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Effect of water temperature and time since of administration of ascorbic acid (AA) on its contents in selected tissues of the common carp *Cyprinus carpio* (L.)

Wpływ temperatury wody i czasu od podania kwasu askorbinowego (AA) na jego zawartość w wybranych tkankach karpia *Cyprinus carpio* (L.)

Summary. The effect of water temperature and time after administration of ascorbic acid (AA) on its content in selected tissues of carp kept in different thermal conditions (15°C and 25°C) was determined. The concentration of AA in the kidney, liver and muscle of common carp before the experiment (control) and on 1, 3, 7 and 14 days after *per os* its administration was determined. The greatest content of AA was found in the kidney, while being smaller in the liver and the smallest in muscles, the average being 14.1 μg kg^{-1}, 12.2 μg kg^{-1} and 8.3 μg kg^{-1}, respectively. On the first day after administration of AA its quantity in the kidney was 2.5 times higher and in the liver, twice as high as in the control sample. During the experiment, the AA content significantly decreased and after 14 days after dosing it fell to the value defined in the control sample. The water temperature did not significantly affect the decline of AA in all tissues, but a two-way analysis of variance revealed significant effects of temperature on the changes of AA content only in the liver.

Key words: *Cyprinus carpio*, ascorbic acid, tissue ascorbic acid concentrations, effect of water temperature

INTRODUCTION

In 1928, Albert Szent-Györgyi isolated from adrenal cortex, and than from oranges, cabbage and red peppers a highly reductive compound (ascorbic acid (AA)), which appeared to be a factor preventing scurvy [Svirbely and Szent-Györgyi 1932]. Vitamin C

(AA) is synthesized at different quantities by plants and the majority of vertebrates owing to an adequate enzymatic system capable of transforming D – glucose into the vitamin. Such ability was not observed in a human being or apes, nor in same birds, guinea pigs, fruit – eating bats, insects or osseous fish (Teleostei), but this ability was observed in two non-teleost fish species: the freshwater stingrey and South American lungfish from South America [Fracalossi *et al*. 2001].

The other animals preserved that ability (they are capable of L – gulonolactone oxidase – LGO) and for them ascorbic acid is not an exogenic compound. In the kidneys of the lampreys (*Petromyzentidae*) the sturgeon family (*Acipenseriae*), a dipnoan fish (*Neoceratodus forsteri*) and marbled lungfish (*Protopterus aethiopicus*), the presence of LGO was observed [Moreau and Dabrowski 1996, 1998b, Dabrowski 2001]. Also, the activity of gulonolactone oxidase in the kidneys of *Acipenser fulvescens* and *Polyodon spathula* was revealed [Dabrowski 1994].

Some authors reported GLO activity in the liver and kidney of common carp [Sato *et al.* 1978, Yamamoto *et al*. 1978, Soliman *et al.* 1985]. However, according to Dabrowski [1990], the biosynthesis of AA-positive results obtained by these authors are problematic and could result from methodological inconsistencies and by the inaccuracy of the used methods.

Vitamin C is found in numerous reactions and biochemical transformations, significant both for mammals and lower vertebrates. Its importance consists in increasing the levels of iron, hemoglobin and erythrocytes [Sterkowicz 1989], it prevents harmful effect and accumulation of cadmium [Kapl *et al.* 1994], influences raising the immunity of an organism against the reaction of pathogenic factors, high temperatures and cold [Pardue and Thaxton 1986], decreases negative effects of stress, destroys oxygenic free radicals (hydroxylic, singlet oxygenic and peroxide). It is also indispensable in the process of collagen creation and proper course of oxidative tyrosine degradation. It co-operates with the hydroxylases taking part in the transformations of steroids, fats and some noradrenalin medicines [Wartanowicz and Ziemlański 1992].

Many studies confirmed the importance of the presence of AA in the diet for fish. According to their research, it may affect for fish growth [Wang *et al.* 2003, Ai *et al.,* 2004, Ai *et al.* 2006], but also increase their survival rate and immunity [Ortuno *et al.* 1999, Kumari and Sahoo 2005, Eo and Lee 2008].

