EFFECT OF PHYTO-ADDITIVES DIETS ON GROWTH PARAMETERS AND BIOCHEMICAL COMPOSITION OF CARP SPECIES (*CYPRINUS CARPIO*) IN RECIRCULATING SYSTEM

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Abstract

This paper presents the study results carried out on the development of intervention products in the prevention of carp species (Cyprinus carpio) disease. The food formulations have been supplemented with phyto-additives enhanced with antiparasitic and / or bacteriostatic qualities with direct benefits on the biological material.

The biological material which was the subject of the experiment was a native fish species represented by one-year old carp (Cyprinus carpio), grown in the Brates Research and Development Farm, from Galati. The carp species was subsequently introduced in the recirculating aquaculture system, while being fed with different types of fish food.

Three types of diets (3 food variants) with different sources of phyto-additives were developed to carry out the experiment.

The growth parameters and biochemical compositions were determined in the population initially studied (farm carp) before being introduced in the recirculating aquaculture system and when the experiment was finished after 90 days of development, feeded with three experimental diets containing garlic, seabuckthorn and combinations thereof.

The results of the study clearly indicate that the use of phyto-additive dietary supplements determined improved feed conversion rates in the experimental groups compared to the control groups. This confirms the positive effect of phyto-additive supplements, as previously demonstrated by other fish species experiments such as Yambo et al. (2007) and Lyons (2007) [7, 17], with the biological materials reaching the retail market required size much quicker.

The existing studies confirm that there is a clear weight gain advantage in different aquaculture species which have been fed with diets containing natural substances which do not pose a risk to fish, human and environmental health (Georgieva K., 2018) [6].

Batch T3, which was fed with a phyto-additive mix (PER = 2.14 and PPV =43.63), was the most efficient protein harvest batch and batch C, which was fed with the standard food (PER = 1.7 and PPV = 35.16) proved to be the most inefficient.

Phyto-additive diets which were used in carp nutrition (Cyprinus carpio) influenced favourably the nutritional quality of the specimens involved in the experiment and the previously reported results are consistent with those reported by Gabor E. et al., 2010 [4].

Key words: phyto-additives, growth parameters, biochemical composition, *Cyprinus carpio*

INTRODUCTION

Aquaculture diseases are a threat to fish stock markets and animal welfare. Intensive aquaculture has led to diseases which requires the use of medicines for any treatment. Excessive use causes side effects that affects both the fish and the environment in which the fish lives.

70-80% of the antibiotics fed to the fish are released in the aquatic environment by urinary and faecal excretion and/or as unused medicated food, thus the antibiotics are affecting the aquatic environment [8,11].

A strategy to reduce the use of antibiotics in aquaculture is to implement growth practices that reduce the stress level of fish

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and the likelihood of infections that require antibiotic treatment.

Several alternatives to antibiotics, including phytoadditives, have been developed.

The application of these alternatives to aquaculture is promising, but studies are needed for a better understanding of the mechanisms of action and to assess their impact on the environment and the biological material.

MATERIALS AND METHODS

The biologic material subject of the experiment was one-year old carp (*Cyprinus carpio*) obtained from the Research and Development Farm Brates, Galati.

The experiment was conducted using 180 carp specimens (*Cyprinus carpio)* split between four tanks. The technological system of development was of recirculating type [3], including one control tank (with control lot) and three experimental tank (with group type 1, type 2 and type 3) (Table 1), with efficient water volume of 0.24 m^3/tank.

The biological material, with an average weight of 24.64±3.97g g/sample was sorted to ensure homogeneity in dimensions of the population and a healthy state.

