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THE EFFECT OF DIETS WITH ADDED GRAPE MARC ON GROWTH PARAMETERS AND MEAT QUALITY OF CARP *(CYPRINUS CARPIO)*

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Abstract

Nutrition is a determining factor in the growth potential of fish. The high costs of fodders have created the need for research of substitutes that will contribute to improving the growth performance and that will achieve high-quality fish products. Many by-products in the food industry are rich in bioactive nutrients, with the potential to serve as functional food ingredients for fish fodder. The aim of this study is to determine how the growth parameters and composition of carp meat (Cyprinus carpio), reared in a recirculating system and fed with diets that have grape marc as a fodder component, are influenced. The inclusion of grape marc in fodders ensures increased growth performance compared to diets without the addition of grape marc, and a feed conversion ratio (FCR) with better values in the experimental lots (1.48 for lot T2, 1.67 for lot T1, 1.62 for lot T3) compared to the control lot C (1.86). Grape marc used as supplement in diets, determined the accumulation of protein and lipids in carp meat, an increased intake of fatty acids, an improvement in the ω3/ω6 ratio, causing an increase in the nutritional value of fish.

Key words*: Cyprinus carpio, fatty acids, grape marc, growth parameters.*

INTRODUCTION

The aquaculture sector is facing diseases that can cause significant losses of biological material. Therefore, strategies focused on disease prevention, rather than treatment of diseased stocks, are recommended. The use of combined immunoprophylaxis may lead to the final protection of the health of aquaculture fish. Many by-products resulting from fruit and vegetable processing are rich in phytonutrients, bioactive nutrients with the potential to be functional food ingredients for fish fodder.

New strategies for rearing technologies are needed for the sustainable development of aquaculture.

The aim of this study is to determine how the growth parameters and composition of carp meat (*Cyprinus carpio*), reared in a recirculating system and fed with diets that have grape marc as a fodder component, are influenced.

By incorporating this by-product, without economic value, the total cost of production in aquaculture can be reduced, knowing that fodder is the largest share in the final cost of fish (Birol & Şennan, 2017).

MATERIALS AND METHODS

The experiment was performed with 1-year-old carp specimens, with an average weight of 22.52±3.92 g/specimen obtained in the Brateș Experimental Laboratory, Galați.

The experiment took place in a 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 8 weeks.

We used 200 carp specimens (*Cyprinus carpio*), divided into four fiberglass tanks with a volume of 240 liters of water, with a feed rate of 4-8 L/min/tank.

At the beginning of the experiment, the biological material was sorted to ensure population homogeneity in the four tanks (control tank and three experimental tanks).

The slaughter of fish at the beginning and end of the experiment, for biochemical analysis of carp meat, was done by bathing the fish in a super concentrated solution of anaesthetic (1 mL of clove oil and 10 L of water with a temperature of 23˚C), until the installation of rigor mortis, according to Law no.43/2014, on the animal protection, used for scientific

purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Fish feeding experiments

For the control lot C, a standard fodder, type ALLER CLASSIC with a grain size of 2 mm, without grape marc, coded FS, was used.

For the experimental lots, the standard fodder was replaced with different percentages of grape marc, as follows:

- 5% of the standard fodder replaced with grape marc (coded with F5% GM), for lot T1;
- 10% of the standard fodder replaced with grape marc (coded with F10% GM), for lot T2;
- 15% of the standard fodder replaced with grape marc (coded with F15% GM), for lot T3.

The amount of fodder administered daily was 2.5% of the weekly measured biomass.

In order to be included in the fodder, fresh grape marc was dried in an oven at a temperature of 600˚C for 18-22 hours, ground and passed through a 1 mm sieve. Fresh grape marc was obtained by processing grapes from the Burgund Mare, Feteasc㠠Neagr㠠and Merlot varieties, after extracting the juice by crushing and pressing, at the Bujoru Research and Development Station for Viticulture and Vinification.

Physical and chemical parameters of the technological water

A portable multiparameter, model HQ40D - Hach, was used to measure pH, temperature, dissolved oxygen and oxygen saturation.

