ORIGINAL ARTICLE

Influence of cyanobacteria on water activity and dry matter of muscles in the common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* W.)

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Abstract

OBJECTIVES: Cyanobacteria are studied from the viewpoint of the issue of risks to water supply, agriculture and recreational activities for a long time. Cyanobacteria produce a wide range of substances which can be toxic and can influence the safety and quality of fish products. The aim of this study was to determine whether the diet with the content of cyanobacteria can affect the water activity and the dry matter of fish muscle and whether this diet can contribute significantly to the shelf life of fish muscles.

METHODS: Common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) were used in this study. Both fish species were divided into two groups. The first group of fish was fed with feed with cyanobacteria (3% of dry matter), the second group with feed without cyanobacteria. The water activity and the dry matter were monitored immediately after sampling of the fish muscle on day 7, 14 and 21 (carp) or on day 10, 20 and 30 (rainbow trout) and seven days after every sampling and cold storage (6–8 °C).

RESULTS: Feed with the content of cyanobacteria significantly decreased the water activity in muscles of both fish species on day 21 (in carp) and on day 30 (in rainbow trout). The dry matter of fish muscle significantly increased on day 7 and 21 (in carp) and on day 10 and 30, but decreased on day 20 (in rainbow trout). The cold storage significantly influenced the dry matter only. While the dry matter was increased in the common carp (7 days of cold storage after sampling on days 14 and 21), the dry matter decreased in the rainbow trout (7 days of cold storage after sampling on day 10).

CONCLUSIONS: The decrease of water activity was found only after longer exposure in the both exposed fish species. The dry matter was influenced far greater and was mostly increased in the both exposed fish species.

Abbreviations:

c -trout	- a group of trouts non-exposed to the effects of cyanobacteria
cya-trout	- a group of trouts exposed to cyanobacteria
c-carp	- a group of carps non-exposed to the effects of cyanobacteria
cya-carp	- a group of carps exposed to cyanobacteria
s 7	- 7 days of cold storage after every sampling
TDI	- tolerable daily intake
WHO	- World Health Organization
LSD test	- the least significant difference test
ANOVA	- analysis of variance

INTRODUCTION

Cyanobacteria are the part of the aquatic ecosystem and can form water blooms (Ressom *et al.* 1994). The development of the cyanobacterial masses and the consequential formation of the water bloom is closely associated with eutrophication, water retention, warming of the water or the change in the height of the water column (Marvan & Maršálek 1996).

Cyanobacteria can produce many substances including cyanotoxins. Microcystins are the most studied and common cyanotoxins. Microcystins in the fish organism represent a potential risk for consumers of the fish muscle (Murény *et al.* 2003). This is the current issue in regions where different fish are common on the dish (Dyble *et al.* 2008). Microcystins are also highly stable compounds and their toxicity is not reduced by boiling (Zhang *et al.* 2010).

Microcystins get into the fish mainly through the gastrointestinal tract (Ibelings & Havens 2008). The peptide bonds with D-amino acids make the microcystins resistant to normal hydrolytic enzymes. They resist the digestive processes in the gastrointestinal tract because of these bonds (Falconer *et al.* 2005, Smith *et al.* 2010). Microcystins mostly accumulate in the liver, about three times more than in the muscle (Xie *et al.* 2004; Ibelings *et al.* 2005).

The water activity is an important parameter that indicates the availability of the water in the food for the development of the microorganisms. The range of the water activity varies around 0.99 (from 0.95 to 0.99) in the fish muscle and therefore is prone to the microbial spoilage relatively easily. The water activity is considered to be the key to the stability of the product (Paakkonen & Roos 1990). The value of the water activity is also influenced by the microbial processes during the food storage (Abbás *et al.* 2009). The water activity is an important criterion for the evaluation of control and food safety. This parameter was used in 1950, when it was clear that the content of water cannot fully explain the degree of storability of foods because of their susceptibility to microbial processes (Berg 1984). Pitombo and Lima (2003) observed the water activity in the fish muscle (*Pseudoplatystoma corruscans*). They found no change in water activity even after 7 days of storage at 6–8 °C.