Carp as a fish of major economic importance, is in many countries, is one of the popular farmed species. However, there is few information on the stability of vitamin C in the tissues of common carp. Therefore an object of this study was to verify whether the water temperature and time after administration of ascorbic acid (AA) *per os* affect on its content in selected tissues of common carp (*Cyprinus carpio* L.).

MATERIAL AND METHODS

The studies were performed in water tanks experiment on 70 carps, of 150 ±10 g body weight. The fish were adapted to laboratory conditions for 2 weeks. They were kept in the aquaria with well – aerated water (6 ±2 mg O_2/l), 19 ±1ºC and pH 7.0–7.3.

Before starting the experiment for carp kidney, liver and muscles the basic levels of AA concentration (control AA) were determined.

The experiment was carried out in two temperature conditions 15ºC and 25ºC. Ascorbic acid (AA) was administered to fish *per os* once (as water solution) at dose 100 mg kg^{-1} b.w. An appropriate amount of ascorbic acid was applied by a rubber tube directly into the esophagus. Before serving AA fish were anesthetized using MS-222. In the studies, Polfa ascorbic acid was used (w.m. 176.0 kDa). After 1, 3, 7 and 14 days after administration AA from randomly selected three fishes, kidney, liver and muscles were collected.

Quantities of ascorbic acid (AA) in selected tissues of control and experimental samples were determined by the method of Omaye *et al*. [1979].

Regression parameters were calculated for changes of the content AA in the selected tissues of carp depending on water temperature and the time since its application. The results of experiment were log transformed to base 10 and the regression equation were determined after following formula: AA = $a - b$ log(x), where: AA – content of vitamin C (AA) in μg kg^{-1}, day – day since AA application, a i b – parameters describing regression, log – logarithm (base 10).

Statistical analysis of data on vitamin C (AA) content in different tissues, depending on water temperature was evaluated by one-way ANOVA analysis. Determination of depending on AA content in different tissues of common carp on temperature and time of its administration was performed using two-way ANOVA indicating effects of the all factors and interaction between them. All statistical analyzes were performed using program SAS [2001] at the level $p \leq 0.05$.

RESULTS

Estimation of the concentration of AA in the tissues of the carp, before the experiment, showed that the higher its content was on kidney (14.10 μg kg^{-1}). Slightly lower in the liver (12.20 μg kg^{-1}) and the least, with significant statistical differences, in muscle (8.30 μg kg^{-1}) (ANOVA, $F = 43.61$, $p < 0.0001$) (Fig. 1).

After the application of AA *per os*, as in the control group, independent of the water temperature at which the fish were kept, the largest concentration of AA was recorded in the kidney, while the lowest in the muscle (Fig. 2). Differences in the AA content in examined tissues, in the fish kept at various thermal condition (15ºC and 25ºC) were statistically significant (ANOVA, temperature: 15ºC, $F = 18.28$, $p = 0.0001$; temperature 25ºC, $F = 4.46$, $p = 0.019$) (Fig. 2).

Analysis of data obtained during the experiment showed that the content of AA in the kidney in fish kept in water at 15ºC decreased from an average of 35.03 μg kg^{-1}, on one day after its application to 15.00 μg kg^{-1} at day 14. In contrast, in fish kept in water at 25ºC from 44.00 μg kg^{-1} to 13.00 μg kg^{-1}, respectively (Fig. 2).

During the period from 1 to 14 days after administration, AA content in the liver reduced from 23.00 μg kg^{-1} to 13.00 μg kg^{-1} in fish kept in water at 15ºC temperature and from 36.00 μg kg^{-1} to 12.00 μg kg^{-1} in the second thermal range (Fig. 2).

One day after AA application, in water at 15ºC, carp muscles had 15 μg kg^{-1} and in the 14-th day after administration, an average 8 μg kg^{-1}. In fish kept in water at 25ºC temperature, the content of AA in the muscles decreased from 25 μg kg^{-1} to 6 μg kg^{-1} (Fig. 2).

