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EFFECT OF DIFFERENT WAYS OF PRIMING TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) SEEDS ON THEIR QUALITY

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K e y w o r d s: tomato, seed germination, seed priming, *Lycopersicon esculentum*.

A b s t r a c t

In the years 2000–2002, the research was done on a possibility of the tomato (*Lycopersicon esculemntum* Mill.) "Etna F_1" seeds priming. Three commercial seed lots produced in China were used. They were hydrated for 6 and 12 hours in the distilled water and water solutions of Cebion (vitamin C), KNO_3 and Gibrescol (GA_3) as well as from 24 hours to 14 days in the solutions of KNO_3 and PEG 8000. Six and 12 hours hydrations of the seeds in KNO_3 solutions improved their germination measured after 72 hours and shortened their mean germination time (MGT) expressed by the Pieper's rate. Seeds of the two tested lots, which without priming germinated after 3 days in 49.7% and 39.7%, respectively, when hydrated for 6 hours in the KNO_3 solution, germinated in 81.3% and 76.7%, respectively. The same seeds, when hydrated for 12 hours in the solution, germinated in 85.0% and 77.0%, respectively. MGT for the seeds of the first tested lot decreased from 3.6 days (check), when hydrating in the KNO_3 solution for 6 and 12 hours, to 3.3 and 3.2 days, respectively. For the second seed lot, the received Pieper's rate was shortened from 3.8 days (check) to 3.4 days for both times of hydration. It was also possible to effectively prime seeds of tomato "Etna F_1" based on the ascorbic acid (vitamin C) solution. Long-term priming (from 1 to 14 days) of the "Etna F_1" seeds in the solutions of KNO_3 and PEG 8000, in comparison with the check seeds, improved both their germination and germination evaluated after 72 hours. It also shortened MGT of the tested seeds. The seeds soaked for 24 hours in the solutions of KNO_3 and PEG 8000 had, after their priming, the germination 60.0% and 58.0%, respectively. After 14 days, this parameter was 67.0% for both solutions in comparison with the germination rate for the non-primed seeds – 38.0%. The germination of the seeds measured after 72 hours increased, after 24 hours of soaking, from 20.0% for the check seeds to 39.0% for the seeds soaked in KNO_3 solution and 51.0% for the seeds soaked in PEG 8000 solution. The maximum values of this parameter for the tested solutions received after 10 days of the primed seeds were 64.0% and 65.0%, respectively. MGT of the check seeds was 5.6 days, whereas after 24 hours of priming in the solutions of KNO_3 and PEG 8000, it lowered to 5.4 and 4.4 days, respectively. MGT of the seeds hydrated in both solutions for 14 days was 3.0 days. The received results of priming of the tomato seeds showed a necessity to describe each time optimal conditions of this technological activity for a given seed lot.

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WPŁYW RÓŻNYCH SPOSOBÓW KONDYCJONOWANIA NASION POMIDORA (*LYCOPERSICON ESCULENTUM* MILL.) NA ICH JAKOŚĆ

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S ł o w a k l u c z o w e: pomidor, kiełkowanie nasion, kondycjonowanie nasion, *Lycopersicon esculentum*.

A b s t r a k t

W latach 2000–2002 wykonano badania nad kondycjonowaniem nasion pomidora (*Lycopersicon esculemntum* Mill.) odmiany Etna F_1. Użyto nasion z trzech komercyjnych partii wyprodukowanych w Chinach. Zastosowano moczenie przez 6 i 12 godzin w wodzie destylowanej, wodnych roztworach Cebionu (witamina C), KNO_3 i Gibrescolu (GA_3) oraz od 24 godzin do 14 dni w roztworach KNO_3 i PEG 8000. Moczenie nasion przez 6 i 12 godzin w roztworze KNO_3 poprawiło ich kiełkowanie oceniane po 72 godzinach i skróciło jego średni czas (MGT) wyrażony współczynnikiem Piepera. Nasiona dwóch badanych partii, które bez kondycjonowania kiełkowały po 3 dobach odpowiednio w 49,7% i 39,7%, po 6-godzinnym moczeniu w roztworze KNO_3, kiełkowały odpowiednio w 81,3% i 76,7%, a po 12-godzinnym – odpowiednio w 85,0% i 77,0%. MGT nasion z pierwszej badanej partii zmniejszył się, dla nasion moczonych przez 6 i 12 godzin w roztworze KNO_3, z 3,6 dnia (kontrola) do odpowiednio 3,3 dnia i 3,2 dnia. W przypadku drugiej partii, uzyskano obniżenie współczynnika Piepera z 3,8 dnia (kontrola) do 3,4 dnia dla obydwu czasów moczenia. Możliwe jest także skuteczne kondycjonowanie nasion pomidora odmiany Etna F_1 w roztworze kwasu askorbinowego (witamina C). Długotrwałe kondycjonowanie (od 1 do 14 dni) nasion pomidora odmiany Etna F_1 w roztworach KNO_3 i PEG 8000, w porównaniu z nasionami nie moczonymi, poprawiło kiełkowanie oceniane po 72 godzinach oraz skróciło także MGT badanych nasion. Nasiona moczone przez 24 godziny w roztworach KNO_3 i PEG 8000 miały energię kiełkowania odpowiednio 60,0% i 58,0%. Po 14 dniach parametr ten wyniósł 67,0% dla obydwu roztworów w porównaniu z energią kiełkowania nasion bez kondycjonowania – 38,0%. Kiełkowanie nasion moczonych 24 godziny, badane po 72 godzinach, wzrosło w porównaniu z kombinacją kontrolną, dla której wyniosło 20%, do 39,0% dla nasion moczonych w roztworze KNO_3 i do 51,0% dla moczonych w roztworze PEG 8000. Maksymalne wartości tego parametru dla badanych roztworów uzyskano po 10 dniach kondycjonowania. Wyniosły one odpowiednio 64,0% i 65,0%. MGT nasienia bez kondycjonowania wyniósł 5,6 dnia, podczas gdy po 24 godzinach kondycjonowania w roztworach KNO_3 i PEG 8000 obniżył się odpowiednio do 5,4 i 4,4 dnia. Po 14 dniach moczenia MGT nasion wyniósł 3,0 dnia dla obydwu roztworów. Uzyskane wyniki kondycjonowania nasion pomidora wykazały konieczność każdorazowego określania optymalnych warunków tego zabiegu dla danej partii nasion.

Introduction

In recent years, there has been in commercial seed production an increasing importance of priming methods for improving seed quality (BRADFORD 1986, CORBINEAU, CÔME 1990, PETRIKOVA 1991, CANTLIFFE 1997, TAYLOR et al. 1998). After years of laboratorial experiments, the method is gradually being introduced into the world's seed industry. For some species, it has become

nowadays a routine enhancement treatment (KUMAR et al. 2002). The biggest problem, however, in applying this method is lack of one universal procedure which could be used for all vegetable or even horticultural seeds. Out of various proposed priming methods, only two have been commonly used: hydro- and osmopriming. The latter is used only in terms of mineral salts solution, i.e. KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, NaCl.

There have been many proofs that priming positively affected vegetable seed germination (HORE et al. 1988, CANTLIFFE 1997). Various compounds were used to improve tomato seed quality such as: PEG, mannitol, KH_2PO_4 + $(NH_4)_2HPO_4$, KNO_3 + K_3PO_4, gibberellic acid, ascorbic acid (ELLSS 1963, BRADFORD 1986, ANDREOLI, KHAN 1999, GROOT et al. 2004). Some of them only improved seed germination, whereas others also sped up the fruit ripening (HAIGH et al. 1986, TULO, DĄBROWSKA 1993, MAUROMICALE, CAVALLARO 1995, 1997, OZBINGOL et al. 1998, 1999). The optimal temperature of priming tomato seeds is 25°C. After priming, they germinated better and in wider range of temperatures (OZBINGOL et al. 1998, 1999).

The main purpose of these experiments was to find out the method for effective priming of tomato seeds.

Materials and Methods

Short-term priming

The seeds used in this experiment came from 3 commercial seeds lots of cultivar "Etna F_1" (described in the previous paper) produced in China. These were: 203G/C from the seed lot 093137/111/203G/C from 2000, 48G from the seed lot 110120/48G from 2001 and 96G from the seed lot 210120/96G from 2002. The seeds were subjected to two various ways of priming at 20°C, in darkness: soaked for 6 or 12 hours in the water solutions of the following compounds: distilled water, the aired distilled water, distilled water with Cebion (0.5 cm^3 in 1 dm^3) (1 cm^3 Cebion has 100 mg of the vitamin C) from MERCK, aired distilled water with 0.5 cm^3 of Cebion, water with KNO_3 (2 g in 1 dm^3), aired water with KNO_3, water with 0.5 g of Gibrescol (Gibrescol has 10% GA_3) and aired water with Gibrescol. In all the tested solutions, 0.2% Funaben T was added to control fungi. The check were untreated seeds.

Long-term priming

The seeds used in this experiment came from the former field experiment (described in the previous paper). They were stored at room temperature (25°C) and RH of the air 60%.

The seeds (15 g for each treatment) were placed into a special reactor from the company "KERAM, Ltd." (Figure 1) fulfilled with 0.5 dm^3 of solutions of the following compounds: distilled water, water solutions of KNO_3 and PEG 8000 with the water potential – 1.2 MPa. All seeds were treated with 0.2% tiuram to control fungi. All the solutions in the reactor were aired. The seeds samples (about 350 seeds) were taken after: 1, 2, 3, 4, 6, 8, 10 and 14 days after priming the seeds. Then, they were rinsed under current stream of water for about 5 minutes and artificially dried in blowing air at 20°C for about 2 days.

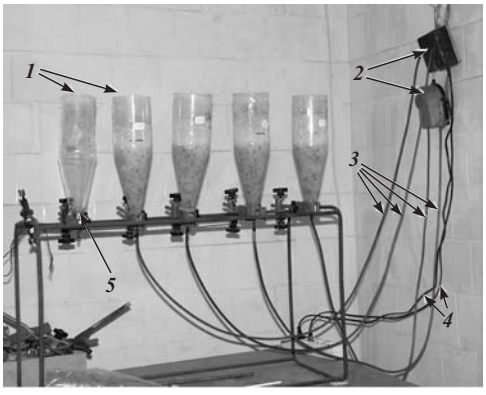

Fig. 1. The cone-shaped reactor used in the experiments with seed priming of the field hybrid cultivar "Etna F_1": *1* – transparent containers with cone-shaped bottom, of 1 dm^3 volume, *2* – air aquarium pomp "Aqua shut" with 0.4 dm^3 min^{-1} effectiveness, *3* – hose, *4* – power cable, *5* – perlator

Seed quality tests

The seeds were tested in the seed laboratory of the seed company "PlantiCo Gołębiew, Ltd.". The following parameters were evaluated: energy of germination – after 5 days, capacity of germination – after 14 days, germination – after 3 days and the mean time of germination (MGT) calculated as the Pieper's rate. The tests were carried out in 3 replications of 100 seeds each.

The received data was statistically processed. The variance was calculated, significant differences were counted using the Duncan's test for $\alpha = 0.05$.

Results and Discussion

Six and 12 hours hydration of seeds in KNO_3 solution improved their germination measured after 72 hours and shortened their MGT expressed by the Pieper's rate (Table 1 and Table 2). Seeds of the two tested lots, which

without priming germinated after 3 days in 49.7% and 39.7%, when hydrated 6 hours in the KNO_3 solution, germinated in 81.3% and 76.7%, respectively. The same seeds, when hydrated for 12 hours in the solution germinated in 85.0% and 77.0% respectively (Table 1 and Table 2). MGT for the seeds of the first tested lot decreased from 3.6 days (check), when hydrated in the KNO_3 solution for 6 and 12 hours, to 3.3 days and 3.2 days, respectively. For the second seed lot, the received Pieper's rate was shortened from 3.8 days (check) to 3.4 days for both times of hydration (Table 1 and Table 2). It was also possible to effectively prime seeds of tomato "Etna F_1" based on the ascorbic acid (vitamin C) solution (Table 1 and Table 2). The used ways of priming did not affect the energy and germination capacity of the seeds (tables 1 and 2).

Long-term priming (from 1 to 14 days) of the "Etna F_1" seeds in the solutions of KNO_3 and PEG 8000, in comparison with the check seeds, improved their germination measured after 72 hours, energy and capacity of their germination (Table 3). It also shortened MGT of the tested seeds. The seeds soaked for 24 hours in the solutions of KNO_3 and PEG 8000 had, after their priming, the germination rate 60.0% and 58.0%, respectively (Table 3).

In the seed industry, in order to improve seed quality, various priming methods have been used. The used in the experiments 6- and 12-hours priming of tomato seeds in water and solutions of various chemical compounds (Cebion, KNO_3 and Gibrescol) did not affect their germination energy. No seed enhancement was also observed for 2 out of the 3 tested seed lots. Only the oldest seeds of the seed lot 091337/111/203G/C, with the lowest starting parameters, responded to priming by increasing their germination capacity after soaking in a solution with Gibrescol. In this case, as mentioned earlier by TULO, DĄBROWSKA (1993) and PERERA, CANTLIFFE (1994), it did speed up their germination. Six and 12-hours priming did affect their germination speed measured after 72 hours, then positively correlated also with the Pieper's rate.

From the practical point of view, the most interesting finding was the positive effect of water and KNO_3 solutions (the cheapest to prepare) on germination of seeds. Similar way of priming was also tested for onion seeds (DORNA, TYLKOWSKA 2001). The received results showed a possibility of practical priming of tomato seeds in KNO_3 solutions. The proposed method in quick and cheap. Another interesting results refer to the use of Cebion (vitamin C) solution to prime the seeds. KARSZNICKA, GRZESIK (2001) reported that the ascorbic acid (vitamin C) in seeds limits appearance of free radicals and lowers their destructive effect of metabolic processes in cells. Moreover, the ascorbic acid additionally disinfects seeds (GROOT et al. 2004) and its natural version could be in the future successfully used in organic farming (GROOT et al. 2004). This will be accompanied by dynamic development of organic seed production as already seen in countries leading in the world's

Table 1

Effect of 6-hours priming of seeds of 3 different seed lots of the field tomato hybrid cultivar "Etna F_1" on their quality

Way of priming	Germination after 72 hours (%)			Germination energy (%)			Germination capacity (%)			The Pieper's rate (days)		
	Seed lot											
	48 G	96G	203G/C	48 G	96G	203G/C	48 G	96G	203G/C	48 G	96G	203G/C
Water	73.3^{c*}	71.7^{c}	5.7^{ab}	90.7^{ab}	91.3^{ab}	72.3^{a}	94.7^{a}	97.3^{a}	88.0^{a}	3.3^{ab}	3.5^{ab}	5.1^{b}
Aired water	63.3^{b}	62.0^{bc}	16.7^{cd}	89.7^{ab}	92.7^{ab}	70.0^{a}	94.3^{a}	98.3^{a}	89.3^{a}	3.4^{bc}	3.6^{ab}	4.7^{ab}
Water with "Cebion"	67.7^{b}	60.0^{bc}	21.3^{d}	92.3^{ab}	92.3^{ab}	71.3^{a}	97.3^{a}	97.7^{a}	86.7^{a}	3.5^{c}	3.5^{ab}	4.4^{a}
Aired water with "Cebion"	80.3^{cd}	69.0^{bc}	11.7^{bc}	92.3^{ab}	89.7^{a}	70.7^{a}	94.3^{a}	97.3^{a}	87.3^{a}	3.2^{a}	3.6^{ab}	4.8^{ab}
Water with KNO_3	81.3^{cd}	76.7^{c}	11.7^{bc}	95.3^{b}	94.0^{ab}	72.0^{a}	97.7^{a}	98.7^{a}	92.7^{a}	3.3^{ab}	3.4^{a}	4.6^{ab}
Aired water with KNO_3	68.3^{b}	67.0^{bc}	11.7^{bc}	92.0^{ab}	96.0^{b}	70.7^{a}	95.7^{a}	98.0^{a}	92.0^{a}	3.4^{bc}	3.4^{a}	4.6^{ab}
Water with "Gibrescol"	69.0^{b}	51.7^{ab}	15.7^{cd}	88.0^{a}	95.0^{ab}	78.7^{a}	93.7^{a}	98.7^{a}	91.3^{a}	3.5^{c}	3.6^{ab}	4.4^{a}
Aired water with "Gibrescol"	72.7^{bc}	62.0^{bc}	15.0^{cd}	92.7^{ab}	95.7^{b}	70.0^{a}	94.7^{a}	98.7^{a}	89.7^{a}	3.3^{a}	3.5^{ab}	4.6^{ab}
Check	49.7^{a}	39.7^{a}	0.0^{a}	93.7^{ab}	93.3^{ab}	74.0^{a}	95.7^{a}	98.3^{a}	86.3^{a}	3.6^{c}	3.8^{b}	4.6^{ab}

