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MICROBIOLOGICAL STUDIES OF CARP (*CYPRINUS CARPIO* L.) FINGERLINGS WINTERED IN COOLING WATERS

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ABSTRACT. The study was carried out in the winter of 1999/2000 at the fishing base of the Olsztyn Fishing Farm. The base was comprised of 48 fish cages (15 m^3 each) suspended in a post-cooling water canal of the Ostrołęka thermal-electric power station. During the 190 days of observations (31 October 1999 - 15 May 2000), the following factors were analysed: bacteriological contamination of skin, muscle and gastric contents of carp fingerlings produced in cages from summer fry (July fry; 3-5 g body weight). Bacteriological analyses consisted of determining the total count of bacteria cultured on agar (TVC 20°C and TłVC 37°C), the total count of colic bacteria (*Escherichia coli*) on Endo medium (Enterobactriacea) and bacteria that is potentially pathogenic to fish and humans *(Aeromonas hydrophila, Pseudomonas fluorescens, P. aeruginosa, Staphylococcus aureus*). The identification of microorganisms was carried out using the biochemical tests Api 20 NE, Api 2E and Api Staph (bioMérieux). Statistical analysis showed significant differences in the number of all the bacteria assayed between the muscle tissue versus digestive tract and skin mucus. The counts of all the groups of bacteria determined in the carp were permissible and did not exceed Polish hygiene norms.

Key words : *CYPRINUS CARPIO*, MICROORGANISM, WINTERING, COOLING WATERS

INTRODUCTION

For a number of years many countries, including Poland, have reared fish in warm post-cooling waters and obtained productivity rates which indicate the great potential of this method for commercial fish production (Steffens 1969, Albrecht 1970, Trzebiatowski et al. 1976, Bertram 1978, Sadowski et al. 1999). Another interesting aspect of the method is that it allows for carp *Cyprinus carpio* L. production in warm waters throughout the year, including in winter (Trzebiatowski et al. 1978, Sadowski et al. 1999). Even at the relatively low temperatures of the cooling waters in winter (8-16°C), carp can gain 50-100% in weight and their weight can increase nearly 20-fold if they are fed on high protein granulates (Trzebiatowski et al. 1978, Ostroumova 1979, Steffens and Rennert 1987). It is, therefore, feasible to produce carp for consumption in the second season of carp fry rearing (Ostroumova 1979, Füllner 1985).

However, commercial fish cultures in cooling waters create conditions that favor the dangerous development of colic bacteria (Cabejszek et al. 1960). The sources of

water contamination are the products of fish metabolism, fish excreta, fodder leftovers, antibiotics, vitamins, etc. (Karpiński 1995). Such contaminants may pollute the waters of the receiver (Alabster 1982, Mäkinen 1991, Crisp and Kelly 1994 - cited in Karpiński 1995, Zmysłowska et al. 2000b, c), and poison the fish produced, thus making them unsuitable for consumption (Zaleski 1985, Svobodová 1992, Guziur et al. 2000a, b). The bacterial microflora living in the mucus on the skin surface, in the gills and in the digestive tracts of fish is varied in terms of quantity and quality (Lèsel 1979, Sugita et al. 1985a, b, Spanggard et al. 1993, Niewolak and Tucholski 1995). Mucus is usually found to contain 10^2 to 10^7 bacteria per cm^2 of skin, whereas in the digestive tract the bacteria count reaches 10^8 per g of gastric contents. The highest bacteria counts for carp, tench *Tinca tinca* L. and grass carp *Ctenopharyngodon indella* (Val.) were determined at the time of the most intensive food consumption in summer. Austin and Allen-Austin (1985) report that Achromobacter, Acinobacter, Bacillus, Corynebacterium, Cytophaga, Flavobacterium, Micrococcus, Moraxella and Pseudomonas are the dominant types of bacteria in water ecosystems, while Aeromonas, Acinobacter, Enterobacter, Pseudomonas prevail in the digestive tracts of freshwater fish. According to Yoshimizu et al. (1976), only two taxa (Enterobacteriaceae and Aeromonas) occur regularly in freshwater fish species, with the microflora of young fish being different from that of adult specimens.