Nitrogen compounds (ammonia, ammonium ions, nitrite ions and nitrate ions) were determined spectrophotometrically according to the Standard Methods for the Examination of Water and Wastewater, 2005, with a Hach Lange DR 1900 spectrophotometer using LANGE kits.

The determination of chemical oxygen consumption (CCO-Mn) was performed using the potassium permanganate method, expressed in mg $KMnO_4$/L, according to the standard SR ISO 6060:1996. A mineralizer, model LT 200 from Hach Lange, was used to determine the organic matter.

Assessment of growth performance and fodder efficiency

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

WGi = Final weight - Initial weight (g/fish);

WGt = Final lot weight - Initial lot weight (kg/total fish);

FCR = feed fed (kg) / weight gain (kg);

SGR = 100 × [(ln Final fish weight) - (ln Initial fish weight)] / experimental days.

Composition of the fodders and fish meat

The analysis of fodder and samples of fish meat was performed using the procedures indicated by the standard methods of analysis for 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 fibre 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.

Increase protein or retained protein (RP) and increase lipid or retained lipid (RL) were calculated using the following formulas:

RP (g protein) = Final specimen mass × [final proteins] - initial specimen mass × [initial proteins]

RL (g lipid) = Final specimen mass × [final lipids] - initial specimen mass × [initial lipids]

Fatty acids profiling

The determination of fatty acids in fish meat and fodder was determined by gas chromatography. 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 and identification by comparison with a chromatography standard.

Statistical analysis

All determinations were made in triplicate from the prepared average sample of 3 specimens (highest, lowest and average mass). Data are presented as mean±standard deviation (SD). The comparison of several samples was done using the ANOVA test - Single factor followed by T test. The differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSIONS

Chemical analysis of fodder

Grape marc had an acidic pH (3.75±0.13%) which may help prevent the development of pathogenic microorganisms. The major component of grape marc flour is fibre (49.35±0.67 g/100 g). Regarding protein content, the identified value (12.76±2.54 g %) was higher than that reported by Sousa et al. (2014), while for lipid content, the identified value (6.11±0.16 g %) was lower than that reported by Sousa et al. (2014). The composition of grape marc used in the present experiment is similar to that used by Nistor et al. (2014), for the nutrition of sheep and dairy cows. Grape marc is characterized by a high content of PUFA (68.88-75.66%) especially linoleic acid, and poor in SFA (12.30-13.37%) and MUFA (10.09-17.95%) according to Renata et al. (2020).

The standard fodder used, Aller type, contains 30 g% protein and 8 g % lipids. Grape marc flour, which replaced 5%, 10% or 15% of standard fodder, did not significantly alter nutrient composition in experimental diets.

Protein concentration ranged from 29.15± 0.22 g % (F 15% GM) to 29.55±0.12 g% (F 5% GM) and 29.65±0.08 g % (F 10% GM) and fat concentration ranged from 8.15±0.10 g % (F 10% GM) to 8.25±0.12 g % (F 15% GM) and 8.35±0.20 g % (F 5% GM).

The energy value for all four types of fodder was in the range of 356.96 kcal/100 g - 364.53 kcal/100 g. The conversion factors used are 4.1 kcal/g for protein, 9.3 kcal/g for lipids and 4.1 kcal/g for carbohydrates.

Substitution of standard fodder with grape marc flour resulted in a change of the fatty acid profile of the experimental feeding diets (Table 1).

Table 1. Fatty acid profile of fodder in which different percentages of grape marc have been incorporated

Fatty acid %	Standard fodder without grape marc (FS)	Fodder with 5% grape marc (F 5% GM)	Fodder with 10% grape marc (F 10% GM)	Fodder with 15% grape marc (F 15% GM)
Total Saturated Fatty Acids (SFA)	36.64	36.47	36.11	35.88
Total Monounsaturated Fatty Acids (MUFA)	31.37	30.75	30.48	30.04
Polyunsaturated Fatty Acids (PUFA)	30.21	31.53	32.25	32.85
Other Fatty Acids	1.78	1.25	1.16	1.23
Total ω-3 fatty acids	2.95	1.95	1.65	1.95
Total ω-6 fatty acids	27.26	29.58	30.60	30.90
ω6/ω3	9.24	15.17	18.55	15.85
ω3/ω6	0.11	0.07	0.05	0.06

Physico-chemical analysis of water

The physico-chemical parameters of the technological water play a significant role in fish biology and physiology. One of the major advantages of a recirculating system is to ensure the technological water quality conditions necessary for rearing the carp species (*Cyprinus carpio*) involved in the experiment.

