Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 1, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

BODY INDICES IN COMMON CARP (*Cyprinus carpio*) JUVENILES FED WITH SLUG (*Arion vulgaris*) MEAL AS AN ALTERNATIVE PROTEIN SOURCE

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Abstract

Research of alternative sources of protein for aquaculture feeds is ongoing for many decades, due to the need to make the aquaculture sector more sustainable. This study explores the incorporation of slug (Arion vulgaris) meal as an alternative protein source in the diets of juvenile common carp (Cyprinus carpio) and examines its impact on body indices. Carp juveniles were reared in a controlled environment and fed using a feed containing slug meal as a protein source. The performances were analysed in relation to a control feed that used standard fish meal as an animal protein source. Body indices are analysed in this research such as Fulton condition factor (K), profile index and meatiness indices. The results presented in this article show the potential of slug meal to substitute fish meal in aquaculture feeds. Along with the rising need for sustainable aquaculture practices, investigating unconventional protein sources is crucial for improving fish growth and health while decreasing dependence on traditional fish meal.

Key words*: aquaculture, diet, feed, growth, nutrition.*

INTRODUCTION

Production of food is of central importance for the world population, and aquatic organisms are well represented as food sources. The production of aquatic organisms is represented by capture fisheries and aquaculture. According to the Food and Agriculture Organization, in the year 2022 the production of aquatic organisms from aquaculture surpassed that of capture fisheries, in a historic first (FAO, 2024). This event comes as confirmation of the rising trends in aquaculture production (FAO, 2022). Rising trends in production come together with a careful approach from customers, who are in search of more qualitative products (Lațiu et al., 2024).

In order to make aquaculture more performant, constant improvement of the utilized feeds is paramount, because feed cost is the biggest of the production costs and the market for feed ingredients is experiencing constant price rises (Rana et al., 2009). Of the ingredients utilized in feed production, protein sources represent a large part of the cost. The mainstay in this industry is fish meal (FAO, 2004), due to its highly nutritive nature, but the cost of fish meal is increasingly higher.

Research on alternative sources of protein is split between protein sources of vegetal origin and protein sources of animal origin (Serra et al., 2024). Research on protein sources of animal origin vary from insects (Vrabec et al., 2015), to animal by-products (Luthada-Raswiswi et al., 2021).

Gastropods (class Gastropoda), represent aquatic and terrestrial slugs and snails. This class of invertebrates was researched across decades regarding their nutritional value and potential as animal protein sources in aquaculture (Muntean et al., 2024a).

Among gastropods, slugs seem to be least studied. Tshernyshev et al. (2022) studied slugs for their rearing productivity as a potential food source for human consumption. Meal obtained from slugs as an alternative protein source in aquaculture was investigated by Muntean et al. (2024b), in a study that analyzed the proximate content, polycyclic aromatic hydrocarbons

(PAHs) content and mineral content of slug meal. The Spanish slug, *Arion vulgaris* Moquin-Tandon, 1855 is an invasive species in Romania, and it is considered a pest for the agricultural sector (Zając et al., 2017; Păpureanu et al., 2014; Pfenninger et al., 2014).

The present research comes as a continuum in slug meal research, as a novel alternative protein source for aquaculture productions. In this study, experimental feed containing slug (*Arion vulgaris*) meal was fed to juvenile carp, *Cyprinus carpio* Linnaeus, 1758. In comparison to a control diet containing standard fish meal, body indices such as the Fulton (K) condition factor, profile index (Pi) and meatiness indices (MI1 and MI2) were analyzed.

MATERIALS AND METHODS

This research took place from July 2024 to January 2025. The bioethics commission of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca approved this research with the 401/21.09.2023 decision, issued to serve for the PhD studies of the first author.

For this research, we developed an experimental feed and a control feed. The experimental feed contained slug (*Arion vulgaris*) meal as an animal protein source and the control feed contained fish meal as an animal protein source, which is the standard for aquaculture feeds.

