

# STIMULATING DISEASE RESISTANCE FOR COMMON CARP (*CYPRINUS CARPIO*) REARED IN RECIRCULATING SYSTEM, BY UTILISING FEEDING DIETS SUPPLEMENTED WITH FATTY ACIDS

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## Abstract

*Fish production carries risks due to infectious diseases, unbalanced diet and environmental challenges which compromise fish welfare. Consumers demand a healthier and safer food and nutritionists recommend a diet low in saturated fat and rich in polyunsaturated fatty acids. The aim of this research is to investigate how diets supplemented with fatty acids affect the disease resistance of the carp species (*Cyprinus carpio*) 1 year of age, reared in a recirculating system. The experiment was performed in the recirculating pilot system, over a period of 2 months, using 120 specimens of carp (*Cyprinus carpio*) and three experimental feeding diets, supplemented with oils from various sources, rich in unsaturated fatty acids. In the experimental groups there were no mortality due to the use of oils rich in fatty acids that have the property to increase the immune system of the fish, compared to the control group where the survival was 86.66%. In the three experimental groups, at the end, a slightly higher level of proteins is observed in group T3 (18.18 g%), compared to group T2 (17.7 g%) and T1 (17.75 g%), the difference being proportional to the weight gain, but the difference is insignificant ( $p > 0.05$ ) which suggests the almost similar evolution of biological material in protein retention, regardless of the source of fatty acids. The feed conversion ratio and protein efficiency improved with the addition of lipids in the feeding diets. The addition of oils to feeding diets resulted in an increase in blood parameters measured in the experimental groups (PCV, Hb, Erythrocyte, MCV, MCH and MCHC), compared to parameters monitored at the beginning of the experiment and at the end of the experiment in the control group fed without oil supplements.*

**Key words:** *Cyprinus carpio*, fatty acids

## INTRODUCTION

Fish disease is a primary constraint for aquaculture and is responsible for impeding economic and socio-economic development in many countries of the world as well as in our country. The increase in the incidence of diseases has led to increased interest in the prevention and treatment of diseases in fish. However, the number of medicines used in aquaculture in Europe has been limited because it has led to a decrease or reduction of the body's immunity, selection of antibiotic-resistant bacterial strains ([1]), environmental pollution and the accumulation in fish tissues of chemical

residues harmful to the health of the consumer. Fish production is at risk due to infectious diseases, an unbalanced diet and environmental challenges that compromise fish welfare. Internationally, there is a growing interest in diversifying fish production, increasing the nutritional value of fish products and obtaining safe and healthy fish products for human consumption. Consumers demand healthier and safer food and nutritionists recommend a diet low in saturated fat and rich in polyunsaturated fatty acids (Buckley and Morrissey, 1998) [9]. Common carp is a popular fish species because it has a specific flavour, is easy to digest and is a valuable source of protein, fat and other nutrients with important roles in human health. The fat content and fatty acid composition of carp meat has been shown to

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vary depending on the technology used for rearing, environmental factors and diet. Fish health can be affected by the quality and quantity of macro and micronutrients, as well as by immunoactive compounds (immunostimulants and probiotics). The aim of the research is to investigate how diets supplemented with fatty acids affect the disease resistance of the carp species (*Cyprinus carpio*) 1 year of age, reared in a recirculating system.

## MATERIALS AND METHODS

The biological material used in the experiment was the one year old carp (*Cyprinus carpio*), obtained in the Research and Development Base Brateș, Galați. The experiment was performed in the pilot system, of recirculating type, which belongs to the Research and Development Institute for Aquatic Ecology, Fisheries and Aquaculture in Galați, for a period of 2 months. The experiment was performed using 120 carp specimens (*Cyprinus carpio*) divided into four fiberglass tanks with a volume of 240 liters of water, with a supply flow of 4-8 l / min / tank. Before populating, the biological material - one summer carp with an average weight of  $73.12 \pm 1.58$  g / specimen was subjected to a treatment against possible parasites or infectious diseases by bathing with potassium permanganate solution 23 ppm, for 5 minutes and sorted to ensure the homogeneity of the population dimensions in the control tank (lot C) and the three experimental tanks (lot T1, lot T2, and lot T3).