Parameters with a significant role in carp biology and physiology were monitored to determine how grape marc diets influence the quality of the aquatic environment (Table 2).

Table 2. Physical and chemical parameters evolution, of the technological water (Average±St. Dev), during the 8 weeks of the experiment

	No. of samples	pH upH	Dissolved oxygen mg/l	Temperature °C	Organic matter mg $KMnO_4$/l	Nitrates, (NO_3^-) mg/l	Nitrites, (NO_2^-) mg/l	Ammonia (NH_3) mg/l	Ammonium (NH_4^+) mg/l
Control tank	56	8.13±0.15	6.65±0.41	25.63±0.73	62.57±12.91	2.85±0.70	0.26±0.17	0.17±0.05	1.73±0.97
Experimental tank 1	56	8.10±0.14	6.38±0.52	25.48±0.60	59.21±13.81	3.74±1.24	0.22±0.09	0.19±0.07	1.95±1.50
Experimental tank 2	56	8.12±0.17	6.52±0.71	25.46±0.56	62.71±12.83	2.94±1.19	0.22±0.07	0.17±0.06	2.11±0.12
Experimental tank 3	56	8.08±0.15	6.42±0.68	25.51±0.91	62.02±16.04	3.86±1.94	0.27±0.12	0.20±0.08	2.18±1.35
CV	56	0.02	0.09	0.03	0.23	0.37	0.45	0.34	0.65

The values of the physical and chemical parameters of water in the four rearing tanks showed that there were no statistically significant differences between the mean values of the parameters measured over the entire experimental period, for any parameter, at the significance threshold of 95% (P- value > 0.05). The values of physico-chemical parameters of water did not register deviations, falling within the optimal interval according to the provisions of Ord. MMGA no. 161/2006 on the classification of surface water quality in order to establish the ecological status of water bodies.

Feeding diets that incorporated different percentages of grape marc, being properly dosed and administered, did not influence the quality of the growing environment, water quality being essential for maintaining the health of fish involved in the experiment.

Analysis of the biologic material involved in the experiment

Growth parameters

At the end of the experiment, the fish in lot T2 fed with 10% added grape marc, had an average mass by 4.58% higher than lot T3 and by 7.63% higher compared to lot T1, results that also influenced the values of specific growth rate (SGR) in the three experimental lots (Table 3).

The amount of fodder consumed during the whole experimental period, to obtain one kg of growth increase (FCR), recorded better values in the experimental groups (1.48 for lot T2, 1.62 for lot T3 and 1.67 for lot T1) compared to control lot C (1.86) (Table 3).

The FCR values for the present experiment are higher than those obtained by Gabor et al. (2012), which studied the effect of phytoadditives on growth parameters and disease resistance in carp fingerlings.

Carp fed with additional grape marc in different percentages, efficiently capitalized on the fodder by reaching a Feed Conversion Rate (FCR) and Specific Growth Rate (SGR) similar to those obtained by Maucieri et al. (2019), in the experimental rearing of carp in conditions of different densities, and by Endut et al. (2016), in the experimental rearing of African catfish in a aquaponic system, but superior to those obtained by Bocioc et al. (2014a), studying the influence of prebiotic pellets on the growth performance of carp fingerlings reared in a recirculating system.

The Feed Conversion Rate (FCR) values obtained in the present feeding experiment of carp with added grape marc for 56 days, are similar to those obtained by Lamichhane et al. (2020), in the 60-day carp breeding experiment, that was fed diets in which *Aloe vera* extract was added, while the values of Specific Growth Rate (SGR) are higher.

The growth performance in the experimental lots showed differences but are not significant (P-value > 0.05), therefore the inclusion of grape marc in fodder ensures a similar or slightly increased growth performance compared to diets without the addition of grape marc.

Mortality was 0% during the experiment, which suggests the possibility of maintaining good health for carp fed with the addition of different percentages of grape marc.

