USING STATISTICAL ANALYSIS TO ASSESS THE EFFECTS OF FEEDING PELLETS ON FISH DIETARY PREFERENCES IN AN INTENSIVE POLYCULTURE SYSTEM
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UTILIZAREA ANALIZEI STATISTICE PENTRU EVALUAREA EFECTELOR PELEȚILOR FURAJERI ASUPRA PREFERINȚELOR ALIMENTARE ALE PEȘTILOR ÎNTR-UN SISTEM INTENSIV DE POLICULTURĂ

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ABSTRACT

The paper proposes a statistical method for assessing the impact of multiple nutritional recipes on fish growth, raised in polyculture systems, a useful tool in selecting diets within small and medium-sized farms. After designing an experimental installation that produces pelletized feed, the study investigated the associations dependent on the feeder composition for three fish species involved in the experiment (Cyprinus carpio, Hypophthalmichthys nobilis and Carassius carassius). The fish relative mass and size variations have been used as performance parameters. The authors also issued hypotheses on the relations created between the studied species and determined the Feed Conversion Ratio.

REZUMAT

Articolul propune o metodă statistică pentru evaluarea impactului mai multor rețete nutritive asupra creșterii peștilor în sisteme de policultură, un instrument util în selectarea dietelor în cadrul fermelor de dimensiuni mici și medii. În urma proiectării unei instalații experimentale specializate pentru producția de furaje peletizate, studiul a investigat asocierile dependente de compoziția furajelor pentru trei specii de pești (Cyprinus carpio, Hypophthalmichthys nobilis și Carassius carassius). Pentru evaluarea performanței, s-au folosit ca parametrii masele relative și creșterea dimensiunii peștilor. Autorii au emis și câteva ipoteze cu privire la legătura creată între structura speciilor studiate și caracteristicile de bază ale dietelor lor preferate. și au calculat Rata de Conversie a Furajelor.

INTRODUCTION

Polyculture is the technology of growing a variety of aquatic species in the same pond, with the aim of improving overall production. This is accomplished by increasing the trophic and spatial niches, by raising different fish species that have complementary feeding preferences. Therefore, using polyculture practices, farmers could increase the production per unit area and successfully contribute to improving the aquatic environment. The presented results can be illustrated in the formulation of an experimental plan to study the dynamics of fish populations in aquaculture, following the systemic description of the phenomenon.

The paper considered a series of general aspects regarding fish feeding, that were presented in a research project (*Research Contract 5N/07.02.2019*), where the essential problems of fish feeding were also highlighted such as: the basic components (proteins, lipids, carbohydrates, vitamins, and minerals), and the connection between energy and proteins. Types of feeding, rate, frequency, and periods of feeding, automatic feeding systems were also addressed in (*Li, et al., 2013; FAO, 2016*).

A study that evaluated feed conversion and efficiency calculation (*Abdel-Twwab, et al., 2008*), corroborated the soundness of the feeding and growing systems, all of which are the main elements of the aquaculture management systems. The authors stated that most fish breeders use complete diets, usually made up of the following components and percentage ranges: protein, 18-50%; lipids, 10-25%; carbohydrates, 15-20%; ash, <8.5%; phosphorus, <1.5%; water, <10%; and traces of vitamins and minerals.

