MICROBIOLOGICAL ASPECTS OF THE CARP POND ECOLOGICAL STATUS DURING THREE YEARS PERIOD

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MIKROBIOLOŠKI ASPEKTI EKOLOŠKOG STATUSA ŠARANSKOG RIBNJAKA TOKOM TROGODIŠNJEG PERIODA

Apstrakt

Šaranski ribnjaci su, pored pastrmskih ribnjaka jedan od dva najzastupljenija vida akvakulture na našim prostorima. Konzumni šaran može da dostigne dužinu od preko 30 cm i masu preko 6 kg. Meso šarana bogato je polinezasićenim masnim kiselinama koje imaju korisno delovanje u ishrani. Šaran iz akvakulture, dobro je prilagođen na uslove životne sredine, koji su slični onima u prirodnom staništu. Naseljava bentos i pelagijal, rastresiti mulj i vegetaciju širokih, usporenih vodotokova. Šaran je omnivor i hrani se rečnim rakovima, insektima, crvima, larvama i submerznim biljkama. Pored makroozooplanktna i makrozoobentosa, mikroskopski vidljive alge, bakterije, gljive i virusi prisutne su u ekosistemu šaranskih ribnjaka. Bakterije mogu biti korisne, bezopasne, štetne ili patogene. *E. coli* i *Enterobacteriaceae* važni su pokazatelji fekalnog zagađenja vode i rizika po zdravlje gajenih životinja. Zdrave jedinke šarana u svom crevnom sistemu imaju korisne bakterije, saprofite, koje u uslovima optimalnog metabolizma, postaju dominantne. Saprofitnu mikrofloru crevnog sistema šarana čine, sa više od 99 % *Bacillus spp., Bifidobacterium spp., Lactobacillus spp., Saccharomycetes*, itd. Aerobne i fakultativno aerobne bakterije čine oko 1 % crevne mikroflore. Različite su bakterije koje izazivaju oboljenja gajene ribe. *Aeromonas spp*. su Gram- negativne bakterije koje naseljavaju prirodno stanište slatkovodnih i morskih ekosistema a i deo su oportunističke saprofitne mikroflore gastrointestinalnog trakta. *Aeromonas spp.* vrlo je čest izolat iz hemoragičnih lezija i nekrotičnih delova tkiva. Kod šarana, *Aeromonas salmonicida* dovodi do oboljenja, poznatijeg kao eritrodermatitis. Septikemije kod šarana mogu izazvati pokretljive aeromonade, *A. hydrophila, A. sobria* i *A.caviae.* U slučaju oboljenja šarana, gde je veoma velika gustina populacije ribe u ribnjaku, lako dolazi do širenja bolesti, a sama bolest teško se iskorenjuje, uz velike ekonomske gubitke.

Enterobakterije obuhvataju rodove *Escherichia, Salmonella, Klebsiella, Edwardsiella* i *Yersinia.* Ukupno je ispitivano po 6 uzoraka ribe (koža i mišić), hrane za ribe, mulja i vode, tokom 4 godišnja doba, tokom trogodišnjeg perioda.

Uzorci kože i mišića šarana ispitivani su prema metodama propisanim u Pravilniku o metodama vršenja mikrobioloških analiza i superanaliza životnih namirnica (Sl. list SFRJ br.25/80). Na ovaj način uzorci su ispitani na prisustvo *Salmonella spp., Proteus spp., E.coli, Clostridium spp. i Staphylococcus aureus.* Odgovarajućim ISO i ostalim mikrobiološkim metodama utvrđivano je prisustvo *Aeromonas hydrophila, Vibrio parachaemoliticus*., *Yersinia enterocolitica*., *P. aeruginosa,* odnosno *E. coli*, koliformnih bakterija i intestinalnih enterokoka. Ukupan broj bakterija izolovanih iz kože i mišića kretao se od 3.11 $\log_{10}$ CFU/g do 4.72 $\log_{10}$ CFU/g. *E.coli* izolovana je iz barem jednog uzorka kože od šest ispitivanih, tokom svih uzorkovanja. Ostali patogeni mikroorganizmi nisu izolovani iz tkiva. Ukupan broj koliformnih bakterija u vodi nije prelazio 198 bakterija/100 ml, dok su u mulju nađeni predstavnici rodova *Aeromonas* i *Clostridium*, pored *E.coli*. Hrana za ribe bila je mikrobiološki ispravna po kriterijumima važećeg Pravilnika. Na osnovu ovih pokazatelja može se zaključiti da je proizvodnja šarana u veštačkom sistemu akvakulture omogućila dobijanje mikrobiološki ispravnog mesa iz ujednačenog ekosistema najsličnijeg prirodnom staništu rečnog šarana *C. carpio.*