Slugs were gathered during July and August 2024 from the UASMV Cluj-Napoca campus using traps with beer as an attractant. Slug meal was prepared according to the method described by Muntean et al. (2024b). Captured slugs were humanely euthanised using a solution of 50% ethanol and 50% water. Slugs were rinsed with cold water and frozen until further processing. Slug meal was obtained after defrosting the slugs and drying them using a drying oven (UFE 400 oven from Memmert, Büchenbach, Germany) at 60ºC. Mass monitoring using an analytical balance (WSP4000/C/2, Partner Radwag, Radom, Poland) was conducted to determine when the dehydration process was finalized. The dried slugs were then ground to a meal using a mill (PM 100, Retsch GmbH, Haan, Germany). Complementary ingredients to the animal protein sources were chosen to develop the experimental and control feed. Slug meal, fish meal and potential vegetal ingredients to the feed were tested for nutrient content following the modified method of Masithoh et al. (2020), with the Fourier transform near-infrared (FT-NIR) technology using Tango Brucker device (Billerica, MA, USA). After testing various vegetal ingredients, the following were chosen to be used along the animal protein sources: sunflower meal, soybean meal, wheat bran, oat bran, corn germ and tiger nut flour.

Two recipes were developed using the selected vegetal ingredients: one containing slug meal as an animal protein source and the vegetal ingredients; another containing fish meal as an animal protein source and the vegetal ingredients. Proportions of the vegetal ingredients differed, to obtain finite feeds with similar nutrient content and that utilized the same amount of animal protein sources. Each recipe contained 1% vitamin and mineral premix Romzeofort (Romvac, Romania). The experimental feed contained: 30% slug meal, 5% sunflower meal, 20% soybean meal, 5% wheat bran, 5% oat bran, 15% corn germ, 19% tiger nut flour, 1% vitamin-mineral premix. The control feed contained: 30% fish meal, 5% sunflower meal, 10% soybean meal, 10% wheat bran, 15% oat bran, 10% corn germ, 19% tiger nut flour, 1% vitamin-mineral premix. In developing the feeds, we aimed for nutrient contents between the feeds as similar as possible. Pellet feeds with the diameter of 2 mm were obtained using a pelleting machine (Agrofortel AGF-150M, Romania). The experimental and control pellets were then tested in triplicate for their nutritional values using the same method we used for testing the nutritional value of ingredients. The proximate composition of the experimental and control feeds are presented in Table 1.

Table 1. Proximate composition of the control and experimental feeds

Nutrient (%)	Control feed		Experimental feed	
	M ± SEM	SD	M ± SEM	SD
Ash (%)	7.91 ± 2.15	3.04	5.8 ± 0.01	0.01
Carbohydrates (%)	18.10 ± 5.50	7.78	7.37 ± 0.28	0.40
Dry matter (%)	95.82 ± 0.07	0.10	95.02 ± 0.04	0.06
Dietary fiber (%)	28.80 ± 0.17	0.24	32.83 ± 0.09	0.12
Fat (%)	7.53 ± 0.07	0.09	6.8 ± 0.10	0.14
Protein (%)	28.53 ± 0.09	0.13	29.76 ± 0.05	0.07
Total sugars (%)	4.9 ± 0	0.00	3.47 ± 0.03	0.05

Legend: M – mean; SD – standard deviation of the mean; SEM – standard error of the mean.

In preparation to the fish rearing experiment, juvenile carp (*Cyprinus carpio*) acquired from a farm with outdoor ponds from Cluj County were acclimated to laboratory conditions for five weeks. At the start of the experiment, four lots of fish were created with 50 specimens each, with fish being individually sampled based on their weight, to obtain lots that are as uniform as possible. Fish weight ranged from 16.1 grams to 27.9 grams, and the mean weight of the specimens for each lot ranged from 20.43 grams to 20.53 grams. The variance of each lot ranged from 6.52 to 8.78. Thus, we ensured the experiment started with similar lots. The experimental treatments were as follows: treatment A, treatment B and treatment C were fed 2% of their biomass daily using the experimental feed, containing slug meal as an animal protein source. Treatment D was set as the control treatment and was fed 2% of the biomass daily using the control feed, containing fish meal as an animal protein source. All treatments had a day of fasting each week.