### Fish feeding experiments

For batch C, standard feed (coded SF) was used for control. For the experimental lots, three experimental feeding diets were realised, supplemented with oils from different sources, rich in unsaturated fatty acids:

-Fodder 1 (coded FT1), with a 5% soybean oil, sprayed at the exit from the extruder, fed to experimental lot T1;

-Fodder 2 (coded FT2), with a 5% olive oil, sprayed at the exit from the extruder, fed to experimental batch T2;

-Fodder 3 (coded FT3), with a 5% fish oil, sprayed at the exit from the extruder, fed to experimental batch T3;

The ration of feed for the fish was 2.5% of body weight. The total quantity of fodder for one day was administered every two hours.

### Physical and chemical analysis

The physico-chemical and biochemical analysis was performed on fish meat and blood, at the beginning of the experiment and at the end of the experiment, after 60 days of growth in an intensive recirculating system, fed with experimental diets enriched in fatty acids.

### The composition of the fodders and composition of the fish meat

The analysis of fodder and samples of fish meat was performed using the procedures indicated by the standard methods of analysis of fodder and fish meat. The moisture was determined by Standard Official Methods of the AOAC (1990).

The total ash was determined by Furnace Incineration described by AOAC (1990). The crude proteins content of the samples was determined using the Kjeldah method of AOAC 17th edition, 2000, Official Method 928.08 Nitrogen in Meat (Alternative II), which involved protein digestion and distillation, where F (conversion factor), is equivalent to 6.25. The total carbohydrate percentage was determined by the difference method.

This method involved adding the total values of crude protein, lipid, moisture, ash and fiber constituents of the sample and subtracting it from 100. The total fats were determined using the Soxhlet method, equipped with Gerhardt Brand Multistate Controller, with modified ether extraction methods AOAC 960.39.

### Fatty acids profiling

The determination of fatty acids in fish meat and fodder was determined by gas chromatography (GC). To extract lipids, the homogenized samples were dried for 1 h at 105°C. The fatty acid methyl esters were analysed with a Clarus-500 gas chromatograph with a Perkin-Elmer mass spectrometry detector, equipped with a system of injection into the capillary column (ratio of 1:100). The change of the fatty acids from the sample to the methyl ester was followed by the separation of the components

on the capillary column, the identification by comparison with a chromatography standard.

The relative concentration of fatty acids was calculated as mass percentage of the identified fats. The working methodology is in accordance with SR CEN ISO / TS 17764-1 / 2008; SR CEN ISO / TS 17764-2 / 2008; EN ISO 661/2005; and SR EN ISO 15304/2003; SR EN ISO 15304 / AC 2005; AOAC 996.01.

Assessment of growth performance and feed efficiency

Individual Weight Growth (WGi, g) and total Weight Growth (WGt, kg), Food Conversion Ratio (FCR, kg/kg), Specific growth rate (SGR, %/day), Protein Efficiency Ratio (PER), Productive Protein Value (PPV) were determined as follows:

WGi=Final weight-Initial weight(g/fish);  
 WGt=Final weight lot-Initial weight lot(kg/total fish);  
 FCR=feed fed(kg)/weight gain(kg);  
 SGR=100×[(ln Final fish weight)-(ln Initial fish weight)]/experimental days;  
 PER=weight gain(g)/protein fed(g);  
 PPV=100[protein gain(g)/fed(g) x protein fed(g)].

Hematological analysis

To study the haematological parameters, blood was collected on anticoagulant (EDTA - etilen-diamino-tetraacetic-acid), at the beginning and at the end of the experiment, through puncturing the caudal vein. To

determine the number of erythrocytes (mil.cel/µl blood) it was used the Neubauer haemocytometer by direct microscope numbering, haemoglobin concentration (Hb, g/dl) was determined by Sahli colorimetric method and haematocrit value (PCV,%) was determined by microhaematocrit method with a Hettich Haematoctit 210 centrifuge.

Mean Corpuscular Volume (MCV, µm<sup>3</sup>), Mean Corpuscular Haemoglobin (MCH, pg) end Mean Corpuscular Haemoglobin (MCHC, (g/dl) were determined as follows:

MCV= Hct\*10/no. erythrocyte;  
 MCH= Hb\*10/ no. erythrocyte;  
 MCHC= Hb\*100/Hct.