The problem of finding optimal diets for Israeli carp is presented in the paper of *Zahra, et al., (2015)* finally recommending a percentage of 40% proteins with 14% lipids. A research done by *Ali et al., (2022)* present the results of preliminary research on the optimal diet of carp. Since for the client the quality of the fish meat is highly important, the problem of the fish diet is also researched by the authors *Ljubojević, et al., (2022)*, who give several feeding recipes, including for carp. Similar problems were researched in other studies *(Shipton, et al., 2022; Al-Jader, et al., 2012; Fan, et al., 2015; Nandeesha, et al., 2000)*. The effects of feeding frequency on carp weight gain are presented in the work of *Ali, et al., (2022)*. The nutritional value of cereals in the diet of Common carp is the topic addressed by the authors *Sultana et al., (2001)*, considering that the local production of quality feeder can bring considerable benefits to the fish farming industry. A concentration of 99.3 g/kg of lipids is found optimal for breeding Crucian carp by the authors *Wang et al., (2014),* while other studies obtained similar results *(Przbyl, et al., 2004; Sun, et al., 2018)*. A research paper presents several problems with the Asian carp, associated with adaptation to the American continent (*Li, et al., 2013)*. The economic value generated by the Asian carp is emphasized, but so is the aggressiveness with which it imposes itself on the native carp. In *FAO, (2016),* several types of feeding for aquaculture fish are proposed. Among the nutritional components indicated by *FAO*, there are GOC, soybean meal, polished rice, fish meal, vegetable oil, vitamins, and minerals. The authors *Abdel-Twwab, et al., (2008*), discuss the effects of various protein diets on Nile tilapia. Effects of farm and commercial inputs on carp polyculture performance are analyzed in the paper of *Hernandez et al., (2022)*, considering the use of additives in feeder recipes.

The formulation of artificial diets for juvenile fish is significantly influenced by various factors, one of the most important being the larvae's capacity to effectively digest the essential nutrients *(Ali, et al., 2022)*. Several studies have undertaken a qualitative exploration of fish nutrition, analyzing in detail aspects related to the real efficiency of the feeder. For example, a research study that evaluated the effect of digestible protein levels in experimental diets for meagre (*Argyrosomus regius*) found that optimal digestible protein intake according to the ECR for rearing juvenile meagre was recorded in 0.8 g DP/100 g fish and day. A substantial literature on the problem of palletization and automation of feed administration in aquaculture systems can be found in various papers such as, *Gageanu, et al., (2019); Voicea, et al., (2021); Nenciu, et al., (2022); Matache, et al., (2021)*. Aquaculture developed under zero-waste conditions was addressed in *Nenciu, et al., (2022)*, where several innovative methods of sludge disposal and ecological food production were identified. The use of some aquaculture plants in fish-feeding diets is also a problem extensively addressed in the literature *(Voicea, et al., 2022)*. Other researchers have as objectives the optimal harvest ages of farmed fish. As an example, *Nguyen et al., (2001),* states that the common carp is optimally harvested at a mass between 0.3 and 0.7 kg. In the experimental process, the common carp was harvested at an average weight of 0.824 kg. In *Pitt et al., (2022)*, it is shown, for example, that carp is harvested at 200 g - 500 g or more (luxury specimens) in Indonesia and at 800 g in Japan, where it grows from April to autumn. The results of using vegetable diets, mixed diets, and synthetic diets are comparatively researched *(Ljubojević, et al., 2022; Byrd, et al., 2022; Kurćubić, et al., 2017; Dobrota, et al., 2022)*. The present study aims to explore the dietary preferences of three fish species (common carp, Asian carp, and Crucian carp) grown in an intensive polyculture system, using statistical analysis. The common challenge addressed involves the difficulty of quantifying the growth rate solely through fish measurements. This difficulty arises due to the varying feeding characteristics of different species and their distinct habitats within the basin. In addition, the introduction of varying numbers of fish from each species adds more complexity to the evaluation process.

MATERIALS AND METHODS

The experiments were conducted in the period 2019 - 2022 within INMA Bucharest institute, utilizing two fishing ponds of different sizes, employing a polyculture approach for fish farming. The large fishing pond, ICP 1 (Figure 1) is a construction made of reinforced concrete with a thickness of 300 mm, a width of 14.4 m, a length of 57 m, and a depth of 4.6 m.

Fig. 1 - Basin no. 1 (ICP1), covered pool and the location with details, the inside of the pool with water quality maintenance devices. The coordinates are 44º 30' 9.51'' N, 26º 4' 21.94'' E

The feeding system is composed of 3 feeders equipped with an auger and a pelletized feed disperser with a diameter between 2 and 10 mm, ensuring automated feeding by sequentially programming the dispersion on the surface of the pool. Two Osaga ORV Aerators were used to provide the optimum aeration-oxygenation level for the water in the fishing tank. The second pond (Figure 2) is an outdoor pilot basin, having a total capacity of 10,000 l. The basin was equipped with a supply system based on green energy (a hybrid wind-photovoltaic system).