Ključne reči: *Cyprinus carpio, carp mikrobiologiaja šarana, ribnjak, ekoistem*

INTRODUCTION

Carp from our aquaculture, is derived from the Asian wild strain that is selected for certain characteristics (Kohlmann et al., 2003). Cultivated carp can reach a length of over 30 cm and weight over 6 kg. Carp meat is rich in polyunsaturated fatty acids (Djinovic et al., 2010) that have beneficial effects in nutrition. Favorable effect of n-3 polyunsaturated fatty acids from fish meat, on human health has been demonstrated in many studies (Von Shacky, 2001, Mozaffarian et al., 2004). Carp inhabits benthos and the pelagic, loose sand and vegetation of broad, slow streams. In terms of reduced concentrations of dissolved oxygen in the water, the fish rises to the surface. Carp is omnivor and feeds on river crabs, insects, worms, larvae and submerged plants. In addition cultivated carp is fed by plant feed. Bacteria from feed and water can be useful, harmless, harmful or pathogenic. Near the habitat of carp there are a number of settlements that are the sources of fecal and other pollution (Harnisz M., et al. 2010.). *E. coli* and *Enterobacteriaceae* are important indicators of faecal contamination of water and health risk of animals. Healthy individuals in their intestine have beneficial bacteria, saprophytes, which in terms of optimal metabolism, become dominant. Saprophytic microflora of carp intestinal systems are, with more than 99%: *Bacillus spp*. *Bifidobacterium spp*., *Lactobacillus spp*. *Saccharomycetes*, etc.. Aerobic and facultative aerobic bacteria are about 1% of the intestinal microflora (Zhou Q., et al. 2009). In carp, *Aeromonas salmonicida* causes disease, known as erythrodermatytis (Gostin I., et al., 2010.). Septicemia in carp can be caused by movable aeromonads, *A. hydrophila, A. Sobria* and *A.caviae,* even when they do not cause disease of fish, can cause gastrointestinal diseases in humans, when eating inadequately prepared meat fish. In the case of carp disease, where there is very high population density of fish in the pond, disease spreads easily and is difficult to eradicate, followed by economic losses. *Enterobacteriaceae* include genera

Escherichia, *Salmonella, Klebsiella, Edwardsiella* and *Yersinia.* Enteropathogenic *E. coli* has been isolated from clinically healthy fish (Kurtovic B. et al., 2001.).

MATERIALS AND METHODS

A total of 6 samples of the fish (skin and muscle), fish feed, mud and water, have been tested during each season, for three years. Samples of skin and muscle of carp were examined by the methods prescribed in the Regulation[1]. In this way the samples were tested for the presence of *Salmonella spp*., *Proteus spp*., *Escherichia coli*, *Clostridium spp*. and *Staphylococcus aureus*. Samples of skin and muscle of fish were tested for the presence of *Aeromonas hydrophila* using method for isolation and identification of moving aeromonads, and method for Isolation of *Vibrio parachaemolyticus* for isolation of *Vibrio* spp.. In order to determine the presence of pseudomonads, and the presences of the genus *Yersinia*, samples were analyzed using Method for isolation and identification of *P. aeruginosa*, or Method for isolation and identification of *Yersinia enterocolitica*.

Samples from the environment, mud and water, were analyzed by the relevant ISO methods the presence of *Enterobacteriaceae*, *Salmonella* spp., *E. coli* and total viable count. Water from the lake was examined by membrane filtration method for determining *E. coli* bacteria and coliforms: EN / ISO 9308-1:2000 Cor 1:2007, and intestinal enterococci EN ISO 7899-2:2000. Samples of complete feeds for carp, were analyzed for the presence of pathogenic bacteria, yeasts and molds, and total aerobic bacteria, the relevant ISO methods.

RESULTS AND DISCUSION

The results are shown in Tables 1, 2, 3 and 4 .

Table 1. The three-year overview of microbiological findings in samples of muscle and skin of carp from aquaculture

				F	I	S	H			
Examination	**sep.08**	**dec.08**	**mar.09.**	**jun.09**	**sep09.**	**dec.09**	**mar.10**	**jun.10**	**sep.10**	**dec.10**
TVC (log CFU/g)	4,72	3,76	3,92	4,08	4,25	3,66	3,45	4,22	4,58	3,11
Salmonella	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E.coli	+ (2/6)	+ (3/6)	+ (3/6)	+ (1/6)	+ (4/6)	+ (2/6)	+ (2/6)	+ (1/6)	+ (4/6)	+ (1/6)
L.monoc.	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Vibrio	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Yersinia	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Aeromonas	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Clostridium	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