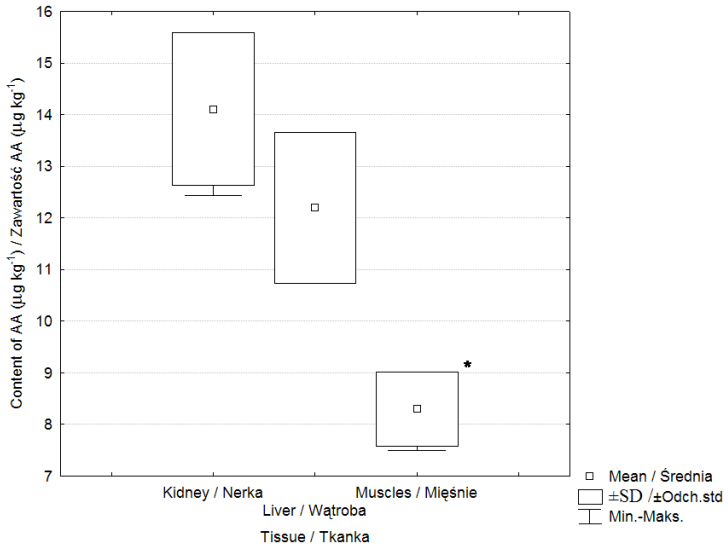

Fig. 1. Contents of ascorbic acid (AA) in tissues of common carp (control) before its application; SE – standard error of mean, SD – standard deviation, * statistical differences at $p \leq 0.05$
Ryc. 1. Zawartość kwasu askorbinowego (AA) w tkankach karpia (kontrola) przed podaniem AA; SE – błąd standardowy średniej, SD – odchylenie standardowe, * statystyczne różnice przy $p \leq 0{,}05$.

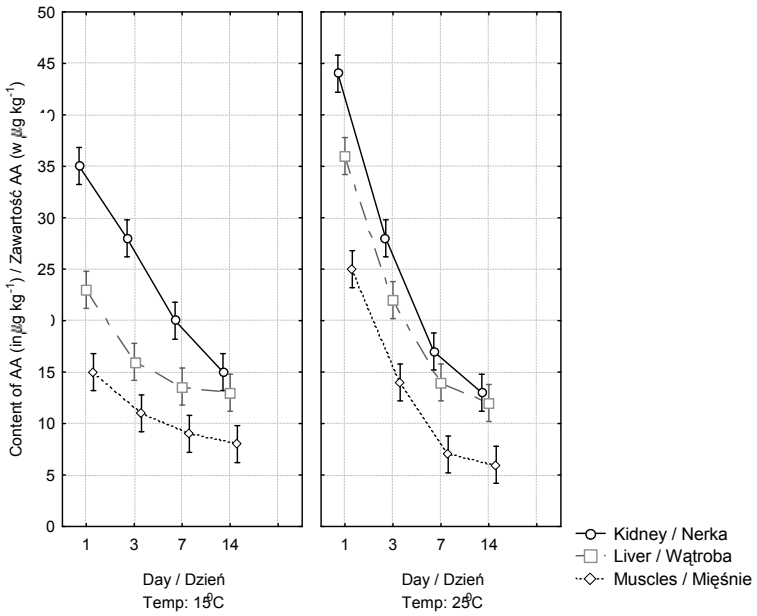

Fig. 2. Mean values and confidence intervals of content of ascorbic acid (AA) in tissues of common carp in two temperature ranges after 1, 3, 7 and 14 day of experiment
Ryc. 2. Średnie wartości oraz przedziały ufności zawartości kwasu askorbinowego (AA) w tkankach karpia w dwóch zakresach temperatur po 1., 3., 7. i 14. dniu eksperymentu