The composition of the fish muscle facilitates this deterioration even more due to chemical reactions which take place in it. The muscle of different species and also substances that are accumulated in the bodies of the fish during the life can have a significant effect on the processes that occur in their muscle. The food intake is one of the important factors. This often depends on the environment which the fish inhabit (Orozco 2000; Abbás *et al.* 2009; Hudecová *et al.* 2010).

The consequences of accumulation of cyanotoxins in various organs, muscles and blood parameters in fish are generally better known (Kopp *et al.* 2008). However, the influence of cyanobacteria in the feed on the water activity of fish muscles has not been studied so far. Therefore, the value of water activity in the fish muscle associated with the content of cyanobacteria in the feed is not known.

MATERIAL AND METHODS

Animals and experimental design

Two experiments were performed using one year old common carp (*Cyprinus carpio* L.) and one year old rainbow trout (*Oncorhynchus mykiss*). Experimental fish were obtained from the Department of Zoology, Fishery, Hydrobiology and Beekeeping of the Mendel University Brno and from the Trout Culture Skalní Mlýn, respectively. Fish specimens were divided into two groups, i.e. control (C) and experimental ones, which were exposed to cyanobacteria. Each group consisted of 30 specimens kept in the laminated circular tanks with its own recirculation with 1 m^3 volume (water temperature 19.6–21.8 °C, oxygen saturation 72–80%, pH 7.35–8.16, 10h light/14h dark cycle). The acclimatisation period lasted 14 days during which fish were fed by commercial granulated food. The initial average body weight and total length of common carp was 352.0±59.8 g and 258.4±16.1 mm, respectively. The initial average body weight and total length of rainbow trout was 292.5±40.7 g and 285.8±10.0 mm, respectively.

Oral exposure to cyanobacterial biomass was performed via feeds. Fish were fed by the same food with addition of 3 % of lyophilised toxic cyanobacterial biomass. The concentration of microcystins was 2 mg/1 kg of food, i.e. 0.4 mg/1kg of fish weight and day. Feeding was three times a day and amounted to 1 % and 0.8% of the fish stock in the common carp and the rainbow trout, respectively. Adaptation of the fish ration was made weekly on the basis of the actual fish weight. The experiments lasted 21 days in the common carp and 30 days in the rainbow trout. Seven specimens from each group were euthanised, necropsied and samples for subsequent evaluations were collected weekly in the common carp on days 7, 14 and 21 and on days 10, 20 and 30 in the rainbow trout.

Experimental procedures and laboratory analyses

Fish muscles from the area of caudal peduncle were used for analyses. The water activity and the dry matter were monitored immediately after sampling of the fish muscle and seven days after every sampling and cold storage. The storage was under temperature 6–8 °C and the muscles were packaged in atmosphere of nitrogen 77%, oxygen 22%, and carbon dioxide 1%. Water activity was determined using Aw meter Novasina, which works on the manometric principle. Dry matter content was determined by drying the samples up to a constant weight at 105°C in the drying room Mettler Toledo HB43.

Statistical analyses.

Differences between experimental groups and controls were tested by Analysis of Variance followed by LSD test. Levels of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Feed with the content of cyanobacteria significantly decreased the water activity in muscles of both fish species on day 21 (in carp) and on day 30 (in rainbow trout) (Tables 1 and 2). Although cyanotoxins affect the water activity in the fish muscle, the values of the water activity were in the range from 0.98 to 1.00 for all samples, which corresponds with the range from 0.95 to 0.99 reported by Orozco (2000) and Abbas *et al.* (2009). Any greater fluctuations in the water activity within 7 days of storage were not observed, as also described by Pitombo and Lima (2003).

