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REDUCTION OF BACTERIAL CONTAMINATION OF COMMON CARP UNDER VARIOUS REARING CONDITIONS

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ABSTRACT. Numbers of bacteria identified on broth agar in 20 and 37°C, coliforms, fecal coliforms, fecal streptococci, anaerobic sulfite-reducing *Clostridium perfringens*, *Pseudomonas aeruginosa*, Aeromonas sp., and Salmonella sp. were evaluated in the muscles, skin, and digestive tract content of common carp (*Cyprinus carpio*). The fish were sampled: 1. from a pond supplied with biologically purified sewage from a sewage treatment plant in Jedwabno (at the age 1+), 2. after 6 months of wintering in an earthen pond supplied with clean lake water, and 3. after subsequent 6 months of rearing in the same water. Wintering and subsequent rearing took place in the Stocking Material Production Centre in Warlity. The fish were aged 2+. Muscles, skin and digestive tract contents of all fish were free from *Clostridium perfringens*, *Pseudomonas aeruginosa*. Salmonella sp. were present only in muscles and skin of fish from sewage-supplied pond. Wintering caused considerable reduction (90.0-98.8%) of coliforms, and fecal coliforms (98.7-100%) in the tissues and digestive tract contents. Numbers of Aeromonas sp. in skin were reduced by 91.1% and concentration of fecal streptococci in the guts - by 74.6%. Further reduction of bacterial contamination was observed after another 6 months of rearing in the pond supplied with clean lake water, but numbers of Aeromonas sp. in the digestive tract increased considerably.

Keywords: FISH FRY, TABLE CARP, AQUACULTURE, WASTEWATER POND, LAKE WATER, PURIFICATION, BACTERIAL INDICES

INTRODUCTION

Fish rearing in ponds receiving waste water is coupled with the danger of contaminating fish flesh with micro-organisms pathogenic to humans. Body surface (skin) and digestive tract are most easily contaminated, and eventually also internal organs and muscles. Strauss (1985) reported that: 1. invasion of fish flesh by pathogenic bacteria was very likely if the fish were reared in water containing over 10^4 of fecal coliforms (*Escherichia coli*) and 10^5 of Salmonella sp. per 100 ml; 2. probability of contamination increased with time of rearing in polluted water; 3. accumulation of intestinal micro-organisms and pathogens on body surface or their penetration into edible tissues was unlikely when bacteria concentration in water was under 10^3 per 100 ml; 4. high concentrations of pathogenic micro-organisms might occur in the digestive tract and in intraperitoneal fluid of the fish even at low numbers of indicatory bacteria. These data base on experimental studies carried out in Israel (Buras et al. 1985)

and the USA (Hejkal et al. 1983, Phelps 1985) and on other data of the same and other authors (Buras et al. 1987, Guttman-Bass et al. 1986, Lesel and LeGac 1983, Slabbert et al. 1989). They enabled WHO (1989) to propose tentative bacterial guidelines in form of the geometric mean number of fecal coliforms of $\leq 10^3$ per 100 ml for fish pond water. These guidelines, based on the existing knowledge on the use of wastewater in aquaculture should, according to the WHO, ensure full protection of fish flesh against bacterial contamination. The possibility of self-purification of fish from bacteria after their transfer into clean water is a yet another practical issue. According to Buras et al. (1987), fish having contaminated muscles did not completely eliminate bacteria even after 6 weeks in clean water. Only fish free from bacteria in the muscles, and containing their small amounts in various internal organs and guts, might get clean in a satisfactory way. Our own results (Niewolak and Tucholski 1995 b) revealed that common carp containing little TC and FC in the muscles eliminated them completely after 14 days in clean flowing river water.

The aim of the present study was to evaluate the reduction of the level of indicatory bacteria and pathogens in the muscles, skin and digestive tract content of common carp reared for 6 months in a wastewater-supplied pond, wintered for another 6 months in a pond supplied with clean lake water, and then reared for 6 other months in the same water. The study was performed in 1996 and 1997.