The experiment lasted for 40 days and was set up in a controlled environment indoors. Each treatment took place in a 750 liters circular tank, filled with 600 liters of water, that amounted to 65 cm of depth, each fitted with its individual system of filtration. Feeding was conducted twice daily, at the beginning of the light period, and before the end of the light period, which lasted for 8 hours daily. Temperature ranged between 19.3ºC and 21.3ºC, and pH ranged between 7.18 and 7.37. Temperature and pH were sampled daily at the beginning of the light period. Dissolved oxygen was sampled weekly and ranged between 7.96 mg O_2/l and 9.08 mg O_2/l. After 23 days an intermediary measurement of all specimens was conducted, scheduled for the nearest fasting day near the middle of the experiment. At the end of the experiment, the final measurement was conducted. The initial measurement at the beginning of the experiment, the intermediary measurement and the final measurement were conducted in the same manner: each fish from each treatment was individually weighed (0.1 grams precision) and photographed in a specially designed fish tank that allows to photograph the fish against the aquarium glass which is fitted with a millimetric scale and is always at the same distance from the camera. Based on the photographs, somatic measurements were determined using the AmScope ToupView 3.7 software. The method of Lațiu et al. (2022) was utilized and the following measurements were determined: total length (cm), standard length (cm), commercial length (cm), fork length (cm), maximum height (cm), minimum height (cm), head length (cm), caudal peduncle length (cm).

Using the weight of the specimens and the somatic measurements, we proceeded to calculating the body indices of the specimens for the purpose of this study.

The Fulton's (K) condition factor was determined using Froese (2006) formula:

$$K = 100 * \frac{W}{Tl^3}$$

where: K is Fulton's condition factor; W = body weight (g); Tl = total length (cm).

The profile index (PI) was calculated based on Cocan and Mireșan (2016) formula:

$$PI = \frac{Sl}{H}$$

where: PI is profile index; Sl = standard length (cm); H = maximum height (cm).

The first meatiness index (MI1) was calculated using the formula from Cocan et al. (2024):

$$MI1 = \frac{Hl * 100}{Sl}$$

where: MI1 is meatiness index 1; Hl = head length (cm); Sl = standard length (cm).

The second meatiness index (MI2) was calculated using the formula from Cocan et al. (2024):

$$MI2 = \frac{Pl * 100}{Sl}$$

where: MI2 is meatiness index 2; Pl = caudal peduncle length (cm); Sl = standard length (cm).

During the experiment, no fatalities were recorded for each treatment.

Data was analysed using Microsoft Excel and GraphPad Prism 8.0.2. The analysis for the indices evolution was determined between the treatments inside the measurement phases, as well as individually for each treatment across the measurement phases. Before testing the data, data were checked for normal distribution using the Shapiro-Wilk test, along with the descriptive statistics (minimum, maximum, mean ±

standard deviation, median, standard error of mean). Data were analysed in conformity with their distribution (non-parametric), using the Kruskal-Wallis test. Where the test showed statistically significant differences, the Dunn post-hoc test for multiple comparisons was utilized, with the purpose of precisely identifying the data that cause the statistically significant differences. The significance threshold was $\alpha = 0.05$.

The indices were calculated for the initial population, which consisted of all the initial lots analysed together (200 specimens), and for each treatment during each phase (measurement): initial, intermediary and final.

Results regarding the body indices are presented graphically and the following notations were utilized in various combinations in text and graphs: K – Fulton's condition factor; PI – profile index; MI1 – meatiness index 1; MI2 – meatiness index 2; pop. start – initial population; A – experimental treatment A; B – experimental treatment B; C – experimental treatment C; D – control treatment D; initial – initial measurement; intermediary – intermediary measurement; final – final measurement.

RESULTS AND DISCUSSIONS

The descriptive statistics for each treatment during each phase (measurement) and for the initial population are presented in Table 2.

For the Fulton's (K) condition factor, no statistically significant differences were found when comparing between treatments inside each phase (Figure 1).