Statistical analysis

All analyses were carried out in triplicate. Statistical analysis was carried out by means of Excel tools. The average values are reported together with standard deviations. The statistical interpretation of the considered data shows a variation within the allowable threshold of P<0.05.

**RESULTS AND DISCUSSIONS**

Chemical analyses of fodder

Four types of fodder with a protein content between 40.95% and 42.15% and a lipid content between 10.35% and 11.25% were used. The composition of the four types of fodders used in the experiment is presented in Table 1.

Table 1 Chemical composition of the fodders

Fodder sample	Moisture, g%	Proteins, g%	Fats g%	Carbohydrates g%	Fiber g%	Ash, g%	Energy value* kcal/100g
SF	6.20	41.15	10.85	35.40	1.5	4.90	414.76
FT <sub>1</sub>	5.90	40.95	11.25	36.90	1.7	3.30	423.81
FT <sub>2</sub>	6.05	41.65	11.15	35.05	2.1	4.00	418.17
FT <sub>3</sub>	6.25	42.15	10.35	36.20	1.2	3.85	417.49

\*calories conversion factors used: for proteins 4.1 kcal/g, for lipids 9.3 kcal/g; for carbohydrates 4.1 kcal/g.

In fully monitored intensive systems, supplementary feed (fodder) is equivalent to distributed feed, with no natural feed [6]. The concentrations of nutrients found in the fodder administered during the experiment are in accordance with the recommendations in the literature for the carp species (*Cyprinus carpio*) and the age of one year

[4]. The concentration of the unsaturated fatty acids of the four fodder types varies very little and can be found in table no.2.

The fodder with FT3 fish oil included has a concentration of polyunsaturated fatty acids with 4.22% higher compared to FT1 and 3.47% higher compared to FT2.



Table 2 The profiles of the fatty acids of the fodders (%)

Fatty acid	SF g/100 g fats	FT <sub>1</sub> g/100 g fats	FT <sub>2</sub> g/100 g fats	FT <sub>3</sub> g/100 g fats
Total Saturated Fatty Acids (SFA)	20.96	20.15	19.65	19.25
Total Monounsaturated Fatty Acids (MUFA)	34.46	35.05	36.85	36.15
Polyunsaturated Fatty Acids (PUFA)	41.15	41.45	41.15	42.20
Other Fatty Acids	3.43	3.35	2.35	2.40
Eicosapentaenoic Acid (C20:5n3) (EPA)	1.12	9.85	9.75	10.00
Docosahexaenoic Acid C22:6n3 (DHA)	13.20	12.80	12.90	13.00
Total ω-3 fatty acids	15.74	14.50	15.85	16.15
Total ω-6 fatty acids	25.41	26.95	25.30	26.05
ω 3/ω6	0.62	0.54	0.63	0.62

### Physico-chemical analyses of water

The evaluation of the physico-chemical parameters of the water was made in order to monitor the aquatic environment, with the

possibility to intervene in case of deviation of the water quality conditions from the allowed limits (table 3).

Table 3 Water chemical parameters in the carp recirculation system

Analysed parameters	U.M.	No. of samples	Average±SD*	Optimum values (according to Order no. 161/2006)
Temperature	°C	40	18.52±1.80	does not normalize
Dissolved oxygen	mg/l	40	8.05±0.75	10
Ph	upH	40	7.75±0.25	6.5-8.5
Organic matter	mg KMnO <sub>4</sub> /l	40	17.50±3.75	<60
Nitrates, (NO <sub>3</sub> <sup>-</sup> )	mg/l	40	2.85±1.40	2.5-3
Nitrites, (NO <sub>2</sub> <sup>-</sup> )	mg/l	40	0.02±0.02	0.03
Ammonia (NH <sub>3</sub> )	mg/l	40	0.15±0.15	0.2
Ammonium (NH <sub>4</sub> <sup>+</sup> )	mg/l	40	0.65±0.25	0,8

\* Standard deviation

The recirculating system was designed to ensure all the technological water quality conditions necessary for the growth of carp species (temperature, dissolved oxygen, pH, organic matter, ammonia, nitrates, nitrite and ammonium), according to Order no. 161/2006 on the classification of surface water quality in order to establish the ecological status of water bodies (table 3).