The present study was undertaken to evaluate bactoriological contamination of skin, muscle and gastric contents of carp fingerlings wintered in cooling waters.

MATERIAL AND METHODS

The research began in October 1999 and terminated in May 2000. The fish were cultured and sampled at the fishing base of the Olsztyn Fish Farm located in the post-cooling water channel of the Ostrołęka power station in northern Poland. Four 15 m^3 cages were stocked with carp summer fry with an average body weight of 3-5 g that were produced in the ponds of the Olsztyn Fish Farm in the spring of 1999 and delivered to Ostrołęka in July 1999. The aim was to rear July carp fry for one year in cages, including wintering in warm waters from the power plant, to produce food fish weighing from 1 to 1.5 kg. Owing to the differences in sizes among the fish produced in the cages, in October 1999 the carps were divided into two groups: S - small carp (average weight 160 g); L - large carp (average weight 700 g) (Table 1). The four cages were stocked to densities of 200 - 500 fish m^{-3} in compliance with the recommenda-

tions of Trzebiatowski et al. (1978), Filipiak (1998) and Sadowski et al. (1999). Monthly control catches were made during the study and a total of 100 fish were caught from each cage. Morphometric measurements of the fishes were taken; total length (LT), standard length (LC) and body height (Alt. max.) were measured to the nearest 0.1 cm and body weight was measured to the nearest 1 g.

TABLE 1

Characteristics of carp fingerlings wintered in cages in cooling waters

Parameters	Group (S)			Group (L)			Body gains of small (S) and large (L) carp	
	October	February	May	October	February	May	S	L
LT (mm)	172.5	198.7	234.2	271.5	288.5	317.7	61.7	45.8
LC (mm)	145.5	164	199.7	230	246.7	270.3	54.2	40.3
Alt. max. (mm)	65.8	75.2	85.4	115	118.4	127	20.4	12.0
Body weight (g)	160	224	325.7	700	820.0	886.6	165.7	186.6

During wintering, automatic feeders were employed to feed the fish ALLER AQUA-SAFIR granulate (90% of the content), supplemented with ALLER AQUA 37/12 and FUTTER-KRAFT feed, which contained, on average, 44.5% protein and 19.6% fat (Table 2). The chemical composition of fish bodies was determined according to methods developed by Trzebiatowski et al. (1976, 1978) and Steffens and Rennert (1987) (Table 3).

TABLE 2

Chemical composition of fish granulates (pellets)

Component	ALLER-AQUA SAFIR	KRAFT-FUTTER	ALLER-AQUA 37/12	Average
Total protein	45.0	44.0	37.0	44.5
Crude fat	20.0	20.0	12.0	19.6
Crude ash	8.0	5.3	1.1	7.5

During the winter rearing of the fish (190 days), the temperature of the discharged waters ranged from 6.2 to 18.0°C and the physicochemical characteristics of the cooling waters and the waters flowing from the cages were satisfactory (Table 4). All the carp that attained commercial size (over 1 kg) were caught on 15 May 2000, while smaller fish (less than 1 kg) were left for further rearing.

TABLE 3

Chemical composition of the body of carp wintered in cages in cooling waters (%)

Component	Group of fish*	Month			Difference between October and May
		October 1999	February 2000	May 2000	
Dry matter	S	26.88	26.40	26.43	- 0.45
	L	28.02	27.39	27.49	- 0.53
Total protein	S	16.95	7.01	17.53	+ 0.58
	L	17.05	17.25	17.38	+ 0.33
Crude fat	S	7.90	7.30	6.79	- 1.11
	L	8.95	8.10	7.96	- 0.99
Crude ash	S	2.00	2.00	2.08	+ 0.08
	L	1.99	2.00	2.01	+ 0.02

** S - small carp * L - large carp*

TABLE 4

Some physicochemical characteristics of cooling waters and post-production waters during the wintering of carp fingerlings