Fig. 2 - Basin no. 2 (ICP 2), open fish-growing system

Fish species employed in the experiment and their dietary regime

Three species of fish were introduced into each of the two experimental basins: common carp (*Cyprinus carpio*), Asian carp (*Hypophthalmichthys nobilis*), and Crucian carp (*Carassius carassius*). The three fish species were raised together in the ponds. A total of 200 kg of juvenile fish from the three species has been introduced into each pool: 120 kg of common carp, 60 kg of Asian carp, and 20 kg of Crucian carp. At each such loading, one of the feeding diets D1, D2, D3, and D4 were administered separately, each consisting of 600 kg of pellets per pool, administered during four months of experimentation.

Feeder production using a simple pelletizing equipment

The production of the feeding pellets was carried out internally, using an extrusion equipment, designed and executed to be easily used by small and medium farms *(Nenciu, et al., 2022)*. Figure 3 presents the equipment used to pelletize fish feeder.

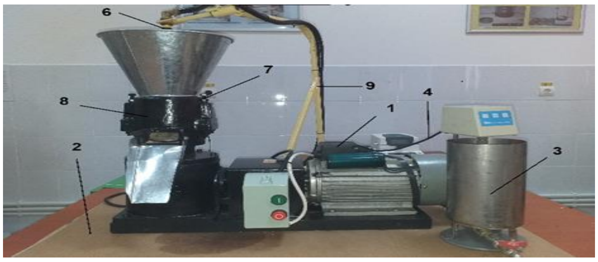


Fig. 3 - Development of a simple equipment design for pellet extrusion in fish feeder production

1 – steam generator; 2 – working stand; 3 – tank for storing blending solutions; 4 – mobile hose; 5 – pipe; 6 – adjusting nozzle; 7 – feeding hopper; 8 – electric heating belt; 9 – arm used to adjust the spraying position;

Four experiments were carried out in different time periods in the two pools, each of them starting with the same number of fish and administering the same amount of food, however in each experiment different type of diets were used: D_1, D_2, D_3, and D_4. The average initial and final masses for each experimental series were recorded. The length, height, and average thickness of the fish of each species have also been recorded. The four feeding recipes (diets) were structurally analyzed, with the data recorded in the sheets (energy value, ash content, moisture content, dry substance content, bulk density, unit density, crude fat content, and pellet durability obtained). The initial characteristics of the fish introduced into the pool, in each experiment, can be found in Table 1.

Table 1

Initial characteristics of fish

Fish species	Initial mass kg	Initial length cm	Initial height cm	Initial thickness cm
Common carp	0.200	16	6.40	1.10
Asian carp	0.150	20	4.20	1.60
Crucian carp	0.125	16	4.48	1.75

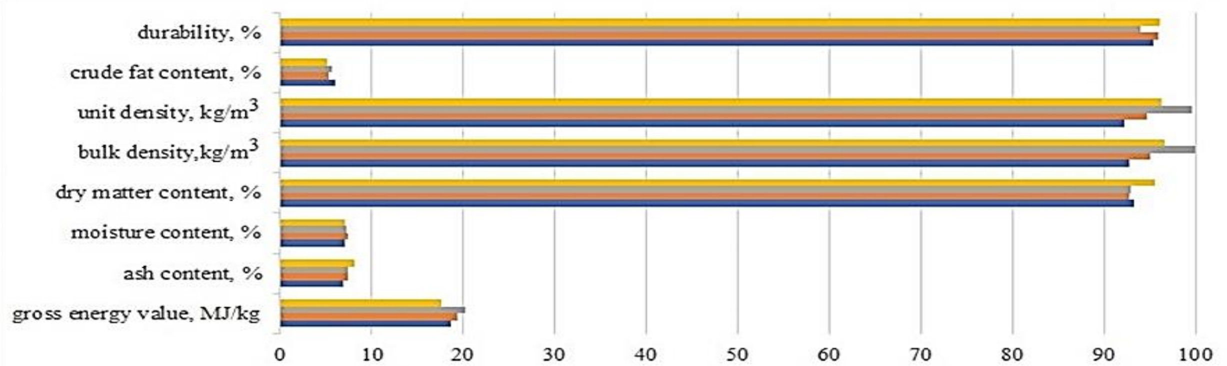
The characteristics of the fish determined at the end of the experiments can be found in Table 2, while the impact of the fish diets (for the four tested recipes) can be seen in Figure 4.