Table 1 Characteristics of the biological material (number of specimens, biomass, average individual mass, density) for the experimental population

Tank /Lot	Number of Specimens	Initial Biomass (g)	Individual average mass±SD* (g/specimen)	Density of the initial population kg/m^3
Control tank / group C	45	1091.25	24.25±3.99	4.55
Experimental tank 1 /Lot T1	45	1147.95	24.51±3.87	4.78
Experimental tank 2 /Lot T2	45	1109.25	24.65±4.07	4.62
Experimental tank 3 /Lot T3	45	1087.20	24.16±3.94	4.53

**Standard deviation*

Fish feeding experiments

The control group was fed with standard feed - Soprofish - Smesa 30/7, without phytoadditives.

The feed given in experimental lots was based on 3 feed diets (3 feed types) suplimented with different phytoadiatives sources:

• Feed 1 (codified with FG), with 4% fresh garlic (FV1) fed to experimental group V1;

• Feed 2 (codified with FB), with 4% fresh seabuckthorn (FV2), fed to experimental group V2;

• Feed 3 (codified with FGB), with 2% fresh garlic and 2% fresh seabuckthorn, fed to experimental group V3.

The fish feed ratio was 2.5% of the body weight. The total feed on a daily basis was given at every two hours.

Physical and chemical analysis

The water chemical parameters in the recirculation system for farmed common carp (*Cyprinus carpio L.*) were determined daily, during the experiment, according the working procedures given by the standard methods of surface water analysis [12].

The pH has been determined following SR ISO 10523:1997 standard with a laboratory pHmetre INO Lab pH 720. with temperature gauge.

The chemical consumption of oxygen has been established based on SR ISO 6060:1996 standard.

The nitrogen and phosphorus compounds have been determined using Standard Methods for wastewater/2005 and water examination with a spectrophotometer DR 2800 using LANGE for water quality kit.

The composition of the feed and composition of the fish meat

The physicochemical and biochemical analysis has been done on the feed and the fish meat at the beginning and the end of the experiment, after 90 days of growth in an intensively recirculated system, fed with three diet feeds with different phytoactive sources.

The feed and fish meat analysis has been made using standard procedures for analysing animal feed and fish meat.

The moisture was determined by Standard Official Methods of the AOAC (1990).

The total ash was determined by incinerating the furnace as described by AOAC (1990).

The crude level of proteins from the samples has been determined using Kjeldahl method 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 content was determined by the difference method.

This method involved adding the total values of crude protein, lipid, moisture and ash constituents of the sample and subtracting it from 100.

The value obtained is the percentage carbohydrate constituent of the sample.

The live weight (g) and linear growth (mm) were individually measured and determined.

At the end of the experimental period, growth parameters were determined to assess the overall development of the biological material.

Statistical analysis

All analyses were carried out in triplicate. Statistical analysis was carried out using Microsoft Excel. The average values are compared with the standard deviations. The statistical interpretation of the considered data shows a variation within the allowable threshold of $P<0.05$.

RESULTS AND DISCUSSIONS

Chemical analysis of feed

The composition of the four types of feed used in the experiment is presented in table 2.

Table 2 Chemical composition of the fodders

Feed sample	Moisture g%	Proteins g%	Fats g%	Total carbohydrates g%	Ash g%	Energy value* kcal/100g
SF	13.20	37.85	9.55	21.40	18.00	331.74
FG	14.20	36.75	8.95	21.90	18.20	323.70
FB	14.15	36.65	8.95	22.00	18.25	323.70
FGB	14.25	36.70	8.50	22.20	18.35	320.54

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

In carp, the crude protein from feed ranges within fairly wide ranges, between 24-45% [10].

Because in intensive and super intensive systems the water is only a simple pfisycal piece without natural food, rich diets rich in proteins (36.65g%-37.85g%) were chosen.

Physicochemical analyses of water

The analysis of the chemical parameters of the water (temperature, dissolved oxygen, pH and organic substance) revealed that during the experimental period they were in optimal ranges for the farmed species.

This also applies to the amounts of ammonia, nitrate, nitrite and ammonium in water which for carp farms should not exceed 2 mg/l and 0.05 mg/l respectively (according to Order no. 121/2006 on the classification of surface water quality) variations being higher than those measured in water during the experiment (table 3).