The analysis of technological indicators highlighted the favourable influence of the addition of grape marc on the growth parameters, grape marc being rich in antioxidants, a similar conclusion stated by Dicu (Stroe) et al. (2013), studying the effect of vitamin C.

Table 3. Bioproductive indicators obtained by rearing carp, fed for 56 days on diets with varying concentrations of grape marc

Growth parameters	UM	Lot C Control tank	Lot T1 Experimental tank 1	Lot T2 Experimental tank 2	Lot T3 Experimental tank 3
		SF	(F 5% M)	(F 10% M)	(F 15% M)
		Initial Parameters			
Number of Specimens	-	50	50	50	50
Mean individual weight	*(g/specimen) mass*±SD*	21.86±3.63	22.45±4.45	23.35±3.79	22.40±4.22
Initial Biomass	kg	1.09	1.12	1.17	1.12
Density of the initial population	kg/m^3	4.55	4.68	4.86	4.67
		Final Parameters			
Number of Specimens	-	50	50	50	50
Mean individual weight	*(g/specimen) mass*±SD*	53.82±13.24	56.01±15.73	60.64±16.61	57.86±15.10
Final *Biomass*	kg	2.69	2.80	3.03	2.89
Density of the final population	kg/m^3	11.21	11.67	12.63	12.05
		Growth parameters			
Number of days	days	56	56	56	56
Weight growth individual (WGi)	g	31.96	33.56	37.29	35.46
Weight growth total (WGt)	kg	1.60	1.68	1.86	1.77
Total Shared Food	kg	2.98	2.81	2.76	2.88
Feed Conversion Rate (FCR)	kg/kg	1.86	1.67	1.48	1.62
Daily growth rate (DGR)	g/day	0.57	0.60	0.67	0.63
Specific growth rate (SGR)	%/day	1.61	1.63	1.70	1.69

* *Standard deviation*

The health of the fish was monitored daily, in order to reduce the incidence of disease and minimize the use of chemicals, medicines and antibiotics.

The biochemical composition of the material involved in the experiment

The results of the biochemical analysis of meat, highlight the accumulation of protein and fat throughout the experiment, to the detriment of moisture content for all lots involved in the experiment. At the end of the experiment, there were differences in protein and lipid accumulation between the control lot and the experimental lots fed with the addition of grape marc.

The protein and lipid increase in fish meat, at the end of the experiment, in lot C, has lower values (535.82 g proteins; 103.50 g lipids), compared to lot T1 (611.83 g proteins; 116.13 g lipids), lot T2 (687.60 g proteins; 168.46 g lipids) and lot T3 (652.41 g proteins; 159.64 g lipids) (Table 4).

Data on the biochemical composition of fodder-fed carp meat incorporating different percentages of grape marc from table 4 are similar to data previously published by Georgieva et al., 2019, on the biochemical composition of common carp meat fed with fodder supplemented with phytoadditives.

Table 4. Biochemical composition (Average±St. Dev), of carp meat fed for 56 days on diets with varying concentrations of grape marc

Biochemical parameters	Fish utilized for population of experiment	After 56 days of experiment. Lot C	Lot T1	Lot T2	Lot T3
		SF	(F 5% M)	(F 10% M)	(F 15% M)
Moisture (g %)	79.80±0.20	78.86±0.38	78.15±0.11	77.30±0.08	77.46±0.10
Proteins (g %)	16.00±0.41	16.44±0.53	17.34±0.29	17.50±0.14	17.47±0.18
Fats (g %)	2.68±0.42	3.01±0.31	3.15±0.38	3.81±0.16	3.80±0.41
Ash (g %)	1.32±0.03	1.38±0.07	1.31±0.27	1.33±0.22	1.27±0.13
M/P	4.99	4.80	4.51	4.42	4.43
RP (g proteins)	-	535.82	611.83	687.60	652.41
RL (g lipids)	-	103.50	116.13	168.46	159.64

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

RP=Retained protein; RL=Retained lipid

The concentration of proteins and lipids has the highest value in the experimental lot fed with fodder with the addition of 10% grape marc. It is noted that the accumulation of these components in carp does not vary proportionally with the addition of grape marc in the fodder. Lot T3, which received fodder with 15% grape marc, has approximately equal values for the accumulation of protein and fat in meat with lot T2, which received fodder with 10% grape marc. The composition of carp meat in this experiment shows higher accumulations of

lipids and proteins compared to the composition of muscle tissue in carp juveniles fed the same type of standard feed supplemented with probiotics in the experiment conducted by Bocioc et al. (2014b). These results create the premises for advanced studies on the addition of grape marc in feeding diets and the extension of studies for carp rearing in soil ponds.