Table 2

Final characteristics of the fish in the two basins

Fish species	Basin	Diets	Mass kg	Length cm	Height cm	Thickness cm
Common carp	Basin 1	Diet 1	0.822	28	11.34	2.7
		Diet 2	0.785	27	10.94	2.5
		Diet 3	0.81	28	11.14	2.7
		Diet 4	0.705	26	10.4	2.65
	Basin 2	Diet 1	0.826	28	11.54	2.8
		Diet 2	0.782	27	10.81	2.72
		Diet 3	0.789	27	10.98	2.75
		Diet 4	0.698	25	9.85	2.57
Asian carp	Basin 1	Diet 1	0.48	48	10.08	1.89
		Diet 2	0.515	46	9.74	1.92
		Diet 3	0.544	47	9.98	1.97
		Diet 4	0.638	51	11.05	2.03
	Basin 2	Diet 1	0.48	48	10.1	1.91
		Diet 2	0.527	50	10.87	1.94
		Diet 3	0.566	51	11.08	1.99
		Diet 4	0.648	54	11.95	2.05
Crucian carp	Basin 1	Diet 1	0.403	22	5.94	2.26
		Diet 2	0.395	21	5.74	2.18
		Diet 3	0.426	23	6.21	2.28
		Diet 4	0.328	17	4.6	2.06
	Basin 2	Diet 1	0.421	23	6.04	2.28
		Diet 2	0.404	22	5.96	2.25
		Diet 3	0.45	23	6.01	2.32
		Diet 4	0.331	17	4.71	2.06

The main characteristics of the pellets in each of the four diets can be seen in figure 4.



D	gross energy value, MJ/kg	ash content, %	moisture content, %	dry matter content, %	bulk density,kg/m^3	unit density, kg/m^3	crude fat content, %	durability, %
D4	17.66	8.06	7.04	95.47	96.58	96.28	5.05	96.06
D3	20.23	7.46	7.19	92.97	99.92	99.52	5.7	93.94
D2	19.29	7.47	7.35	92.79	94.98	94.69	5.27	95.96
D1	18.62	6.95	7	93.27	92.8	92.19	5.9	95.29

Fig. 4 - The main characteristics of the pellets in each of the four diets

In the growing conditions described above and applying the described feeding diet, at the final measurement performed by random selection of a batch of fish, it was found that:
1) the fat content for the common carp species was 22.5% compared to the normal standard, which would be somewhere around 13-15% (with normal growth with controlled feeding, but also body growth would be lower by 10-12 % compared to what was obtained in the experiments referred to);
2) the fat content in Asian carp species was 12.5%, compared to the normal standard, which is 10% in a normal fish feed, but in this case, the body weight is 20% lower than what have been obtained in experimental

research. The Asian species have assimilated very well the intensive feeding without taking a large surplus of fat;

3) the crucian carp had a 20% higher fat intake than the normal standard, which would be somewhere around 10-12% of its body mass.