When analysing the evolution of the Fulton's (K) condition factor for the same treatment across all measurement phases, the following statistically significant differences were encountered between initial measurement and intermediary measurement and between initial measurement and final measurement: $K_{A\ initial}$ vs. $K_{A\ intermediary}$ (***, p=0.0001); $K_{A\ initial}$ vs. $K_{A\ final}$ (****, p<0.0001); $K_{B\ initial}$ vs. $K_{B\ intermediary}$ (****, p<0.0001); $K_{B\ initial}$ vs. $K_{B\ final}$ (****, p<0.0001); $K_{C\ initial}$ vs. $K_{C\ intermediary}$ (****, p<0.0001); $K_{C\ initial}$ vs. $K_{C\ final}$ (****, p<0.0001); $K_{D\ initial}$ vs. $K_{D\ intermediary}$ (****, p<0.0001); $K_{D\ initial}$ vs. $K_{D\ final}$ (****, p<0.0001). The drop in values of the condition factor can be attributed to antinutritional factors (i.e. feed structure) or environmental conditions (i.e. water quality), that occurred in the accommodation of the carp juveniles to the experimental conditions, although they were acclimated for five weeks to laboratory conditions from outdoor pond conditions prior to the experiment. No statistically significant differences were found when analysing the evolution of the Fulton's (K) condition factor for the same treatment between the intermediary and the final measurement.

Hwang et al. (2016) obtained during their research condition factor values between 1.93±0.20 and 2.48±0.37 for juvenile carp (*Cyprinus carpio*) that were reared from hatching in a controlled environment at 23-25°C. A mean value of Fulton's condition factor of 1.82±0.011 for adult wild common carps was obtained by Cocan et al. (2024) and mean values for three populations of adult common carps between 2.04±0.18 and 3.81±0.39 were obtained by Savin et al. (2022).

Lower mean values were recorded in this study, between 1.4 ± 0.16 and 1.88 ± 0.22, compared to the literature.

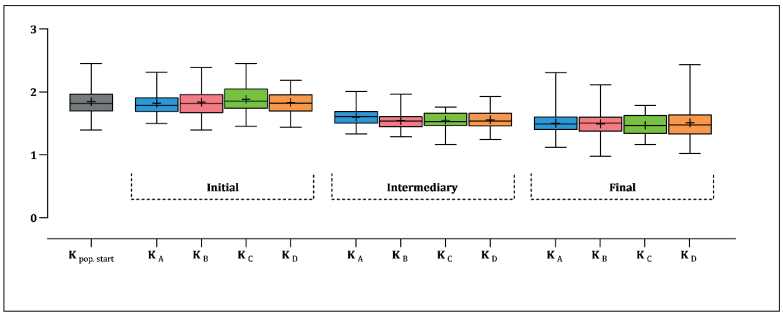

Figure 1. Fulton's (K) condition factor during each phase of the experiment and for the initial population

Table 2. Descriptive statistics for the initial population and each treatment during each phase

