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MACRONUTRIENTS MODIFICATION IN THE MUSCLE OF COMMON CARP (*CYPRINUS CARPIO*) DURING WINTER

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Abstract

Climate change can affect the life cycle of fish reared in earthen ponds, especially in winter, when the metabolism is reduced and the fish no longer feeds due to low temperatures. In Romania, in recent years, winter temperatures have been higher than normal, justifying the need to assess biochemical changes in fish meat. The biochemical characterization of macronutrients in carp meat was performed by monitoring during the winter, the following parameters: moisture, proteins, lipids, ash, fatty acids in fish meat, temperature and oxygen of the water. The experiment was conducted between November 2020 - March 2021, the biological material being represented by common carp (Cyprinus carpio), aged one summer. At the beginning of winter, saturated fatty acids (25.80%) and monounsaturated fatty acids (49.03%) were found in a higher proportion compared to the end of winter, when polyunsaturated fatty acids had the highest percentage (55.38%) of the entire amount of lipids. The amount of protein, fat and ash during the winter period decreased but insignificantly, while the water content of the meat increased. The biological material recorded a physiological loss of 27.28% of the initial average mass.

***Key words**: biochemistry, Cyprinus carpio, fatty acids, macronutrients.*

INTRODUCTION

Fisheries and aquaculture, worldwide, are threatened by climate change through rising water temperatures and levels, melting glaciers, significant variations in rainfall, changes in abundant ichthyological stocks and changes in water salinity and acidity. Fish meat plays an important role in human nutrition. The effects of consuming fishery products are to reduce the incidence of cardiovascular diseases, to lower the total cholesterol level, by reducing the level of triglycerides, to moderate the inflammatory response and to improve the metabolism of carbohydrates.

Among the potential sources of winter mortality, heat stress and starvation have received the most scientific attention. Other sources, such as trophic chains and pathogens, have a significant impact but there are not enough studies to date. Recent experimental designs highlight the effects of the interaction between these stressors on end-of-winter mortality (Hurst, 2007).

Taking into account the demands on water temperature, carp is a eurythermic species, preferring higher temperatures and wide seasonal variations, this category includes representatives of the cyprinid family. Knowing the optimal thermal range for each species and age category is of particular importance in rearing and production technologies.

Jobling (1993) investigated the growth rate of carp in relation to the increase in water temperature. The results are directly proportional to the increase in water temperature.

In the case of low temperatures and those exceeding the upper limit of thermal comfort, the metabolic activity of fish is greatly diminished. The tolerated variation limits of the aquatic environment temperature for the species *Cyprinus carpio* are between 3 - 35˚C, according to Froese & Pauly (2022). The optimal development temperature being in the thermal range 20 - 25˚C.

This paper evaluated winter losses and changes in the biochemical composition of carp meat between November 2020 and March 2021. During the years 2020-2021, our studies were carried out within the fish farm Brateș Experimental Base belonging to the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, Galați.

MATERIALS AND METHODS

The experiment took place in 3 earth ponds, belonging to the Brateș Experimental Base, Galați County. In these ponds, carp in monoculture was monitored from the beginning of November 2020 until the end of March 2021. Water temperatures were measured daily at 8 o'clock in all 3 ponds, in the same point, at a depth of 1 meter, using a multiparameter model HQ40D - Hach.

The fish were kept in a concentrated solution of clove oil to be anesthetized before slaughter. This procedure complies with law no. 43/2014 on the protection of animals used for scientific purposes and with the 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.

Once a month, 100 specimens were fished from each pond for measurement and weighing, followed by biochemical analysis of the meat for 5 specimens from each weight category. The biochemical analysis of the meat was performed by weight categories, as follows: under 20 g, between 21 and 100 g, between 101 and 200 g.

The analysis of fish meat samples was performed using the procedures indicated by the standard methods of analysis for 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 Kjeldahl 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 fats were determined using the Soxhlet method, equipped with Gerhardt Brand Multistate Controller, with modified ether extraction methods AOAC 960.39.

Fatty acids in meat were determined at the beginning and at the end of the experiment. A homogeneous sample was achieved from the meat of 10 specimens of *Cyprinus carpio* fished from the 3 ponds.

The determination of fatty acids in fish meat was determined by gas chromatography (GC). For lipid extraction, 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 fatty acids from the sample to methyl ester was followed by separation of the components on the capillary column and the identification by comparison with a chromatography standard.

