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EFFECT OF DIETARY PROTEIN LEVEL ON GROWTH, HEMATOLOGICAL PROFILE, AND MEAT BIOCHEMICAL COMPOSITION OF JUVENILE EUROPEAN CATFISH

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Abstract

This study aimed to evaluate the influence of the protein level from fish feed on the growth dynamics, hematological profile, and meat biochemical composition of juvenile European catfish, Silurus glanis (Linnaeus, 1758). In this context, 508 juvenile European catfish, with an average weight of 33±1.80 g/fish, were reared in a recirculating aquaculture system (RAS). Fish were fed with two different proteins: VE 41- fish feed with 41% protein content, and VE 50- fish feed with 50% protein content. After 35 days, the results regarding the growth performance revealed a better feed conversion ratio (FCR-1.03 g/g) and specific growth rate (SGR - 2.02 %/day) in the VE50P. Also, fish meat's hematological profile and biochemical composition showed a similar trend. In conclusion, the fed protein content plays an essential role in the digestibility and efficiency of nutrient utilization, on the welfare and nutritional quality of the final product.

***Key words**: biochemical composition, European catfish, growth.*

INTRODUCTION

Silurus glanis L. is considered important in aquaculture due to its growth abilities and high nutritional values, such as: high growth rate, white meat with consistency, few bones, pleasant flavor also high feed utilization efficiency (Linhart et al., 2002; Jankowska et al., 2006; Adamek et al., 2015). Also, *S. glanis* has good abilities to adapt to different environmental conditions, and it is suitable for growing in recirculating aquaculture systems (RAS) (Bud et al., 2004). The aquaculture production in ten European countries registered an increase from 600 tonnes in 1993 to 2,000 tonnes in 2002, as reported by Copp et al. (2009), and Linhart et al. (2002).

Several researchers have studied the dietary protein requirements for young catfish in various rearing conditions. Specifically, these studies indicate that for the growing of catfish in troughs, the optimal range for food proteins is between 43% and 45% (Meske, 1987). Similarly, in fishponds, the recommended protein levels range from 40% to 42%, as observed (Krasznai et al., 1980), while for catfish raised in cages and silos, the suggested protein content varies from 40% to 45% (Bogut et al., 2002). Regarding the research on catfish dietary requirements for lipids, there is limited information, with partial investigations conducted by Bogut et al. (2002).

The composition, quality, and quantity of components in fish feed have a crucial role in influencing the growth and survival of fish, affecting both feed costs and meat quality. These factors ultimately have a significant impact on the consumer's experience regarding the fish product. The nutritional profile of fish varies depending on the species, the stage of development of the species, the environmental conditions, etc. (NRC, 2011).

Protein is an important nutrient in fish feeds (Wilson, 2002) necessary for the growth, maintenance as well as production of hormones, enzymes, and antibodies required for many vital processes of fish. Also, lipids are considered an important source of energy and essential fatty acids for fish (Mir et al., 2020; Chen et al., 2023).

Numerous studies have consistently indicated that the optimal dietary protein requirements for young catfish vary depending on the rearing environment. Therefore, in this context, this study aimed to evaluate the influence of the fed protein level on the growth dynamics, hematological profile, and meat biochemical composition of juvenile European catfish, *Silurus glanis* (Linnaeus, 1758), reared in the conditions of a recirculating aquaculture system (RAS).

MATERIALS AND METHODS

Experimental design. The present study was conducted, for six weeks, in the recirculating aquaculture systems of the Faculty of Food Science and Engineering, Dunărea de Jos, University of Galați, România.

Two experimental variants were created: $V1_{50P}$, where fish were fed with commercial feed with a protein content of 50 % crude protein, and $V2_{41P}$ where fish were fed with commercial feed with a protein content of 41 % crude protein (Table 1). The feeding intensity during the period experimental was 3% per body weight per day(BW/day), while the feeding frequency was four meals/day. The photoperiod was maintained on a light/dark (12:12-h) condition during the feeding trial.

In this context, 508 juvenile European catfish with an average individual weight of 33 ± 1.80 g/fish were distributed in the rearing units of the recirculating system. The trial was conducted in duplicate. The constructive description of the RAS system was previously described by Vasilean et al. (2008), Plăcintă et al. (2012).