* means for a seed lot followed in a column by the same letter are not significantly different according to the Duncan's test for $\alpha = 0.05$

Table 2

Effect of 12-hours priming of seeds of 3 different seed lots of the field tomato hybrid cultivar "Etna F_1" on their quality

Way of priming	Germination after 72 hours (%)			Germination energy (%)			Germination capacity (%)			The Pieper's rate (days)		
	Seed lot											
	48 G	96G	203G/C	48 G	96G	203G/C	48 G	96G	203G/C	48 G	96G	203G/C
Water	74.0^{bc*}	83.7^{b}	38.0^{cde}	90.3^{a}	96.7^{a}	73.3^{a}	95.3^{a}	98.7^{a}	90.0^{ab}	3.6^{bc}	3.2^{a}	4.3^{a}
Aired water	86.3^{c}	71.0^{b}	38.3^{de}	91.7^{ab}	93.3^{a}	68.0^{a}	96.0^{a}	99.0^{a}	86.0^{a}	3.3^{abc}	3.6^{abc}	4.3^{a}
Water with "Cebion"	74.3^{bc}	69.3^{b}	36.0^{cde}	93.0^{ab}	92.0^{a}	73.3^{a}	97.0^{a}	97.3^{a}	90.3^{ab}	3.4^{ab}	3.5^{abc}	4.3^{a}
Aired water with "Cebion"	86.7^{c}	47.7^{a}	29.0^{bc}	92.3^{ab}	92.0^{a}	67.3^{a}	96.7^{a}	98.7^{a}	88.0^{ab}	3.3^{abc}	3.8^{c}	4.8^{a}
Water with KNO_3	85.0^{c}	77.0^{b}	29.7^{bcd}	97.0^{b}	92.3^{a}	75.3^{a}	99.0^{a}	96.7^{a}	91.3^{ab}	3.2^{a}	3.4^{ab}	4.4^{a}
Aired water with KNO_3	88.0^{c}	68.0^{b}	30.7^{bcd}	95.3^{ab}	91.3^{a}	69.0^{a}	98.0^{a}	96.7^{a}	89.7^{ab}	3.2^{a}	3.5^{abc}	4.4^{a}
Water with "Gibrescol"	75.7^{bc}	73.3^{b}	42.0^{e}	94.3^{ab}	95.0^{a}	72.7^{a}	98.0^{a}	98.0^{a}	94.0^{b}	3.4^{abc}	3.4^{ab}	4.4^{a}
Aired water with "Gibrescol"	80.7^{bc}	49.7^{a}	25.3^{b}	92.3^{ab}	91.0^{a}	71.3^{a}	97.0^{a}	97.3^{a}	93.7^{b}	3.3^{abc}	3.7^{bc}	4.7^{a}
Check	49.7^{a}	39.7^{a}	0.0^{a}	93.7^{ab}	93.3^{ab}	74.0^{a}	95.7^{a}	98.3^{a}	86.3^{a}	3.6^{bc}	3.8^{c}	4.6^{a}

* means for a seed lot followed in a column by the same letter are not significantly different according to the Duncan's test for $\alpha = 0.05$

Table 3

Effect of long-term priming of seeds of 3 different seed lots of the field tomato hybrid cultivar "Etna F_1" on their quality

Length of soaking (days)	Germination after 72 hours (%)			Germination energy (%)			Germination capacity (%)			The Pieper's rate (days)		
	Way of priming											
	water	water with KNO_3	PEG 8000	water	water with KNO_3	PEG 8000	water	water with KNO_3	PEG 8000	water	water with KNO_3	PEG 8000
Check	20^{d*}	20^{a}	20^{a}	38^{d}	38^{a}	38^{a}	72^{d}	72^{bc}	72^{cd}	5.6^{b}	5.6^{f}	5.6^{e}
1	39^{e}	39^{cd}	51^{d}	48^{e}	60^{de}	58^{b}	73^{d}	73^{bcd}	72^{cd}	4.6^{a}	5.4^{e}	4.4^{d}
2	13^{c}	30^{b}	53^{d}	31^{c}	48^{b}	64^{c}	65^{c}	72^{bc}	80^{f}	5.6^{b}	5.5^{ef}	4.5^{d}
3	8^{b}	36^{c}	48^{c}	17^{b}	52^{c}	65^{c}	63^{c}	80^{e}	76^{e}	6.2^{d}	5.4^{e}	4.4^{d}
4	5^{b}	40^{d}	45^{b}	16^{b}	57^{d}	65^{c}	50^{b}	76^{d}	74^{de}	6.6^{e}	4.8^{d}	4.4^{d}
6	0^{a}	45^{e}	45^{b}	7^{a}	60^{de}	66^{c}	16^{a}	74^{cd}	72^{cd}	6.0^{c}	4.4^{c}	4.2^{c}
8		59^{f}	61^{e}		63^{ef}	67^{c}		72^{bc}	71^{bc}		3.3^{b}	3.4^{b}
10		64^{g}	65^{f}		64^{fg}	67^{c}		70^{ab}	69^{ab}		3.1^{a}	3.0^{a}
14		60^{f}	60^{e}		67^{g}	67^{c}		67^{a}	67^{a}		3.0^{a}	3.0^{a}

* means followed in a column by the same letter are not significantly different according to the Duncan's test for $\alpha = 0.05$

seed industry. In only one country, the UK, in 2005, there were over 7000 ha of organic crops and its share in the EU's agriculture in 2005 exceeded 3% of the total land used for agriculture. The use for organic seeds of vegetables in Poland is still small, however it is predicted to increase soon and become a profitable product line (BRALEWSKI, HOŁUBOWICZ 2004).

The long-term priming of the tomato seeds resulted in lowering their sowing value. In the carried out experiment, already after 4 days the germinating seeds were observed. Only 24 hours soaking improved their germination measured after 72 hours as well as MGT. Therefore, practical use of this procedure should be connected with precise keeping the described regime in terms of both time and temperature. If not so, opposite effect or even loss of seed germination could happen. The long-term priming in KNO_3 and PEG 8000 solutions is free of such risk. They improved their both speed of germination after 3 days and MGT expressed as the Pieper's rate. The improvement was recorded after 1 day and was the biggest after 8 and 14 days of the soaking, which is slightly longer than described by OZBINGOL et al. (1998). Longer seed soaking lowered the parameter. The received results are in agreement with the earlier findings of BRADFORD (1986), ODELL et al. (1992), TULO, DĄBROWSKA (1993), MAUROMICALE, CAVALLARO (1995, 1997). Priming increased seed quality because already 6-hours of it, as reported by KARSZNICKA, GRZESIK (2001), initiated the regeneration processes of damaged cell membranes, genetic apparatus and activates enzymes acting against appearing of free radicals.

The received results of priming of the tested tomato seeds showed a necessity to describe each time optimal conditions of this technical procedure for a given seed lot.

The tested in this experiment methods of tomato seed priming were immediately applied into practice. A small commercial installation for tomato seed priming to the seed company needs was built. First seeds improved this method were put onto the market in the spring of 2007.

Conclusions

1. Six and 12 hours soaking of tomato seeds of the cultivar "Etna F_1" in the KNO_3 solutions improved their germination evaluated after 3 days and shortened their mean germination time (MGT) in comparison with non-primed seeds.

2. Twenty four hours long or longer (up to 14 days) priming of the seeds of tested cultivar in the solutions of KNO_3 and PEG 8000 improved their energy of germination and germination evaluated after 72 hours and shortened their MGT in comparison with the unsoaked seeds.

3. It is possible to effectively prime tomato seeds of the cultivar "Etna F_1" based on the solution of ascorbic acid (vitamin C) and use this element in the organic seed production.

4. The received in the experiments results of short tomato seed priming in the solutions of Cebion, KNO_3 and Gibrescol as well as long tomato seed priming in the solutions of KNO_3 and PEG 8000, showed that there is a need each time for a given seed lot to describe its optimal conditioning conditions.

Translated by Roman Hołubowicz

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CADMIUM AND LEAD CONTENT IN OAT AND SOIL FERTILIZED WITH COMPOSTS

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K e y w o r d s: composts, cadmium, lead, oat, soil.

A b s t r a c t

Application of waste substances in agriculture, even after their processing involves various kinds of hazards, among others concerning supply of heavy metal load to the soil environment. Therefore, the investigations aimed at determining the effect of composts of various origin on bioavailability of cadmium and lead in soil and their concentrations in oat. Applied fertilization, irrespectively of the kind of compost, caused an excessive cadmium accumulation in oat aboveground parts. Plants accumulated the largest quantities of the studied metals in their roots system. After a three-year period of research a considerable increase in the content of bioavailable cadmium forms in soil was found, whereas the content of bioavailable lead forms underwent only slight changes. The conducted investigations revealed a progressive soil acidification process, which may condition increasing bioavailability of the studied heavy metals.

ZAWARTOŚĆ KADMU I OŁOWIU W OWSIE I GLEBIE NAWOŻONEJ KOMPOSTAMI

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S ł o w a k l u c z o w e: komposty, kadm, ołów, owies, gleba.

A b s t r a k t

Wykorzystywanie do celów rolniczych substancji odpadowych, nawet po ich przetworzeniu, niesie ze sobą różnego rodzaju zagrożenia m.in. dotyczące wprowadzenia metali ciężkich do środowiska glebowego. Celem badań było określenie wpływu kompostów rożnego pochodzenia

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na dostępność kadmu i ołowiu w glebie oraz ich zawartość w owsie. Zastosowane nawożenie, niezależnie od rodzaju kompostu, spowodowało nadmierną kumulację kadmu w częściach nadziemnych owsa. Najwięcej badanych pierwiastków rośliny gromadziły w systemie korzeniowym. Po trzyletnim okresie badań stwierdzono znaczące zwiększenie się zawartości dostępnych dla roślin form kadmu w glebie, niewielkim zmianom natomiast uległa zawartość dostępnych form ołowiu. Wykazano postępujący proces zakwaszenia gleby, co może warunkować coraz większą dostępność dla roślin badanych metali ciężkich.

Introduction

Parent rock is the natural source of heavy metals in soil. In case of agricultural lands these elements may be additionally supplied in result of fertilization, e.g. with composts. However, heavy metal supply to the soil does not mean their uptake by plants. This process is considerably influenced by soil properties, such as: soil pH, sorption capacity, organic matter contents and soil heavy metal concentrations (BASTA et al. 2005, BORUVKA, DRABEK 2004, GORLACH, GAMBUŚ 2000, TLUSTOS et. al 2006).

The mechanism of heavy metal uptake by plants is complicated and usually constitutes a resultant of such processes as: cation exchange by cell membranes, inter cellular transport and biochemical reactions occurring in the rhisosphere.

There are two basic mechanisms of metal uptake by roots: passive and active. Heavy metal absorption by plants does not prove the indispensability of these elements but results from their soil concentrations (GONDEK, FILIPEK-MAZUR 2003).

There are numerous works discussing the effect of individual soil environment agents on heavy metal sorption and mobilization (BALIK et al. 2002, BARROW et al. 1981, CHANEY 1982, GERRITSE, VAN DRIEL 1984, PAVLIKOWA et al. 2002) but the results of presented experiments pertain to the soils into which heavy metals were supplied as solutions or readily soluble salts. Soils, where waste substances containing heavy metals were used as fertilizers pose another problem. Therefore presented research aimed at determining the effect of compost of various origin on availability of these elements from soil and their content in oats.

Material and Methods

Three-year studies were conducted as a pot experiment in a vegetation hall of the Department of Agricultural Chemistry. PVC pots contained 5.5 kg of air-dried soil material. Detailed characteristics of soil material have been presented in Table 1. Composts from Cracow (two different batches) based on

green wastes were used – composts (*A*) and (*B*) and also compost prepared from plant materials and produced in Nitra (Slovakia) – compost (*C*) and compost based on straw and poultry manure and produced in Prague (Czech Republic) – compost (*D*). Heavy metal concentrations in oats from pots where the composts were applied were compared with concentrations in plants from objects fertilized with mineral salts and farmyard manure.

Table 1

Physical-chemical properties of experimental soil

Parameters							
Fraction < 0.02 (%)	pH (H_2O)	pH (KCl)	Hh* (mmol(+) kg^{-1})	Organic C (g kg^{-1})	Total N (g kg^{-1})	Available	
						P (mg kg^{-1})	K (mg kg^{-1})
32	6.27	5.75	11.2	11.0	1.10	75.21	294.73
Total content mg kg^{-1} d.m.							
Cu	Zn	Mn	Fe	Pb	Ni	Cr	Cd
9.68	73.49	232.99	11 585	30.75	17.35	8.29	0.55

* Hh – hydrolytic acidity

In the vegetative experiment doses of compost and farmyard manure were determined on the basis of their nitrogen concentrations. Nitrogen dose was 0.8 g per pot. Phosphorus and potassium were balanced in all treatments to the highest level supplied with organic fertilizers – phosphorus to 1.41 g pot^{-1} as phosphorite meal, potassium to 1.21 g pot^{-1} – as water KCl solution.

Oat (*Avena sativa* L.) was the test plant each year (Dragon c.v. in the first year and Kasztan c.v. in the second and third year). Plant density, supplementary fertilization rates and the length of growing period are given in Table 2. The plants were harvested at full maturity and the obtained biomass yield was separated into grain, straw and roots.

Table 2

Cultivar oat and doses of nutrient

Year	Cultivar	Amount of plants in pot	Day of vegetation	Fertilization (g kg^{-1})		
				N	P	K
1st year	Dragon	14	82	0.15*	0.26**	0.22**
2nd year	Kasztan	14	90	0.15	0.10	0.22
3rd year	Kasztan	14	109	0.15	0.10	0.22

* in form of organic fertilizers
** supplemented by mineral

Dry mass content was assessed in the composts and farmyard manure after samples drying at 70° in a dryer with hot air flow, and total nitrogen concentrations (in fresh material) after sample mineralization in a concentrated sulphuric acid(V) using Kjeldahl method. In dried and crushed fertilizer samples phosphorus, potassium, magnesium, calcium, sodium and trace elements (Cu, Zn, Mn, Cr, Pb, Cd and Ni) were assessed after sample dry mineralization in a muffle furnace (at 450°C for 5 h) and ash solution in nitric acid(V) (1:2). Phosphorus was determined by Beckman DU 640 spectrophotometer at the wave length 436 nm; potassium, calcium and sodium were assessed by flame photometry (FES), magnesium and trace elements by atomic absorption method (AAS) using Philips PU 9100X spectrometer (OSTROWSKA et al. 1991). Results of compost chemical composition were given in Table 3 and Table 4 and discussed in detail in the other publication (GONDEK, FILIPEK-MAZUR 2005).

Table 3

Macroelements content in FYM and composts used in experiment

Fertilizer	Dry matter (g kg^{-1})	Total N (g kg^{-1} d.m.)	P (g kg^{-1} d.m.)	K (g kg^{-1} d.m.)	Ca (g kg^{-1} d.m.)	Na (g kg^{-1} d.m.)	Mg (g kg^{-1} d.m.)
Farmyard manure (FYM)	205	20.9	21.4	18.7	23.8	5.0	4.6
Compost (*A*)	514	20.8	4.9	25.4	38.6	2.2	4.5
Compost (*B*)	483	17.5	4.5	26.7	38.0	0.9	4.1
Compost (*C*)	941	30.8	10.8	11.7	16.0	1.8	2.0
Compost (*D*)	424	24.7	43.6	30.4	14.4	0.5	1.9
SD	237	5.7	18.7	8.2	13.4	0.8	1.4
CV	40	24	117	35	50	58	44

SD – Standard deviation
CV – Coefficient of variation

Yields of individual parts were dried in a dryer with hot air flow (at. 70°), weighed and dry matter contents were assessed separately for grain, straw and roots. Results of oats yielding were discussed in an earlier publication (GONDEK, FILIPEK-MAZUR 2005). Dried and crushed plant material samples were dry mineralized in a muffle furnace (at 450°C for 5 h) (OSTROWSKA et al. 1991). Obtained ash was dissolved in nitric acid (1:2) and still hot replaced to volumetric flaks. In such prepared samples Cd and Pb contents were assessed by ICP-AES method in JY 238 Ultrace apparatus. In the soil material (sampled separately from each pot) pH was assessed in a suspension of soil and

Table 4

Trace elements content in FYM and composts used in experiment

Fertilizer	Cu (mg kg^{-1} d.m.)	Zn (mg kg^{-1} d.m.)	Mn (mg kg^{-1} d.m.)	Fe (mg kg^{-1} d.m.)
Farmyard manure (FYM)	411.00	419	314	1405
Compost (*A*)	35.15	291	245	4550
Compost (*B*)	33.20	290	316	5345
Compost (*C*)	8.45	72	104	727
Compost (*D*)	58.30	495	113	10 850
SD	20.37	173	104	4026
CV	60	60	53	88
Fertilizer	Cr (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)	Cd (mg kg^{-1} d.m.)	Ni (mg kg^{-1} d.m.)
Farmyard manure (FYM)	2.81	2.76	0.90	9.62
Compost (*A*)	13.35	23.40	2.00	6.66
Compost (*B*)	18.00	25.90	1.60	7.19
Compost (*C*)	3.03	1.08	0.10	2.79
Compost (*D*)	57.25	15.45	0.92	9.22
SD	23.73	11.18	0.86	2.69
CV	104	68	76	42

SD – Standard deviation
CV – Coefficient of variation

water, and in soil and 1 mol dm^{-3} KCl suspension, where soil to solution ratio was 1:2.5. Organic carbon was determined after sample mineralization in potassium dichromate by Tiurin method. Selected heavy metals (Cd and Pb) were extracted (for one hour) from soil with 1 mol dm^{-3} HCl solution at soil to solution ratio 1:10 (OSTROWSKA et al. 1991).