Parameter	Unit	October 1999		February 2000		May 2000	
		E*	S*	E*	S*	E*	S*
Temperature	(°C)	18.0	17.5	11.2	10.8	15.3	15.0
Oxygen	mg O_2 dm^{-3}	10.2	9.9	13.9	13.7	13.1	13.0
N-NH_4	mg dm^{-3}	0.11	0.15	0.33	0.46	0.66	0.90
BZT_5	mg O_2 dm^{-3}	3.7	4.7	3.2	3.9	3.6	4.5
N-NO_3	mg dm^{-3}	1.2	2.1	1.1	1.3	1.7	1.6
N-NO_2	mg dm^{-3}	0.002	0.003	0.001	0.002	0.007	0.010
N_{total}	mg dm^{-3}	1.7	2.6	0.08	0.91	0.02	0.98
P_{total}	mg dm^{-3}	0.65	1.69	0.31	0.36	0.41	0.51
P-PO_4	mg dm^{-3}	0.44	1.06	0.11	0.13	0.13	0.16
pH	-	8.10	8.17	7.79	7.96	7.98	8.08

**E - cooling waters (from the surface)*
**S - post-production waters (from the inside of the cages)*

Specimens for bacteriological tests were chosen randomly three times during the season in autumn (October), winter (February) and spring (May). Five to six specimens of both fish-size groups were taken from each of the four cages. The results obtained were calculated as the mean of four samples. The bacteriological analyses entailed determining the total number cultured on agar (TVC 20°C and TVC 37°C) and the total number of coliform bacteria on Endo medium (Enterobacteriaceae). The bacteria which is potentially pathogenic to fish and humans *Aeromonas hydrophila* was

cultured on mA medium, *Pseudomonas fluorescens* on Kinga B medium, *Pseudomonas aeruginosa* on Kinga A medium, and *Staphylococcus aureus* on Chapman medium. The results obtained were calculated as the mean of four samples (Guziur et al. 2000b, Zmysłowska et al. 2000a, b).

The bacteriological tests consisted of the quantitative analyses of the bacterial composition in 1 l of gastric contents and muscles and in the mucus from 1 cm^2 of skin cultured on suitable media at the optimum incubation temperature according to methods devised by Rodina (1968), Burbianka and Pliszka (1983) and the modified method of Zmysłowska et al. (1987, 2000a, b).

The identification of microorganisms was carried out using Api biochemical tests (bioMérieux) 20 NE, 2E and Staph.

Analysis of variance and Tukey's tests ($\alpha = 0.05$) were applied (Łomnicki 1995) to determine the statistical significance of differences in the microorganism counts found in the digestive tract, skin mucus and muscle tissues of fish.

RESULTS AND DISCUSSION

The condition and health of the fish were good at the beginning of wintering (October). The fat level in both groups of carp (L and S) was high and sufficient (7.90 - 8.95%, Table 3) according to the recommendations of Filipiak (1998).

The results revealed differences in the numbers of the microorganisms determined depending on the date and place of sampling and the method of collecting samples from the carp body. The highest count of the groups and species of the bacteria analysed were always determined in the gastric contents. They were lower in the skin surface mucus and the lowest, with some bacteria absent, in the fish muscle tissues (Figs. 1, 2, 3). The total count of bacteria in 1 l of gastric contents ranged from 3.5×10^5 to 9.6×10^5 (TVC 20°C) and from 5.8×10^5 to 6.5×10^5 (TVC 37°C). The bacterial microflora of the digestive tract and the skin mucus may reflect its content in the feed granulate and its dynamic balance with the environment (Lèsel 1979). The results reported in this paper correspond with results obtained by other authors (Zaleski 1985, Sugita et al. 1985a, b, Zmysłowska et al. 2000a, c, d).

With regard to the season of the year when the tests were performed (October, February, May), the lowest counts of the microorganisms determined were usually attained in winter, while higher counts were recorded in autumn and spring. This observation can be related not only to the lower water temperature in winter, but also

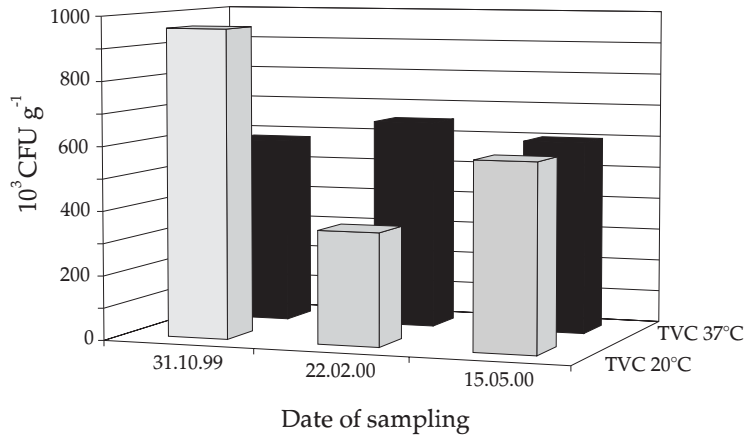

Fig. 1. Count of bacteria cultured on agar at 20 and 37°C in 1 g of gastric contens (CFU - colony forming units).