Table 1. Proximate composition of diets used in the experiment

Composition	UM	Nutra Mp-T	Classic 1P
Crude protein	%	50.0	41.0
Crude fats	%	20.0	12.0
Crude cellulose	%	0.7	3.0
Ash	%	0.7	6.5
Phosphorus	%	1.3	0.9
Digestible energy	MJ/kg	19.7	14.2
Vitamin A	UI/kg	12000	10000
Vitamin D_3	UI/kg	1800	1250
VitaminaE	mg	180	150
Vitamin C	mg	500	75
Lysine	%	-	2.4
Methionine	%	-	0.75
Cystine	%	-	0.6

Water quality parameters. Throughout the experiment, water quality parameters (temperature, pH, and dissolved oxygen) were monitored daily. The nitrogen compounds were measured twice/week with the help of Spectoquant Nova 400 and Merck kits. Mean water temperature, dissolved oxygen, and pH, based on daily measurements, over the experimental periods were: 25.6 ± 1.9 °C, 5.9 ± 1.7 mg/L, respectively 7.58 ± 0.54 unit pH. The nitrogen compounds ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4$) were: 20.16 ± 6.56 mg/L, 0.04 ± 0.01 mg/L, respectively 13.04 ± 1.16 mg/L. These values fall within the established normal range for the optimal rearing conditions of *Silurus glanis*, as indicated by a study conducted by Copp et al., 2009.

Fish Growth Performance. At the end of the experimental period, all the fish were weighed, and the following growth parameters were calculated: fish survival, specific growth rate (SGR), protein efficiency ratio (FCR) protein efficiency ratio (PER), through the following formulas:

- Survival rate (SR, %) is calculated as (Nt/N0) × 100, where Nt represents the fish number at the end of the experiment, and N0 is the number of fish at the beginning of the experiment.
- Weight gain (WG, %) is determined by [(BWf−BWi)/BWi] × 100, with BWi and BWf representing the initial and final average body weight (g) of fish sampled from each tank, and t indicating the experimental period in each trial (days).

- Relative growth rate (RGR, g/g/day) is calculated as (BWf - BWi) / t / BW, where BWf and BWi are the final and initial body weights (g) of fish, and t is the experimental period in days;
- Specific growth rate (SGR, %/day) is determined by [(lnBWf−lnBWi)/t] × 100;
- Feed conversion ratio (FCR) is expressed as FI (g)/BG (g), where FI represents food consumption (food provided – uneaten food), and BG is the biomass gain per rearing unit;
- Protein efficiency ratio (PER) is defined as BG divided by the amount of protein consumed.

Fish proximate composition. The proximate analyses of fish muscle were analyzed according to standard methods (AOAC, 2012) at the beginning of the experiment and the end of the trial.

Dry matter content was determined by heating at a temperature of 105 ± 2 ºC using the Sterilizer Esac oven. The crude protein content was assessed using the Kjeldahl method employing Gerhardt-type equipment. Fats were determined through the Soxhlet method, involving petroleum ether extraction, utilizing Raypa extraction equipment. The ash content was evaluated by combustion at temperatures of 550 ± 20°C using a Nabertherm furnace.

To evaluate the retained proteins and lipids we calculate the retained protein and the retained lipids:
- Retained protein (RP): PR=final individual weight x Pf –initial individual weight x Pi.
- Retained lipids (RL): LR= final individual weight x Lf – initial individual weight, where: Lf=final body lipids (%), Li=initial body lipids (%).

Blood sample collection. Blood samples were extracted from the fish caudal vein using heparinized syringes after deep anesthesia of fish with 0.3 mL/L of 2-phenoxyethanol (Velisek et al., 2004).

Hematological parameters, such as red blood cell count (RBC × 10^6/µL), hematocrit (Ht, %), and hemoglobin levels (Hb, g/dL), were evaluated using established methods. For the RBC we use a Neubauer hemocytometer, glass blood diluting pipette, and Vulpian diluting solution (Svobodova et al., 2012). The cyanmethemoglobin method with Drabkin's reagent was employed for Hb determination, with absorbance measured at 540 nm using a Specord 210 UV–Vis spectrophotometer. Hematocrit (Ht, %) measurements involved transferring 30 µL of heparinized blood into hematocrit microcapillary tubes and subsequent centrifugation. Other hematological indices, including mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL), were calculated from the obtained values of Ht, Hb, and RBC.