Analyses of plant and soil material from the experiment were conducted in four simultaneous replications whereas on organic materials and initial soil in two replications. A sample of laboratory materials with known parameters was added to each series of the analyzed material and the result was considered reliable if relative standard deviation (RSD) did not exceed 5%.

The obtained results were elaborated statistically using one factor ANOVA and differences estimation by Duncan test at significance level $\alpha < 0.05$ (STANISZ 1998). Standard deviation (SD) and variability coefficient (V%) were computed for the analyzed parameters.

Results and Discussion

Results concerning chemical composition of composts, particularly heavy metal concentrations indicate a potential use of these materials as fertilizers (Table 3 and Table 4). Therefore vegetative studies were undertaken to determine the effect of compost supplement to the soil on heavy metal availability to plants and their concentrations in oat.

Yields of oat grain in the first year of research were the largest on the object fertilized with mineral salt. The increase was statistically significant in comparison with yields from all treatments. From among the applied composts, those from Nitra (*C*) and Prague (*D*) – Figure 1, markedly better affected the grain yield than the composts produced in Cracow. In the second year of the experiment the biggest grain yields were obtained on the object where mineral salts and composts from Cracow (*B*) were used as fertilizers. On the other treatments grain yields were significantly smaller at non-significant differentiation between objects. In the third year of research comparable yields of oat grains were registered on treatments with mineral salts, farmyard

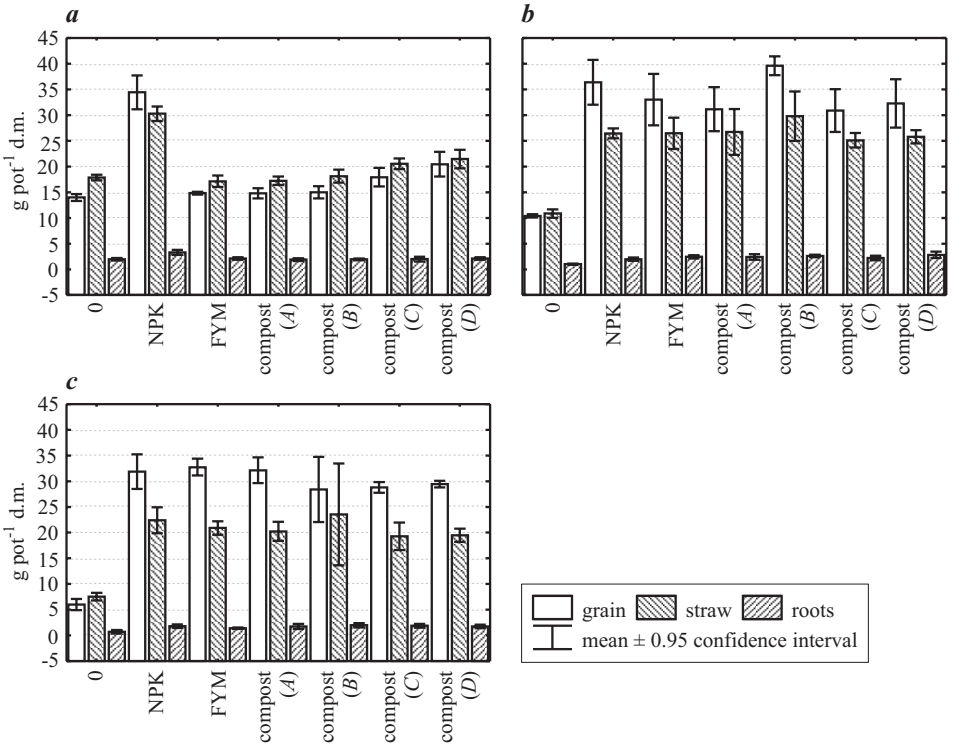

Fig. 1. Yield of biomass oat: *a* – 1st year, *b* – 2nd year, *c* – 3rd year

manure and compost (*A*). Summary yield of oat grains for three years was the largest, like in individual years, on mineral salt treatment (102.74 g pot^{-1}) – Table 5. On the objects where composts were used for fertilization summary oat grain yield was comparable with registered on farmyard manure treatment (80.66 g pot^{-1}).

Table 5

Yield of dry matter biomass oat (g pot^{-1}) planted in treated soil

Fertilization	Grain	Straw	Roots
0	30.38^{a*}	36.28^{a}	3.69^{a}
NPK	102.74^{d}	79.18^{d}	7.15^{c}
Farmyard manure (FYM)	80.66^{bc}	64.54^{b}	5.96^{b}
Compost (*A*)	77.90^{b}	64.27^{b}	6.03^{b}
Compost (*B*)	83.05^{c}	68.42^{c}	6.55^{bc}
Compost (*C*)	77.66^{b}	64.94^{b}	6.09^{b}
Compost (*D*)	82.24^{bc}	66.77^{bc}	6.71^{bc}
SD	22.03	13.07	1.12
CV	29	21	19

* homogeneous groups according to the Duncan test, $\alpha < 0.05$
SD – Standard deviation
CV – Coefficient of variation

The yield of oat straw in the first year of research, like the yield of grain was significantly the largest on mineral salt treatment. Fertilization with farmyard manure and composts from Cracow (*A*) and (*B*) shaped oat yield on a level similar to detrminated on the control (Figure 1). In the second year of investigations the largest straw yield was obtained on the object receiving compost from Cracow (*B*) (29.82 g pot^{-1}). The yields of straw from the other treatments did not differ significantly. A similar relationship was found in the third year of the experiment. Summary yield of oat straw was the largest on mineral treatment, whereas on the other compost treatments (except the compost *B* treatment) it was similar to obtained on farmyard manure (Table 5).

Oat root biomass after the first year of the experiment was not significantly different (Figure 1), except the mineral salt treatment. In the second year markedly more oat roots (in relation to mineral salt treatment) was noted on all objects receiving organic fertilization, except the object where the compost from Nitra (*C*) was used. In the third year of the experiment significantly less roots were found only on the object fertilized with farmyard manure, whereas on the other treatments the differences were not statistically significant. The summary amount of root biomass was the largest on mineral salt treatment,

while compost fertilization shaped the root biomass yield on a level similar to registered on farmyard manure treatment (Table 5).

Mean cadmium content in oat dry matter (from the three years of the experiment) depended on plant organ and applied fertilization (Table 6). The highest cadmium content was detected in the roots. Oat grain and straw on individual treatments contained comparable amounts of this element but there was a significant diversification among treatments. In comparison with the value of 0.15 mg kg^{-1} dry matter (GORLACH, GAMBUŚ 2000) assumed as permissible for grain destined for consumption, a considerably exceeded content was registered in plants from all treatments except the control. In view of fodder utilization, cadmium concentrations in the studied biomass did not raise objections. Significantly largest cadmium quantities, irrespective of plant organ, were assessed in plants fertilized with mineral salts. Presented results are in agreement with these reported by LOGAN, CHANEY (1983), GONDEK, FILIPEK-MAZUR (2003), who demonstrated a more intensive cadmium uptake in a pot experiment conditions than in field cultivation. According to CHANEY (1982), cadmium is the element which is not affected by so called soil-plant barrier, which means that plants tolerate in their organs (and do not reveal any toxicity symptoms) the amounts of cadmium which are normally noxious for animals consuming the plants. The fact mentioned above explains why no decline in plant yield was noted on treatment receiving mineral salts where this element concentration was the highest. On the other hand KABATA--PENDIAS, PENDIAS (1984) report that at its increased uptake by plants,

Table 6

Average weighted content of cadmium and lead in dry matter of oat (average for three years)

Fertilization	Cd (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)	Cd (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)	Cd (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)
	grain		straw		roots	
0	0.15^{a*}	0.19^{a}	0.17^{a}	0.61^{abc}	0.76^{a}	2.77^{bc}
NPK	0.59^{d}	0.16^{a}	0.69^{d}	0.73^{cd}	1.45^{d}	3.05^{c}
Farmyard manure (FYM)	0.48^{c}	0.25^{b}	0.54^{c}	0.47^{a}	1.23^{c}	2.88^{bc}
Compost (*A*)	0.42^{b}	0.17^{a}	0.50^{c}	0.68^{bc}	1.00^{b}	2.59^{abc}
Compost (*B*)	0.40^{b}	0.19^{a}	0.41^{b}	0.62^{abc}	1.13^{bc}	2.17^{a}
Compost (*C*)	0.57^{d}	0.18^{a}	0.52^{c}	0.55^{ab}	1.17^{c}	2.53^{ab}
Compost (*D*)	0.57^{d}	0.19^{a}	0.55^{c}	0.92^{d}	1.11^{bc}	2.89^{bc}
SD	0.1	50.03	0.16	0.14	0.21	0.29
CV	34	15	33	22	19	11

* homogeneous groups according to the Duncan test, $\alpha < 0.05$

SD – Standard deviation

CV – Coefficient of variation

cadmium is mostly accumulated in roots and probably forms so called phytochelatines with sulfhydrol groups and proteins.

Lead concentrations in oat grain did not exceed 0.25 mg kg^{-1} dry matter and slight diversification among treatments was observed (Table 6). This amount did not raise any objections in view of grain destination for consumption or forage (GORLACH 1991). Straw lead concentrations proved between 2 and 5 times higher and the largest quantities were registered in the biomass from the object receiving mineral fertilizers and compost from Prague (*D*). The largest amounts of lead were assessed in oats roots and significant diversification among treatments was noted. This metal concentrations in oats roots depended on its availability in the substrate and plant organ. Like cadmium and chromium, lead was retained in oats roots which resulted from forming in the root system of sparingly soluble lead forms.

Soil fertilization with composts led to significant increase in its pH (determined in H_2O and KCl), particularly after the first year of the experiment, in comparison to the soil from unfertilized object and mineral treatment (Figure 2, Figure 3). Statistically proved de-acidifying effect was demonstrated

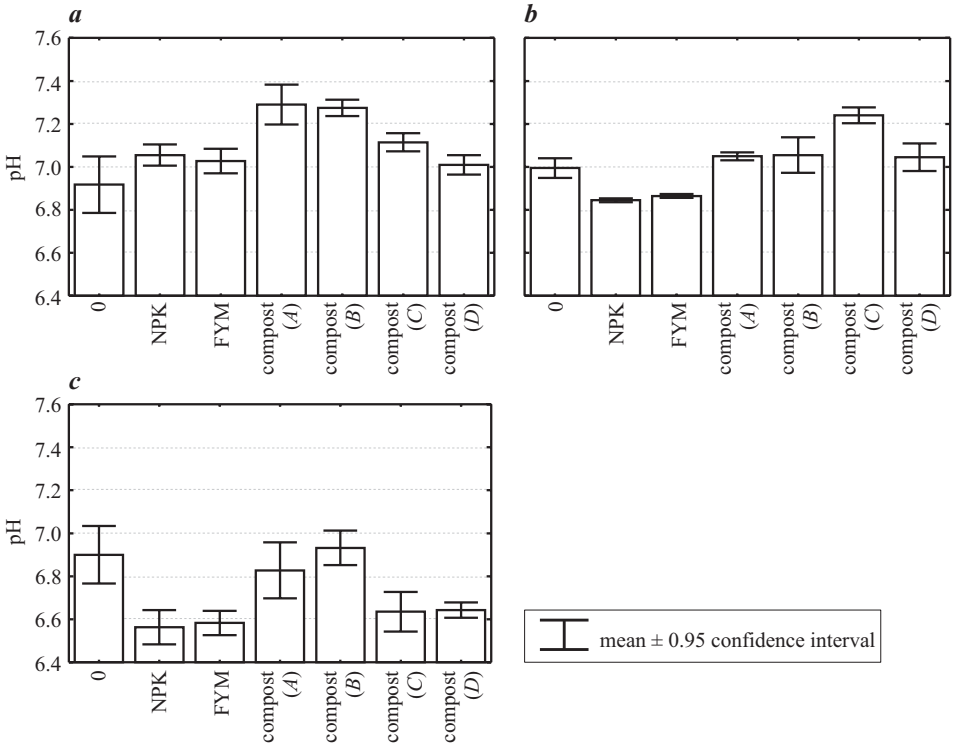

Fig. 2. Soil reaction (pH H_2O): *a* – 1st year, *b* – 2nd year, *c* – 3rd year

for composts from Cracow (*A*) and (*B*). Progressively decreasing pH value was registered in the subsequent years, irrespective of the fertilization applied and the highest rate of soil acidification was noted on mineral treatment and compost from Nitra (*C*). An increase in pH value under the influence of compost application was also found by LEKAN and KACPEREK (1990), PINAMONTI (1998) and SZULC et al (2003). Positive effect of composts, mainly those from Cracow, on the soil pH resulted primarily from higher concentrations of calcium in comparison with quantities assessed in farmyard manure and higher in comparison with the other composts. A relatively transitory positive effect of compost fertilization on soil pH (observed only in the first year) was due to the dose of these materials, as has been corroborated by results of research conducted by SZULC et al (2003), who revealed increasing pH value with increasing compost dose.

Compost application caused a raise of organic carbon content, however the increase was not statistically significant in comparison with the control and mineral treatment (except for the objects fertilized with composts *A* and *B* from Cracow) – Figure 4. According to SZULC et al (2003) application of composts based on urban wastes influenced organic carbon content and the

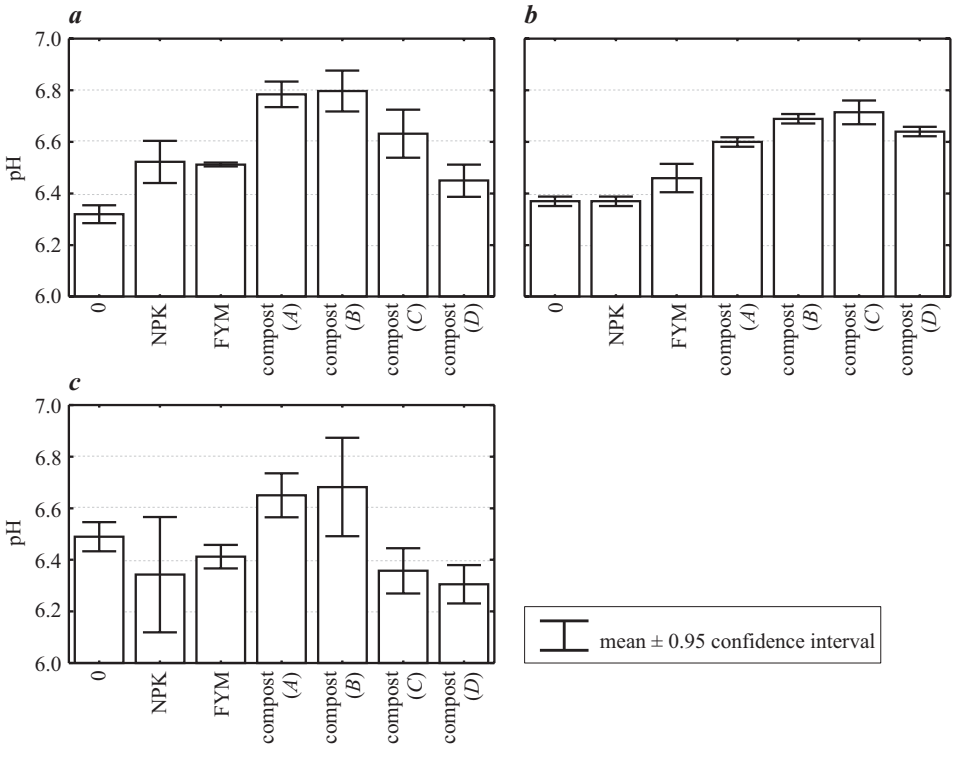

Fig. 3. Soil reaction (pH KCl): *a* – 1st year, *b* – 2nd year, *c* – 3rd year

increase in this component content in soil was proportional to applied compost dose. Authors' own investigations revealed a decline in organic *C* content in soil of each treatment and the content of this element in soil fertilized with composts from Nitra (*C*) and Prague (*D*) proved the most stabile.