All analyses were performed in triplicate. Data are presented as mean ± standard deviation (SD). Comparison of several samples was done using the ANOVA test - Single factor followed by T test. Significance was defined as $p<0.05$.

RESULTS AND DISCUSSIONS

The water temperature values, monitored daily are presented as weekly averages and are found in Table 1.

Table 1. Evolution of water temperature starting with November 1st 2020 until March 31st 2021, in the wintering ponds of the common carp

	U.M.	Pond 1	Pond 2	Pond 3	Average ± SD
	˚C	Average±SD	Average±SD	Average±SD	
Week 1 (1-7.11.2020)	˚C	12.53±0.63	13.64±0.65	13.99±0.77	13.39±0.76
Week 2	˚C	10.96±0.32	11.60±0.35	12.83±0.14	11.80±0.95
Week 3	˚C	10.30±0.02	11.01±0.03	12.26±0.04	11.19±0.99
Week 4	˚C	9.61±0.10	10.10±0.09	11.39±0.09	10.37±0.92
Week 5	˚C	7.90±1.02	8.30±0.97	9.11±0.81	8.44±0.62
Week 6	˚C	6.60±0.38	6.86±0.44	7.87±0.52	7.11±0.67
Week 7	˚C	5.86±0.11	6.04±0.08	7.01±0.12	6.30±0.62
Week 8	˚C	5.41±0.45	5.19±0.68	6.93±0.25	5.84±0.95
Week 9	˚C	6.44±0.55	6.57±0.62	7.51±0.46	6.84±0.59
Week 10	˚C	7.17±0.45	7.16±0.35	8.04±0.16	7.46±0.51
Week 11	˚C	4.64±0.58	4.61±0.53	7.29±0.41	5.51±1.53
Week 12	˚C	3.89±0.35	3.80±0.37	4.11±0.97	3.93±0.16
Week 13	˚C	4.31±0.17	4.30±0.19	5.44±0.63	4.69±0.66
Week 14	˚C	3.69±0.12	3.41±0.27	5.10±0.24	4.07±0.91
Week 15	˚C	4.49±0.33	3.87±0.38	5.40±0.26	4.59±0.77
Week 16	˚C	3.99±0.13	3.50±0.45	5.47±0.10	4.32±1.03
Week 17	˚C	4.46±0.16	3.84±0.32	5.61±0.30	4.64±0.90
Week 18	˚C	5.43±0.49	4.60±0.18	5.81±0.28	5.28±0.62
Week 19	˚C	5.64±0.17	5.16±0.19	5.51±0.29	5.44±0.25
Week 20	˚C	5.93±0.26	5.20±0.13	5.06±0.22	5.40±0.47
Week 21	˚C	6.40±0.28	5.69±0.25	6.17±0.33	6.09±0.36
Week 22 (28-31.03.2021)	˚C	7.28±0.17	5.85±0.13	7.08±0.35	6.73±0.77

*SD = standard deviation

The temperature in the 3 ponds varied both due to their location on the farm and the dynamics of the fish population associated with each pond. However, the range of values was close, the average difference between the data collected for each pond was 0.92˚C. These values were compared with the water temperature measured at the Brateș Experimental Base over the past 10 years. Table 2 shows the differences between the average monthly temperature measured between

01.11.2020 - 31.03.2021 and the average monthly water temperature calculated from the data collected in the Brateș Experimental Base. The average monthly temperatures in the experimental period were higher than the average values recorded in previous years, the largest temperature difference being in December. Statistically speaking, the differences are significant, p>0.05.

This increase in temperature, amid global warming, influences the metabolic processes of reared fish species.

Table 2. Average monthly water temperature for the 5 experimental months compared to the averages from the same months from the past 10 years

	Average water temperature in the experiment	Average water temperature in the past 10 years
November	11.04±1.84	10.22±1.65
December	6.91±1.09	6.13±1.11
January	5.69±1.52	2.95±1.86
February	4.46±0.77	3.18±1.14
March	5.79±0.72	5.07±1.27

*Values presented as average ± standard deviation

The beginning of the winter period is considered when the water temperature drops below 10˚C, any feeding being superfluous. At this water temperature, the carp no longer feeds, entering the hibernation phase.