In essence, the carp and the crucian carp ate excessively and, as a result, at the evaluation, it could be seen that their livers were covered in fat. The working statistical evaluation method was based on the given equations (1)-(10). To highlight the performances of the four types of fish-feeding diets, the relative estimators have been be used:

- the average relative increase in fish mass:

$$\delta m_{rel}(p,b,R) = \frac{m(p,b,R) - m_0(p,b)}{m_0(p,b)} \cdot 100 \tag{1}$$

where $m(p,b,R)$ is the average final mass (after four months of growth) of the fish, *p* is the type of fish (common carp, Asian carp, and Crucian carp), b is the rearing pool (1 or 2), $m_0(p,b)$ is the average initial mass of the fish, and $\delta m_{rel}(p,b,R)$ is the relative average mass growth of the fish, which is expressed in percentages, R being the type of feeding diet used;

- the average relative increase in the length of the fish:

$$\delta L_{rel}(p,b,R) = \frac{L(p,b,R) - L_0(p,b)}{L_0(p,b)} \cdot 100 \tag{2}$$

where $L(p,b,R)$ is the average final length (after four months of growth) of the fish, $L_0(p,b)$ is the average initial length of the fish, and $\delta L_{rel}(p,b,R)$ is the increase in length relative to the average of all fish, expressed in percentages;

- the average relative increase in the height of the fish:

$$\delta h_{rel}(p,b,R) = \frac{h(p,b,R) - h_0(p,b)}{h_0(p,b)} \cdot 100 \tag{3}$$

where $h(p,b,R)$ is the average final height (after four months of growth) of the fish, $h_0(p,b)$ is the average initial height of the fish, and $\delta h_{rel}(p,b,R)$ is the increase in height relative average of the fish, which is expressed in percentages, R being the type of feeding recipe used;

- the average relative increase in the thickness of the fish:

$$\delta g_{rel}(p,b,R) = \frac{g(p,b,R) - g_0(p,b)}{g_0(p,b)} \cdot 100 \tag{4}$$

where $g(p,b,R)$ is the average final thickness (after four months of growth) of the fish, $g_0(p,b)$is the average initial thickness of the fish, and $\delta g_{rel}(p,b,R)$ is the increase in the average thickness relative to the fish, which is expressed in percentages.

A measure of the approximate volume growth of fish can be formulated, according to the following formula:

$$\delta V_{rel}(p,b,R) = \frac{V(p,b,R) - V_0(p,b)}{V_0(p,b)} \cdot 100 \tag{5}$$

where:

$$V_0(p,b) = L_0(p,b) \cdot h_0(p,b) \cdot g_0(p,b) \tag{6}$$

and

$$V(p,b) = L(p,b) \cdot h(p,b) \cdot g(p,b) \tag{7}$$

Similarly, the approximate density before the monitoring time interval can be defined according to the formula (8)

$$\rho_0(p,b) = \frac{m_0(p,b)}{V_0(p,b)} \tag{8}$$

respectively, the approximate density at the end of the experimental time interval (of treatment application or monitoring):

$$\rho(p,b,R) = \frac{m(p,b,R)}{V(p,b,R)} \tag{9}$$

A relative increase in density can be defined as follows:

$$\delta\rho(p,b,R) = \frac{\rho(p,b,R) - \rho_0(p,b)}{\rho_0(p,b)} \cdot 100 \tag{10}$$

Since in the two pools, the feeding recipes and medical treatments were identical, the study continued with the average values per pool. In this way, in formulas (1)-(10), the variable b disappears, and the functions become dependent on one or two variables, p (type of fish) and R (type of treatment).

RESULTS

The equations for calculating the increases in mass, length, height, and thickness, and, subsequent calculations of the density of the fish, allow some graphic representations that lead to some immediate conclusions. In Figure 5, the graphics depict the relative increases, in accordance with the experimental data and the defining equations (1)-(10), related to the type of diet (or recipe) administered. Diets are marked with D_1, D_2, D_3, and D_4. The calculations were performed by averaging over the two aquaculture basins.