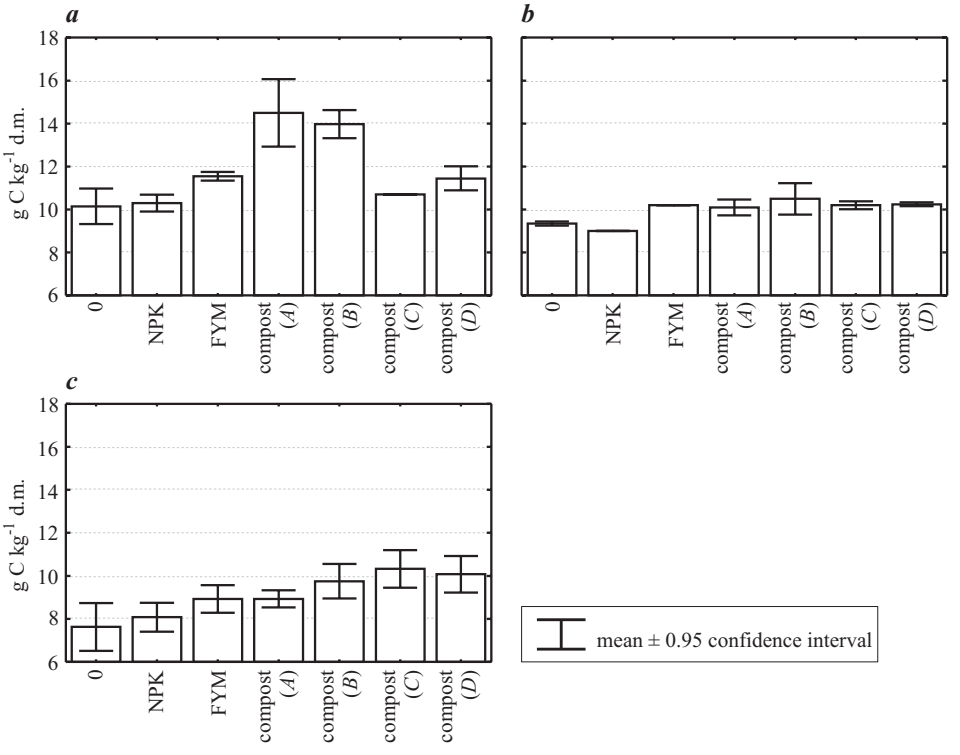

Fig. 4. Content of organic C in soil: *a* – 1st year, *b* – 2nd year, *c* – 3rd year

Contents of cadmium forms available to plants in soil of treatments increased in the subsequent years of the experiment and after the third year was on an average 12.3% higher in relation to the contents assessed after the first year (Table 7). Beside soil fertilized with compost (*D*) from Prague, after the first year of the experiment this element concentrations did not reveal any significant diversification. In the second and third year a greater diversification among objects was found for cadmium and the largest amounts of this element were extracted from soil fertilized with minerals.

The content of lead forms available to plants did not grow significantly (in the 1st and 2nd year) in soil where composts were applied, except for soil fertilized with compost (*D*) from Prague (Table 7). In the third year of the research the largest quantities of lead extracted with HCl solution were assessed in soil receiving mineral fertilizers and compost (*A*) from Cracow.

Table 7

Cadmium and lead content in soil

Fertilization	Cd (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)	Cd (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)	Cd (mg kg^{-1} d.m.)	Pb (mg kg^{-1} d.m.)
	1st year		2nd year		3rd year	
0	0.39^{a*}	16.85^{ab}	0.38^{a}	16.60^{ab}	0.62^{d}	16.60^{b}
NPK	0.39^{a}	16.55^{a}	0.40^{ab}	16.90^{ab}	0.51^{c}	16.95^{b}
Farmyard manure (FYM)	0.39^{a}	16.38^{a}	0.42^{c}	16.60^{ab}	0.45^{b}	16.68^{b}
Compost (*A*)	0.39^{a}	16.52^{a}	0.41^{b}c	15.95^{a}	0.43^{ab}	16.92^{b}
Compost (*B*)	0.39^{a}	16.57^{a}	0.40^{ab}	16.80^{ab}	0.41^{ab}	15.23^{a}
Compost (*C*)	0.38^{a}	16.30^{a}	0.41^{b}c	16.20^{a}	0.38^{a}	15.10^{a}
Compost (*D*)	0.41^{b}	17.33^{b}	0.39^{a}	17.20^{b}	0.45^{b}	16.80^{b}
SD	0.01	0.35	0.01	0.42	0.08	0.80
CV	2	2	3	3	17	5

* homogeneous groups according to the Duncan test, $\alpha < 0.05$
SD – Standard deviation
CV – Coefficient of variation

According to RUTKOWSKA et al (2003) compost supplement to soil in the first place caused a release of elements, which are bound in the compost in largest amounts in exchangeable and carbonate fractions. On the basis of investigations conducted a significant increase in the content of available cadmium, whereas the content of available lead forms in soil changed only slightly after 3 year period of the experiment.

It should be emphasized that a raise in soil heavy metals available to plants is not the same for all elements. Heavy metal passing into soil solution depends on soil properties and the element itself (GORLACH, GAMBUŚ 2000). The investigations revealed a proceeding process of soil acidification, which undoubtedly greatly influenced increased availability of some elements, mainly cadmium, to plants (CHRISTENSEN 1984, MCBRIDE et al. 1997). After three years of the experiment organic carbon content decreased visibly, which might have also contributed to better availability of cadmium and lead.

Conclusion

1. Applied fertilization, irrespectively of the kind of compost, caused an excessive cadmium accumulation in oat aboveground parts.

2. Plants accumulated the largest quantities of the studied metals in their roots system.

3. After a three-year period of research a considerable increase in the content of bioavailable cadmium forms in soil was found, whereas the content of bioavailable lead forms underwent only slight changes.

4. The conducted investigations revealed a progressive soil acidification process, which may condition increasing bioavailability of the studied heavy metals.

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INFLUENCE OF LOW TEMPERATURE ON EXPRESSION OF MnSOD GENE IN POLISH BARLEY CULTIVARS

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K e y w o r d s: barley, mitochondrial manganese superoxide dismutase (MnSOD), Real-Time PCR, cold tolerance.

A b s t r a c t

The first line of live cells defense against reactive oxygen species activity are superoxide dismutases (SOD). Molecular examinations, using Real-Time PCR method proved, that expression of genes coding for superoxide dismutases is changed, as a result of exposing plants to low temperature. The aim of our study was to characterize changes of mitochondrial manganese superoxide dismutase (MnSOD) gene expression under the influence of low temperature in five (3 winter and 2 spring) Polish barley cultivars, using Real-Time PCR method. As a result of examinations, we affirmed that for all tested barley cultivars expression of mitochondrial manganese superoxide dismutase gene increased after cold treatment. Cultivars with spring growth habit: Blask and Poldek were characterized by slow MnSOD gene expression growth, whereas cultivars with winter growth habit: Bażant, Bursztyn and Horus responded with fast MnSOD gene expression growth under the influence of low temperature.

WPŁYW NISKIEJ TEMPERATURY NA EKSPRESJĘ GENU MnSOD W POLSKICH ODMIANACH JĘCZMIENIA

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S ł o w a k l u c z o w e: jęczmień, mitochondrialna manganowa dysmutaza ponadtlenkowa (MnSOD), Real-Time PCR, tolerancja na chłód.

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Abstrakt

Dysmutazy ponadtlenkowe (SOD) stanowią pierwszą linię obrony żywych komórek przed działaniem reaktywnych form tlenu. Badania molekularne z wykorzystaniem metody Real-Time PCR dowiodły, że ekspresja genów je kodujących ulega zmianom w wyniku poddawania roślin działaniu niskiej temperatury. Celem pracy była charakterystyka zmian ekspresji genu kodującego mitochondrialną manganową dysmutazę ponadtlenkową (MnSOD) pod wpływem niskiej temperatury w pięciu (3 ozimych i 2 jarych) polskich odmianach jęczmienia z użyciem metody Real-Time PCR. We wszystkich analizowanych odmianach jęczmienia, po działaniu na nie niską temperaturą, stwierdzono wzrost ekspresji genu mitochondrialnej manganowej dysmutazy ponadtlenkowej. Odmiany jare – Blask i Poldek charakteryzowały się powolnym wzrostem ekspresji genu MnSOD, a ozime – Bażant, Bursztyn i Horus – szybkim.

Introduction

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), and the hydroxyl radical ($OH^{\cdot}$), which are often generated in live cells as a by-products of natural metabolic processes and under the influence of environmental stresses, are serious danger, because of their high oxidative potential (BOWLER et al. 1992, WU et al. 1999). Among threats connected with theirs activity in cell, the most important are: lipid membranes peroxidation, damage of DNA strands and enzymes inactivation (WU et al. 1999). The first line of live cells defense against reactive oxygen species activity are superoxide dismutases (SOD) (EC 1.15.1.1) (FINK, SCANDALIOS 2002). These enzymes dismutate two superoxide anions into H_2O_2 and O_2 (BAEK, SKINNER 2006). In plants superoxide dismutases can be classified by cellular location and the catalytic metals required, into 3 groups: Cu/ZnSOD in the cytosol and in chloroplasts, MnSOD in mitochondria and FeSOD in chloroplasts (FINK, SCANDALIOS 2002, BAEK, SKINNER 2006). Manganese superoxide dismutase in wheat, despite the fact of their location in mitochondria, are encoded by nuclear genes (BAEK, SKINNER 2006). Molecular researches, using Real-Time PCR method proved, that expression of these genes changed as a result of exposing plants to low temperature (WU et al. 1999, BAEK, SKINNER 2003). Real-Time PCR technique, described also as a quantitative PCR (QPCR), is one of the most accurate method of gene expression analysis. Utilization of that method allows to describe and compare amounts of mRNA transcripts derived from different genes. QPCR method is now often use in identification and expression characterization of cereal genes. In molecular examinations of barley, Real-Time PCR method allowed to characterize the expression profile of genes encoding B-hordeins (PISTÓN et al. 2005), to describe some genes, which are active during embryogenesis and caryopsis development (LIU et al. 2005). That method was used to examine changes of *HvC2d1* and

HVA1 genes expression under the influence of abiotic stresses, too (OUELHADJ et al. 2006, QIAN et al. 2007).

The aim of this study was to characterize changes of manganese superoxide dismutase (MnSOD) gene expression under the influence of low temperature in Polish barley (*Hordeum vulgare* L.) cultivars, using Real-Time PCR method.

Material and Methods

Five Polish barley cultivars were examined. Among these cultivars Bażant, Bursztyn and Horus showed winter growth habit, while Blask and Poldek showed spring growth habit. Five days old seedlings, derived from sterile kernels, were placed in 4°C. Fragments of leaves were collected after 2 and 7 days. Material was collected from control forms, placed in 24°C, too. RNA was isolated using "Total RNA" kit (A&A Biotechnology), according to manufacturer's protocol. Reverse transcription to cDNA were carried out using TaqMan® Reverse Transcription Reagents (Applied Biosystems), according to protocol suggested by producer. Real-Time PCR reactions were carried out using Mx3005P system (Stratagene) kindly provided by Biomedica Poland company. For these analysis Brilliant® SYBR® Green QPCR Master Mix reagents (Stratagene) were used. Reactions were prepared according to the protocol enclosed with reagents. Every sample was analyzed in 3 replications. As a reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used (BURTON et al. 2004). In Real-Time PCR reaction were used HPLC purified primers obtained by BAEK, SKINNER (2003) for wheat and designed by means of Primer3 (http://primer3.sourceforge.net) for barley. The primers sequences were as follows: MnSOD-F: 5'-CAG AGG GTG CTG CTT TAC AA-3' and MnSOD-R: 5'-GGT CAC AAG AGG GTC CTG AT-3'. Analysis of obtained results were carried out using MxPro application (Stratagene). In these analysis MnSOD gene expression was compared between control and tested forms.

Results and Discussion

As a result of examinations we affirmed that for all tested barley cultivars expression of mitochondrial manganese superoxide dismutase gene increased after cold treatment. Cultivars with spring growth habit: Blask and Poldek were characterized by slow MnSOD gene expression growth. Expression level measured after 2 days of cold treatment was not much higher than in control forms. However expression measured after 7 days of cold treatment was much

higher in comparison to forms did not treat by low temperature (Figure 1). Barley cultivars with winter growth habit: Bażant, Bursztyn and Horus responded with fast MnSOD gene expression growth under the influence of low temperature. For cultivars Bażant and Bursztyn, in spite of further activity of low temperature, expression of examined gene decreased, and in material collected after 7 days was considerably lower that in sample collected after 2 days of cold treatment (Figure 2). Among winter barley cultivars only cv. Horus showed continued increase of MnSOD gene expression with prolonged low temperature activity (Figure 2). That cultivar turned out to be the most sensitive for cold, too – the increase of MnSOD gene expression was the highest. The least sensitive for cold was cv. Bażant (Figure 2). Obtained results suggest, that MnSOD gene expression profile in barley cultivars is connected with their growth habit. The faster expression growth in winter cultivars suggests that level of their cold tolerance is higher in comparison with spring cultivars, because of ability to immediate reaction to stressful conditions.

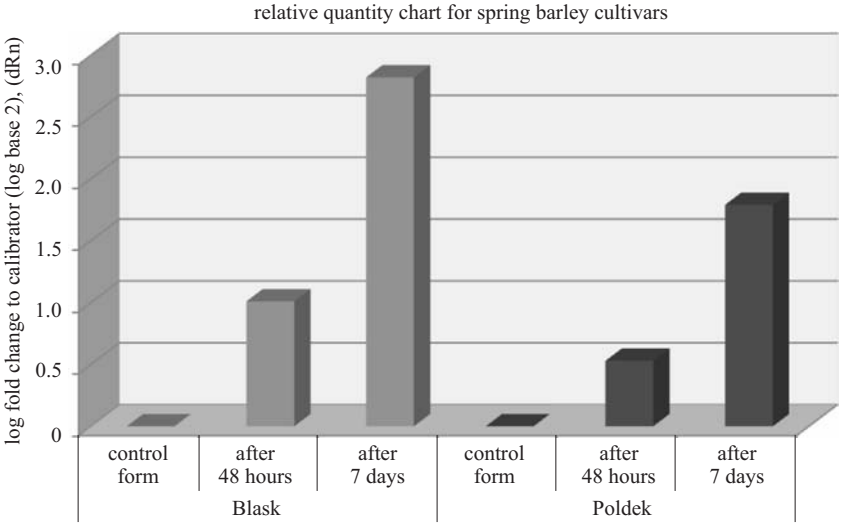

Fig. 1. Changes of MnSOD gene expression in spring barley cultivars under the influence of low temperature, shown as a fold change in comparison to control forms

Changes in expression of genes encoding superoxide dismutases, which appears during low temperature treatment, are so far described for wheat. BAEK and SKINNER (2003) characterized changes in expression of these genes during four weeks of low temperature activity in two near isogenic lines of wheat with different growth habit: 442 (winter) and 443 (spring). In line 442 expression of MnSOD gene increased during 4 weeks of cold treatment, while