MATERIAL AND METHODS

FISH

Three groups of common carp were used in the experiment. Group one (A), aged 1+, was reared from April 21 to October 18, 1996, in the pond supplied with biologically treated sewage in Jedwabno Sewage Treatment Plant. The initial individual body weight of the fish was 39.3 g. Stocking material (aged 0+) was obtained from the Stocking Material Production Centre in Warlity (Niewolak and Tucholski 2000 b). Carp reared in the same pond and wintered from October 18, 1996, to April 11, 1997, in 1-6°C in a pond supplied with clean lake water in Warlity, were used as group B. Group C consisted of the fish aged 2+, harvested from the wintering ponds and reared for another 6 months in a similar pond with the same water, from April 11 to October 24, 1997, fed fish pellets. The ponds were supplied with water from Szeląg Wielki Lake (Masurian Lake District). Fish body weight and

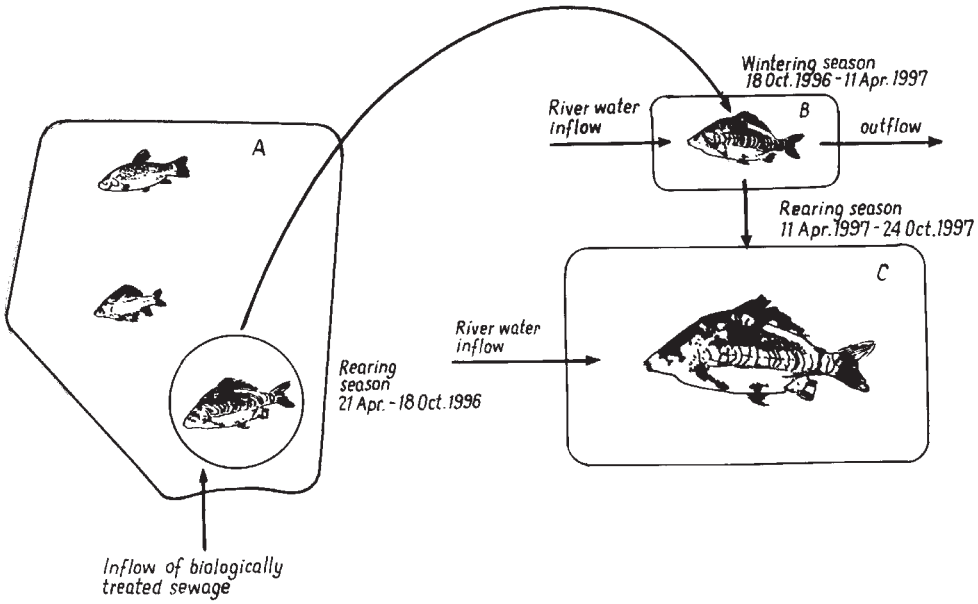

Fig. 1. The design of the experiment on common carp cleaning (explanation in the text).

length before and after wintering, and weight of table carp, are shown in Tab. 1. Experimental design is shown in Fig. 1.

TABLE 1

Weight and length of carp body used in sanitary and bacteriological investigation

No. of fish	Group of fishes					
	A (18 Oct.1996)[a]		B (11 April 1997)		C (24 Oct. 1997)	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
1	348.7	22.4	317.7	25.2	2.31	45.1
2	422.3	23.6	359.9	25.6	2.07	43.1
3	378.4	23.3	245.4	25.1	2.16	44.2
4	375.3	22.2	253.6	24.5	1.45	40.3
5	373.2	21.5	218.4	21.5	2.23	43.7

a – in brackets date of sampling

SAMPLING

Microbiological analyses were carried out of carp fingerlings harvested on Oct. 18, 1996, the same fish after 6 months of wintering (Apr. 11, 1997), and table carp after another 6 months of rearing. Five fish from each group were sampled. Fish aged 1+

harvested in autumn 1996 and spring 1997 were transported to the laboratory in tanks with pond water, and dissected according to Buras et al. (1987). Skin (sampled above the lateral line, under the dorsal fin), muscles, and digestive tract contents were placed in sterile Petri dishes. The tissues were weighed under sterile conditions, ground in a mortar with sea sand, and suspended in physiological solution (NaCl; 10 ml of solution per 1 g of the tissue). The suspensions were homogenised using a Universal Laboratory Aid Type MPW-309 homogenizer, at 1000 rpm for 10 minutes. The homogenates were diluted 1:10-1:10000, depending on the tissue, and inoculated into culture media. Time lag from fish harvest to the analysis did not exceed 6 hours. Table carps aged 2+, harvested in autumn 1997, were kept in a flow-through plastic tank supplied with lake water for 3 days before the analyses.