Most commonly isolated bacteria in fish muscle is *E. coli*, during all the seasonal study. In raised carp, the majority of the members of the species E. coli and 64%, following 4.2% of *Salmonella spp*., 17.6% *Vibrio spp*. and 3.3% *L. monocytogenes* (Basti A., et al., 2004). According to other findings mostly representatives were isolated of the

genus *Flavobacterium* 37% of isolates, following family *Enterobacteriaceae* (15.6%) and *Vibrionaceae* (37.8%) and others families with less than 1% of the total number of isolates (Mahmoud B., et al., 2004.). As for the total number of aerobic bacteria, its maximum value is 4.72 $\log_{10}$CFU/g in autumn sampling. Similar growth of TVC in the fall was recorded with other authors, where the TVC was 9.4 $\log_{10}$CFU / g (Al-Harbi A.A. et al., 2004). Bacteria of the genus *Aeromonas*, *Yersinia* and *Vibrio*, were not isolated during any season or year of sampling. Species of the genus *Vibrio*, they are much more common isolates from aquaculture brackish water ecosystems, which are as high as 58% of total isolates (Al-Harbi A.A. et al., 2004). In water and sludge, other than coliforms, intestinal enterococci, *E. coli* and *Aeromonas* spp., as is the case with our samples, it is possible to isolate *Pseudomonas spp*., *Vibrio spp*. and *Bacillus* spp., also nonsporogenic and Gram- positive bacteria (Kennedy B. et al, 2006). The total number varies from 10^1 to 10^5 cfu / ml, slightly more than the total number of coliform bacteria in water (Table 2 and Table 3).

The most common isolate in our case is *E. coli*, when it comes to mud, while in some findings, the most frequent isolate of Gram-negative bacteria is *Aeromonas*. The number of bacteria *Vibrio* ranged from 10^1/ml to 10^3/ml. (Kennedy B. et al, 2006).

Table 2. The three-year overview of microbiological status of sludge from the carp pond

					M	U	D			
Examination	**Sep-08**	**Dec-08**	**mar.09.**	**Jun-09**	**sep09.**	**Dec-09**	**Mar-10**	**Jun-10**	**Sep-10**	**Dec-10**
Salmonella	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E.coli	+ (4/6)	+ (3/6)	+ (5/6)	+ (2/6)	+ (3/6)	+ (4/6)	+ (4/6)	+ (5/6)	+ (6/6)	+ (3/6)
L.monoc	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Vibrio	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Yersinia	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Aeromonas	+ (2/6)	+ (3/6)	+ (3/6)	+ (2/6)	+ (4/6)	+ (4/6)	+ (3/6)	+ (2/6)	+ (4/6)	+ (5/6)
Clostridium	+ (2/6)	+ (5/6)	+ (4/6)	+ (4/6)	+ (5/6)	+ (3/6)	+ (4/6)	+ (1/6)	+ (5/6)	+ (1/6)

Table 3. The three-year overview of microbiological status of water from the carp pond

			W	A	T	E	R			
Examination*	**Sep-08**	**Dec-08**	**mar.09.**	**Jun-09**	**sep09.**	**Dec-09**	**Mar-10**	**Jun-10**	**Sep-10**	**Dec-10**
Total col. number	115	101	129	187	88	97	122	120	101	108
Intestinal enterococci	47	33	104	94	39	45	15	9	25	42
E.coli	98	82	67	122	74	86	54	14	75	55

Table 4. Microbiological safety of fish feed during three year period

Examination	Sep-08	Dec-08	mar. 09.	Jun-09	sep 09.	Dec-09	Mar-10	Jun-10	Sep-10	Dec-10
	F			E		E	D			
Salmonella	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
L. monoc.	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E.coli	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Yeasts and moulds	2.95	2.85	2.7	4	2.95	4.3	5	3.25	2.87	3.14
TVC	3.85	4.7	3	4.9	4.48	4.77	7	5.52	6.82	4.14
C.botulinum/ C.perfringens	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
S.pyogenes	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Proteus	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

When it comes to feed that has been used for carp (Table 4.), microbiological safety of the analyzed samples corresponds to the current Regulation[2]. Pathogenic microorganisms were not detected, and the total number of aerobic bacteria did not exceed 6.82 $\log_{10}$ cfu/g.

It is very important not to have *Salmonella* and other pathogens in feed, because they are cause of numerous outbreaks, that can not be controlled easily (Moretro T. et al., 2004.). Once it enters into a factory for the fish food production, these bacteria are very difficult to eradicate, and it becomes so called "domestic strain" (Lunestad B.T., et al., 2007.)

CONCLUSIONS

Regarding obtained results, it can be cocncluded that during three year long period of examination, common carp from aquaculture was microbiologicaly safe, its water habitat was microbiologicaly safe and it wasn`t threatened with pathogens. Based on these results, it could be concluded that the production of carp in artificial aquaculture system allowed obtaining microbiologicaly safe food from balanced ecosystem most, that is most similar to natural habitats of river carp *C. carpio*.

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