Data analysis. All data are presented as mean ± standard deviation. The statistical analyses were conducted utilizing the SPSS statistical software for Windows, Version 26.0, Chicago, IL, USA, SPSS Inc. The differences in the means of hematological parameters and proximate composition of fish meat at the two sampling time points (before and after the experimental period) were compared using a T-dependent test ($p < 0.05$) for each treatment diet.

RESULTS AND DISCUSSIONS

Fish growth performance. Feeding fish feeds with different levels of protein had a notable impact on the growth of the fish body. The best results, regarding the fish growth performance, were observed in the group of fish that received a diet consisting of 50% (Table 2).

Table 2. Growth performance of juveniles *of European catfish*

Variant	V150P	V241P
Initial mean weight (g/fish)	32.65 ±0.02^a	32.63 ±0.02^a
Final mean weight (g/fish)	66.71 ±2.62^a	60.12 ±3.71^a
SGR (%/day)	2.02 ±0.11^a	1.74 ±0.18^b
FCR (g feed/g fish)	1.03 ±0.08^a	1.26 ±0.17^b
PER (g/g)	1.95 ±0.15^a	1.96 ±0.27^a

Values are means ± standard deviation from duplicate groups of fish. Values with different letters on the rows indicate significant differences (T-test, p < 0.05) among experimental variants

No statistically significant differences ($p>0.05$) were found between the mean values of the protein efficiency ratio (PER). The growth rate (SGR) and feed conversion ratio (FCR) were significantly better in the $V1_{50P}$ variant. Thus, the $V1_{50P}$ has obtained an SGR value of 2.02 g%/day and an FCR of 1.03 g fodder/g weight gain, while in variant $V2_{41P}$ value of 1.74 g%/day SGR respectively FCR of 1.26 g fodder/g weight gain. This meant that fish feeding with high-protein diets (50% protein)

use dietary protein more efficiently than fish fed a low-protein diet (41%). Also, regarding the final mean weight of fish, higher values were recorded in the experimental variants where the protein level was higher (50% proteins).
Mareš et al., 2003 and Has-Schön et al., 2004, indicate that the optimal level of total protein in the feed for rearing European catfish fry should be within the range of 40–45%. These findings were further supported by Bekcan et al., 2006, who observed that dietary protein content of 40% led to the highest growth performance in European catfish (*Silurus glanis*) compared to other levels such as 30, 35, and 44%.
Florczyk et al., 2014, reported that the fish's growth rate and final body weight were significantly higher when it is used a diet with a protein content of 45% and lipid content of 20% (referred to as the 45/20 diet). The specific growth rate (SGR) in this variant was measured at 2.6% BW/day, highlighting the positive impact of this specific dietary composition on the growth of juvenile European catfish. Similarly, Grecu et al., 2019, identified an analogous outcome in their study. They found that the highest SGR was achieved when the fish were fed a diet containing 54% proteins and 18% lipids, with a feeding level set at 2.5% BW/day. This further supports the concept that specific combinations of protein and lipid content in the diet can significantly influence the growth performance of catfish, as evidenced by the observed higher SGR in the mentioned dietary variant.
Fish proximate composition. The nutritional profile of the feed, the feeding rate, age, size, sex, and habitat features may all play a role in determining the meat composition of the different fish species, and the first indication of the fish's commercial standards that are required for food regulations (Marichamy et al., 2012).
The results of the body proximate composition, at the beginning and the end of the experimental period, are presented in Figure 1.
Regarding the meat's biochemical composition, the obtained results showed significant differences ($p<0.05$) in the levels of crude proteins, lipids, and ash, between the initial moment and experimental variants, respectively between experimental variants (V1$_{50P}$ and V2$_{41P}$). Analyzing the results presented in Figure 1, it is observed that the water content in the V1$_{50P}$ variant is notably lower ($p<0.05$), in comparison with the experimental variant V2$_{41P}$.