MICROBIOLOGICAL ANALYSES

The following analyses of fish tissues and digestive tract contents were performed:

1. total numbers (CFU g^{-1} wet weight) of bacteria on broth agar, after 72 hours of incubation at 20°C (TVC 20°C);
2. total numbers (CFU g^{-1} wet weight) of bacteria on broth agar, after 24 hours of incubation at 37°C (TVC 37°C);
3. numbers (CFU g^{-1} wet weight) of coliform bacteria (TC) on Endo medium, after 48 hours of incubation at 37°C;
4. numbers (CFU g^{-1} wet weight) of fecal coliform bacteria (FC) on Endo medium, after 24 hours of incubation at 44.5°C;
5. numbers (CFU g^{-1} wet weight) of fecal streptococci (FS) on m-Enterococcus Agar, after 72 hours of incubation at 37°C;
6. numbers (CFU g^{-1} wet weight) of anaerobic sulfite-reducing bacteria (*Clostridium perfringens*) on Wilson-Blair medium, after 18 hours of incubation in 37°C (in the samples pasteurised at 80°C for 10 min.);
7. numbers (CFU g^{-1} wet weight) of *Pseudomonas aeruginosa* (Pa) on mPa Agar, after 48 hours of incubation at 41.5°C;
8. numbers (CFU g^{-1} wet weight) of Aeromonas sp. (Ae) on Rimler-Shotts medium, after 24 hours of incubation at 37°C;
9. presence or lack of Salmonella sp. on selective Kauffman's medium with sodium tetrathionate, after 24 hours of incubation at 37°C, followed by separation medium

with xylose, lysine, and sodium desoxycholate (XLD), under the same incubation conditions (Bordner et al. 1978).

Numbers of bacteria indicatory of sanitary state (1-8) in the muscles, skin and digestive tract contents of common carp were evaluated after bacteria culture in Petri dishes, in 3 replicates. In the case of bacteria evaluated on broth agar in 20 and 37°C, all colonies were counted. In the case of all other indicators and pathogenic bacteria, only typical colonies were taken into consideration and randomly confirmed according to the techniques described by Niewolak and Tucholski (2000 a). Occurrence of Salmonella sp. on XLD medium was confirmed on Kligler's medium, on urea broth, and in glass agglutination test for HM flagellar antigen, according to Burbianka et al. (1983). Simultaneously with the evaluation of the concentration (presence) of bacteria in fish tissues, bacteriological analyses of water from Jedwabno and Warlity ponds were performed. Numbers of bacteria identified on broth agar in 20 and 37°C, of coliforms, fecal coliforms and fecal streptococci, were evaluated. The results were compared with the guidelines published in the Decree of the Minister of Environment Protection, Natural Resources, and Forestry of Nov. 5, 1991, concerning classification of waters, and with the WHO data (1989) on the use of sewage in agriculture and aquaculture. The results were also compared with the data of Albinger (1992) and Cabejszek (1960) concerning water pollution. The study included bacteriological analyses of the muscles, skin and digestive tract contents of 15 common carp individuals, and of 1 sample of water from each pond: the pond in Jedwabno supplied with wastewater and lake water-supplied pond in Warlity.

RESULTS

WATER

Data concerning numbers of bacteria determined on broth agar in 20 and 37°C, of coliforms, fecal coliforms, and fecal streptococci, i.e. of the micro-organisms which indicate the level of bacteriological contamination of Jedwabno sewage treatment plant water in 1995 and 1996, were reported by Niewolak and Tucholski (2000 a). These values were typical for highly contaminated waters (class III of purity or beyond any class). Numbers of bacteria in water of the wintering pond at the Stocking Material Production Centre in Warlity, determined on broth agar in 20 and 37°C, amounted to 170 and 28 CFU ml^{-1} respectively. Concentrations of coliforms, fecal coliforms, and

fecal streptococci were: 9, <3, and 7 MPN 100 ml^{-1} respectively. According to Cabejszek (1960) and the Decree of the Minister of Environment Protection, Natural Resources and Forestry of Nov. 5, 1991, such values indicate class I of water quality, having low level of organic matter easily decomposable by heterotrophic bacteria (TVC 20°C < 300 CFU ml^{-1}), free from fecal contamination (fecal coliforms < 2 MPN 100 ml^{-1}) (Albinger 1992). According to the WHO (1989), this water met the requirements of fish culture (Tab. 2).