Table 3 Water chemical parameters in the carp recirculation system

Analyzed parameters	U.M.	No of samples	Average ± *SD**	Optimum values (according to Order nr. 121/2006
Temperature	°C	90	24.82±0.55	does not normalize
Dissolved oxyge	mg/l	90	7.04±0.23	10
Ph	uPh	90	7.66±0.02	6.5-8.5
Organic matter	mg $KMnO_4$/l	90	23.40±0.75	<60
Nitrates, (N-NO_3)	mg/l	90	0.56±0.15	2.5-3
Nitrites, (N-NO_2)	mg/l	90	0.005±0.001	0.03
Ammonia (NH_3)	mg/l	90	0.2±0.01	0.2
Ammonium [(N-$NH)^{4+}$]	mg/l	90	0.83±0.05	0.8

**Standard deviation*

Maintaining physicochemical parameters at optimal values was possible due to the technological water treatment system (containing mechanical filters, biological filters, UV sterilization plant), which is meant to ensure the chemical and physical quality of the water supply of the growing tanks, corresponding to the physiological requirements of the culture species involved in the experiment.

The water treatment is efficiently removing the most important parameter that determinates the water quality which is the total quantity of non-ionizat nitrogen (TAN=Total Ammonia Nitrogen).

Analysis of the biologic material involved in the experiment

Following the biometric data analysis, the mean average mass values of the carp species had an ascending evolution in all lots (C, T1, T2, T3) (Table 4).

Table 4 Bioproductive indicators obtained in the case of the carp *(Carpino carpio*) growth in the pilot recirculatory system fed with phytoadditive diets

Growth parameters	***UM***	***Lot C***	***Lot T1***	***Lot T2***	***Lot T3***
Initial Parameters					
Number of fish	-	45	45	45	45
Mean individual weight	g/fish	24.25±3.99	24.51±3.87	24.65±4.07	24.16±3.94
Initial biomass	g	1091.25	1102.95	1109.3	1087.2
Initial stocking density	kg/m^3	4.5	4.6	4.6	4.5
Final Parameters					
Number of fish	-	43	45	45	45
Mean individual weight	g/fish	80.4	89.2	86.1	89.9
Final biomass	g	3457.2	4014	3874.5	4045.5
Final stocking density	kg/m^3	14.4	16.7	16.1	16.9
Growth parameters					
Number of days	zile	90	90	90	90
Individual growth	g	56.15	64.69	61.45	65.74
Total increase in growth (Sr)	kg	2.4	2.91	2.8	2.96
Total Shared Food	kg	3.79	4.37	4.42	3.55
Feed Conversion Rate (FCR)	g	1.6	1.5	1.58	1.2
Daily growth rate	g/zi	0.61	0.75	0.71	0.76
Specific growth rate (SGR)	% zi	0.69	0.80	0.76	0.81

Total weight gain, calculated by the difference between the final and initial biomass of the lot, was higher in group T3 of 2.96 kg compared to 2.91 kg in group T1, 2.8 kg in group T2 and 2.4 kg in group C.

Comparing the Food Conversion Ratio, (calculated as report between given feed (kg) and the difference between the final and initial biomass of the group [kg]), in the four tanks, it was observed that the lowest value recorded (1.20) was in the T3 lot, corresponding to the diet with 2% garlic and 2% seabuckthorn. The highest value was recorded in group C (1.6), whilst group T1 and group T2 returned intermediate values (1.5 and 1.58) [15].

In this experiment, as in other experiments reported by various authors [6], phytoadiative supplements have positively influenced the biomass accumulation in carp brood from the experimental groups compared to group C, the highest value being recorded at the group fed with the combined FGB diet supplemented with garlic and seabuckthorn which is almost equal to the accumulation of biomass gathered in the group fed with FG garlic diet.