The physiological losses were assessed at the end of the winter period, when the control fishing was carried out. In Table 3, the losses recorded, both in number of specimens and in mass, are presented in comparison with the losses allowed in the technological norms.

At the end of March, the biomass of the biological material was 27.28% lower compared to the biomass parked in the winter basins at the beginning of November. This value is higher than the technological losses and physiological declines allowed for the fish material during its winter hibernation, for each weight category, determined within the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, Galați. Technological norms in the field of fish farming for the eastern and south-eastern areas of Romania were carried out in the Brateș Experimental Base. The set of rules was established in 2009 based on measurements made in previous years.

Mortality due to energy consumption from pre-winter reserves is considered the main cause of declining stocks of young fish at the end of the winter period, according to Pratt & Fox (2002). Resistance to starvation, which depends on the size of the fish, is the main mechanism considered for mortalities at the end of hibernation.

Table 3. Technological losses and physiological declines recorded at the end of winter in the experiment, as well as the values allowed in the technological norms

Average Weight Category g/fish	Technological Norms		Experimental Values	
	No. Specimens	Physiological Declines	No. Specimens	Physiological Declines
0 – 20 g	Up to – 35%	Up to – 30%	36.12%	33.27%
21 – 100 g	Up to – 25%	Up to – 25%	23.33%	26.44%
101 – 250 g	Up to – 20%	Up to – 20%	18.51%	22.13%
Average	26.6%	25%	25.99%	27.28%

In periods of prolonged drought and excessive temperatures compared to the normal of that period, losses of 3-5% are allowed, above the norm of technological losses for that period, for fish specimens up to 1 kg inclusive.

Technological losses during the winter period 01.11.2020 - 31.03.2021 were 1.12% higher than the allowed value for carp under 20 g, but lower by 1.67% for carp weighing between 21 - 100 g and 1.49% for carp weighing between 101 and 250 g. Fish biomass recorded weight losses higher than the allowed values, for all weight categories.

The values of macronutrients in the meat of common carp, *Cyprinus carpio*, were influenced by the degree of activity of the biological material, triggered by winters with higher temperatures than in previous years. Table 4 shows the biochemical parameters measured in the experiment, at the beginning, at the end and monthly during the experiment, from the beginning of November to the end of March. The water content showed a tendency to increase in the muscular tissue of carp, during the winter, in all the weight categories. At the end of March, the moisture content of common carp meat was declining, coinciding with the end of the hibernation period.

The amount of lipids and proteins decreased during the winter period, but the range of variation was not large. This change in biochemical composition is due to the energy consumption necessary for fish to survive the winter. The differences are not statistically significant between the values of macronutrients in fish meat obtained between December 1st, 2020 and March 1st, 2021 (p<0.05), but are statistically significant compared to the values of macronutrients in fish meat obtained on November 2nd, 2020 and March 31st, 2021 ($p>0.05$).

The heavier fish also showed higher amounts of lipids at the beginning of the winter period. The energy value of carp meat highlights the loss of fat and protein in muscle tissue during the winter. Fish use lipid reserves both to survive hibernation and for activity triggered by high winter temperatures, considering that the fish does not have a food source to supplement energy consumption.

Takeuchi et al. (1986), in the experiment of feeding carp before winter with diets low in protein but rich in energy, obtained values of moisture and lipids lower but the amount of protein higher, compared to the carp used in the present experiment, both at the beginning and at the end of the winter period.

Table 4. Variation of the biochemical profile of the common carp meat, *Cyprinus carpio*, during the winter period November 2020 - March 2021