TABLE 2

Concentrations of indicatory bacteria in water of ponds used for common carp rearing and cleaning. 1. Pond supplied with biologically treated sewage in Jedwabno. 2. Earthen pond supplied with clean water from Szeląg Lake in Warlity.

Water	[1]TVC 20°C	[2]TVC 37°C	[3]TC	[4]FC	[5]FS
	[6]CFU ml^{-1}		[7]MPN 100 ml^{-1}		
Pond supplied with biologically treated wastewater in Jedwabno	10500	7000	15000	14000	140000
Earthen pond supplied with clean water (from Lake Szeląg) in Warlity Station	170	28	9	< 3	7

1 – TVC 20°C - total viable count at 20°C
2 – TVC 37°C- total viable count at 37°C
3 – TC - total coliforms
4 – FC - fecal coliforms
5 – FS - fecal strepcococci
6 – CFU - colony forming unit
7 – MPN - most probable number

FISH

BEFORE WINTERING

Muscles of common carp (1+) reared in the wastewater-supplied pond from Apr. 26 to Oct. 18, 1996, (together with crucian carp and tench), contained bacteria identified on broth agar at 20 and 37°C, coliforms, fecal coliforms, fecal streptococci, and Aeromonas sp. Muscles of 1 individual contained also Salmonella sp. No pathogenic *Pseudomonas aeruginosa* or anaerobic sulfite-reducing *Clostridium perfringens* were observed. Concentrations of indicatory bacteria in skin and digestive tract contents of common carp (1+) (except *Pseudomonas aeruginosa* and *Clostridium perfringens*) were several fold higher than in the muscles. Salmonella sp. was present in skin of 1 fish only and was absent in the digestive tract contents (Tab. 3 A).

TABLE 3

Recovery of bacteria from carp organs before and after wintering and rearing season in pond; mean (for five fish) and range for the number of bacteria in CFU per 1 g wet wt. (in brackets per cent reduction)

	Carp	TVC 20ºC	TVC 37ºC	TC	FC	FS	Aeromonas sp.	Salmonella sp.
	Before wintering							
	Fish organs:							
	Muscle	$1.6x10^3$ $0.5x10^3$ - $5.7x10^3$	$0.5x10^3$ $0.2x10^3$ - $0.9x10^3$	$0.045x10^3$ 0 - $0.1x10^3$	$0.04x10^3$ 0 - $0.1x10^3$	$0.08x10^3$ $0.01x10^3$ - $0.2x10^3$	$0.03x10^3$ 0 - $0.045x10^3$	+(1)
A	Skin	$16.2x10^3$ $3.9x10^3$ - $47x10^6$	$18.9x10^3$ $2.1x10^3$ - $38.0x10^3$	$9.1x10^3$ $0.35x10^3$-$40.8x10^3$	$2.8x10^3$ $0.3x10^3$ - $8.5x10^3$	$0.26x10^3$ $0.07x10^3$ - $0.6x10^3$	$0.9x10^3$ $0.05x10^3$ - $3.5x10^3$	+(1)
	Intestine tract	$16.2x10^6$ $7.5x10^6$ - $28.5x10^6$	$6.3x10^6$ $2.0x10^6$ - $10.0x10^6$	$3.3x10^6$ $0.35x10^6$ - $7.5x10^6$	$1.9x10^6$ $0.1x10^6$ - $4.8x10^6$	$5.9x10^3$ $0.33x10^3$-$14.5x10^3$	$0.17x10^3$ 0 - $0.4x10^6$	NF
	After wintering							
	Fish organs:							
	Muscle	$1.87x10^3$ $0.7x10^3$ - $4.6x10^3$ (0)	$5.0x10^3$ $0.11x10^3$ -$24.0x10^3$ (0)	$0.18x10^3$ 0 - $0.33x10^3$ (98.8)	$0.03x10^3$ 0 - $0.15x10^3$ (99.9)	$0.7x10^3$ 0 - $2.5x10^3$ (0)	$0.04x10^3$ 0 - $0.14x10^3$ (0)	NF
B	Skin	$63.3x10^3$ $5.0x10^3$ - $0.21x10^6$ (0)	$14.6x10^3$ 0.2x10 - $65.0x10^3$ (32.8)	$0.97x10^3$ $0.05x10^3$-$2.27x10^3$ (94.2)	0 0 (100)	$13.7x10^3$ $0.05x10^3$-$65.6x10^3$ (0)	$0.08x10^3$ 0 - $0.25x10^3$ (91.1)	NF
	Intestine tract	$10.7x10^6$ $2.4x10^6$ - $26.0x10^6$ (47.8)	$4.08x10^6$ $0.51x10^6$-$11.6x10^6$ (35.3)	$0.3x10^6$ $0.07x10^6$ -$0.62x10^6$ (90.0)	$24.9x10^3$ $0.2x10^3$ - $0.11x10^6$ (98.7)	$1.5x10^3$ 0 - $6.5x10^3$ (74.6)	$230x10^3$ $68.0x10^3$ -$450x10^3$ (0)	NF
	After rearing season							
	Fish organs:							
C	Muscle	$0.35x10^3$ $0.035x10^3$-$1.4x10^3$ (78.2)	$0.31x10^3$ $0.03x10^3$ - $1.3x10^3$ (38.0)	0 0 (100)	0 0 (100)	$0.03x10^3$ 0 - $0.08x10^3$ (62.5)	0 0 (100)	NF
	Skin	$1.03x10^3$ $0.13x10^3$ - $3.5x10^3$ (93.7)	$0.10x10^3$ $0.03x10^3$ - $0.2x10^3$ (99.4)	$0.24x10^3$ 0 - $1.1x10^3$ (97.4)	0 0 (100)	$0.03x10^3$ $0.005x10^3$-$0.08x10^3$ (88.5)	$0.1x10^3$ 0 - $0.45x10^3$ (88.9)	NF
	Intestine tract	$1.3x10^6$ $1.4x10^3$ - $3.3x10^6$ (92.0)	$0.23x10^6$ $0.35x10^3$-$0.81x10^6$ (98.6)	$0.52x10^6$ $0.42x10^3$ - $2.0x10^6$ (84.2)	0 0 (100)	$0.08x10^3$ $0.02x10^3$ - $0.1x10^3$ (86.5)	$204x10^3$ $0.3x10^3$ - $460x10^3$ (0)	Nf

