2 and VC, at a p-value of 0.05. However, there was no significant difference between groups VC and V1. *Hermetia illucens* larval meal can partially replace the traditional diet based on fish meal for feeding *Silurus glanis.*

CONCLUSIONS

The experiment's results, which involved replacing 30% of the standard feed (containing 40% crude protein) with either soybean meal or full-fat black soldier fly larvae meal, indicate varying outcomes from this partial replacement. Specifically, the growth rate for the diet containing soybean meal was lower, while the growth rate for the diet with insect meal was higher compared to the control group that received 100% of the standard feed.

The study indicates that it is feasible to replace 30% of the protein source in current fish feed formulations (based on fish meal) with insect meal for feeding European catfish (*Silurus glanis*). Updating fish feeding recipes is essential to reduce costs for sustainable aquaculture and support the circular economy, where insect farming is a key protein source to replace plant-based proteins and decrease the fish meal percentage in fish diets. This is especially important given climate change and human activities, which affect soybean crops and fish populations in natural waters. It is advisable to continue experiments with defatted BSFL flour, which is high in protein, to meet nutritional needs and promote European catfish well-being.

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