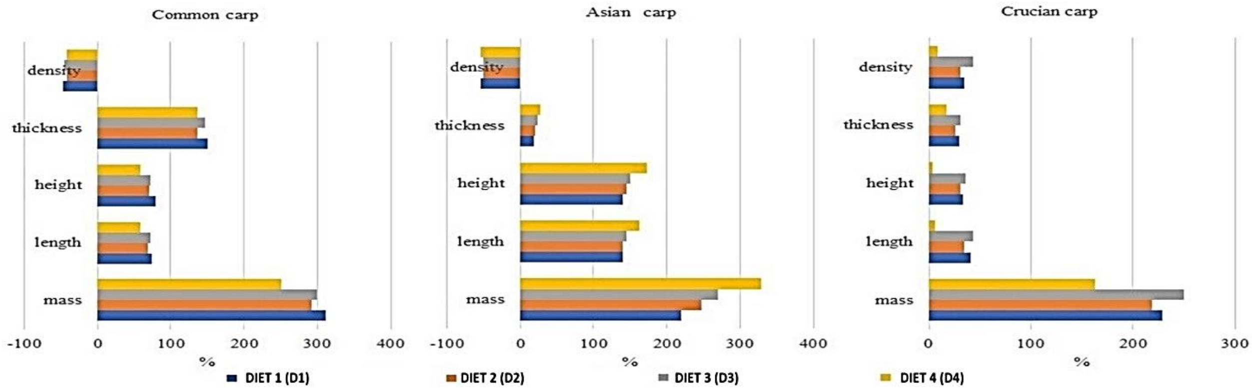


Fig. 5 - The relative increases in mass, length, height, width, and density of fish

From Figure 5, can be drawn the following results:

R1) the highest *relative mass increase* occurs as follows: in the common carp, it corresponds to the D_1 recipe and reaches the value of 312%; for the Asian carp, it corresponds to the D_4 diet and reaches the value of 329%; while for the Crucian carp, it corresponds to the D_3 diet and reaches the value of 250%;

R2) the highest value of the *relative increase in length* occurs as follows: for the common carp, it corresponds to the D_1 diet, with a value of 75% (with a small difference from the D_3 diet, with a value of 72%), for the Asian carp, it corresponds to the D_4 diet, with a value of 162.5%, and for the Crucian carp, it corresponds to the D_3 diet, with a percentage of 43.75%;

R3) the highest *relative increase in height* occurs in the common carp for the D_1 diet, with a value of 78.75%, for the Asian carp for the D_4 diet, with a value of 173.81%, and for the crucian carp for the D_3 diet, with a value of 36.38%;

R4) the *relative increase in width* (or *thickness*), with the highest intensity, occurs as follows: for the common carp for the D_1 diet, with a value of 150%, for the Asian carp for the D_4 diet, with a value of 27.5%, and for the Crucian carp for the D_3 diet, with a value of 31.43%;

R5) the *increase relative to the approximate density* of fish, with the highest magnitude, occurs as follows: for the common carp for the D_1 diet, with a value of -47.32%, for the Asian carp for the D_1 diet, with a percentage of -53.26% (a very small difference compared to the D_4 treatment, with the percentage of 53.22%), and for the Crucian carp for the D_3 diet, with a value of 43.67%.

R6) combining the performances of each diet for each category of fish, it can be observed that the best score is recorded: for common carp, diet D_1, Asian carp, diet D_4, and for Crucian carp, diet D_3.

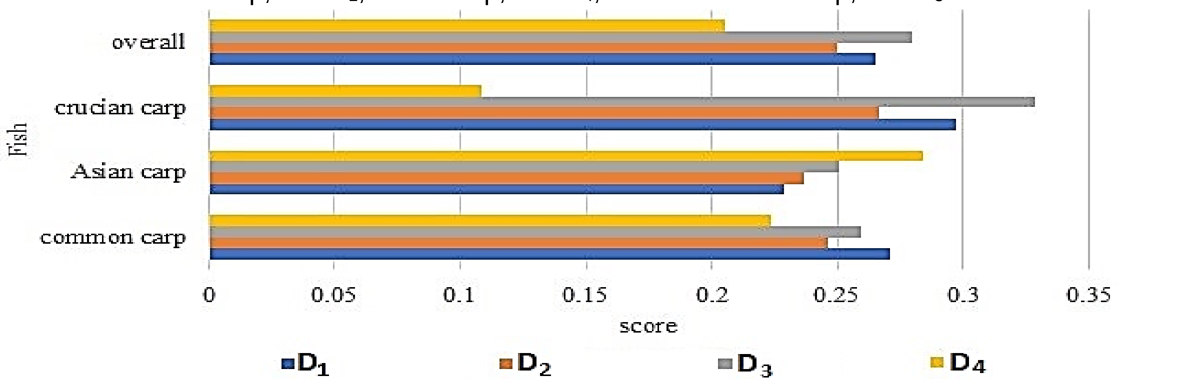


Fig. 6 - Graphic representations of the normalized scores recorded by the four diets for each fish category and globally (overall fish categories)