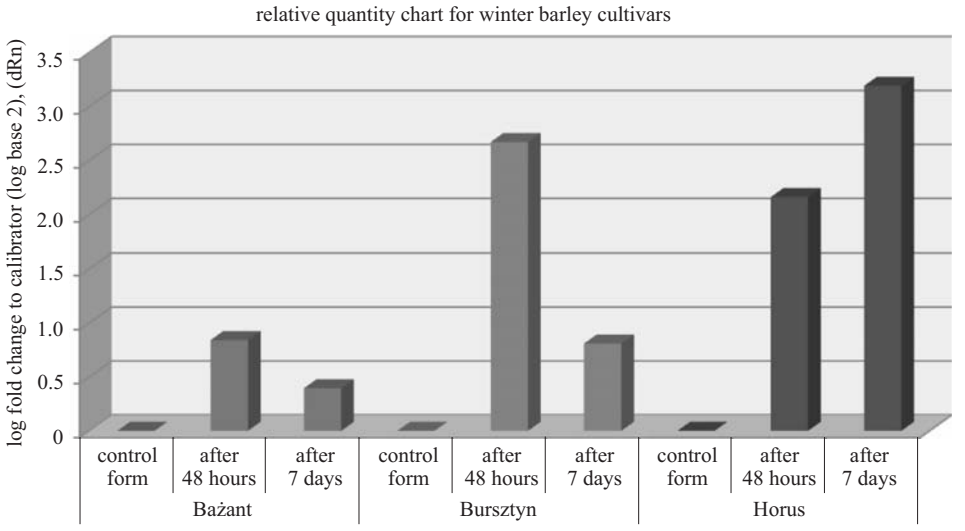

Fig. 2. Changes of MnSOD gene expression in winter barley cultivars under the influence of low temperature, shown as a fold change in comparison to control forms

in line 443 expression reached the highest level after 2 weeks and then decreased (BAEK, SKINNER 2003). The same authors examined influence of differences between various forms of MnSOD genes on its expression in low temperature. Examined gene forms characterized changes in encoded amino acid in position 166 and in 3' untranslated region (UTR). They showed that change in encoded amino acid do not have an significant influence on reaction for cold, however changes in 3' UTR caused changes in MnSOD gene expression in low temperature conditions (BAEK, SKINNER 2006). Changes in MnSOD gene expression in winter and spring wheat cultivars were studied also by WU et al. (1999). Objects of their examinations were spring wheat cv. Katepwa and winter wheat cv. Norstar. As a result of that studies, they showed that in artificial conditions, for winter wheat expression of MnSOD gene increased till 49 day of experiment, while for spring cultivar reached maximum level in a sample collected after 7 days of cold treatment. In natural conditions they found that MnSOD gene expression increase was much faster for cv. Norstar in comparison to cv. Katepwa (WU et al. 1999). That reaction was similar to obtained for Polish barley varieties tested in our study. In spite of differences between studied objects, all cited publications confirm increase of manganese superoxide dismutase gene expression in wheat during low temperature treatment. Our results presented in this paper confirmed that similar dependence is true for barley cultivars, too. This information indicates, that analyzing of MnSOD gene expression could be helpful in breeding programs, during development of new barley cultivars with improved cold tolerance.

Conclusions

1. After low temperature treatment, for all examined barley cultivars were showed intensification of mitochondrial manganese superoxide dismutase gene expression, what indicates at participation of this enzyme in response to cold of barley tissues and determination their cold tolerance.

2. In these studies were showed that exist differences in MnSOD gene expression changes between spring and winter Polish barley cultivars.

3. Because of faster growth of MnSOD gene expression in stressful conditions, winter barley cultivars seem to be better prepared for immediate reaction to stress caused by low temperature.

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EFFECTS OF POTASSIUM FERTILIZATION AND STORAGE AT 4°C ON THE CONTENT OF SOME ORGANIC ACIDS ACTIVE IN DARKENING OF EDIBLE POTATO TUBERS

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K e y w o r d s: organic acids, potassium fertilization, tuber darkening, storage.

A b s t r a c t

The aim of 6-year field experiment carried out with split-plot design method was to define the impact of Cl^- and SO_4^- potassium fertilizer rates on organic acid content in potato tubers. An effect of organic acid proportion on enzymatic tuber darkening was also evaluated. The results revealed and essential role of vitamin C in decreasing tuber tendency to darkening. The role of citric acid in tuber darkening was much lower. SO_4^- potassium fertilizer affected tuber darkening in a higher degree, especially following after harvest. The effect of chlorogenic acid on the process under study was observed to be significantly negative in the tubers were fertilized with potassium in the sulphate form.

WPŁYW NAWOŻENIA POTASOWEGO NA ZAWARTOŚĆ WYBRANYCH KWASÓW ORGANICZNYCH ODDZIAŁUJĄCYCH NA CIEMNIENIE BULW ZIEMNIAKÓW JADALNYCH PRZECHOWYWANYCH W TEMPERATURZE 4°C

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S ł o w a k l u c z o w e: kwasy organiczne, nawożenie potasowe, ciemnienie bulw, przechowywanie.

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Abstrakt

Celem 6-letnich badań eksperymentu polowego, który założono w układzie split-plot, było określenie wpływu formy Cl^- i SO_4^- nawozu potasowego na zawartość kwasów organicznych w bulwach ziemniaka. Badano również oddziaływanie kwasów organicznych na enzymatyczne ciemnienie bulw. Wpływ kwasu cytrynowego na podatność bulw na ciemnienie był niewielki. Nawóz potasowy w formie SO_4^- powodował wzrost ciemnienia bulw, zwłaszcza po zbiorach. Kwas chlorogenowy istotnie negatywnie wpływał na proces ciemnienia bulw nawożonych potasem w formie siarczanowej.

Introduction

A tendency for dark-brownish colouring of both raw and boiled potato tubers is considered an important qualitative parameter. The market requirements qualify this phenomenon as a "defect" which may disqualify the tubers for processing (MÜLLER 1988, ROGOZIŃSKA 2002). Enzymatic darkening is caused by oxidation of phenolic compounds, especially thyrosine, with catalytic activity of polyphenyloxidase. Synthesis and concentration of tyrosine over plant vegetation period is in 55% conditioned by genetic potential of the variety (DEAN et al. 1992). It increases at the end of vegetation (CORSINI et al. 1999). Many studies confirmed the relationship between the tendency for darkening and polyphenyloxidase activity taking part in oxidation of phenols to dark--coloured products (LAERKE et al. 2000). Chlorogenic acid and coffee acid, appearing mainly at the moment of infection with *Ervinia carotovora*, are the main phenolic compounds being the natural antibacterial product (GHANEKAR et al. 1984). It is also the result of stress caused by excessive moisture or draught during plant vegetation, mechanical impairment during harvest and transportation as well as activities connected with storage technology (PAWELZIK et al. 1999, LAERKE et al. 2002, ROGOZIŃSKA et al. 1986). Phenols and polyphenyloxidase are separated by intracellular membranes, therefore increasing tuber turgor reduces the susceptibility to darkening affecting the structure of potato tissue (DEAN et al. 1993, PAWELZIK et al. 1999). Darkening of boiled tubers proceeds because of combination of chlorogenic acid to dark-coloured complexes of ferric-dichlorogenic acid via its Fe^{2+}. This process stops at the presence of compounds chelating iron, such as phosphates, but mainly citric acid (GRIFFITHS, BAIN 1997, ROGOZIŃSKA et al. 2002). The darkening depends considerably on the content of two macroelements nitrogen and potassium and their ratio in the tubers (MÜLLER 1988, KOLBE, HAASE 1997, ROGOZIŃSKA 2002). Potassium stimulates the process of synthesis of proteins, simple and composed (starch, cellulose) sugars as well as organic acids, especially citric acid, and vitamin C. At a good status of this element in plants it can lower the tendency for tuber darkening (ORLOVIUS 1996).

The relationship between this phenomenon and concentrations of N, K, Mg and Ca as well as organic acids was shown by many authors (among others LAERKE et al. 2000, 2002, CIEĆKO et al. 1993, ROGOZIŃSKA 2000, KOLBE, HAASE 1997).

Potato tubers are consumed and processed in the period far away from the harvest and that is why even under optimum conditions of storage there exist changes mainly caused by biochemical processes which modify chemical composition of the tubers. As results from the literature data an increase of temperature over 10°C is beneficial for a decrease of chlorogenic acid content (GHANEKAR et al. 1984). The same effect was observed in case of storage time (ROGOZIŃSKA et al. 1986, ROGOZIŃSKA, PIŃSKA 1991). According to DEAN et al. (1992) the latter factor can reduce the tendency for darkening, while LAERKE (2002) observed that it did not change after harvest. In turn results of an other group of scientists (including MÜLLER 1988, KOLBE et al. 1997, ROGOZIŃSKA et al. 1991, ROGOZIŃSKA 1986), indicated significant increase of potato tuber flesh darkening what is connected with a decrease of vitamin C concentrations.

Our results has not proved univocally which organic acid has the strongest impact on limiting or even lack of the tendency for darkening of potato tubers and what dose of potassium fertilizer lowers or strongly reduces this negative parameter of potato tubers.

Material and Methods

The field experiment was carried out for 6 years on the area of the University of Technology and Life Sciences Experimental Station as two-factor one in a split-plot system with three replications. A medium-early variety Mila was chosen for investigations. Mineral fertilization was applied shortly before the potato seeding. Two forms (KCl and K_2SO_4) of potassium fertilizer were used in the doses of 0, 80, 160 and 240 kg K_2O ha^{-1} on the background of constant nitrogen dose of nitrogen and potassium, respectively – 120 kg ha^{-1} (ammonium nitrate) and $P_2O_5^-$ 120 kg ha^{-1} (triple super phosphate). After the end of vegetation, i.e. full maturity of tubers, tubers of the 30–60 mm were collected from all combinations to make combined samples of 10 kg. 5 kg were used for immediate analysis, while another 5 kg after storage for 6 months at 4°C and 95% relative air moisture. The range of laboratory analyses both after harvest and after storage included concentrations of vitamin C according to Tillmans, citric acid as described by Reifen and chlorogenic acid with Mapson. The assessment of raw and boiled tubers was done by visualisation with the use of Danish tables, where 9 indicates lack of darkening and 1 – black tubers. The data after standardization were evaluated by calculation of correlation coefficients and multi-factor regression analysis in order to obtain a synthetic picture of relationship between the parameters under study.

Results

Standardization of the results allowed comparing parallel features of different descriptions as well as evaluation of the magnitude of quantitative changes of the parameters under study on the background of changing doses of two forms of potassium fertilization (Figure 1). It was found that independently from the form of the fertilizer and time of evaluation (Figure 2) increasing of doses caused increases of citric acid concentrations and decreases of chlorogenic acid ones. The content of vitamin C changed both under the influence of the K_2O fertilizer forms and storage. Potassium used in the chloride form caused a tendency of increasing vitamin C concentration in the fresh potato tubers fertilized with 160 and 240 kg KCl ha^{-1}, while this parameter changed in the opposite way after application of potassium in the sulphate form. The occurrence of dark spot of the raw tuber parenchyma was significantly and positively lowered by the use of potassium in the chloride form (Figure 1). The storage time of the tubers fertilized with potassium in the sulphate form (dose 240 kg ha^{-1}) applied before the tuber seeding gave a positive effect because it reduced (after 4 h) the darkening of the tuber flesh. Darkening of boiled tubers (Figure 3, Figure 4) fertilized with potassium in the chloride form was the most intense at the dose of 80 kg ha^{-1}, while an increase of fertilization changed this parameter in a positive way, the other way around than the sulphate form, independently from the time of investigations.

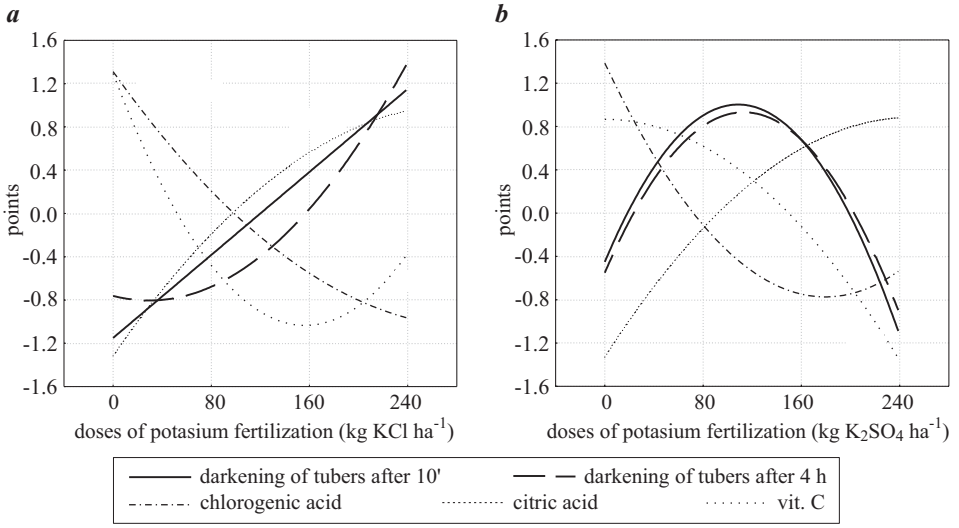

Fig. 1. Darkening of raw tubers in dependent on doses of potassium fertilization after harvest: *a* – in chloride form , *b* – in sulphate form

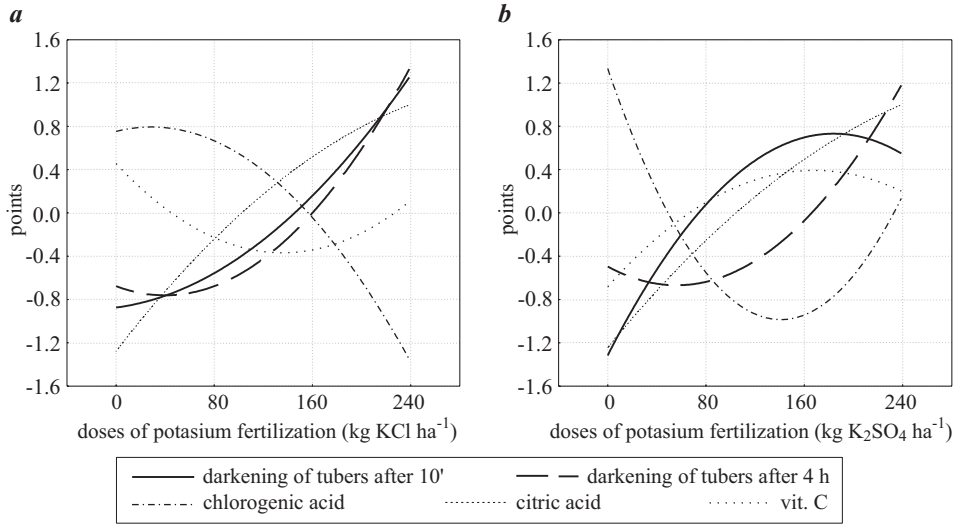

Fig. 2. Darkening of raw tubers in dependent on doses of potassium fertilization after storage: *a* – in chloride form, *b* – in sulphate form

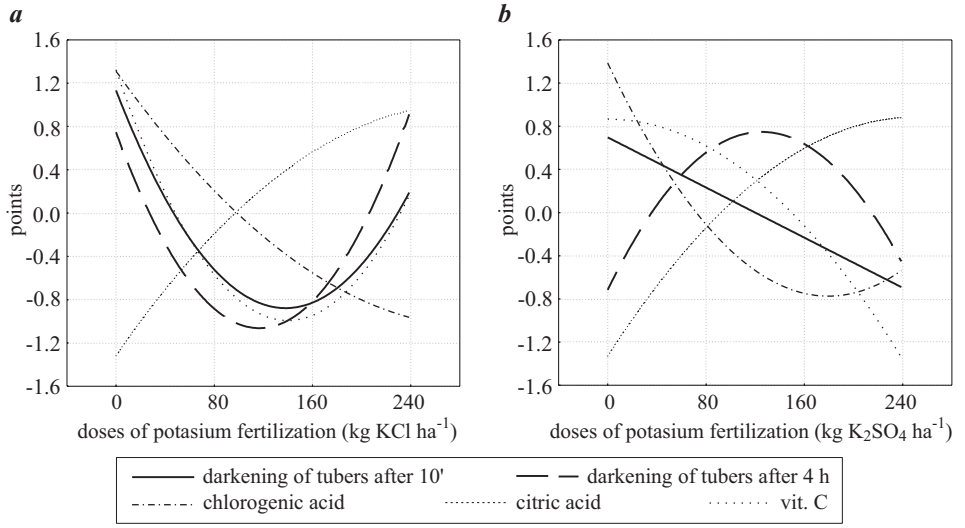

Fig. 3. Darkening of boiled tubers in dependent on doses of potassium fertilization after harvest: *a* – in chloride form , *b* – in sulphate form

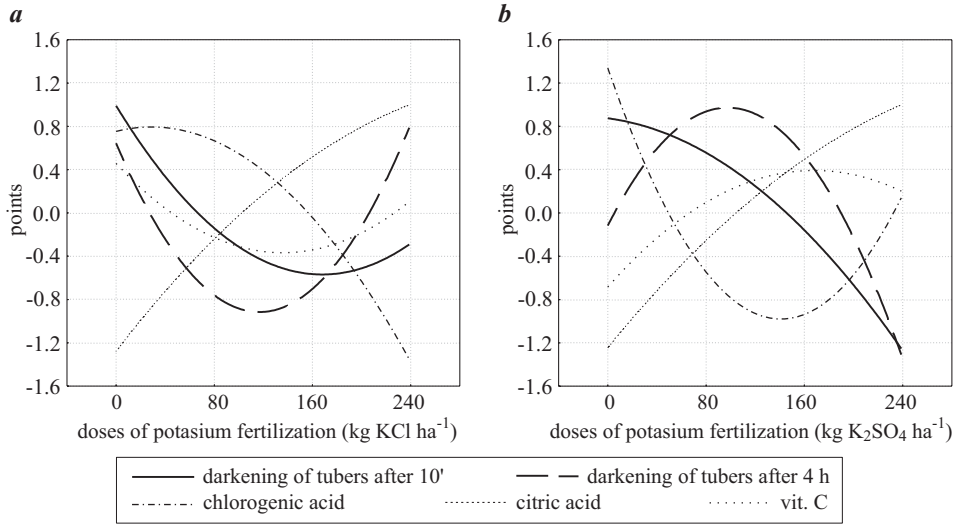

Fig. 4. Darkening of boiled tubers in dependent on doses of potassium fertilization after storage: *a* – in chloride form , *b* – in sulphate form

Correlation coefficients among the investigated parameters are shown in Table 1. The data indicated a crucial role of vitamin C played in the limiting of the tendency for darkening of the parenchyma of fresh tubers. The activity of citric acid is smaller, while chlorogenic acid acts negatively.