Weight	Standard length		**November 2nd 2020**	**December 1st 2020**	**January 1st 2021**	**February 2nd 2021**	**March 1st 2021**	**March 31st 2021**
Under 20 g	Between 8 - 11cm	Moisture	79.01±0.73	80.38±0.04	81.14±0.01	80.69±0.34	80.83±0.01	79.73±0.03
		Proteins	17.67±0.08	16.89±0.01	16.77±0.10	16.58±0.29	16.98±0.21	17.63±0.32
		Lipids	1.36±0.04	1.22±0.03	0.76±0.18	0.86±0.01	0.95±0.02	1.35±0.06
		Ash	1.19±0.04	1.17±0.02	1.11±0.07	1.08±0.08	1.03±0.06	1.05±0.06
		Energy value* kcal/100g	85.095	80.595	75.825	75.976	78.453	84.838
Between 21-100g	Between 11 - 15cm	Moisture	78.42±0.40	80.19±0.49	80.53±0.90	80.48±1.12	80.51±0.37	79.84±0.43
		Proteins	17.51±0.35	16.78±0.19	16.69±0.12	16.37±0.64	16.66±0.45	17.11±0.43
		Lipids	1.90±0.41	1.55±0.41	1.41±0.95	1.16±1.26	1.49±0.44	1.69±0.32
		Ash	1.17±0.04	1.16±0.02	1.05±0.03	1.05±0.06	1.02±0.08	1.05±0.08
		Energy value* kcal/100g	89.461	83.213	81.542	77.905	82.163	85.868
Between 101-200g	Over 15 cm	Moisture	78.71±0.01	80.35±0.25	80.58±0.45	80.14±0.81	80.82±0.67	80.31±0.81
		Proteins	17.17±0.13	16.65±0.03	16.96±0.34	16.97±0.28	17.08±0.04	17.52±0.22
		Lipids	1.99±0.23	1.46±0.49	0.98±0.09	0.93±0.71	0.86±0.66	0.82±0.52
		Ash	1.19±0.04	1.21±0.03	1.08±0.01	1.03±0.02	1.03±0.08	1.03±0.16
		Energy value* kcal/100g	88.904	81.843	78.65	78.226	78.026	79.458

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

**Values presented as average ± standard deviation

Larger fish in the *Cyprinus carpio* species, considered in the experiment, had higher lipid reserves than small fish in early winter. Post & Parkinson (2001) demonstrated the dependence between fish size and specific metabolic rate. The larger the fish size is, the lower the specific metabolic rate will be. This phenomenon, coupled with higher lipid reserves at the beginning of winter, leads to a higher degree of survival of larger specimens. Therefore, smaller specimens of fish will have a higher mortality rate during the winter due to reduced energy reserves and higher metabolism, in conditions of starvation.

High mortality depending on lipid content and biological mass at the beginning of the winter period was also recorded by Biro et al. (2004), in a study conducted to determine lipid consumption and survival rate of the species *Oncorhynchus mykiss*. The initial weight and starvation during the winter represent important selective pressures for the one-year-old *Oncorhynchus mykiss* specimens, leading to the depletion of lipid reserves up to a minimum critical amount depending on the initial state of maintenance of the biological material.

Cho (2005), for specimens of *Puvulichthys olivuceus* starving for 4 weeks, obtained protein and lipid content values from meat, similar to those in the present experiment.

Table 5 shows the percentage distribution of fatty acids, measured before and after winter, in the meat of *Cyprinus carpio*.

Of the saturated fatty acids, the largest amount is palmitic acid (C16:0), both at the beginning of the winter period and at the end of it. All saturated fatty acids had lower values at the end of winter.

The main share of monounsaturated fatty acids is oleic acid (C18:1), before and after winter. Myristoleic (C14:1), 11-eicosenoic (C20:1), erucic (C22:1), and nervonic (C24:1) acids are found in higher percentages at the end of winter than at the beginning of this period.

Eicosapentaenoic (C20:5) and linoleic (C18:2) acids represent the largest share of polyunsaturated fatty acids at the beginning of the winter period. This percentage distribution changes considerably at the end of the winter period, when linoleic acid (C18:2) and α-linoleic acid (C18:3) are the majority of polyunsaturated fatty acids.
Overall, during the winter, the fatty acid profile changes, the carp preferentially consuming the reserves of monounsaturated and saturated fatty acids. Thus, at the end of March, polyunsaturated fatty acids, which have been used less in basal metabolism, are found in the largest amount. This evolution of fatty acid changes is similar to that from the experiment conducted by Kminkova et al. (2001), in which the fatty acid content of carp tissues over a year was evaluated.
In a study conducted by Guler et al. (2008), related to fatty acid changes in common carp meat, from a lake in Turkey, over the course of a year, palmitic acid (saturated fatty acid) had a lower value in early winter but a higher value in early spring than in the present experiment. The same phenomenon is found for the percentage of oleic acid (monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid), the main representatives of each category of fatty acids.
Guler et al. (2011), repeated the experiment, using common carp *Cyprinus carpio*, from another natural environment, where the fatty acid values were lower for palmitic and oleic acid but higher for docosahexaenoic acid (C22:6).
The ratio of saturated to unsaturated fatty acids is an indicator of the importance of nutritional fat for humans. Ratio values above 0.35 indicate fats with a beneficial quality for consumption. In Table 5, this ratio (n-3)/(n-6) has values greater than 0.35, both before and after wintering, which means that this species, *Cyprinus carpio*, can be introduced into diets for humans, at any time of the year.
In a 2004 study by Bauer & Schlott, common carp activity level was monitored during the winter. The carp was active during the winter, and it began to feed when the water temperature exceeded 3.1˚C, justifying the need for in-depth studies on the feeding behaviour and population densities of the carp in wintering ponds.