	$K_{pop.\ start}$	$K_{A\ initial}$	$K_{B\ initial}$	$K_{C\ initial}$	$K_{D\ initial}$	$K_{A\ intermediary}$	$K_{B\ intermediary}$	$K_{C\ intermediary}$	$K_{D\ intermediary}$	$K_{A\ final}$	$K_{B\ final}$	$K_{C\ final}$	$K_{D\ final}$
N	200	50	50	50	50	50	50	50	50	50	50	50	50
Minimum	1.40	1.50	1.40	1.46	1.44	1.33	1.29	1.17	1.24	1.12	0.98	1.16	1.02
Maximum	2.45	2.32	2.39	2.45	2.18	2.01	1.97	1.76	1.93	2.31	2.11	1.79	2.43
M ± SEM	1.84±0.01	1.82±0.03	1.83±0.03	1.88±0.03	1.83±0.03	1.59±0.02	1.55±0.02	1.55±0.02	1.56±0.02	1.50±0.03	1.4±0.02	1.47±0.02	1.5±0.04
Median	1.82	1.79	1.82	1.85	1.82	1.61	1.54	1.53	1.54	1.49	1.51	1.47	1.48
SD	0.20	0.02	0.22	0.22	0.18	0.14	0.13	0.13	0.14	0.17	0.16	0.16	0.30
	$PI_{pop.\ start}$	$PI_{A\ initial}$	$PI_{B\ initial}$	$PI_{C\ initial}$	$PI_{D\ initial}$	$PI_{A\ intermediary}$	$PI_{B\ intermediary}$	$PI_{C\ intermediary}$	$PI_{D\ intermediary}$	$PI_{A\ final}$	$PI_{B\ final}$	$PI_{C\ final}$	$PI_{D\ final}$
N	200	50	50	50	50	50	50	50	50	50	50	50	50
Minimum	2.25	2.42	2.25	2.41	2.40	2.38	2.31	2.44	2.47	2.44	2.34	2.42	2.48
Maximum	3.33	3.00	3.33	2.90	3.04	3.14	3.10	2.99	3.00	3.17	3.34	3.07	2.90
M ± SEM	2.68±0.01	2.68±0.02	2.70±0.02	2.66±0.02	2.69±0.02	2.71±0.02	2.72±0.02	2.70±0.02	2.70±0.02	2.72±0.02	2.73±0.02	2.72±0.02	2.67±0.01
Median	2.70	2.70	2.75	2.67	2.69	2.72	2.74	2.71	2.68	2.74	2.70	2.74	2.66
SD	0.14	0.12	0.17	0.12	0.14	0.13	0.13	0.12	0.11	0.16	0.17	0.15	0.09
	$MI1_{pop.\ start}$	$MI1_{A\ initial}$	$MI1_{B\ initial}$	$MI1_{C\ initial}$	$MI1_{D\ initial}$	$MI1_{A\ intermediary}$	$MI1_{B\ intermediary}$	$MI1_{C\ intermediary}$	$MI1_{D\ intermediary}$	$MI1_{A\ final}$	$MI1_{B\ final}$	$MI1_{C\ final}$	$MI1_{D\ final}$
N	200	50	50	50	50	50	50	50	50	50	50	50	50
Minimum	26.12	26.26	26.12	26.55	27.38	28.23	28.57	28.08	31.00	28.02	29.17	29.79	29.55
Maximum	40.25	39.24	37.64	40.25	36.81	39.04	36.66	38.88	37.72	37.44	38.34	39.29	37.12
M ± SEM	32.57±0.2	33.04±0.42	32.61±0.39	32.29±0.4	32.35±0.36	33.02±0.35	33.25±0.28	33.28±0.34	34.38±0.21	33.41±0.26	33.66±0.29	34.81±0.27	33.65±0.28
Median	32.94	33.32	32.99	32.43	32.93	33.08	33.30	33.71	34.39	33.85	33.90	35.09	33.99
SD	2.77	2.95	2.75	2.82	2.54	2.47	2.01	2.37	1.47	1.82	2.02	1.91	1.95
	$MI2_{pop.\ start}$	$MI2_{A\ initial}$	$MI2_{B\ initial}$	$MI2_{C\ initial}$	$MI2_{D\ initial}$	$MI2_{A\ intermediary}$	$MI2_{B\ intermediary}$	$MI2_{C\ intermediary}$	$MI2_{D\ intermediary}$	$MI2_{A\ final}$	$MI2_{B\ final}$	$MI2_{C\ final}$	$MI2_{D\ final}$
N	200	50	50	50	50	50	50	50	50	50	50	50	50
Minimum	15.31	15.86	15.31	17.21	16.28	17.18	16.34	16.21	15.88	17.89	16.45	16.34	14.74
Maximum	22.87	22.87	22.67	20.72	22.64	21.86	20.67	20.85	19.43	22.25	21.44	21.15	20.82
M±SEM	18.95+0.09	18.84+0.19	18.87+0.18	19.02+0.13	19.08+0.21	19.25+0.17	18.57+0.14	18.52+0.15	17.81+0.15	19.83+0.14	18.86+0.17	17.83+0.14	18.36+0.17
Median	18.97	18.77	18.85	19.00	19.04	19.34	18.64	18.55	17.89	19.81	18.83	17.89	18.36
SD	1.26	1.36	1.30	0.90	1.45	1.18	0.95	1.09	1.04	0.97	1.21	1.00	1.21

Legend: N – sample size; M – mean value; SD – standard deviation of the mean; SEM – standard error of the mean; pop. start – initial population; initial – refers to the initial measurement; intermediary – refers to intermediary measurement; final – refers to final measurement; K – Fulton's condition factor; PI – profile index; MI1 – meatiness index 1; MI2 – meatiness index 2.