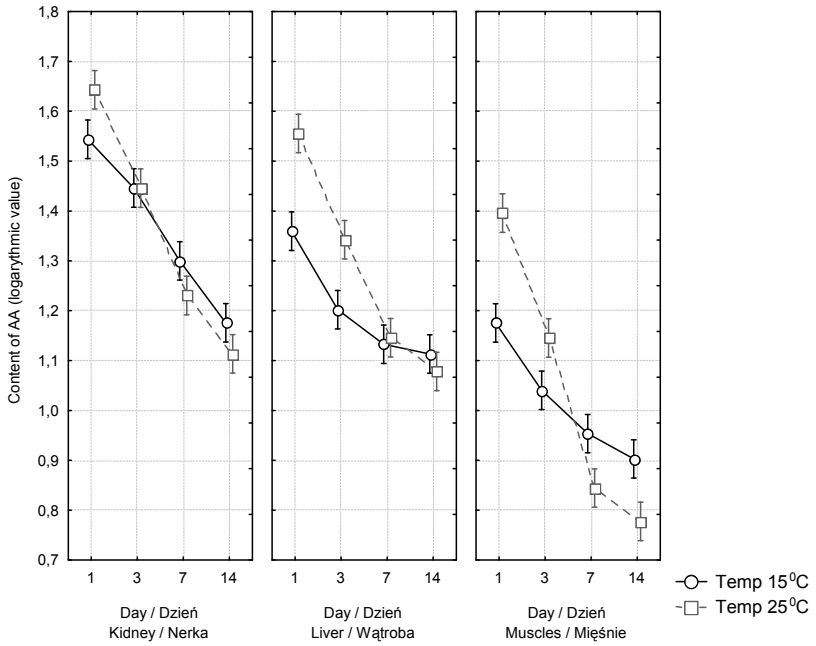

Fig. 3. The relationship of content AA in different tissues of common carp on water temperature (logarithmic value); *statistical differences at $p \leq 0.05$
Ryc. 3. Zależność zawartości AA w poszczególnych tkankach karpia od temperatury wody (wartości logarytmiczne); *statystyczne różnice przy $p \leq 0,05$

Analysis of interaction between water temperature and types of tissue of carp on the content of AA, in 15°C and 25°C showed, that both of this parameters does not significantly influence the content of AA in selected tissues of carp (ANOVA, $F = 0.522$, $p = 0.606$).

Table 1. The values of statistical parameters for experimental factors affecting on the content of AA in selected tissues of common carp
Tabela 1. Wartości parametrów statystyki dla czynników doświadczenia wpływających na zawartość AA w wybranych tkankach karpia

Tissue / Tkanka	Factor / Czynnik	F	p
Kidney / Nerka	temperatura / temperatura	0.45	0.5120
	day / dzień	257.48	< 0.0001
	temp. × day / temp. × dzień	10.05	0.0006
Liver / Wątroba	temperatura / temperatura	28.43	< 0.0001
	day / dzień	121.84	< 0.0001
	temp. × day / temp. × dzień	13.35	0.0001
Muscles / Mięśnie	temperatura / temperatura	2.80	0.1120
	day / dzień	225.00	< 0.0001
	temp. × day / temp. × dzień	39.10	< 0.0001

Table 2. Values of regression parameters (*a* and *b*), the correlation coefficient (r) and value of p parameter describing the changes of content of ascorbic acid (AA) in selected tissues of common carp during experiment fallowing formula: AA = $a - b \log(\times)$
Tabela 2. Wartości parametrów regresji (*a* i *b*), współczynnik korelacji (r) i prawdopodobieństwo (p) opisujące zmiany zawartości AA w wybranych tkankach karpia podczas eksperymentu według wzoru AA = $a - b \log(\times)$

Tissue / Tkanka	Temperature Temperatura	*a*	*b*	r	p
Kidney / Nerka	15°C	35.50	17.81	-0.926	0.00001
	25°C	42.62	27.74	-0.866	0.0003
Liver / Watroba	15°C	21.83	8.81	-0.734	0.0066
	25°C	34.28	21.51	-0.830	0.0008
Muscles / Mięśnie	15°C	14.55	6.16	-0.837	0.0007
	25°C	23.63	17.23	-0.816	0.0012

Analysis of test results for each of the tissues, showed, that with the passage of time (from 1 to 14th) AA content decreased significantly ($p < 0.0001$). Fish-holding temperature had only a significant effect on AA content only in the liver (ANOVA, $F = 28.43$, $p < 0.0001$). In the other two tissues (kidney and muscles), this parameter had no significant effect on AA content (Fig. 3). Simultaneously, a statistically significant interaction effect on the temperature x day on AA content for each of the tissues, was confirmed (Table 1).