NT – not tested
NF – not found
+(1) – found in one fish

AFTER WINTERING

Numbers of indicatory coliforms in muscles, skin and digestive tract contents of the fish decreased after 6 months of wintering in the pond supplied with clean lake water. Numbers of bacteria determined on broth agar at 20 ^{o}C decreased only in the

gut, and of those determined at 37 °C – in the skin and guts. Concentrations of fecal streptococci decreased in the digestive tract, and of Aeromonas sp. – in the skin. In other cases (Tab. 3 B) numbers of bacteria determined on broth agar in 20°C and 37°C, of fecal streptococci and Aeromonas sp. more or less increased. Muscles, skin and digestive tract contents did not contain pathogenic Salmonella sp. and *Pseudomonas aeruginosa* or *Clostridium perfringens* (Tab. 3 B).

AFTER THE REARING SEASON

Carp harvested from the wintering pond and reared for 6 more months (until Oct. 24, 1997) in the pond supplied with lake water at the Stocking Material Production Centre in Warlity, and fed fish pellets, eliminated most indicatory bacteria. Their muscles were free from coliforms, fecal coliforms and Aeromonas sp. No pathogenic Salmonella sp., and potentially pathogenic *Pseudomonas aeruginosa* were found, nor anaerobic sulfite-reducing *Clostridium perfringens*. Concentrations of bacteria determined on broth agar at 20 °C and 37 °C, and of fecal streptococci, decreased by 78.2, 38.0, and 62.5% respectively. Skin of the fish contained no fecal coliforms, Salmonella sp., *Pseudomonas aeruginosa*, or *Clostridium perfringens*. In some individuals also coliforms and Aeromonas sp. were not found. The average numbers of bacteria determined on broth agar at 20 °C and 37 °C decreased by 93.7 and 99.4%, and concentrations of coliforms, fecal streptococci, and Aeromonas sp. – by 97.4, 88.5, and 88.9% respectively. Digestive tract contents were free from Salmonella sp., *Pseudomonas aeruginosa*, and *Clostridium perfringens*. Concentrations of bacteria determined on broth agar at 20 °C and 37 °C dropped by 92.0 and 98.6%, and numbers of coliforms, fecal coliforms, and fecal streptococci – by 84.2, 100, and 86.5% respectively. The average concentration of Aeromonas sp. increased about twice (Tab. 3 C).