Figure 1. The biochemical composition of *Silurus glanis*, fed with different proteins and lipid level

Regarding the protein content, a significant ($p<0.05$) higher value was obtained in the variant V150P. In terms of the protein content of the V2$_{41P}$ variant and the recorded protein value at the initial moment of the experiment, no significant differences ($p<0.05$) were recorded.
Overall, the obtained results indicate a trend toward higher lipid and protein content in the V150P variant. This variant corresponds to a feed with higher nutrient levels. In comparison with the initial moment of the experiment, the lipid content registered a significantly lower ($p<0.05$) value in V2$_{41P}$.
As shown in Figure 2, the smallest amount of retained protein was recorded in fish from V1$_{50P}$,
where it also retained the largest amount of lipids.

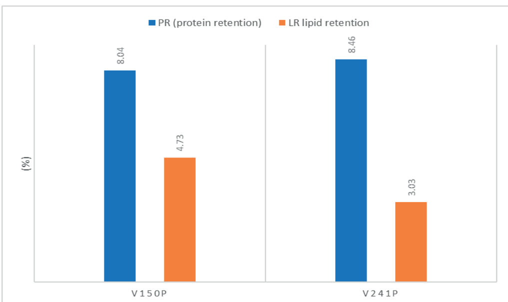

Figure 2. Graphical representation of protein and lipids retention

Similar findings have been reported by other researchers in studies involving other freshwater carnivorous fish species. For instance, Nyina-wamwiza et al., 2005 observed comparable results in pikeperch (*Sander lucioperca*), while Aliyu-Paiko et al. (2010) reported analogous trends in snakehead. Additionally, Bezbaruah & Deka (2021), in their investigation into the variation of protein content in the meat of three catfish species, identified protein content values ranging from 14.49% to 18.14%.

Indeed, the dietary energy provided to fish has a significant impact on their nutrient requirements. Mohanta et al. (2013) underscored the importance of carefully managing energy sources, particularly lipids and dietary proteins, to meet the nutritional needs of fish effectively. The balance and composition of these dietary components play a crucial role in supporting the overall health, growth, and performance of fish.

This meant that the dietary energy of fish has a major impact on the nutrient requirements by proper use of energy sources, particularly lipid and dietary protein (Mohanta et al., 2013).

These findings showed the fact that, although the nutritional profile of fish varies from species to species, size, age, and environmental conditions, the knowledge of the proximate composition of meat of fish can give us an idea regarding developing a more cost-effective and nutritionally balanced feed for the culture of European catfish.

***Hematological profile*.** The hematological profile of fish provides valuable insights into their physiological well-being and overall health. When analyzed in conjunction with other indicators, hematological results can serve as useful tools for identifying and evaluating conditions that induce stress or diseases, ultimately influencing the growth performance of fish.

Table 3 displays the recorded values of hematological parameters at both the beginning and the end of the experiment, offering a comprehensive view of the changes in the fish's physiological state.

The statistical comparison revealed higher values of red blood cell count, PCV, Hb, and MCV at the beginning of the experiment ($p < 0.05$). When we compare the hematological parameter values between the two experimental variants, no significant differences ($p > 0.05$) were observed at the end of the feeding experiment.

Table 3. Variation of hematological and biochemical parameters during the experiment

Parameter	Before the growth test	$V1_{50P}$	$V2_{41P}$
RBCc ($x10^6/\mu L$)	$2.15 \pm 0.18^{*}$	$1.55 \pm 0.34^{**a}$	$1.28 \pm 0.37^{**b}$
PVC (%)	$27.46 \pm 2.37^{*}$	$22.12 \pm 1.74^{**a}$	$20.25 \pm 1.79^{**a}$
Hb (g/dL)	$8.51 \pm 0.43^{*}$	$7.27 \pm 0.44^{**a}$	$7.16 \pm 0.89^{**a}$
MCV (μm^3)	$157.72 \pm 1.6^{*}$	$142.30 \pm 10.9^{**a}$	$113.63 \pm 10.65^{**a}$
MCH (pg)	$39.58 \pm 5.05^{*}$	$46.90 \pm 8.16^{**a}$	$57.89 \pm 8.38^{**a}$
MCHC (g/dL)	$31.83 \pm 3.0^{*}$	$33.96 \pm 3.96^{**a}$	$36.59 \pm 3.26^{**a}$

Note: Values with different symbols */** on the row differ significantly at the comparison of initial values and values after feeding with feeds containing different protein content ($p < 0.05$). Values with different letters in a row differ significantly in the comparison between the two experimental variants. RBC - red blood cell (erythrocyte) count; Hb - hemoglobin; Ht - hematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin content.