The parameters *a* and *b* describing the regression equation reached the highest values for kidney while the lowest for the muscles, regardless of water temperature. Generally, higher values of these parameters, in all types of tissues, were obtained for fish kept in water temperature 25°C (Table 2). Moreover, for all tissues, regardless of the temperature at which the fish were kept of the correlation coefficients (r) had the similar, minus values ranging from -0.926 for kidney (T = 15°C) to -0.734 for liver (T = 15°C) (Table 2).

DISCUSSION

Ascorbic acid (AA) is one of the essential vitamins for the proper functioning of animal organisms, including fish. AA is a potent antioxidant and is essential for many synthesis reactions, supports the action of many enzymes and participates in their bio-synthesis [Wilson 1973, Henrique *et al.* 1998, Wang *et al.* 2003, Eo and Lee 2008].

Since the 60's possibilities of natural synthesis of vitamin C were tested in some fish. Several authors have indicated that in some species, including in the common carp, activity GLO was found in the liver, so they can produce of AA [Sato *et al*. 1978; Yamamoto *et al*. 1978, Soliman *et al.* 1985]. This problem is still controversial and unclear, although, according to Fracalossi *et al*. [2001] since 1985 no study published detected GLO activity in Teleostei fishes.

The necessary presence and importance of AA in fish feed has already established in the 60s the last century, Kitamura *et al*. [1965] and it has been confirmed by Eo and Lee [2008] studies. Simultaneously a number of observations has shown that higher concentrations of AA in the feed can promote growth and lead to higher growth rate of fish [Wang *et al.* 2003, Ai *et al*. 2004, Ai *et al.* 2006]. Supportive action is based not only on the impact on fish growth, but also results in greater survival of young fish, and non-specific immune responses. The studies of Ai *et al.* [2006] shows that increasing the AA content in the feed caused a significantly increased survival rate of yellow croaker (*Pseudosciaena crocea*). In addition, as reported Eo end Lee [2008] dose from 82 mg AA kg^{-1} resulted in improved immune tiger puffer – *Takifugu rubripes*, but increasing the dose above 160 mg kg^{-1} did not result in improvement of immunity. However, Ortuno *et al*. [1999] showed that reinforcement the immunity of gilthead seabream (*Sparus aurata* L.) occurred 2 weeks after administration of increased doses of AA and maintained until 10 weeks.

On the other hand, McLaren *et al.* [1947] observed growth inhibition and anatomicpathologic changes in trout fed with fodder devoid of vitamin C, which was also confirmed later by other authors [Yamamoto *et al*. 1978, Dabrowski 1990]. In addition there are some contradictory reports that dietary AA did not improve the growth performance of seabream [Henrique *et al.* 1998] and yellow croaker [Ai *et al*. 2006].

The content of ascorbic acid (AA) in body depends on the degree of absorption from the digestive tract and of synthesis, taking place in the tissues and by intestinal flora. After absorption from the intestine, vitamin C is stored in greater quantities in tissues with extensive metabolisers. The greatest of its contents were found in other vertebrate animals and man in the eye lens, brain, thymus, liver, pancreas, adrenal gland, intestinal wall and leukocytes [Lewin 1976, Wartanowicz and Ziemlański 1992, Wolf 1993, Lechowski and Nagórna-Stasiak 1995]. In our experiment, after administration *pre os* of AA, 2.5-fold increase of its concentration in the common carp kidney and 2 times higher in the liver, than in the control after 24 hours (1 day) was detected (Fig. 1 and 2). Similarly, when administered different doses of AA in the feed, several times higher concentration of AA in tissues of carp reported by Dabrowski [1990] and Wang *et al.* [2003]. The greatest concentration of AA, Dabrowski [1990] was found in the intestines, but as well as in this experiment the large his quantity also had kidney and liver. Other researchers reported, when synthesis of AA in fish occurs, a large its concentration are in the kidneys and livers [Ikeda and Sato 1964, Sato *et al*. 1978, Yamamoto *et al.* 1978, Soliman *et al.* 1985] or kidneys only [Dabrowski 1990, 1994, Moreau and Dabrowski 1996, Moreau and Dabrowski 1998a]. Moreover, differences in biosynthesis of AA in fish in different parts of kidneys were observed. An example is the white sturgeon (*Acipenser transmontanus*), in which greater activity of GLO in the caudal portion of the kidney by Moreau and Dabrowski [1996] has been determined.