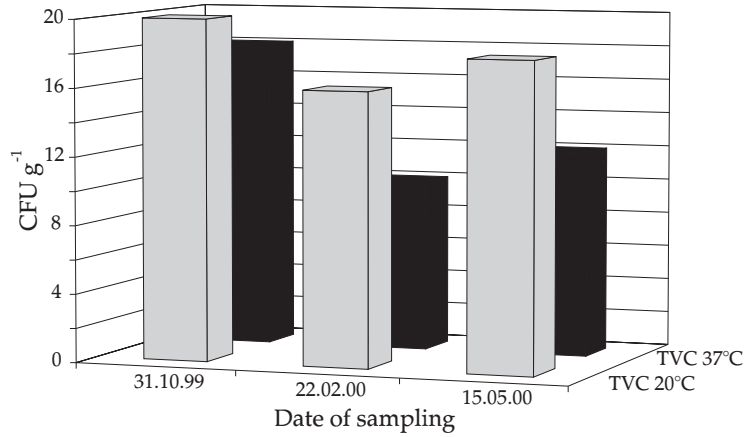

Fig. 2. Count of bacteria cultured on agar at 20 and 37°C in 1 g of muscle tissue.

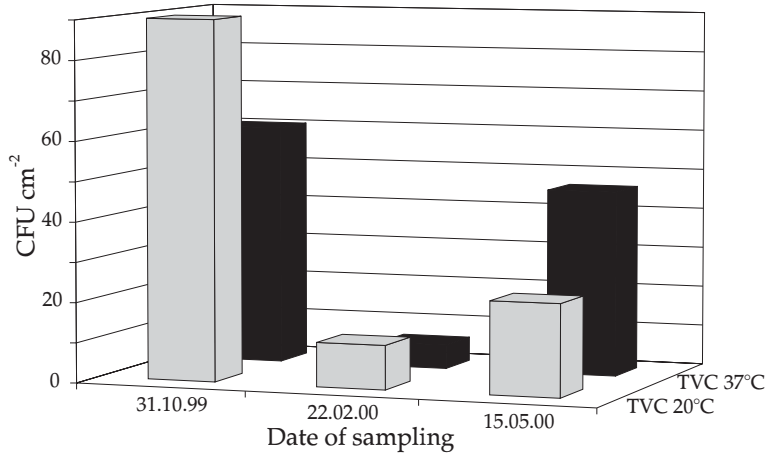

Fig. 3. Count of bacteria cultured on agar at 20 and 37°C in mucus (per 1cm^2 of skin).

to the fact that feeding the fish granulate was discontinued. Similar relationships were detected by Zmysłowska et al. (2000d) in their bacteriological tests on tench reared in a tank culture at various water temperatures. Likewise, the results correspond with those from investigations by Niewolak and Tucholski (1995), who proved that a more advanced trophic state of water, and communal sewage in particular, favored higher numbers of heterotrophic microorganisms in such environments.

The highest count of Enterobactriacea analysed were always in the gastric contents and were usually attained in autumn and spring, while in winter they were absent (Figs. 4, 5, 6). The species *Aeoromonas hydrophila* and *Pseudomonas fluorescens*, which are potentially pathogenic to fish and have a strong affinity with them, were more numerous in the analysed samples than bacteria that are pathogenic to humans. From 50 to 750 cells of *Aeoromonas hydrophila* were determined in 1 g of gastric contents and from 0 to 410 in the mucus from 1 cm^2 of skin (Figs. 7, 8, 9). The figures for *Pseudomonas fluorescens* were from 0 to 58 and from 0 to 62, respectively (Table 5).