Table 5. Percentage distribution of fatty acids in common carp meat, at the beginning and end of the winter period

	Start of the experiment 01.11.2020	End of the experiment 31.03.2021
C 14:0	2.03	1.92
C 15:0	0.69	0.50
C 16:0	16.85	12.10
C 17:0	1.07	1.00
C 18:0	5.16	4.89
∑SFA	**25.80**	**20.41**
C 14:1 (n-5)	0.57	0.62
C 15:1 (n-5)	0.96	0.44
C 16:1 (n-7)	12.01	6.63
C 17:1 (n-8)	2.40	0.95
C 18:1 (n-9)	30.70	9.46
C 20:1 (n-9)	1.63	2.29
C 22:1 (n-9)	0.70	1.11
C 24:1 (n-9)	0.06	0.24
∑MUFA	**49.03**	**21.74**
C 18:2 (n-6)	5.06	26.86
C 18:3 (n-6)	0.63	0.51
C 18:3 (n-3)	3.42	10.54
C 20:2 (n-6)	0.58	0.40
C 20:3 (n-6)	0.07	2.96
C 20:3 (n-3)	0.31	0.77
C 20:4 (n-6)	2.64	2.32
C 20:5 (n-3)	5.52	6.21
C 22:4 (n-6)	0.48	0.39
C 22:5 (n-3)	1.34	0.93
C 22:6 (n-3)	2.81	3.49
∑PUFA	**22.86**	**55.38**
Other fatty acids	2.19	2.47
n-3	13.40	21.94
n-6	9.46	33.44
(n-3)/(n-6)	**1.42**	**0.66**

Steffens (1996), determined the efficiency of diets enriched with lipids and a high energy intake, for the efficient growth and food conversion of common carp. These lipid-supplemented diets lead to an increase in the amount of fat in the fish tissues, carp juveniles with higher fat deposits showing greater resistance to winter conditions.
This correlation between the state of maintenance of the biological material and the survival rate during the winter was also analysed by Sogard & Olla (2000), in the species *Theragra chalcogramma*, aged one summer in the absence of food. Fish survival followed the same bioenergetic pattern, high survival rates after 200 days at average temperatures below 3°C were observed in fish that gained sufficient body weight and a good state of maintenance during the summer growing season. Biochemical analyses performed on the meat of *Theragra chalcogramma* showed a substantial

increase of the water amount in the tissues to the detriment of lipids during the winter, a phenomenon also observed in the present experiment.

For a higher survival rate of the fish during the winter period, methods are required to ensure the best possible maintenance of the biological material at the beginning of winter. In the context of climate change, the reassessment of the norms of technological losses and physiological declines allowable during the winter, must be taken into account in order to ensure the efficiency of fish production.

CONCLUSIONS

The temperature of the aquatic environment between November 2020 and March 2021, recorded higher values than the monthly average measured in previous years, which characterizes the influence of climate change on aquatic ecosystems.

Winter mortality is inversely proportional to the energy value of the weight groups considered in the experiment. The risk of starvation due to a winter with higher temperatures in which the fish are active is much higher.

The physiological losses during the winter period are 2.28% higher than the normative ones, motivated by the increased activity of the carp during the hibernation period, a value that is placed at the upper allowed limit.

The percentage of moisture has increased to the detriment of proteins and lipids in common carp meat over the winter.

Saturated and monounsaturated fatty acids decreased during the winter in the meat of *Cyprinus carpio*. Polyunsaturated fatty acids accounted for a larger share of carp meat at the end of winter, both compared to the beginning of fish hibernation and compared to other categories of fatty acids at the end of the experiment.

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