During the study, in the four experimental basins there were no deviations of the concentrations of the physico-chemical parameters of the water outside the optimal spread.

### Analysis of the biologic material involved in the experiment

In lot C there was the smallest increase in growth (0.92 kg). In experimental lots T2, T2

and T3 total growth increase was 80.43%, 90.2% and 109.7% higher compared to lot C, which indicates the stimulatory effects, in the accumulation of biomass, of fatty acids from oils added to feed diets (Table 4). The results are comparable to those obtained by Bharathi S., et al., 2019 [2]. Food Conversion Ratio, represented by the amount of fodder consumed to obtain one kg, the growth increase registered better values in the experimental lots (1.43 for lot T3, 1.72 for lot T2, 1.96 for lot T1) compared to control lot C (2.4) (Table 4). Functional additives can lead to higher productivity and increased resistance to infectious diseases, which would lead to sustainable aquaculture.

Table 4 Bioproductive indicators obtained in the carp (*Cyprinus carpio*) growth in the pilot recirculating system fed with fatty acids diets

Growth parameters	UM	Lot C	Lot T1	Lot T2	Lot T3
		Control tank	Experimental tank 1	Experimental tank 2	Experimental tank 3
<b>Initial Parameters</b>					
Number of Specimens	-	30	30	30	30
Mean individual weight	(g/specimen) mass±SD*	75.32±3.15	73.15±2.35	72.25±3.52	71.75±4.25
Initial Biomass	Kg	2.26	2.19	2.17	2.15
Density of the initial population	kg/m <sup>3</sup>	9.42	9.14	9.03	8.97
<b>Final Parameters</b>					
Number of Specimens	-	26	30	30	30
Mean individual weight	(g/specimen) mass±SD*	122.30±6.24	128.4±8.22	130.5±9.15	135.6±5.89
Final Biomass	kg	3.18	3.85	3.92	4.07
Density of the final population	kg/m <sup>3</sup>	13.25	16.05	16.31	16.95
<b>Growth parameters</b>					
Number of days	days	60	60	60	60
Weight growth individual (WGi)	g	46.98	55.25	58.25	63.85
Weight growth total (WGt)	kg	0.92	1.66	1.75	1.92
Total Shared Food	kg	2.208	2.249	3.006	2.739
Feed Conversion Rate (FCR)	g	2.4	1.96	1.72	1.43
Daily growth rate (DGR)	g/day	0.59	0.69	0.73	0.80
Specific growth rate (SGR)	%/ day	0.43	0.70	0.74	0.80

\* Standard deviation

The survival of the biological material, an important indicator in any rearing system, was monitored throughout the experiment, recording a value of 100% in lot T1 where the biological material is fed with soybean oil, in lot T2 where the biological material is fed with fodder with olive oil and in lot T3 where the biological material is fed with fodder with fish oil. In the experimental lots there were no mortality due to the use of fatty acid-rich oils that have the property to increase the immune system of the fish, compared to the control lot where survival was 86.66% (Table 4). The survival rate was

within normal limits for one summer carp, however, the differences being significant ( $p < 0.05$ ) between lot C and experimental lots. The addition of oils stimulated growth parameters and survival rate, the results being more favorable compared to those obtained by Sashi B. et al., 2014 [6].

#### **The biochemical composition of the material involved in the experiment**

The effect of fatty acid diets on the biochemical components of the carp species can be assessed from the study of the data presented in table 5.