The coefficient of variability is below 15%, so the sample is homogeneous and the average is representative.

At the end of the experimental period, the carp brood mass recorded insignificant differences (P>0.05%) between experimental groups T1, T2, T3 and notable differences (P<0.05%) between control group C and the groups fed with diets supplemented with phytoadditives.

Fulton's Condition Factor (K), used for assessing the degree of well-being of the fish [8]was calculated using the equation:

$K=mc/L^3x100$, abbreviations-Fulton condition factor (K); mc-body weight; L-total length.

Group T3 which was fed a supplemented died with a combination of phytoadditives has shown at the end of the experimental period a better condition (3,68) than the biologic material from group T1 (3,5) and T2 (3,4). All three groups (fig. 1) have been registered with higher values compared to the control group (2.95) fed with standard feed [14].

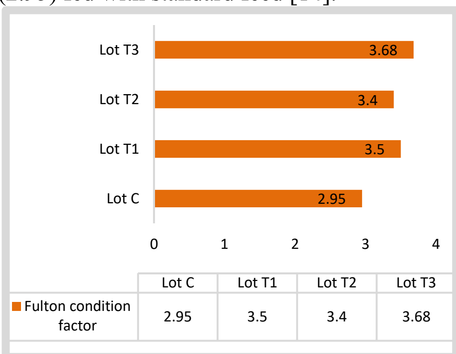

Fig. 1 Fulton condition factor recorded in experimental lots

Adding phytoadditives in the feed for the experimental groups has influenced the body bionetry. The values obtained are compared with the data from the industry [5].

The biochemical composition of the material involved in the experiment

The effect of diets with different phytoadiative sources on the biochemical components of the carp species from the study of data is shown in table 5.

Table 5 Initial composition of fish meat and after 90 days of differential feed experiment with phytoadiative addition from different sources

	Fish sample				
	Initial	Lot C	Lot T_1	Lot T_2	Lot T_3
Moisture, (g%)	82.38±0.14	72.14±0.35	72.60±0.02	74.30±0.21	72.30±0.32
Proteins, (g%)	14.35±0.35	18.55±0.07	19.45±0.11	19.00±0.22	20.18±0.02
Fats, (g%)	1.13±0.86	6.53±0.02	6.10±0.07	5.50±0.10	6.00±0.02
Ash, (g%)	1.51±0.26	1.78±0.02	1,65±0.04	1.65±0.22	1.65±0.11
M/P	5.74	3.89	3.73	3.91	3.58

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

Analyzing the data on **protein content**, there were significant differences between the onset of the experiment and after 90 days of experiment and between the control group C and the three experimental groups at the end of the experiment.

Between the three experimental groups at the end of the study, a slightly higher level in the T3 group fed with FGB (feed with phytoadditive mix) was observed, the difference being proportional to the weight gain, but the difference is insignificant as suggested by the almost similar evolution of the biological material in retaining the protein, regardless of the source of phytoadditives.

Analyzing the data referring to the **fats content**, it was found that there are no differences between the four batches, suggesting similar evolution of the biological material in the accumulation of fats, regardless of their nutritional spectrum.

The values obtained for the protein and the carp fat in all four groups studied are in the same parameters as the values quoted by the literature[1, 2].

At the start of the experiment, the biological material was characterized by a U/P ratio of 5.74. During the experiment, this ratio decreased in all four batches to 3.89 in group C, 3.58 in group T3, 3.91 in group T2 and 3.73 in group T1.

During the experiment, the use of phytoadiative feed resulted in a decrease in moisture, in favor of increasing body fat and lipid concentrations, along with weight gain.

At the end of the experiment, the highest protein and lipid content in fish meat was determined in T3 specimens (20.18g% protein and 6.00g% lipids) where the diet was improved feed with a mixture of phytoadditives (FGB), and in the T1 group (19.45g% protein and 6.10g% lipids), where the diet was the improved garlic (FG) feed, the biological material being characterized by a better growth increase compared to the T2 group where the diet was improved with seabuckthorn.