Overall, the values of hematological parameters obtained in our study ranged with the normal values for European catfish according to the limits found in the literature. In a study conducted by Docan et al. in 2010, focusing on the hematological response of European catfish under various conditions, including different stocking densities and feeds with extruded pellets containing 41% protein and 12% lipid, the erythrocyte count was reported to range between $1.57 \pm 0.21 \times 10^6 \mu l$, respectively to $1.65 \pm 0.24 \times 10^6 \mu l$. In our study, no significant differences ($p > 0.05$) were recorded in the number of erythrocytes, ranging from $1.28 \pm 0.37 \times 10^6 \mu l$ in the $V2_{41P}$ variant to $1.55 \pm 0.34 \times 10^6 \mu l$ in the V1 50P.

Docan et al., 2010, found in a study on the hematological response of European catfish maintained in different conditions stocking density and feed with extruded pellets containing 41% protein and 12% lipid, that the number of erythrocytes in *S. glanis* varies between $1.57 \pm 0.21 \times 10^6 \mu l$ and $1.65 \pm 0.24 \times 10^6 \mu l$.

Regarding the values of the erythrocyte constants, there can be a significant decrease ($p < 0.05$) in the experimental variants in comparison with the initial moment of the experiment. Also, the statistical comparison of the erythrocyte count between the two experimental variants recorded significant differences, with lower values in the $V2_{41P}$.

In our study, the values of blood hemoglobin content (Hb) of *Silurus glanis* non-significantly differed among the experimental variants ($p>0.05$), but there was a significant decrease ($p<0.05$) of hemoglobin concentration in comparison with the initial moment of the experiment. However, the hemoglobin values fall within the recommended optimal range (7.17 and 7.80 g/mL) recommended by Köprücü et al. (2006), quoted by Docan et al. (2010).

The value of hematocrit percentage showed no statistical differences ($p>0.05$) between the experimental variants recording the highest value in the variant $V1_{50P}$ (22.12±1.74 %) and the lowest value in the $V2_{41P}$ (20.25±1.79%). Comparing the hematocrit values after 6 weeks of feeding with high-protein feeds to the initial moment, a significant decrease ($p<0.05$) can be observed.

The increase of hemoglobin and hematocrit values corresponding to increased dietary protein levels suggested efficient oxygen transport within the body, consequently enhancing the growth performance of fish. This aspect underscores the significance of protein intake and its impact on the growth and health of fish (Ahmed & Ahmad, 2020).

Regarding the values of the Red blood cell constants, obtained after six experimental weeks, it can be observed a significant decrease ($p<0.05$) of MCV, respectively a significant increase ($p>0.05$) of MCH, MCHC in both experimental variants. Higher values of MCH and MCHC were recorded in the experimental variant $V2_{41P}$. The MCV values obtained in the $V2_{41P}$ variant correlate with the lower values of erythrocyte count in this experimental variant, being influenced by the inhibition of erythropoiesis or the destruction of RBC.

CONCLUSIONS

The findings of this investigation reveal the significant impact of dietary protein levels on fish growth, feed conversion ratio, and the hemato-biochemical composition of fish. Based on our results, it is recommended that a dietary protein of 50% crude protein is suitable for promoting the growth and efficient feed utilization of European catfish fingerlings, with a weight of 30-70 g. These data contribute valuable insights for formulating nutritionally balanced diets for the intensive and semi-intensive cultivation of this fish species.

Additional researches are required to determine the ideal protein and lipid levels in dietary content, as these factors are crucial for the successful intensive rearing of *Silurus glanis* in recirculating systems.

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