The results in the dry matter were far more significant and are presented in the Tables 3 and 4. The statistically significant increase in the carp was observed after 7 ($p<0.05$) and 21 ($p<0.001$) days. The statistically significant increase ($p<0.05$) in the rainbow trout was observed after 10 ($p<0.01$) and 30 ($p<0.001$) days. Sta-

Tab. 1. Average values of water activity in the rainbow trout (mean±SD).

day	10	S 7	20	S 7	30	S 7
c-trout	1.003±0.001	0.986±0.001	0.993±0.002	0.99±0.003	0.987±0.004	0.986±0.004
cya-trout	1.004±0.002	0.985±0.006	0.994±0.004	0.99±0.005	0.982±0.006	0.985±0.006
p-value	>0.05	>0.05	>0.05	>0.05	<0.01	>0.05

Tab. 2. Average values of water activity in the common carp (mean±SD).

day	7	S 7	14	S 7	21	S 7
c-carp	0.984±0.01	0.984±0.01	0.984±0.01	0.990±0.01	0.985±0.009	0.985±0.01
cya-carp	0.984±0.01	0.986±0.01	0.985±0.01	0.984±0.01	0.952±0.01	0.986±0.01
p-value	>0.05	>0.05	>0.05	>0.05	<0.001	>0.05

Tab. 3. Average values of dry matter (%) in fish muscles in the rainbow trout (mean±SD).

day	10	S 7	20	S 7	30	S 7
c-trout	24.22±0.34	28.28±0.78	26.64±0.98	26.02±0.89	22.67±1.17	25.1±1.04
cya-trout	25.61±1,05	24.36±1.09	25.95±1.41	26.25±1.40	25.92±1.60	24.93±0.60
p-value	<0.01	<0.001	<0.05	>0.05	<0.001	>0.05

Tab. 4. Average values of dry matter (%) in fish muscles in the common carp (mean±SD).

day	7	S 7	14	S 7	21	S 7
c-carp	32.16±0.55	25.45±1.11	22.65±0.51	24.24±0.53	23.70±1.24	28.43±0.77
cya-carp	32.63±0.01	25.46±0.01	22.82±0.01	35.4±0.01	34.03±0.01	34.69±0.01
p-value	<0.05	>0.05	>0.05	<0.001	<0.001	<0.001

tistically significant decrease ($p<0.05$) was observed after 20 days.

The cold storage significantly influenced the dry matter only. While the dry matter was increased in the common carp (7 days of cold storage after sampling on days 14 and 21), the dry matter decreased in the rainbow trout (7 days of cold storage after sampling on day 10).

Mareš *et al.* (2009) evaluated the effect of naturally developing cyanobacteria on the composition of muscles of two commercially important freshwater fish, *Cyprinus carpio* and *Hypophthalmichthys molitrix*. The cyanobacterial water bloom induced statistically significant effects only in the content of fatty acids in the common carp (decrease of the ratio of n-3 and n-6), while all studied parameters including the content of dry matter and fat, proteins, fatty acid composition and some amino acids were affected in the silver carp. These authors however found decrease of the dry matter in silver carp. On the other hand, Palikova *et al.* (2011) did not find substantial changes in basic parameters of dietetic quality including dry matter in the Nile tilapia (*Oreochromis niloticus*) affected by the exposure to cyanobacteria. The only significant difference was observed during second week of exposure, when higher levels of dry matter were found in the exposed fish. We found mostly the increase of dry matter in our study. Differences among the studies and various fish species might be attributed to a number of internal and external factors, for example by different used biomass with other concentration of toxins, by the type of exposure (controlled feeding vs. the exposure to a complex bloom) and also by variable sensitivities of used fish species.

CONCLUSION

The effect of cyanobacteria on the values of water activity was low. Some decrease was found only after longer exposure (on day 30 in the rainbow trout and on day 21 in the common carp) in the exposed fish. The values in the range from 0.98 to 1.00 are commonly reported in the fish muscle. The effect of cyanotoxins was far greater on the dry matter of fish muscles and was mostly increased in the exposed fish. We suppose that the increase of dry matter positively corresponds with the decrease of water activity in the muscle.

More extensive research should be done in the future to verify more parameters of quality that could be affected by cyanobacteria. The comparison with other fish species would also be suitable.

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Potential Conflicts of Interest: *None disclosed.*

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