Table 1

Correlation coefficients intensity of darkening from content of organic acids the potato's raw tubers

Organic acids	After harvest				After storage			
	KCl		K_2SO_4		KCl		K_2SO_4	
	darkening of raw tubers							
	after 10 min	after 4 h	after 10 min	after 4 h	after 10 min	after 4 h	after 10 min	after 4 h
Vitamin C	+0.87	+0.81	+0.57	–	+0.90	+0.76	–	–
Citric acid	+0.53	–	–	–	+0.67	–	+0.93	–
Chlorogenic acid	–0.54	–	–	–0.62	–	–	–	–

Discussion

There is no doubt that basic biochemical and physiological processes undergoing in potato tubers need aqueous medium, secured mainly by the cellular juice. An increase concentration of organic compounds, especially vitamin C and a lesser extent also citric acid, in this juice in the situation of full

potassium supply provided by the chloride fertilizer form causes a lowering tendency for parenchyma darkening. The results corroborated with the findings of numerous authors, among others ORLOVIUS (1996), ROGOZIŃSKA, PIŃSKA (1991), ROGOZIŃSKA (2002), KOLBE, HAASE (1997), MÜLLER (1988), PAWELZIK et al. (1999), CIEĆKO et al. (1993), who reported on a beneficial situation occurring in potato tuber fully supplied with potassium, with a parallel concentration of those organic acids reducing and/or limiting creation of chinons and their further polymerisation to melanins (grey dye).

Biochemical processes play the most important role in darkening processes in which the secretion of phenolic compounds, such as tyrosine and chlorogenic acid, takes place (DEAN et al. 1992). Synthesis and concentration of these compounds proceeds during vegetation and increase after infection of the tubers (GHANEKER et al. 1984). Chlorogenic acid is involved in the formation of coloured complex with iron (non-enzymatic darkening). This process is slowed down mainly by citric acid (GRIFFITHS, BAIN 1997, ROGOZIŃSKA 2002). The use of sulphate form of the fertilizer in the dose of more than 80 kg kg^{-1} acted toward lowering of the darkening of raw and boiled tubers. It was caused by a lower concentration of chlorogenic acid, despite the fact that the content of vitamin C remained the same, independently from the form of the fertilizer. In practise darkening of tubers depends on the ratio citric acid : chlorogenic acid (MÜLLER 1988, KOLBE, HAASE 1997, ROGOZIŃSKA 2002), what in turn is related to concentrations of N:K in the tubers. A wide range of the ratio of these elements the amount of free organic acids, among them citric acid, decreases and therefore boiled tubers show a higher tendency for darkening. Similar relationship was shown in this study where the most needed set of fertilizers were the doses of 120 kg N ha^{-1} and 160 kg K ha^{-1}. Correlation coefficients demonstrated a negative relationship between vitamin C and chlorogenic and citric acids. Concentration of the latter acid increased with growing doses of K_2O independently from the timing of investigations. The study showed also that the content of chlorogenic acid was stimulated both by potassium concentration in the tubers and their storage at 4°C.

Numerous investigations carried out by other authors, including CORSINI et al. (1999), MÜLLER (1988), KOLBE, HAASE (1997), ROGOZIŃSKA, PIŃSKA (1991), ROGOZIŃSKA et al. (1986), and PAWELZIK, DELGADO (1999), demonstrated that tuber darkening increases during storage. According to LAERKE et al. (2000, 2002) this process is more visible in the early phase of storage. This is connected with decreasing vitamin C concentration and stress caused, among others, by mechanical impairments of the tubers, what is in the contrary to the results reported by DEAN et al. (1993).

Conclusions

1. Line regress analyses illustrating the relationships among the content of studied organic components and potassium fertilizers indicated that the most profitable dose K_2SO_4 in limitation of tendency to darkening was 80–160 kg h^{-1}, however for chloride form enlargement the doses decrease the degree of enzymic and nonenzimatic darkening of potato tubers.

2. Results of investigations show unambiguously, that on decrease or lack of darkening flesh of the potato tubers the most influences the vitamin C, the less citric acid.

3. The opinion about unfavourable influence of chlorogenic acid on susceptibility to darkening, under influence applying the high doses of potassium fertilizer in sulphate form particularly (> 160 the identifier K_2O h^{-1}) has confirm. In this form of fertilizer conditions, the darkening of tubers increased together with the content of chlorogenic acid.

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EFFECT OF THE FEEDING AND HOUSING SYSTEM ON PIG FATTENING RESULTS

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K e y w o r d s: fattening, welfare, housing system, slaughter quality, lucerne green forage.

A b s t r a c t

The experiment was performed on 40 hybrid growing-finishing pigs divided into four equal groups and placed in pens (4.2 m x 3.6 m). The experimental design was as follows: group I (control) – litterless housing (solid floor), a complete diet offered *ad libitum*; group II – litterless housing (solid floor), a complete diet supplemented with lucerne green forage offered *ad libitum*; group III – straw-litter housing, a complete diet offered *ad libitum*; group IV – straw-litter housing, a complete diet supplemented with lucerne green forage offered *ad libitum*. Complete diet, green forage and water intake, as well as the quantity of straw used as litter were monitored during the experiment. Very good production results were reported in all experimental groups. Group I pigs (raised on a litterless floor and fed a complete diet) were characterized by the best fattening results and slaughter quality. Over the entire experimental period, lower water intake was recorded in the groups fed a diet supplemented with lucerne green forage.

WYNIKI TUCZU ŚWIŃ W ZALEŻNOŚCI OD ZASTOSOWANEGO SYSTEMU ŻYWIENIA I UTRZYMANIA

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S ł o w a k l u c z o w e: tucz, dobrostan, systemy utrzymania, wartość rzeźna, zielonka z lucerny.

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Abstrakt

Badania przeprowadzono na 40 tucznikach mieszańcach. Podzielono je na 4 grupy doświadczalne (po 10 sztuk w każdej grupie) i umieszczono w kojcach, o wymiarach 4,2 m x 3,6 m, zgodnie z układem: grupa I (kontrolna) – utrzymanie bezściołowe (na podłodze litej), żywienie *ad libitum* mieszanką pełnoporcjową; grupa II – utrzymanie bezściołowe (na podłodze litej), żywienie *ad libitum* mieszanką pełnoporcjową i dodatkowo zielonką z lucerny; grupa III – utrzymanie ściołowe (płytka ściółka), żywienie *ad libitum* mieszanką pełnoporcjową; grupa IV – utrzymanie ściołowe (płytka ściółka), żywienie *ad libitum* mieszanką pełnoporcjową i dodatkiem zielonki z lucerny. W doświadczeniu kontrolowano spożycie paszy, lucerny, zużycie słomy oraz ilość pobranej wody.

Świnie wszystkich grup doświadczalnych osiągnęły bardzo dobre wyniki produkcyjne. Najlepszymi wynikami tucznymi oraz rzeźnymi charakteryzowały się tuczniki z grupy I utrzymywane bezściołowo i żywione mieszanką pełnoporcjową. Tuczniki z grup otrzymujących dodatkowo zielonkę z lucerny charakteryzowały się niższym pobraniem wody w okresie całego doświadczenia.

Introduction

A variety of animal housing systems, feeding and care regimes coexist in the modern pig production industry. Most of them fulfill animal welfare requirements. The presence of litter is the main criterion for classifying animal housing systems. Recent years have witnessed a growing number of research studies investigating other, advanced pig housing, feeding and management systems. Those studies aim to improve animal welfare and increase the animals' immunity to infections (HERBUT, WALCZAK 2004, KAPELAŃSKI et al. 2004, KOZERA 2007). New systems are also proposed to support the production of organic food with high nutritional value to meet increasing consumer demand (DYRCZ et al. 1997, FALKOWSKI, WERNIK 1998, FALKOWSKI, RAUBO 2007, KOZERA 2007). Systems where in addition to a complete diet, pigs have access to roughage and are provided with larger pen area per animal are increasingly often referred to as organic pig production farms. Research results (HOEGES 1984, TRITTHART 1986, DYRCZ et al. 1997, FALKOWSKI, WERNIK 1998) validate the beneficial effect of litter housing on the performance and health of sows, piglets and growing-finishing pigs. Moreover, litter housing reduces the threat of cannibalism and often shortens the fattening period.

The objective of this study was to evaluate the effect of the applied pig housing and feeding system on fattening results and carcass quality.

Materials and Methods

The experiment was carried out between 12 July and 28 October 2005, on an individual farm located in the Province of Warmia and Mazury (NE Poland). The observations involved 40 hybrid growing-finishing pigs produced

by simple four-breed crossing [♀(♀Polish Landrace x ♂ Polish Large White) x ♂(♀Pietrain x ♂Duroc)]. The animals were selected by the analogue method, subject to body weight, age and sex. Pigs were divided into four equal groups and were placed in pens (4.2 m x 3.6 m). The experimental design was as follows:

– group I (control) – litterless housing (solid floor), a complete diet offered *ad libitum*;

– group II – litterless housing (solid floor), a complete diet supplemented with lucerne green forage offered *ad libitum*;

– group III – straw-litter housing, a complete diet offered *ad libitum*;

– group IV – straw-litter housing, a complete diet supplemented with lucerne green forage offered *ad libitum*.

Two complete diets used in the experiment, growing diet (1) and finishing diet (2) – Table 1, contained 17.0% and 15.0% of total protein respectively. Farm-produced feed was composed of wheat, triticale, barley, oat and rye. The main component, wheat grain, accounted for 59.0% and 58.0% of diets 1 and 2 respectively. Ground grain was supplemented with high-protein components,

Table 1

Composition of experimental diets

Specification	Diets	
	1 (30–70 kg of body weight)	2 (70–110 kg of body weight)
Ground wheat	59.00	58.00
Ground barley	–	10.00
Ground triticale	14.00	–
Ground rye	–	10.00
Ground oat	7.00	5.00
Soybean oil meal	5.00	5.00
Protein concentrate	15.00	12.00

i.e. soybean oil meal (5.0% in both diets) and protein concentrate (15.0% in diet 1 and 12.0% in diet 2). Complete grain-soybean diets, balanced according to *Pig Nutrient Requirements* (1993), were offered *ad libitum* via automatic feeders. In addition, two groups received freshly cut lucerne green fodder, in the amount of 0.8 kg per animal daily, which was placed on the floor each morning and evening. Daily gains, feed consumption, water intake ("Metron" water meters were mounted in pens) and the quantity of straw used as litter were monitored during the experiment. Pigs were weighed individually on the farm before transportation, and again at the

meat plant. Meat content was determined on hanging hot right half-carcasses with the use of an ultrasound probe, ULTRAFOM 300. The carcasses were classified into grades according to the EUROP scheme (*Mięso...* PN-91/A--82001/A1/1995), based on the percentage content of lean meat. pH was measured on hot half-carcasses, in the dorsal muscle *(musculus longissimus dorsi),* 45 minutes (pH_{45}) after bleeding and after 24 hours (pH_{24}) of chilling. Back fat thickness was measured on chilled half-carcasses, at five points:
– at the thickest point over the shoulder;
– on the back, behind the last rib;
– over the cranial edge of *m. gluteus medius* (loin I);
– over the middle of *m. gluteus medius* (loin II);
– over the caudal edge of *m. gluteus medius* (loin III).

The results were verified statistically and the significance of differences between mean values in groups was determined by a two-factorial analysis of variance in an orthogonal design and by Duncan's test, using STATISTICA PL 7.0 software.

Results and Discussion

The total protein content of diets was consistent with the pigs' demand for protein at the first and second stage of the fattening period, i.e. 16.9% and 14.8% respectively, as recommended by *Pig Nutrient Requirements* (1993). Lucerne green forage applied in the experiment had a total protein content of 3.74% (Table 2).

Table 2

Chemical composition (%) of experimental diets

Specification	Diets	
	1 (30–70 kg of body weight)	2 (70–110 kg of body weight)
Dry matter	84.21	85.55
Crude protein	16.9	14.8
Crude fat	0.88	1.04
Crude fiber	4.01	4.29
Crude ash	3.60	3.78
Metabolizable energy MJ kg^{-1} (calculated)	13.5	13.3
N-free extractives	58.82	61.64
Organic matter	80.61	81.77

Pigs of all experimental groups had similar initial body weight which ranged from 31.6 kg in group III to 31.9 kg in group II (Table 3). On the day of slaughter, pigs of groups II, III and IV had similar weight (from 108 to 108.6 kg), while group I pigs weighed 110.2 kg on average. The average fattening period of group I and 4 pigs was 92 days, of group II and III pigs – 103 days. The highest daily gains were reported in respect of control group I (852 g) and group IV (830 g) animals. Group III (747 g) pigs gained weight at a much slower rate, and the lowest gains were noted in group II (739 g). The differences in the daily gains of group II and III animals were statistically significant. In a similar experiment carried out in the same pig house on animals produced by an identical commercial crossing method, higher daily gains ranging from 881 g to 909 g were reported (FALKOWSKI, RAUBO 2007). In a study on hybrid growing-finishing pigs produced by simple four-breed crossing [(Polish Landrace x Polish Large White) x (Duroc x Pietrain)], WIĘCEK and SKOMIAŁ (2000) noted daily gains within a similar range to that observed in this experiment (778 g to 816 g). In a study investigating the effect of the

Table 3

Results of fattening of experimental pigs

Specification	Statisticsls	Type of housing			
		litterless		straw-litter	
		concentrate diets	concentrate diets + green forage	concentrate diets	concentrate diets + green forage
		group I (control)	group II	group III	group IV
Initial body weight (kg)	$\bar{x}$ s	31.8 4.45	31.9 3.94	31.6 3.65	31.8 3.48
Final body weight (kg)	$\bar{x}$ s	110.2 10.05	108.0 6.13	108.6 6.33	108.2 8.99
Average daily gain (g)	$\bar{x}$ s	852^a 81	739^b 108	747^b 113	830 109
Days of fattening	$\bar{x}$ s	92 16.0	103 20.0	103 20.0	92 16.0
Daily intake of experimental diets (kg)	$\bar{x}$	2.67	2.48	2.56	2.65
Daily intake of green forage (kg)	$\bar{x}$	–	0.8	–	0.8
Feed conversion ratio (feed/gain) (kg/kg)	$\bar{x}$	3.17	3.08	3.20	3.19
Feed conversion ratio (green forage/gain) (kg/kg)	$\bar{x}$	–	1.08	–	0.96
Daily water intake dm^3/head	$\bar{x}$	5.80	4.03	5.64	4.48

a, b ≤ 0.05

housing system (confinement, free range) on fattening results, KOZERA (2007) reported daily gains in the range of 709 g to 823 g.