The M/P ratio, that characterizes the biological material in terms of nutritional value and maintenance status, decreased in all four lots, evolution determined by the accumulation of protein. The values of this ratio reached 4.80 in lot C, 4.51 in lot T1, 4.42 in lot T2 and 4.43 in lot T3. The proportion of biochemical components in carp meat in this experiment coincides with that identified by Raesen et al. (2017), in the common carp (*Cyprinus carpio*) caught in the wild.

The fatty acid composition of carp meat at the beginning and end of the experiment is shown in Table 5. MUFA monounsaturated fatty acids were predominant in the starter lot and at the end of the experiment in all four lots, the values representing about half of the total fatty acid content (~51.12%).

Table 5. Composition of fatty acids (%) in the meat of carp fed for 56 days with fodder, including varied concentrations of grape marc

Fatty Acids, %	Fish utilized for population of experiment	Lot C Control tank	Lot T1 Experimental tank 1	Lot T2 Experimental tank 2	Lot T3 Experimental tank 3
		SF	(F 5% M)	(F 10% M)	(F 15% M)
Saturated Fatty Acids (%)	28.64	27.97	27.51	26.88	27.02
Monounsaturated Fatty Acids (%)	52.07	51.25	50.58	50.54	51.18
Polyunsaturated Fatty Acids (%)	19.21	20.23	21.65	22.30	21.75
Other Fatty Acids (%)	0.08	0.55	0.26	0.28	0.05
Eicosapentaenoic Acid (C20:5n3) (EPA) (%)	3.18	3.05	4.24	4.42	3.98
Docosahexaenoic Acid C22:6n3 (DHA) (%)	4.38	4.39	5.155	5.82	5.42
Total ω-3 fatty acids (%)	7.55	7.43	9.40	10.25	9.41
Total ω-6 fatty acids (%)	11.66	12.80	12.25	12.05	12.34
ω-3/ω-6	1.54	1.72	1.30	1.18	1.31
ω-6/ω-3	0.65	0.58	0.77	0.85	0.76

The concentration of fatty acids in carp used to start the experiment is similar to that of common carp specimens analysed by Matos et al. (2019), except for the PUFA ω6/ω3 ratio which is 3.5 times lower, which highlights an increased nutritional quality of the specimens with which the experiment was started.

At the end of the experiment, the concentration of polyunsaturated fatty acids showed higher values in the experimental lots (21.65% lot T1; 22.30% lot T2; 21.75% lot T3), compared to the control lot (20.23%) (Table 5).

The PUFA values in the experimental lots are similar, with a slight increase in the lot fed with 10% added grape marc, but the differences are insignificant ($p > 0.05$). The data obtained are similar to those previously presented by Ljubojević et al., 2017, for the wild carp caught in the Danube.

The grape marc used as a supplement in feeding diets comes with an increased intake of fatty acids, leading to an improvement in the ω3/ω6 ratio, an increase in the nutritional value of the fish and a higher resistance of the biological material to environmental conditions and disease.

These results indicate that the incorporation of grape marc into the feeding diets of the carp species (*Cyprinus carpio*) reared in a recirculating system is feasible, with the recommendation to extend the rearing studies in soil basins.

The use of grape marc in aquaculture feeding diets creates an opportunity with great potential, not yet exploited.

CONCLUSIONS

Fish fed with added grape marc has a better fodder conversion ratio and improved weight gain.

This by-product rich in unsaturated fatty acids has the potential to be used as a functional fodder ingredient in carp feeding, as it stimulates the accumulation of polyunsaturated fatty acids in fish meat.

The introduction of grape marc as a source of bioactive compounds in feeding diets is a support for the development of "organic" products, the application of organic technologies, as well as the improvement of the supply with fish of superior quality.

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