Investigating the dependence of the dietary preferences of fish on the food component

In order to obtain an assessment of the dependence of the dietary preferences of fish on the main characteristics of each diet, first are calculated the correlations between the scores of the diets for each fish species and the percentages of the components of the diets for the four types of diet. Table 4 gives the values and graphic representations for the main characteristics of the pellets in each diet. The correlations between the scores recorded for each fish species (including all types of fish) and the main characteristics of the four diets used in the experiment are given in Table 4.

Table 4

Correlations between the scores achieved by the three categories of fish and the average category, with each of the four feeding diets

Correlations	gross energy value	ash content	moisture content	dry matter content	bulk density	unit density	crude fat content	durability
Common Carp Score	0.583	-0.96	-0.052	-0.786	-0.222	-0.256	0.969	-0.6
Asian Carp Score	-0.511	0.936	-0.274	0.888	0.474	0.491	-0.723	0.229
Crucian Carp Score	0.859	-0.817	0.321	-0.939	0.066	0.04	0.827	-0.711
Overall score	0.863	-0.792	0.244	-0.891	0.141	0.111	0.858	-0.789

Table 5 shows the results of the univariate linear regression analysis *(Moineagu, et al., 1976; www.statistikindom.com)*. The main results are the coefficient of determination R^2 and the probability of rejecting the regression coefficients, *p* which show whether the respective independent variable (here, the characteristics of the feeding diets) is significant or not for the relative increases of the studied fish species or for all three species together.

Table 5

The main results of the study on the variation of the scores of the diets were applied to the main characteristics of the diets

Fish species	Common Carp		Asian Carp		Crucian Carp		Overall	
Features of the diet	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**
gross energy value	0.34	0.417	0.26	0.489	0.74	0.141	0.74	0.137
ash content	0.92	0.04	0.88	0.064	0.67	0.183	0.63	0.208
moisture content	0.00	0.948	0.08	0.726	0.10	0.679	0.06	0.756
dry matter content	0.62	0.214	0.79	0.112	0.88	0.061	0.79	0.109
bulk density	0.05	0.778	0.22	0.526	0.00	0.934	0.02	0.859
unit density	0.07	0.744	0.24	0.509	0.00	0.960	0.01	0.889
crude fat content	0.94	0.031	0.52	0.277	0.68	0.173	0.74	0.142
durability	0.36	0.400	0.05	0.771	0.51	0.289	0.62	0.211

3.2. The average food consumption of all fish involved in the experiment, FCR calculation

An important parameter for raising fish in farms for any farm animals, is the Feed Conversion Ratio (FCR), defined, for example, in *(Wenk, et al., 1980; https://www.aquaneo-techna.com)*.

For the calculation of an estimated value of this performance parameter (quality) of the feeder, have been considered the following experimental data: 100 kg of fish were introduced into the basins at the beginning, whose average initial and final masses were 0.158333 kg and 0.772167 kg, respectively. Taking into account the initial average mass and the number of fish introduced into the basins, the result is an initial number of approximately 631 fish. The total amount of food administered in 120 days was 600 kg, and the estimated final mass of fish was 387.3288 kg. Dividing the amount of food by the final mass of the fish results in an average FCR of 1,549.

DISCUSSION

The results show that each of the three types of fish species registers maximum performance (increase in mass, length, height, and thickness as well as relative density), on one type of diet: *common carp* on diet D_1, *Asian carp* on diet D_4, and the *Crucian carp* on diet D_3. The D_2 diet does not determine maximum performance in any of the three types of fish.