Ascorbic acid is very unstable, quantity decreases shortly and any excess is removed from the body. According to Blom and Dabrowski [1996] concentration of AA in young fish fell rapidly in the first 14 days after first feeding. Also in rainbow trout, the same authors a decrease in AA in the period of 30–40 days in the whole body observed. But reducing time was varied depending on the individual tissues and ranged from 17 to 142 days.

On the other hand Thed and Erickson [1992] exposed channel catfish to a 0.3% ascorbic acid solutions for 8 h, that a decrease in its content in tissues after only 16 hours after fish transfer to clean water was obtained. How the authors explained,

that sharp fall could be the result of stress and metabolic changes caused by environmental changes. In the present study in the all times after administration (1, 3, 7 and 14 days) concentration of AA in all examined tissues: kidneys, liver and muscle were decreased (statistically significant). And at day 14 after administration *per os* her content dropped to a level close to that obtained for the control sample (Fig. 1 and 3).

A many factors influence the degradation rate of ascorbic acid. And apart from high concentration of oxygen, heavy metals (eg Cu and Fe), alkaline pH, the thermal parameters are very important [Wartanowicz and Ziemiański 1992]. According Kubiński [1997] very low content of AA in the feed is caused mainly by thermal treatment. In present study, temperature as an experimental factor had a statistically significant effect on the decrease of AA content only in the liver (Table 2, Fig. 3). However, for other organs (kidney and muscles), there was no significant effect of this parameter. In addition, the effect of temperature on the rate of change of concentration of AA in tissue can provide regression parameters describing the decline in AA content in all examined tissues. For fish kept at higher experimental temperature (25^{o}C) they had higher values (Table 2).

In conclusion, the greatest AA content in both the control sample and during the experiment was reported in the kidney, somewhat less in the liver and in muscle. The content of AA in the tissues decreased with time after application *per os* and within 14 days after the application came up to the quantity defined in the control sample. The rate of decrease in AA in the tissue does not depend on the temperature of water in which fish were kept, but it had an impact on reducing the quantity this vitamin in the liver.

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Streszczenie. W badaniach określono wpływ temperatury wody i czasu po podaniu kwasu askorbinowego (AA) na jego ilości w wybranych tkankach karpi utrzymywanych w różnych warunkach termicznych: 15 i 25^{o}C. Ustalono stężenie AA w nerce, wątrobie i mięśniach karpia przed rozpoczęciem doświadczenia (kontrola) oraz w 1, 3, 7 i 14 dniu po podaniu AA *per os*. Największą ilość AA stwierdzono w nerkach, nieco mniejszą w wątrobie, a najmniejszą w mięśniach, odpowiednio 14,1 μg kg^{-1}, 12,2 μg kg^{-1} oraz 8,3 μg kg^{-1}. W pierwszym dniu po podaniu AA *per os* jego ilość w nerce była 2,5-krotnie większa, a w wątrobie 2-krotnie większa niż w próbie kontrolnej. W kolejnych dniach zawartość AA zmniejszała się istotnie i po 14 dniach od podania zmniejszyła się do wartości określonej w próbie kontrolnej. Temperatura wody nie wpływała istotnie na spadek zawartości AA we wszystkich tkankach, ale analiza dwuczynnikowa wariancji wykazała istotny wpływ temperatury na zmiany zawartości AA tylko w przypadku wątroby.

Słowa kluczowe: *Cyprinus carpio*, kwas askorbinowy, zawartość witaminy C w tkankach, wpływ temperatury wody