TABLE 5

Count of some species and groups of bacteria in 1 g of gastric contents, 1 g of muscle tissue and mucus (per 1 cm^2 of skin) of the wintered carp fingerlings (total number of bacteria)

Group / species of bacteria *	Date of sampling								
	31 October 1999			21 February 2000			15 May 2000		
	Gastric contents	Muscle tissue	Mucus from skin	Gastric contents	Muscle tissue	Mucus from skin	Gastric contents	Muscle tissue	Mucus from skin
TVC 20°C	960,000	20	90,000	350,000	16	11,000	580,000	18	23,000
TVC 37°C	580,000	18	602,000	650,000	10	6,000	600,000	12	46,300
Enterobacteriaceae	200	2	120	0	0	0	165	3	95
Aeromonas hydrophila	50	6	0	750	2	410	460	10	323
Pseudomonas fluorescsens	58	0	62	0	0	0	45	0	36
Pseudomonas aeruginosa	10	0	0	0	0	0	15	0	0
Staphylococcus aureus	0	0	0	40	0	0	12	0	0

**TVC - total count of bacteria on agar at 20 and 37°C*

The carp sampled for analyses (which, together with the food fish, constituted 80% of the stocks of the cage culture - Guziur et al. 2000b) showed a high frequency of pathogenic bacteria which could cause mass fish losses. Therefore, it is recommended to monitor fish continuously during both the summer and winter seasons of fish rearing in cooling waters.

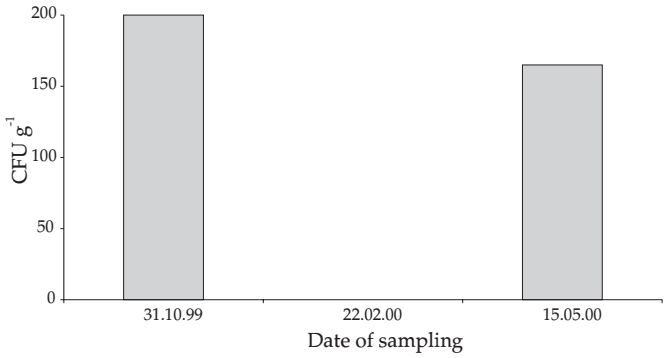

Fig. 4. Count of Enterobacteriaceae in 1 g of gastric contens.

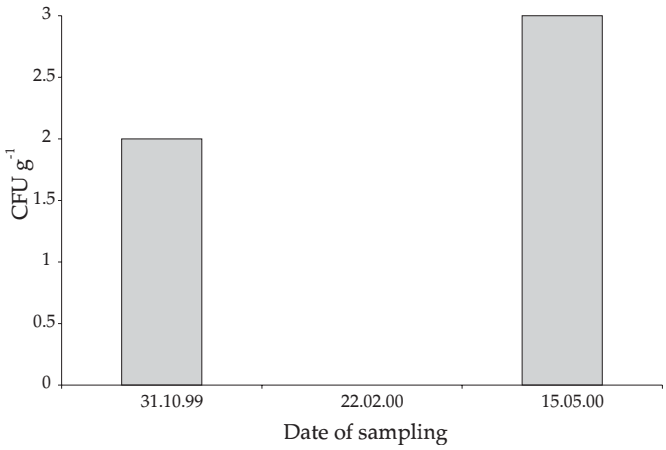

Fig. 5. Count of Enterobacteriaceae in 1 g of muscle tissue.

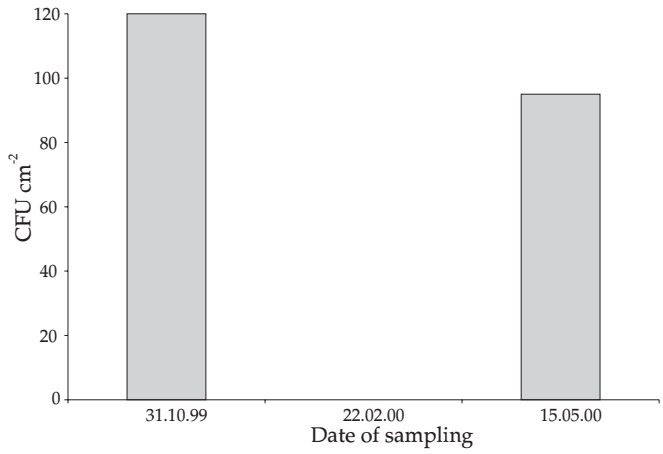

Fig. 6. Count of Enterobacteriaceae in mucus (per 1 cm^2 of skin).