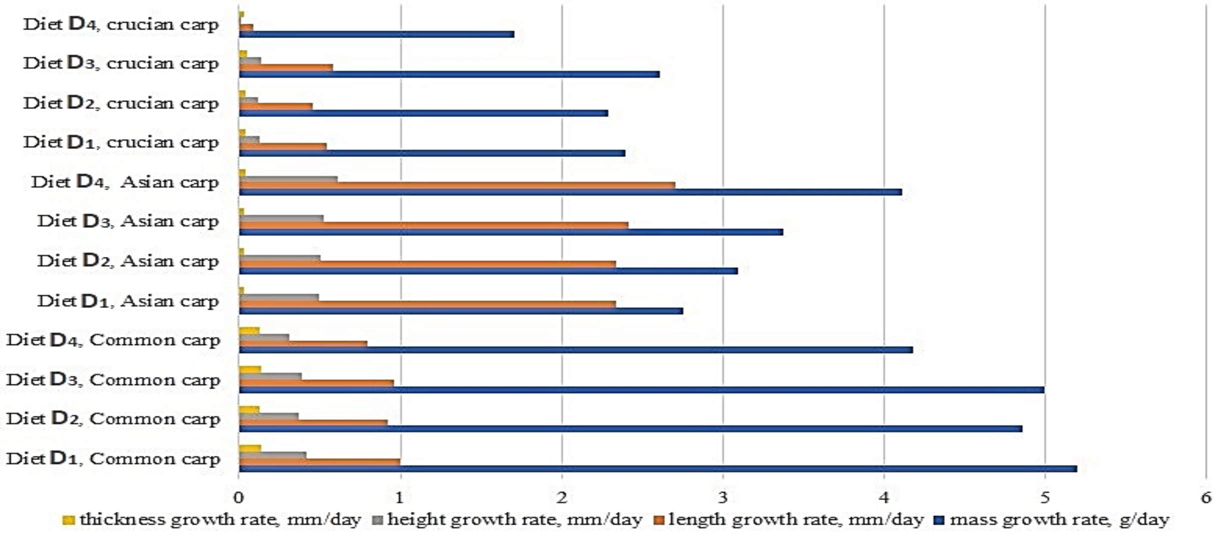


Fig. 7 – The mass growth rates and linear dimensions of the three fish species were monitored in the experiment

The average FCR value for all three species of fish involved in the experiment was 1,549, in accordance with the analyzed literature *(Singh, et al., 2011; Przbyl, et al., 2004; Saleh, et al., 2014)*. This FCR value is in accordance with the specialized literature for fish: Atlantic salmon, 1.0; tilapia, 1.5 to 4.6 for vegetable feeding; farmed salmon, 1.0; and Chinese and Asian carp, 4.9, with vegetable feeding, according to *(Saleh, et al., 2014; Sahzadi, et al., 2006)*. For the common carp, the authors *Przbyl, et al., (2004)*, who followed the evolution of FCR over time, indicate a minimum value of 2.07. The authors indicate that for common carp subjected to four diets, FCR values were between 1.43 and 1.5. For bream, the authors *Singh, et al., (2011)* use the term Feed Conversion Efficiency (FCE), calculated similarly to FCR, and indicate values between 0.37 and 0.76. For the pikeperch, *Dobrota, et al., (2022)* indicate FCR values between 1.56 and 2.55.

CONCLUSIONS

CG1. The tested feeding diets in the preferences of the different fish species is deduced from the scores achieved by each diet, for each of the three fish species and as a whole. Common carp prefer the D_1 diet, Asian carp prefer the D_4 diet, and Crucian carp prefer the D_3 diet. Overall, the preferences of the entire fish population go towards the D_3 diet.

CG2. From the analysis of the correlations between the dietary preferences of the fish (the scores recorded on the four diets) and the composition of the diets, it is observed that for the Common carp, the crude fat content and the energy value are the elements that best explain the growth of the fish both in mass and in length, width, and thickness.

CG4. The results obtained in the examined experiment regarding the cost of growth, used the FCR indicator. The values calculated from the experimental data for the FCR indicator fit very well with the results given in all the literature.

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