Average daily feed intake in all experimental groups ranged from 2.48 kg in group II to 2.67 kg in group I. Complete diet consumption per kg weight gain reached on average 3.17 kg, 3.08 kg, 3.20 kg and 3.19 kg in the experimental groups, respectively. The consumption of lucerne green forage amounted to 0.96 kg in group IV and 1.08 kg in group II. In a previously cited experiment, FALKOWSKI and RAUBO (2007) reported similar feed consumption values per kg weight gain, at 3.10 kg to 3.23 kg. Similar results were also noted by WIĘCEK, SKOMIAŁ (2000) in whose study feed consumption per kg weight gain reached 2.87–3.00 kg.

Group III and IV pigs kept on litter used an average of 0.40 kg of straw daily per animal.

As demonstrated by the data in Table 3 and Figure 1, the average daily water intake of pigs fed lucerne green forage was lower than that of animals fed solely a complete diet. The highest water intake was reported in respect of pigs fed solely a complete diet, at 5.80 and 5.64 dm^3/animal/day in groups I and III, respectively. In groups kept in litter and litterless pens and additionally fed lucerne, water intake reached 4.48 and 4.03 dm^3/animal/day, respectively. As indicated in Figure 1, the water intake of growing-finishing pigs was similar only in the initial period of the experiment. Differences in water intake were observed in the successive fattening weeks subject to the applied feeding

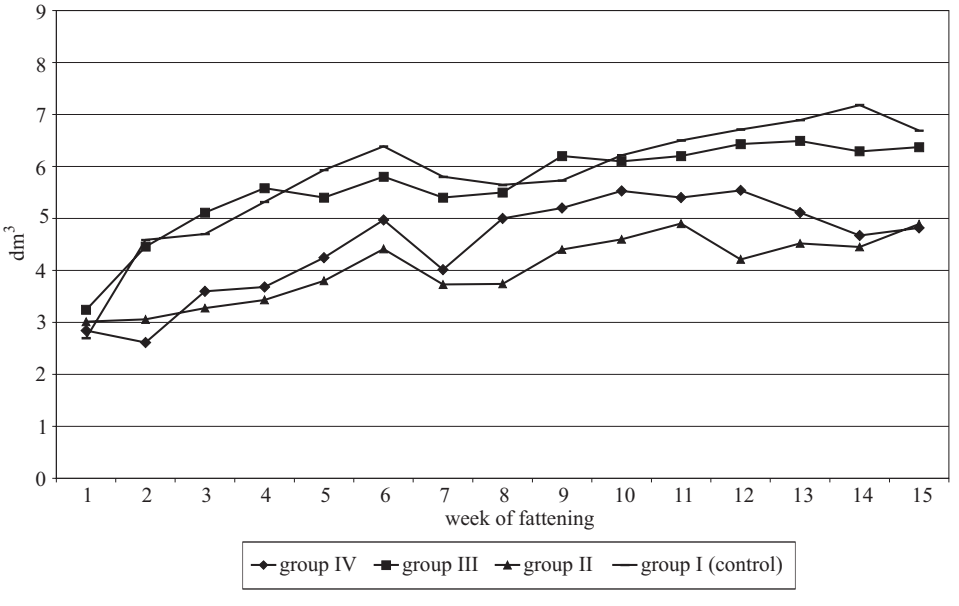

Fig. 1. Water intake dm^3/head

regime. It can be deduced that lower water intake reported in pigs fed lucerne green forage (groups II and IV) resulted from the high water content of the consumed lucerne.

According to GADD (2005), the water demand of pigs in different weight categories is as follows: 15–40 kg – 2.25 dm^3/day/animal, 40–60 kg – 5.00 dm^3/day/animal, 60 kg ± 6.00 dm^3/day/animal. In line with National Research Council standards (1998), the water intake of growing pigs with free access to water is around 2.5 dm^3 per kg of consumed feed. In a study investigating the effect of water intake and water serving methods on fattening results, PASCHMA (2002) noted that water intake varies significantly on a seasonal basis. The above author did not observe significant differences induced by various feeding regimes (dry or wet feed). MOUNT et al. (1971) reported only minor variations in the water intake of growing pigs raised at a temperature of 7°C to 22°C. According to ENGLISH et al. (1988), growing--finishing pigs with the body weight of 15 kg to 90 kg consume from 2 to 6 dm^3 of water daily, but their water intake can increase by more than 100% with a rise in ambient temperature.

Group I animals (Table 4) had the highest body weight. The carcasses of group I pigs were 2.5 kg heavier on average than those in the remaining three groups. All carcasses were characterized by very good meatiness. The highest average meat content (57.02%) was reported in respect of pigs kept in a litterless system and fed solely a complete diet (control group). The meat content of carcass in the remaining experimental groups was similar, at 55.17%, 55.20% and 55.06% in groups II, III and IV respectively. KAPELAŃSKI et al. (2004) noted lower performance values, with a meat content of 55.06% and average back fat thickness from 5 measurements of 29.3 mm. In the above study, pigs were fed lucerne green forage and were kept in a free housing system.

At four out of five measurement points, the highest back fat thickness was determined in group IV pigs whose back fat was thinner than in group I and III pigs only behind the last rib (Table 4). As regards the above parameter, the back fat of group I and III pigs was significantly thicker in comparison with group II animals. The average back fat thickness measured at "loin I" showed a highly significant difference between group IV (22.2 mm) and group II (14.9 mm), and a significant difference between group III (19.3 mm) and group II. Back fat thickness measured at "loin II" was the highest in group IV (14.8 mm) and the lowest in group II (11.7 mm). The differences in back fat thickness measured at "loin III" were highly significant between group IV (20.8 mm) and group II (13.6 mm) pigs. These relationships were confirmed by average back fat thickness from five measurements. The highest back fat thickness was recorded in group IV (21.2 mm), followed by group III

Table 4

Carcass characteristics

Specification	Statisticsls	Type of housing			
		litterless		shallow-litter	
		concentrate diets	concentrate diets + green forage	concentrate diets	concentrate diets + green forage
		group I (control)	group II	group III	group IV
Carcass weight (kg)	$\bar{x}$	87.7	85.9	86.0	85.4
	s	9.49	6.72	6.96	8.84
Meatiness (%)	$\bar{x}$	57.02	55.06	55.20	55.17
	s	3.76	2.65	5.19	3.04
Back fat thickness over the shoulder (mm)	$\bar{x}$	30.5	27.1	30.5	30.5
	s	6.71	4.84	6.70	3.50
Back fat thickness on the back (mm)	$\bar{x}$	18.9^{a}	17.3^{b}	19.3^{a}	17.9
	s	2.51	4.29	6.66	4.38
Back fat thickness over loin I (mm)	$\bar{x}$	18.3	14.9^{Bb}	19.9^{a}	22.2^{A}
	s	3.40	4.53	6.74	4.44
Back fat thickness over loin II (mm)	$\bar{x}$	13.9	11.7	14.6	14.8
	s	3.48	4.50	5.21	4.82
Back fat thickness over loin III (mm)	$\bar{x}$	17.0	13.6^{B}	18.4	20.8^{A}
	s	3.97	4.27	6.09	5.75
Back fat thickness mean of 5 measurements (mm)	$\bar{x}$	19.7	18.9^{b}	20.5^{a}	21.2^{a}
	s	2.38	3.67	5.88	3.66
pH_{45}	$\bar{x}$	6.14	6.19	6.14	6.18
	s	0.23	0.28	0.21	0.18
pH_{24}	$\bar{x}$	5.73	5.68	5.74	5.76
	s	0.12	0.08	0.09	0.10

A, B ≤ 0.01
a, b ≤ 0.05

(20.5 mm), while the lowest back fat thickness was noted in group II (18.9 mm). Significant differences were validated statistically only between groups III and IV and group II.

Based on active acidity results (pH_{45} from 6.14 to 6.19), PSE meat and partially PSE meat was not determined. pH_{24} is measured to identify DFD meat whose pH exceeds 6.2. After 24 hours of storage, the pH of normal-quality meat is usually in the range of 5.5–5.8 to 6.0 (KORTZ 2001). The results of pH_{24} measurements indicate that the meat of the investigated pigs had normal properties (pH_{24} from 5.68 in group 2 to 5.76 in group IV).

Conclusions

1. All of the investigated housing and feeding systems provided animals with a favorable growing environment and the fattening results in all experimental groups can be described as satisfactory or highly satisfactory. The highest daily gains were observed in respect of growing-finishing pigs kept in litterless pens and fed a complete diet *ad libitum*.

2. The water intake of pigs fed a complete diet *ad libitum* and additionally offered green forage was lower than that of pigs fed solely concentrated feed.

3. The analyzed management conditions, i.e. housing and feeding systems, did not significantly differentiate the meat content of the produced carcasses. All carcasses were characterized by very good meatiness and most of them were graded in class E of the EUROP scheme.

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PRODUCTION EFFICIENCY OF SLAUGHTER TURKEY-TOMS FED DIETS SUPPLEMENTED WITH CHOLECALCIFEROL AND 25-HYDROXYCHOLECALCIFEROL

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K e y w o r d s: slaughter turkeys, 25-OH-D_3 feed supplementation, tarsometatarsal bones.

A b s t r a c t

Young slaughter male turkeys of BUT 9 type (108 birds) were divided into 3 groups and reared until 24 weeks of age. Two forms of vitamin D_3 were applied as the experimental factor in feed premixes. Group I turkeys were administered cholecalciferol (D_3), group II turkeys – cholecalciferol and 25-OH-D_3 in a 1:1 ratio, group III turkeys – only 25-OH-D_3. The complete feed program covered 6 periods (feed was changed every 4 weeks). Basic performance parameters and leg bone growth were examined every 4 weeks, and meat quality traits were evaluated in week 24.

Turkeys' health condition was satisfactory. The periodic evaluation of feed consumption per kg of body weight showed certain variations (± 1.3%) between the investigated groups, but no statistically significant differences were found. The experimental factor had no impact on the body weights of turkeys (in week 24–19.9 kg to 20.1 kg). Similar results were also reported as regards meatiness (carcass dressing percentage of 84.4% to 84.6%, breast muscle – 26.7% to 27.9%, leg muscles – 18.4% to 19.3%). The addition of 25-OH-D_3 to premixes significantly contributed to an increase in the length of the tarsometatarsal bone in turkeys aged 12 and 16 weeks.

EFEKTYWNOŚĆ ODCHOWU INDORÓW RZEŹNYCH ŻYWIONYCH PASZĄ Z DODATKIEM CHOLEKALCYFEROLU LUB 25-HYDROKSYKALCYFEROLU

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S ł o w a k l u c z o w e: indyki rzeźne, żywienie 25-OH-D_3, kości stępowo-śródstopne.

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Abstrakt

Młode indory rzeźne BUT 9 (108 szt.) podzielono na 3 grupy i odchowywano do 24 tygodnia życia. Jako czynnik doświadczalny w premiksach zastosowano dwie formy witaminy D_3. Indyki z grupy I otrzymywały cholekalcyferol (D_3), z grupy II – cholekalcyferol i 25-OH-D_3 w stosunku 1:1, a z grupy III – wyłącznie 25-OH-D_3. Program żywienia mieszankami pełnoporcjowymi obejmował 6 okresów (zmiana mieszanki co 4 tygodnie). Co 4 tygodnie oceniano też podstawowe wskaźniki produkcyjne oraz rozwój kości nóg, a w 24. tygodniu dokonano oceny cech mięsnych indyków.

Stan zdrowotny ptaków nie budził zastrzeżeń. Okresowa ocena zużycia paszy na 1 kg masy ciała wykazywała pewne zróżnicowanie między grupami, ale ostatecznie istotnych różnic nie potwierdzono (różnice ± 1,3%). W masie ciała indorów (w 24 tyg. – 19,9–20,1 kg) również nie stwierdzono działania czynnika doświadczalnego. W ocenie ich mięsności obliczone wskaźniki także były zbliżone (wydajność rzeźna – 84,4–84,6%, mięsień piersiowy – 26,7–27,9%, mięśnie nóg – 18,4–19,3%). Dodatek do premiksów 25-OH-D_3 wpłynął wyraźnie na zwiększenie przyrostów długości kości stępowo-śródstopnej indorów do 12 i do 16 tygodnia życia.

Introduction

The continuous improvement in the performance traits of slaughter turkeys has nearly doubled the weight of contemporary international hybrids, in particular male turkeys (20–25 kg in week 22–24), in comparison with turkeys raised twenty or even ten years ago. One of the main breeding goals was to increase body weight and the weight of breast and thigh muscles (VELLEMAN et al. 2003). A very fast growth rate and, consequently, increased body weight, pose a significant load on the skeleton, in particular the lower limbs in turkeys. Due to the above, various types of leg anomalies are a common problem in the breading of slaughter turkeys, in particular males (PRYSZCZ et al. 1996, *Choroby drobiu*. 2005). Yet another problem is posed by dyschondroplasia which affects long bones, in particular those located in the drumstick (tibia), which are marked by the most intense growth between week 8 and 12, with daily increment of up to 1.0–1.9 mm (HESTER, FERKET 1998, ŁADOŃ 1998, SELL 1996). The causes of motor system anomalies have not yet been fully explained. Some authors believe that the main reason is an inadequate diet and recommend that effective remedies be sought in this area (WHITESIDE 1996).

Since calcium phosphate is the main building block of bones, growing turkeys have an increased demand for vitamin D_3, calcium and phosphorus (WILLIAMS et al. 2000, RAO, RAGHURAMNLU 1999). It should also be noted that the only available form of vitamin D_3 is its active form, 25-OH-D_3, and the above applies not only to birds (LANENGA et al. 1999, RAO, RAGHURAMNLU 1999, GERTNER et al. 1997). By way of complex biochemical reactions in various bodily organs, vitamin D_3 is transformed into its active form which reaches its point of destination, including the bones (MOSZCZYŃSKI, PYĆ 1999). Vitamin D_3 is transformed into an active 25-OH-D_3 metabolite in the course of various

reactions which lower the efficiency of the process (KUTNER 1993). During research efforts to produce a synthetic active form of vitamin D_3, it was split into hydroxyl derivatives: 25-OH-D_3, 1,25-$(OH)_2$-D_3 and 24,25-$(OH)_2$-D_3. The first two forms actively participate in building the skeletal system. Those forms of vitamin D_3 are not subject to the hydroxylation process and they do not have an adverse effect on the liver (HEALY et al. 2003, PANDA et al. 2001, ECAROT, DESBARATS 1999). Vitamin D_3, added to the feed in the form of the 25-OH-D_3 hydroxyl derivative, is absorbed into the bloodstream from the intestine, and it is a more effective and more available form of vitamin D_3 in the body (WRÓBEL 2000ab, TSANG et al. 1990).

Bone calcification is a complex process and new research attempts are continuously made to investigate this process (TURNER 2002). For this reason, further research is required to examine the use of various vitamin and mineral supplements, including different forms of vitamin D_3, which support the process of bone mineralization in growing slaughter turkeys and minimise mortality over the rearing period.

The objective of this study was to determine the effectiveness of feeding slaughter turkeys a diet supplemented with premixes containing vitamin D_3 and its hydroxyl derivative, 25-OH-D_3, based on rearing performance and leg bone growth data.

Materials and Methods

For the needs of the study, 108 one-day-old young male turkeys of medium-heavy type (BUT 9) were purchased and were randomly divided into 3 experimental groups of 36 birds each, with 3 analogues per group. Turkeys were raised in an experimental farm at the University of Warmia and Mazury in Olsztyn, in accordance with the recommendations of the Turkey Testing Station (FARUGA 1997).

The experimental factor involved two forms of vitamin D_3: standard vitamin D_3 and its hydroxyl derivative of 25-OH-D_3 (Table 1). Group I turkeys were fed cholecalciferol (vitamin D_3), group II turkeys – cholecalciferol and 25-OH-D_3 in a 1:1 ratio, and group III turkeys – only 25-OH-D_3.

Feed mixes for all turkey groups had the same energy value and nutrient content (Table 2). Basal feed mixes – friable without premixes, were combined with an experimental premix dedicated for every group and produced by Polsanders in Pruszcz Gdański. The vitamin and mineral content of premixes was consistent with NRC recommendations (1994) and Poultry Nutrient Requirements (1996). The applied premixes differed with regard to the content of the analysed experimental factors, i.e. the form of vitamin D_3 (Table 1).