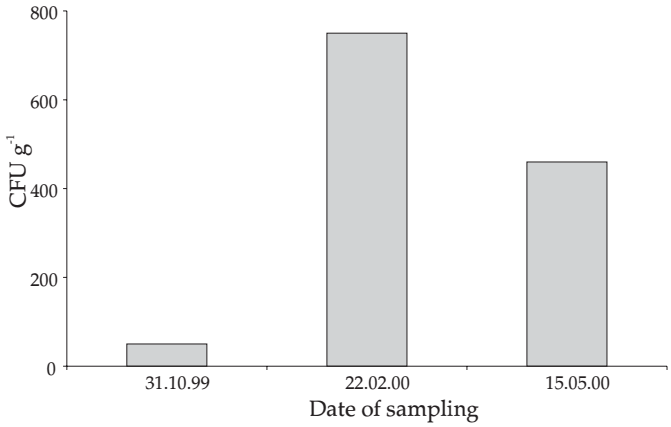

Fig. 7. Count of bacteria *Aeromonas hydrophila* in 1 g of gastric contens.

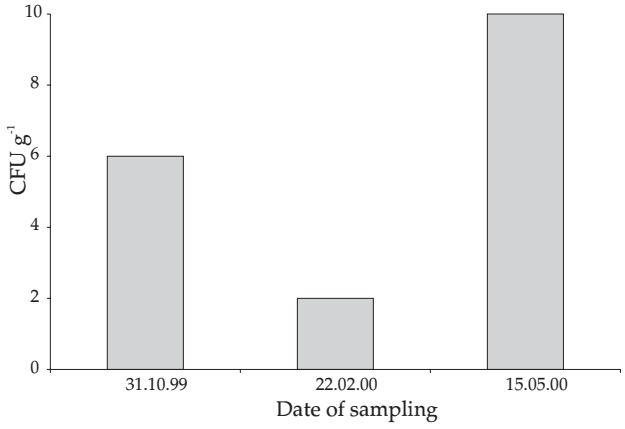

Fig. 8. Count of bacteria *Aeromonas hydrophila* in 1 g of muscle tissue.

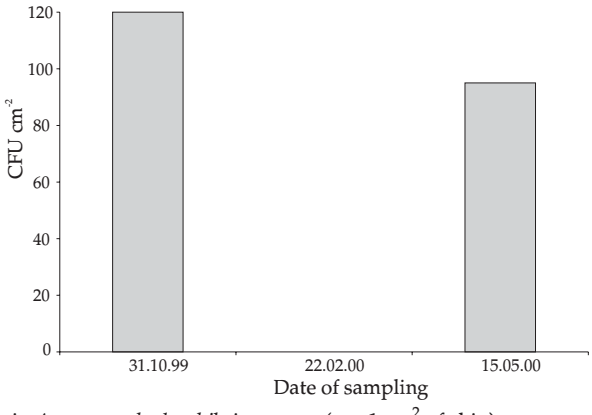

Fig. 9. Count of bacteria *Aeromonas hydrophila* in mucus (per 1 cm^2 of skin).

Determining the statistical significance of differences in the count of microorganisms in the digestive tract, mucus sampled from the skin and muscle tissue was obtained by analysis of variance. At a significance level of 0.05, it was demonstrated that the number of bacteria depended on the type of sample analysed (α = 0.05, F = 2.797). Following this, Tukey's test (T method) was used, and it was shown that differences in the count of all the groups of bacteria assayed were statistically significant in each of the three environments (digestive tract, skin mucus and muscle tissue). Statistically significant differences were also observed between the number of bacteria present in the digestive tract versus muscle tissue as well as the skin mucus versus muscle tissue.