DISCUSSION

Considerable differences in the numbers of indicatory bacteria in the muscles, skin and digestive tract contents of common carp reared in the wastewater-supplied pond in Jedwabno, and wintered and reared in a pond supplied with clean lake water in Warlity, are consistent with the results of other studies. Similar differences were observed between bacteria content in the tissues of common carp reared in the pond supplied with biologically purified sewage in Olsztynek, and the same fish reared using traditional technique in Pasym Fish Farm (Niewolak and Tucholski 1995 a).

These differences may be related to fish heath, individual condition, former feeding intensity, and retention of bacteria in the posterior part of the digestive tract where they may proliferate (Geldreich 1966, Del Rio Rodriguez 1997). Strong contamination of some individuals may affect the average numbers of bacteria in the muscles, skin and digestive tract of the entire fish group. Such situation took place in fish after wintering in lake water supplied pond in Warlity. Average TVC 37°C in the muscles of these fish increased 10 fold compared to the fish reared in the wastewater-supplied pond in Jedwabno; this was due to considerable contamination of 1 individual bearing 24000 CFU g^{-1} of fresh weight. TVC 37°C in the muscles of the other 4 fish were 115, 130, 220, and 660 CFU g^{-1}. Generally, however, there was a gradual decrease of indicatory bacteria concentrations in the muscles, skin and gut of carps wintered and then reared for 6 months in lake water supplied pond. This was probably related to low level of bacteria in lake water. Decrease of digestive tract contamination of the fish after wintering might have also resulted from starvation, while reduced numbers of bacteria in the muscles and skin might have been due to low water temperature (1-6°C). Fish body temperature is similar to that of the environment, thus the bacteria were unable to proliferate. Low concentrations of indicatory bacteria in the muscles, skin and digestive tract contents of the fish (2+) after 6 months of rearing in clean lake water in Warlity were related to very low level, or absence, of these bacteria in the environment. This resulted in an elimination of the majority of indicatory bacteria, not only from the body surface and gut, but also from the muscles. The average numbers of bacteria determined on broth agar in 20 and 37°C, and of fecal streptococci in the muscles of table carp (2+) obtained from the fingerlings reared in the wastewater-supplied pond in Jedwabno and wintered in the pond with clean lake water, were at least 10 fold lower compared to the concentrations of these bacteria in carp reared in a traditional pond in Pasym (Niewolak and Tucholski 1995 a). Coliforms and *Clostridium perfringens*, present in the muscles of carp reared in Pasym, were not found in the fish reared in Warlity.

CONCLUSIONS

1. Muscles of common carp (1+) reared for 6 months (from fry stage) in wastewater-supplied pond of high bacteriological contamination, and then, after 6 months of wintering, in a pond supplied with clean lake water, were able to eliminate 98.8% of coliforms and fecal coliforms. No pathogenic Salmonella, potentially pathogenic *Pseudomonas aeruginosa*, or anaerobic sulfite-reducing

Clostridium perfringens were found. Numbers of bacteria determined on broth agar at 20 and 37°C, of fecal streptococci and Aeromonas sp. increased.

2. Concentrations of coliforms and fecal coliforms in the skin and digestive tract contents of the fish (1+) after 6 months of wintering in clean water decreased by 90.0-100%. TVC 20°C and TVC 37°C were reduced up to 50%. Numbers of fecal streptococci were reduced only in the digestive tract contents, and concentration of Aeromonas sp. – only in skin.
3. The muscles of carp 2+ reared for 6 months (from 1+ age) in the pond supplied with clean lake water were entirely free from coliforms, fecal coliforms, and Aeromonas sp. Fecal coliforms were also absent from the skin and guts. Numbers of bacteria determined on broth agar in 20 and 37°C decreased by from 92.0 to 99.4%, and concentrations of coliforms, and fecal streptococci – from 84.2-97.4%. Numbers of Aeromonas sp. in skin decreased by 90%, and in the digestive tract - considerably increased compared to the fish from wastewater-supplied pond in Jedwabno.
4. The results of the present study indicate a possibility of considerable reduction of bacteriological contamination of the muscles and skin of common carp reared from fry stage in wastewater-supplied pond and then transferred for a long period to clean water. This period involved in the present study 6 months of wintering and 6 more months of rearing until 2+ age. Bacteria, especially those evaluated on broth agar at 20 and 37°C, were not, however, entirely eradicated from the muscles.