Table 5 Composition of the fish meat before and after 60 days of differential feeding experiments

Biochemical parameters	Fish sample				
	Initial	Lot C	Lot T <sub>1</sub>	Lot T <sub>2</sub>	Lot T <sub>3</sub>
Moisture, (g%)	81.55	74.84	72.55	72.45	72.15
Proteins, (g%)	14.55	16.85	17.75	17.70	18.18
Fats, (g%)	2.15	6.10	6.55	6.75	6.85
Ash (g%)	1.60	2.20	3.05	2.95	2.75
M/P	5.60	4.44	4.09	4.09	3.97

M/P= Moisture, (g%)/ Proteins, (g%)

Analysing the data referring to the protein content, significant differences are observed between the beginning of the experiment and after 60 days of experiment and between the control lot C and the three experimental lots at the end of the experiment ( $p < 0.05$ ). At the end of the experiment, a slightly higher protein level was observed in group T3 (18.18 g%), compared to group T2 (17.7 g%) and T1 (17.75 g%), the difference being proportional to the weight gain, but the difference is insignificant ( $p > 0.05$ ) which suggests the almost similar evolution in protein accumulation of the biological material, regardless of the source of fatty acids.

Analysing the data on fat content, it was found that there are no differences at the end of the experiment between the three experimental lots, suggesting a similar evolution in fat accumulation of the

biological material, regardless of the source of fatty acids. The results indicate the beneficial effects of incorporating oils from different sources in the diet of common carp and are similar to the values obtained by Manjappa K. et al., 2002 [5], in the experiment performed on carp (*Cyprinus Carpio*) with an individual weight of 2.13–2.21 g, over a period of 120 days, fed diets in which the concentration of lipids varied.

When populating, the biological material was characterized by a U / P ratio equal to 5.6. At the end of the experiment this ratio decreased in all four lots reaching 4.4 in lot C, 4.09 in lot T1 and T2 and 3.97 in lot T3. The incorporation of oils in feed diets has led to a decrease in water content, in favour of increasing the concentration of lipids and proteins in body mass, along with weight gain.

Table 6 Recovery of protein from fodder with the addition of oils used to feed carp reared in a recirculating system for 60 days

	Fish sample			
	Lot C	Lot T <sub>1</sub>	Lot T <sub>2</sub>	Lot T <sub>3</sub>
Protein Efficiency Ratio (PER)	51.70	49.14	46.53	55.30
Productive Protein Value (PPV)	2.28	2.84	3.02	3.69

Protein Efficiency Ratio (PER) represents the ratio between body mass gained and ingested proteins. The highest value of this ratio is in the T3 lot (55.30), therefore the fish oil diet determined a more efficient recovery of the fodder protein (table 6). The feed conversion ratio and protein efficiency improved with the addition of lipids in feed diets, results similar to those obtained by Sajed S. et al., 2014 [8]

Productive Protein Value (PPV) is calculated based on the accumulation of protein in fish meat, without considering the increase in body mass of biological material.

A high value of PPV is found in lot C (3.69). This result indicates a good utilization of proteins in diets with added oils from different sources (Table 6). The protein efficiency coefficient (PER) and the productive value of protein (PPV) evolve in direct proportion to the accumulation of protein in carp meat, the accumulation of protein being stimulated by the addition of oils in feeding diets.

The fatty acid composition of the carp utilized to populate the experiment (initial) is illustrated in table 7.

Table 7 Percent composition of fats in the carp meat (*Cyprinus carpio*) (Wmed:73.12±1.58 g/specimen) – specimens utilized for population experiment

Fish sample	Saturated fatty acids (SFA)	Monounsaturated fatty acids (MUFA)	Polyunsaturated fatty acids (PUFA)		
			Total ω-3 fatty acids	Total ω-6 fatty acids	n-3/n-6
Fish utilized for population experiment	25.14	37.56	15.85	18.99	0.83

The concentration of fatty acids in carp meat fed for 2 months with diets supplemented with oils from different sources is illustrated in Table 8. The concentration of saturated fatty acids (SFA)

in the four lots at the end of the experiment varied from 26.15% in the control lot to 26.15% in lot T1, 27.55% in lot T2 and 26.35% in lot T3.