CONCLUSION

1. Diversification in the quantities of the determined groups (TVC 20°C, TVC 37°C and Enterobacteraceae) and species of bacteria (*A. hydrophila*, *P. fluorescens*, *P. aeruginosa*, *S. aureus*) was observed depending on the date of fish sampling (autumn, winter, spring) and part of the fish body sampled (skin mucus, muscle tissue, digestive tract contents).
2. The analysed samples of carp produced higher bacteria counts in winter and lower ones in autumn and spring.
3. The groups and species of bacteria determined during the study were most numerous in the gastric contents of carp, less frequent in mucus from the skin surface and scarce or absent in muscle tissue.
4. Sporadically, cells of bacteria pathogenic to humans were determined (*Pseudomonas aeruginosa*, *S. aureus*); bacteria that is potentially pathogenic to fish (*A. hydrophila*, *P. fluorescens*) were scarce.
5. The hygienic quality of the carp reared in the cage culture, especially the muscle tissue as a fish raw product (fillets), can be regarded as satisfactory and safe for human consumption.
6. Statistical analysis showed significant differences in the number of all bacteria assayed between the muscle tissue versus digestive tract and skin mucus.

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STRESZCZENIE

MIKROBIOLOGICZNE BADANIA NARYBKU KARPIA *(CYPRINUS CARPIO L.)* ZIMOWANEGO W WODACH POCHŁODNICZYCH

Terenem badań w zimie 1999/2000 roku była baza 48 sadzy (á 15 m^3) Gospodarstwa Rybackiego Olsztyn, usytuowana na kanale wód pochłodniczych elektrociepłowni w Ostrołęce. W trakcie 190 dni obserwacji (31.10.1999 - 15.05.2000 r.) analizowano stopień zanieczyszczenia bakteriologicznego skóry, mięśni i treści pokarmowej narybku karpia (początkowa średnia masa 435 g x szt^{-1}, tab. 1), wyprodukowanego w sadzach z narybku letniego "lipcówki" (3-5 g) (tab. 1-4).

Badania bakteriologiczne obejmowały oznaczenia ogólnej liczby bakterii wyhodowanych na podłożu agarowym (TVC 20°C i TVC 37°C), ogólnej liczby bakterii z grupy pałeczki okrężnicy (*Escherichia coli*) na podłożu Endo (Enterobactriacea*)* oraz bakterii potencjalnie chorobotwórczych dla ryb i człowieka *(Aeromonas hydrophila* - na podłożu mA, *Pseudomonas fluorescens* - podłoże Kinga B, *P. aeruginosa* - podłoże Kinga A, *Staphylocccus aureus* - podłoże Chapmana). Identyfikację tych drobnoustrojów przeprowadzano na testach biochemicznych Api 20 NE, Api 2E oraz Api Staph firmy bioMérieux.

Uzyskane wyniki wykazały, że przewód pokarmowy i śluz z powierzchni skóry posiadają znacznie większą liczebność drobnoustrojów, aniżeli tkanka mięsna (tab. 5, rys. 1-9). Ogólna liczba TVC 20°C w treści pokarmowej wynosiła 350000 - 950000 jtk x g^{-1}, a TVC 37°C nie przekroczyła 650000 jtk x g^{-1}. Liczebności tych grup bakterii w śluzie pobieranym z 1 cm^2 skóry wyniosły od 46300 do 65200 jtk , natomiast w tkance mięsnej - zaledwie od 12 do 18 jtk x g^{-1}, przy czym najmniejsze ilości drobnoustrojów wystąpiły w zimie, w najniższych temperaturach wody (5-6°C - luty 2000 r.). Najwyższą liczebnością wśród bakterii chorobotwórczych charakteryzowały się *Aeromonas hydrophila*, a nieco niższą - *Pseudomonas fluorescens* (tab. 5) i to zarówno w treści pokarmowej, jak i śluzie ze skóry.

Analiza statystyczna wykazała istotne różnice w liczebnościach badanych bakterii między tkanką mięsną a przewodem pokarmowym i śluzem z powierzchni skóry.

Pomimo że stwierdzony poziom liczebności badanych bakterii u karpi jest dopuszczalny i nie przekracza polskich norm higienicznych, to jednak ze względu na ryzyko masowych śnięć ryb wskazane jest stałe bakteriologiczne monitorowanie ryb i zrzutowych wód pochłodniczych, w których są one hodowane.

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