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STRESZCZENIE

REDUKCJA LICZEBNOŚCI BAKTERII O ZNACZENIU SANITARNYM U KARPIA W RÓŻNYCH WARUNKACH CHOWU

Badano stopień zanieczyszczenia bakteriologicznego mięśni, skóry i treści przewodu pokarmowego: 1) karpia (1+) hodowanego w stawie zasilanym biologicznie oczyszczonymi ściekami przy oczyszczalni w Jedwabnie (od 21 kwietnia do 18 października 1996 r.); 2) tej samej ryby po zimowaniu (od 18 października 1996 r. do 11 kwietnia 1997 r.) w zimochowie zasilanym czystą wodą jeziorową w Ośrodku Zarybieniowym w Warlitach na Pojezierzu Mazurskim oraz 3) karpia (2+) hodowanego dodatkowe 6 miesięcy (od 11 kwietnia do 24 października 1997 r.) w stawie zasilanym czystą wodą jeziorową w tym samym ośrodku zarybieniowym przy dokarmianiu ryb sztuczną paszą granulowaną. Stawy w Ośrodku Zarybieniowym w Warlitach zasilane są wodą z jeziora Szeląg Wielki. Badania bakteriologiczne przeprowadzano każdorazowo na 5 rybach przed i po okresie zimowania oraz po okresie hodowli w stawie zasilanym czystą wodą

jeziorową w ww. ośrodku zarybieniowym. Obejmowały oznaczenie liczebności bakterii na agarze bulionowym w temperaturze 20 i 37ºC, liczby bakterii z grupy pałeczki okrężnicy, liczby kałowych bakterii z grupy pałeczki okrężnicy, liczby paciorkowców kałowych, Aeromonas sp., *Pseudomonas aeruginosa*, Salmonella sp. oraz beztlenowych bakterii przetrwalnikujących, redukujących siarczyny (*Clostridium perfringens*). W mięśniach, skórze i treści przewodu pokarmowego karpia (1+) hodowanego w stawie zasilanym biologicznie oczyszczonymi ściekami przy oczyszczalni w Jedwabnie brak było *Pseudomonas aeruginosa* i *Clostridium perfringens*; Salmonella sp. występowała tylko w mięśniach i skórze 1 ryby. Wszystkie inne ww. bakterie występowały w dużych ilościach. Po okresie zimowania w zimochowach zasilanych czystą wodą jeziorową w Ośrodku Zarybieniowym w Warlitach, liczba bakterii oznaczana na agarze bulionowym w temperaturze 20ºC, paciorkowców kałowych i Aeromonas sp. w mięśniach tych ryb nie uległa większym zmianom; liczba bakterii oznaczanych na agarze bulionowym w temperaturze 37ºC wzrosła mniej więcej 10-krotnie. Natomiast liczba bakterii z grupy pałeczki okrężnicy i liczba kałowych bakterii z grupy pałeczki okrężnicy zmniejszyła się o około 99% i nie przekroczyła odpowiednio 330 i 150 kolonii w 1 g.św.m. Większy stopień redukcji liczebności wszystkich badanych grup bakterii wskaźnikowych, występujących w badanych tkankach i treści przewodu pokarmowego, nastąpił dopiero po okresie hodowli ryb w stawie zasilanym czystą wodą jeziorową w Ośrodku Zarybieniowym w Warlitach. W mięśniach tej grupy ryb redukcji podlegało średnio 78,2% bakterii oznaczanych na agarze bulionowym w temperaturze 20ºC, 38,0% bakterii oznaczanych na agarze bulionowym w temperaturze 37ºC, 62,5% paciorkowców kałowych oraz wszystkie bakterie z grupy pałeczki okrężnicy i z rodzaju Aeromonas. W skórze stopień redukcji liczebności badanych bakterii wskaźnikowych wahał się od 88,5 do 100%, zależnie od grupy tych drobnoustrojów. W treści przewodu pokarmowego jedynie liczba Aeromonas sp. nie uległa redukcji; stopień redukcji innych grup bakterii wskaźnikowych wahał się od 84,2 do 100%. W żadnej z badanych tkanek ryb przetrzymywanych w zimochowie i hodowanych dalej w stawie zasilanym czystą wodą jeziorową w Ośrodku Zarybieniowym w Warlitach nie stwierdzono *Clostridium perfringens*, *Pseudomonas aeruginosa* i Salmonella sp.

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