The survival rate of the biological material is a very important index in any growth system, it has been continuously monitored and calculated with the following formula:

Survival Rate (SR)%=No. of animals survived (fish)/No. of animals leased (fish)x100

Analyzing the values of the losses, we note that a survival rate of 100% was recorded for the experimental lots receiving phytoadditives, compared with the control group where the survival rate was 95%, which received standard feed without phytoadiatives.

This result can be explained by the immunostimulatory effect of garlic due to the presence of allicin, which has an antibiotic effect and stimulates the digestive activity as well as due to the vitamin complex that the seabuckthorn contains.

The recirculation system offers the possibility to always have the biological material under observation and to intervene at any time to avoid losses.

The increased survival rate recorded in the experimental groups of the increased summer carp in the recirculating system confirms the positive effect of phytoadditives on carp [16, 13].

Protein Efficiency Ratio (PER) represents the ratio between body mass gain and ingested proteins. The highest value of this report is in T3 (4.7), therefore the phyto-additive diet resulted in a more efficient use of the protein in the feed.

Productive protein value (PPV) does not consider increasing body mass itself, but adding protein to fish meat.

A high PPV value is found in T3 (95.73), which indicates good protein utilization in the FGB diet (with 2% garlic and 2% seabuckthorn)

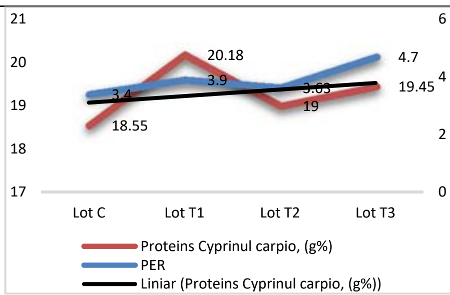

Fig. 2 Dependence of the protein content of carp meat, fed with diets supplemented with phytoadditives and the Protein Efficiency Ratio (PER)

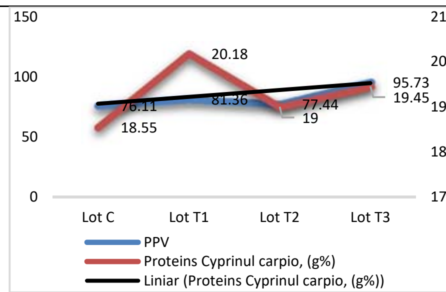

Fig. 3 Dependence of the protein content of meat carp, fed diets supplemented with phytoadditives and Protein Production Value (PPV)

The protein efficency ratio (PER) and the productive protein value (PPV) are directly linked to the protein gain in carp meat. However, the protein gain is stimulated by the phytoadditive feed diets. (Fig. 2, 3).

CONCLUSIONS

•Phytoadditive feed used in carp diet *(Cyprinus carpio)* does not alter the quality of the aquatic environment, with monitored parameters falling within the permissible and recommended limits for the waters used in fish farming, being in the second and third quality class, in accordance with the provisions of Order MMGA no. 161/2006;

•Using phytoadditives (4% garlic, 4% seabuckthorn in carp brood feed *(Cyprinus carpio)* in a recirculating system leads to an improvement of the main growth index by stimulating a better assimilation of feed.

•The phytoadditive diets used in carp nutrition (*Cyprinus carpio*) have favourable influenced the nutritional quality of the specimens involved in the experiment.

•The phytoadditive addition is favourably influencing the survival rate and the general health of the bilogical material through the biostimulating and immunomodulating effects of the phytoadditives.

•The positive results obtained in this experiment could be used to develop further research regarding the phytoadditive dies for carp or any other important species from the economic point of view, but with a longer time frame of the culture. Establishing the action mechanisms and the optimal inclusion levels is a topic that is reccomended for further research.

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