The profile index (PI) analysis showed no statistically significant differences when comparing between treatments inside each phase (Figure 2).
Statistically significant differences were not encountered for the profile index (PI) when analysing the evolution of the same treatment across the initial, intermediary and final measurement phases.

Lower values of the profile index (PI) show a greater amount of meat on the carcass. Cocan et al. (2024) obtained a mean value of 2.4 ± 0.013 for the profile index of adult wild common carp, which is a close value to the mean values obtained in this study for juvenile carp, between 2.66 ± 0.12 and 2.73 ± 0.17, considering the age difference from the studies.

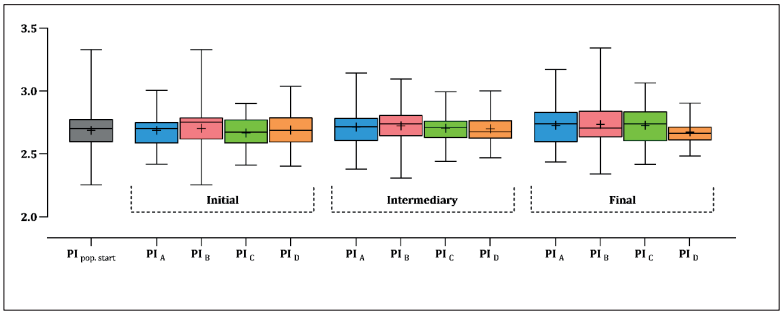

Figure 2. Profile index (PI) during each phase of the experiment and for the initial population

The analysis of the meatiness index 1 (MI1) showed no statistically significant differences when comparing the treatments inside each phase (Figure 3).
When comparing the evolution of the same treatment for the meatiness index 1 (MI1), statistically significant differences were encountered for treatment C, between $MI1_{C\ initial}$ vs. $MI1_{C\ final}$ (****, $p<0.0001$) and $MI1_{C\ intermediary}$ vs. $MI1_{C\ final}$ (*, $p = 0.0466$), and for treatment D, between the initial and intermediary measurement, $MI1_{D\ initial}$ vs. $MI1_{D\ intermediary}$ (**, $p=0.004$). The value of the meatiness index 1 (MI1) did not improve for the mentioned treatments.
The results from this study regarding the meatiness index 1 (MI1) show mean values between 32.29 ± 2.82 and 34.81 ± 1.91, which is a higher value than the mean obtained by Cocan et al. (2024) for adult wild common carp. A higher value of the meatiness index 1 translates to a lower amount of meat on the carcass.

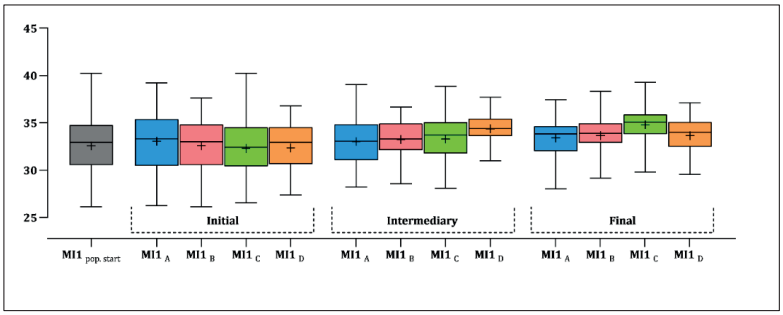

Figure 3. Meatiness index 1 (MI1) during each phase of the experiment and for the initial population