Table 8 Composition of fatty acids (%), in the meat of carp fed for 60 days with diets with varied concentrations of lipids based on fatty acids

<b>Fatty Acids (%)</b>	<b>Lot C</b>	<b>Lot T<sub>1</sub></b>	<b>Lot T<sub>2</sub></b>	<b>Lot T<sub>3</sub></b>
<i>Saturated Fatty Acids</i>	25.14	26.15	27.55	26.35
<i>Monounsaturated Fatty Acids</i>	37.56	35.01	34.25	35.05
<i>Polyunsaturated Fatty Acids</i>	35.84	36.45	36.80	37.6
<i>Other Fatty Acids</i>	1.46	2.39	1.40	1.05
<i>Eicosapentaenoic Acid (C20:5n3) (EPA)</i>	0.35	0.52	1.40	2.35
<i>Docosahexaenoic Acid C22:6n3 (DHA)</i>	1.05	1.25	1.85	2.55
<i>Total ω-3 fatty acids</i>	16.85	17.95	19.35	20.00
<i>Total ω-6 fatty acids</i>	18.99	18.50	17.45	17.60
<i>ω-3/ω-6</i>	0.89	0.97	1.11	1.14

The saturated fatty acids quantity accumulated in the experimental lots had higher values than the control lot.

The monounsaturated fatty acids are prevalent only in the control lot (41.56%). In the experimental lots, polyunsaturated fatty acids are prevalent in lot T3(37.60%) > lot T1(36.45%) > lot T2(36.80%). The results obtained show that the fat content and fatty acid composition of carp also vary due to diet and are consistent with the conclusions of Dragana Ljubojević et al. in 2017 [3]. Equally important is the quality of polyunsaturated fatty acids, highlighted by the ratio of the two constituent groups ω3 and ω6. In the experimental lots, the value of the ω-3 / ω-6 ratio is higher compared to the

control lots, the results being similar to those reported by M. Bohm., Et al., 2014 [8].

#### Haematological analysis

Haematological parameters are used as an indicator of the health of fish, detecting physiological changes in the biological material involved in the experiment, following various stress conditions [10]. This study showed that the addition of oils to feeding diets resulted in an increase in blood parameters measured in the experimental groups (PCV, Hb, Erythrocyte, MCV, MCH and MCHC), compared to parameters monitored at the beginning and end of the experiment. the control batch fed without the addition of oils.

Table 9 Variation of haematological indicators at the population (Ti) and at the end of the experiment (Tf)

<b>Lot</b>	<b>Haematocrit (Hct) %</b>	<b>Haemoglobin (Hb) (g/dl)</b>	<b>Erythrocyte (mil/μl)</b>	<b>Mean Corpuscular Volume (MCV) (μm<sup>3</sup>)</b>	<b>Mean Corpuscular Haemoglobin (MCH) (pg)</b>	<b>Mean Corpuscular Haemoglobin (MCHC) (g/dl)</b>
Ti Initial	30.95±1.40	6.53±1.15	1.33±0.10	233.77±19.39	48.42±4.70	21.19±1.47
Tf Lot C	32.48±2.25	7.35±1.60	1.40±0.60	238.11±27.41	60.74±13.2	22.78±3.15
Lot T <sub>1</sub>	34.50±3.50	8.05±1.75	1.45±0.90	250.74±22.53	59.52±5.3	22.94±5.13
Lot T <sub>2</sub>	34.73±2.40	8.35±0.90	1.48±1.05	258.87±31.74	56.53±6.6	23.96±1.56
Lot T <sub>3</sub>	35.98±2.20	8.50±1.20	1.50±0.80	270.01±26.30	63.42±11.1	23.36±3.10

Ti= Beginning of the experiment;  
Tf= After 60 days of experiment.

The results are similar to those obtained by Siyavash Soltanian & Mohammad Saeid Fereidouni in 2016 who studied the effect of *Lawsonia inermis* on the immunity and

survival of the common carp, *Cyprinus carpio* [11] (Table 9).

The results of this study can be explained by the adaptive response of fish to nutrition

supplemented with fatty acids from various sources, there are no toxic reactions to the detriment of fish. From a statistical point of view, the haemoglobin quantity increased, presenting significant differences ( $p < 0.05$ ) both between the control lot and the experimental lots, and between the start and the end of the experiment.

## CONCLUSIONS

- Fodder enriched with fatty acids from different sources contributes to improving production parameters by obtaining a higher feed conversion factor.
- The nutritional value of carp meat depends on the existence of a balanced mixture of fodder components and is positively influenced by diets supplemented with fatty acids.
- This experiment highlights the importance of fatty acids in the feeding diets used for common carp, to prevent many diseases and in maintaining proper health, which leads to sustainable aquaculture.

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