The analysis of the meatiness index 2 (MI2) showed statistically significant differences for the intermediary measurement between $MI2_{A\ intermediary}$ vs. $MI2_{D\ intermediary}$ (****, $p<0.0001$) (Figure 4). For the final measurement statistically significant differences were found

between $MI2_{A\ final}$ vs. $MI2_{B\ final}$ (**, p=0.0047), between $MI2_{A\ final}$ vs. $MI2_{C\ final}$ (****, p<0.0001), between $MI2_{A\ final}$ vs. $MI2_{D\ final}$ (****, p<0.0001) and between $MI2_{B\ final}$ vs. $MI2_{C\ final}$ (**, p = 0.0014). These differences can be attributed to the fact that the measurement of the caudal peduncle is the most sensible to human error, because it is subjective to establish which is the anterior point of the measurement.

The evolution of the same treatment across measurement phases shows statistically significant differences for treatment A, between $MI2_{A\ initial}$ vs. $MI2_{A\ final}$ (**, p = 0.0022), for treatment C, between $MI2_{C\ initial}$ vs. $MI2_{C\ final}$ (****, p<0.0001), and for treatment D, between $MI2_{D\ initial}$ vs. $MI2_{D\ intermediary}$ (***, p = 0.0003).

In the case of treatment A, the value of meatiness index 2 (MI2) did not improve. For treatment C there was a significant improvement in value between the initial measurement and the final measurement, and for treatment D there was a significant improvement in value between the initial measurement and the intermediary measurement.

Mean values of the meatiness index 2 (MI2) from this research are between 17.81 ± 1.04 and 19.83 ± 0.97, which is a slightly lower value than that obtained by Cocan et al. (2024) of 20.04 ± 0.16 for adult wild common carp. The lower the value of the meatiness index 2 (MI2) is, the bigger is the meat content on the carcass.

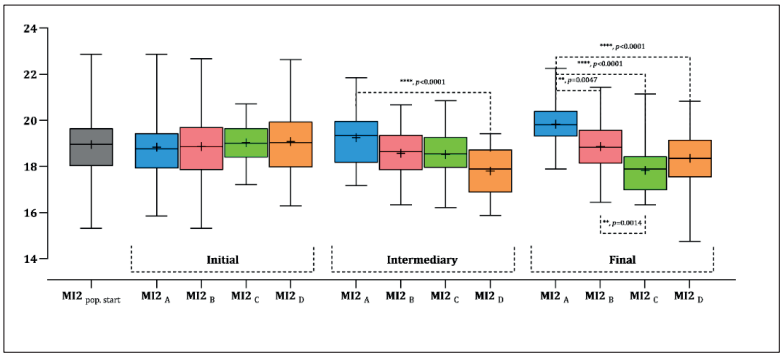

Figure 4. Meatiness index 2 (MI2) during each phase of the experiment and for the initial population (**, p<0.005; ****, p<0.0001)

To our knowledge, this is the first experimental application of slug meal in fish feeding trials assessing morphometric performance. This investigation broadens the scope of alternative proteins in aquaculture and highlights the unexplored potential of non-insect invertebrates in feed innovation.

The use of terrestrial gastropods collected from agricultural environments could serve as a biological control measure while simultaneously contributing to protein circularity. This dual role adds value to what is traditionally considered a pest, reinforcing the principles of resource efficiency and waste minimization.

CONCLUSIONS

The analysis of body indices can determine how different approaches to diet can influence the growth of aquatic organisms. Based on the results of this research on body indices of common carp (*Cyprinus carpio*) juveniles reared in a controlled environment using an experimental feed containing slug meal (*Arion vulgaris*) in comparison to standard fish meal, as an animal protein source, it can be concluded that slug meal can replace fish meal in feed with similar results.

The research demonstrates the feasibility of integrating unconventional protein sources, such as slug meal, into aquaculture feed without compromising fish condition. Our findings support future investigations into local, low-cost protein alternatives for sustainable aquaculture.

ACKNOWLEDGEMENTS

This work was carried out through the Core Program within the National Research

Development and Innovation Plan 2022–2027, with the support of MCID, project no. PN 23 05.

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