Table 1

Supplemental vitamin doses subject to turkey age

Group	Age, in weeks	D_3 (cholecalciferol) IU/kg of feed	25-OH-D_3 IU/kg of feed
I	1–4	4500	–
	5–8	4000	–
	9–12	3750	–
	13–24	3400	–
II	1–4	2250	2250
	5–8	2000	2000
	9–12	1850	1850
	13–24	1700	1700
III	1–4	–	4500
	5–8	–	4000
	9–12	–	3750
	13–24	–	3400

In control group I and experimental group IV, vitamin D_3 content was consistent with the recommendations of the parental stock producer, i.e. British United Turkeys (*BUT* 1998). In group II, the recommended vitamin D_3 dose was halved. To supplement the deficiency in the recommended D_3 dose for this group, the premix was enriched with its hydroxyl derivative – 25-OH-D_3 which is marked by greater availability in birds. In experimental group III, the recommended dose of vitamin D_3 was replaced in its entirety with its hydroxyl form of 25-OH-D_3. It is believed that the optimal dose of 25-OH-D_3 is 68.9 μg kg^{-1}, i.e. 2756 IU kg^{-1} of feed (1 μg 25-OH-D_3 corresponds to 40 IU of vitamin D_3) – WRÓBEL 2000b.

Table 2

Nutritive value of (basal) feed mixes for slaughter turkeys

Nutrients	IU	Mix – feeding period (in weeks)					
		A	B	C	D	E	F
		0–4	5–8	9–12	13–15	16–20	> 20
ME kg^{-1}	kcal. kg^{-1}	2800	2850	3050	3150	3200	3250
Protein	%	28	26.5	21.5	18.5	16.5	15.8
Lysine	%	1.8	1.7	1.4	1.2	1.0	0.8
Methionine	%	0.66	0.60	0.50	0.48	0.44	0.36
Threonine	%	1.1	1.05	0.85	0.65	0.57	0.52
Phosphorus	%	0.75	0.70	0.55	0.50	0.46	0.43
Calcium	%	1.35	1.25	1.1	1.00	0.90	0.70
Sodium	%	0.15	0.16	0.16	0.17	0.17	0.17

Turkeys were bred until 24 weeks of age. In the course of rearing:
- group feed consumption was determined every 7 days;
- birds were weighed individually in week 4, 8, 12, 16, 20 and 24;
- feed consumption per kg of body weight was determined for periods marked by successive weighing dates;
- the length, width and circumference of tarsometatarsal bones (*os tarsometatarsale* II–IV) were measured with the use of a slide calliper and a ruler in the above weeks. The length of the tarsometatarsus was measured between the tarsometatarsal process and the trochlea of the third metatarsal bone. The width and circumference of the tarsometatarsus was measured in the central part of the bone shaft (at $^1/_2$ bone length);
- mortality and culling (for health reasons) rates were recorded regularly with an indication of the cause.

Turkeys' health condition was monitored by a specialist veterinary physician.

After the 24-week rearing period, 9 birds with similar average body weight were selected of each group, fasted for 12 hours and sacrificed. The carcasses were eviscerated and weighed. After chilling for 24 hours at 4°C, the carcasses were weighed again and subjected to a simplified slaughter analysis. The carcasses were examined for: carcass dressing percentage, giblet content (including the heart, liver, gizzard), breast muscle content (including the fillet), thigh and drumstick muscle content, reserve fat content (periintestinal and depot fat).

The obtained results were statistically verified with Duncan's test based on an analysis of variance with the use of Excel 8.0 and WinSTAT software.

Results and Discussion

The reported indicators of feed consumption per kg of body weight (Table 3) corresponded to poult production standards (*BUT* 1998) for medium-heavy turkeys. As regards nutritional effectiveness, the experimental factor had a statistically non-significant impact on feed conversion (differences of ± 1.3%). Other sources (WRÓBEL 2000ab) point to much higher (4 to 12%) feed utilisation by birds fed premixes with 25-OH-D_3.

The evaluation of turkey body weight (Table 4) showed that the experimental factor had no significant impact on body weight gains. In other studies, feed supplementation with the hydroxyl derivative of vitamin D_3 increased the final body weights of turkeys by 2.0–3.3% (WRÓBEL 2000ab, YARGER et al. 1995). The body weights of BUT 9 experimental turkeys reported in this study are highly satisfactory, ranging above the standard (19.6 kg in week 24) anticipated by the producer (*BUT* 1998).

Table 3

Average periodic feed consumption per kg of body weight

Period, weeks	Unit of measure	Group		
		I	II	III
1–4	kg	1.57	1.54	1.59
	%	100	98.1	101.3
1–8	kg	1.76	1.79	1.80
	%	100	101.7	102.3
1–12	kg	2.09	2.08	2.15
	%	100	99.5	102.9
1–16	kg	2.51	2.47	2.30
	%	100	98.4	91.6
1–20	kg	2.87	2.81	2.80
	%	100	97.9	97.6
1–24	kg	3.16	3.13	3.12
	%	100	99.1	98.7

Table 4

Body weight, kg

Age, weeks	Statistical measure	Group		
		I	II	III
4	x	0.81	0.79	0.78
	v %	11.65	11.83	10.99
	%	100	97.8	95.6
8	x	3.45	3.40	3.32
	v %	9.65	9.77	11.90
	%	100	98.5	96.1
12	x	7.53	7.57	7.23
	v %	9.70	10.11	12.35
	%	100	100.6	96.0
16	x	11.80	11.81	11.66
	v %	9.01	7.90	7.85
	%	100	100.1	98.8
20	x	16.04	16.02	16.35
	v %	10.85	7.84	8.49
	%	100	99.9	101.9
24	x	20.10	19.92	20.07
	v%	9.27	8.17	8.69
	%	100	99.1	99.9

x – mean values

The health condition of experimental turkeys was satisfactory. No deaths were reported until 16 weeks of age. In the entire production cycle of 24 weeks, there were only two sudden deaths (circulatory failure) and two birds were culled due to large goitres. Motor system anomalies were not found during periodic examinations of the limbs and joints.

Changes in the length, width and circumference of tarsometatarsal bones of turkeys at a different age are presented in Tables 5–7. Statistically significant differences were reported in older birds (from week 12) only in respect of the length of the tarsometatarsus (Table 5). A much higher increase (2.4–3.1%) in the length of the tarsometatarsal bone was observed in group II and group III turkeys aged 8 to 12 weeks. In view of the average periodic changes in talus width (Table 6), the widest talus bones were reported in turkeys of control group I (1.10 cm) in the first month (up to 4 weeks of age). In successive control periods, excluding week 20, very similar results were obtained in respect of talus width (Table 6). With the exception of the youngest birds aged 4 and 8 weeks, the circumference of talus bones (Table 7) was not marked by clear differences resulting from the administered experimental diet. The best results were noted

Table 5

Changes in the length of the tarsometatarsal bone in turkeys, cm

Age, weeks	Statistical measure	Group		
		I	II	III
4	x v %	6.86 8.21	7.05 5.91	6.85 7.47
	%	100	102.8	99.9
8	x v %	12.61 6.21	12.76 3.86	12.79 4.58
	%	100	101.2	101.4
12	x v %	16.89^{B} 2.53	17.42^{A} 3.45	17.29^{A} 4.31
	%	100	103.1	102.4
16	x v %	17.31 4.63	17.45 3.22	17.30 3.37
	%	100	100.8	99.9
20	x v %	17.63^{a} 3.10	17.50^{a} 5.81	17.39^{b} 3.18
	%	100	99.2	98.6
24	x v %	17.99 2.51	18.06 2.47	18.13 3.07
	%	100	100.4	100.8

x – mean values

Means with different superscripts differ significantly: *A, B* at $P \leq 0.01$; *a, b* at $P \leq 0.05$

Table 6

Changes in the width of the tarsometatarsal bone in turkeys, cm

Age, weeks	Statistical measure	Group		
		I	II	III
4	x v % %	1.10^{Aa} 10.36 100	1.04^{b} 9.52 94.5	1.02^{B} 8.04 92.7
8	x v % %	1.73 6.50 100	1.72 5.75 99.4	1.78 7.78 102.9
12	x v % %	2.06 4.41 100	2.07 5.53 100.5	2.04 6.22 99.0
16	x v % %	2.29 4.39 100	2.32 4.28 101.3	2.33 4.75 101.7
20	x v % %	2.48^{Aa} 4.37 100	2.46^{b} 4.25 99.2	2.43^{b} 4.77 98.0
24	x v % %	2.58 4.90 100	2.55 3.93 98.8	2.52 5.36 97.7

Explanations as in Table 5

Table 7

Changes in the circumference of the tarsometatarsal bone in turkeys, cm

Age, weeks	Statistical measure	Group		
		I	II	III
4	x v % %	3.59^{Aa} 11.76 100	3.44^{b} 6.30 95.8	3.36^{B} 6.41 93.6
8	x v % %	5.04 4.35 100	5.05 3.92 100.1	5.18 5.64 102.8
12	x v % %	6.24 2.52 100	6.34 3.16 101.6	6.28 4.89 100.6
14	x v % %	6.79 4.36 100	6.67 2.78 98.2	6.68 3.72 98.4
16	x v % %	7.23 4.67 100	7.19 4.62 99.4	7.13 5.46 98.6
24	x v % %	7.49 4.44 100	7.43 3.90 99.2	7.41 4.52 98.9

Explanations as in Table 5

in respect of turkeys fed a diet supplemented with vitamin D_3 (100%) until 4 weeks of age. Similarly as in the case of talus width, compensatory growth was also observed in respect of talus circumference between weeks 4 and 8.

In the conducted study, the most dynamic growth of long bones in turkeys was observed in the period from the first day to 10–12 weeks of age, similarly to the results reported by other authors (TURNER, LILBURN 1992, KONCICKI 1993, SELL 1996, HESTER, FERKET 1998, ŁADOŃ 1998). The obtained results do not clearly support the conclusion that an experimental diet supplemented with a hydroxyl derivative of vitamin D_3 directly affects changes in the analysed parameters of the talus in turkeys from particular age groups. Similarly to an analysis of body weight, a general evaluation of the carcass dressing percentage (Table 8) after 24 weeks of rearing is not indicative of significant differences resulting from an experimental diet. The reported carcass dressing percentage values exceeded the results obtained by other authors (PUCHAJDA, FARUGA 2000), and they significantly exceeded (by 6.73%) the standards recommended by British United Turkeys (*BUT* 1998).

Table 8

Results of slaughter analysis (live body weight = 100%)

Specification	Statistical measure	Group		
		I	II	III
Carcass dressing percentage	x	84.63	84.57	84.45
	v %	0.92	2.60	0.86
Giblets:				
Heart	x	0.29	0.30	0.29
	v %	9.64	8.16	7.82
Liver	x	0.83	0.85	0.86
	v %	13.45	14.68	7.60
Gizzard	x	0.64	0.67	0.61
	v %	11.30	10.99	9.68
Breast muscle	x	27.98	27.48	26.76
	v %	5.20	5.08	4.80
including fillet	x	4.25	4.35	4.20
	v %	8.10	8.09	3.77
Total leg muscles	x	19.43	18.74	19.34
	v %	7.31	9.12	7.29
including: thigh muscle	x	11.03	10.80	11.03
	v %	5.85	7.96	4.32
Drumstick muscle	x	8.40	7.94	8.31
	v %	8.77	10.28	10.25
Total reserve fat:	x	1.29	1.46	1.45
	v %	37.25	28.40	30.56
including:				
periintestinal fat	x	0.53	0.59	0.61
	v %	37.94	31.10	23.96
depot fat	x	0.76	0.87	0.84
	v %	36.56	25.70	37.16

Conclusions

1. In comparison with the control group of birds, significant differences in body weight and feed consumption per kg of body weight, which resulted from the applied experimental diet, were not reported in any of the investigated dietary periods. The health condition of the investigated birds was satisfactory.

2. The highest leg bone increment was recorded in turkeys aged 4 to 8 weeks.

3. Diet supplementation with 25-OH-D_3 visibly increased the length of the tarsometatarsal bone in turkeys aged 8 to 12 weeks.

4. The results of a periodic evaluation of the experimental diet's impact on turkey performance and final changes in the dimensions of the tarsometatarsal bone (talus) do not clearly support the need to modify feed supplementation of young slaughter turkeys through the replacement of vitamin D_3 (100%) with its hydroxyl derivative (25-OH-D_3).

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AIRBORNE MOLDS IN THE AIR OF CIECHOCINEK SPA*

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K e y w o r d s: airborne molds, microbiological air pollution, Ciechocinek health resort.

A b s t r a c t

This study was conducted in the Ciechocinek, which is the largest lowland health resort in Poland. Measurement sites were located around open inhalators, in the recreation areas, and in typical urban areas. The survey consisted of enumeration of molds, according to Polish Standard (*Ochrona czystości powietrza...* PN89/Z-04111/03), and their identification. September was the month with the highest abundance of molds; their concentrations were highest in stations located in the urban section of Ciechocinek, with the lowest around the open inhalators. Molds of the *Cladosporium* genus were the most common in the tested air, particularly in summer.

GRZYBY PLEŚNIOWE W POWIETRZU UZDROWISKA CIECHOCINEK

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S ł o w a k l u c z o w e: grzyby pleśniowe, mikrobiologiczne zanieczyszczenia powietrza, uzdrowisko Ciechocinek.

A b s t r a k t

Badania prowadzono na terenie Ciechocinka – największego uzdrowiska nizinnego w Polsce. Stanowiska badawcze zlokalizowano w otoczeniu otwartych inhalatoriów, na terenach uzdrowiskowych oraz typowo miejskich. Badania obejmowały oznaczenie liczebności grzybów zgodnie

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z polską normą (*Ochrona czystości powietrza...* PN89/Z-04111/03) oraz ich identyfikację. Najwyższe liczebności grzybów pleśniowych stwierdzono we wrześniu. Wyższe liczebności grzybów pleśniowych notowano na stanowiskach zlokalizowanych w części miejskiej Ciechocinka, natomiast najniższe – w otoczeniu otwartych inhalatoriów. W badanym powietrzu, szczególnie latem, dominowały grzyby z rodzaju *Cladosporium*.

Introduction

Molds are omnipresent; they occur in all environments. Spores of molds and fragments of mycelium attached to dust or floating in the air constitute an essential and often even dominant component of bioaerosols polluting the air (KRZYSZTOFIK 1992, FILIPIAK et al. 2004, KOŁWZAN et al. 2005).

In general, inhalation of mold spores is not harmful for people without health problems. However, due to their small dimensions, which fall between 2 and 100 μm, but usually do not exceed 10 μm, mold spores may penetrate deep into the respiratory tract and may even reach air sacs (KÄMPFER, WEIßENFELS 1997, MÜCKE, LEMMEN 1999). In addition to pollen, mold spores are considered the chief respiratory allergens. Molds from the *Alternaria, Cladosporium, Penicillium,* and *Aspergillus* genera are the most important sources of mold allergens. Upon entering the organism, mold spores may cause various allergic reactions from allergic nasal congestion to bronchial asthma or even infections, particularly in individuals with seriously impaired immune system (GRAJEWSKI, TWARUŻEK 2004).

Furthermore, certain species of molds are capable of producing mycotoxins. These substances are products of secondary metabolism and could be very harmful to living organisms, including humans. According to GRAJEWSKI and TWARUŻEK (2004), mold spores absorbed through respiratory tracts may contain one or even several "hidden" mycotoxins due to the fact that some secondary metabolites of mycelium also accumulate in spores. Literature describes cases of inhalation poisoning caused by mycotoxins from the mold spores *Stachybotrys chartarum, Aspergillus sp., Penicillium sp., Trichoderma sp*, and *Paeciliomyces sp*., among others (MÜCKE, LEMMEN 1999, GRAJEWSKI, TWARUŻEK 2004).

For molds, the air is only a transporting medium and is unsuitable for their growth or reproduction. The abundance of mold spores in the air is affected by numerous factors, such as: geographical location, weather conditions (temperature, amount of precipitation, air motion, and humidity), microclimatic conditions, and human activities (LACEY 1994).

The purpose of this study was to determine the abundance of molds in the Ciechocinek air and to check for presence of allergenic and toxinogenic molds. Due to the scenic surroundings and diverse flora, the entire town functions as

a health resort. Therefore, the microbiological purity of the air, not only around the graduation towers, but also in the entire town, is essential for the patients.

Materials and Methods

Object of the study

The town of Ciechocinek is located in Kujawy, on the left bank of the Vistula river, 25 kilometers from Toruń (52°52' N, 18°47' E). The Ciechocinek area is characterized by exceptionally high annual mean temperature (ca. +8°C) and is among the warmest regions in the lowland belt. The region is also characterized by low air humidity and unusually low precipitation; its annual total rarely exceeds 500 mm (LEBIEDZIEWICZ 2001).

Ten measurement sites were established in the Ciechocinek area (Figure 1).

Four stations were located within the zone affected by the natural inhalators: 1 m (I1) and 10 m (I2) from the "Grzyb" fountain (brine source no. 11), 1 m from graduation tower no I, on the side of the brine flow (I3), and 1 m from

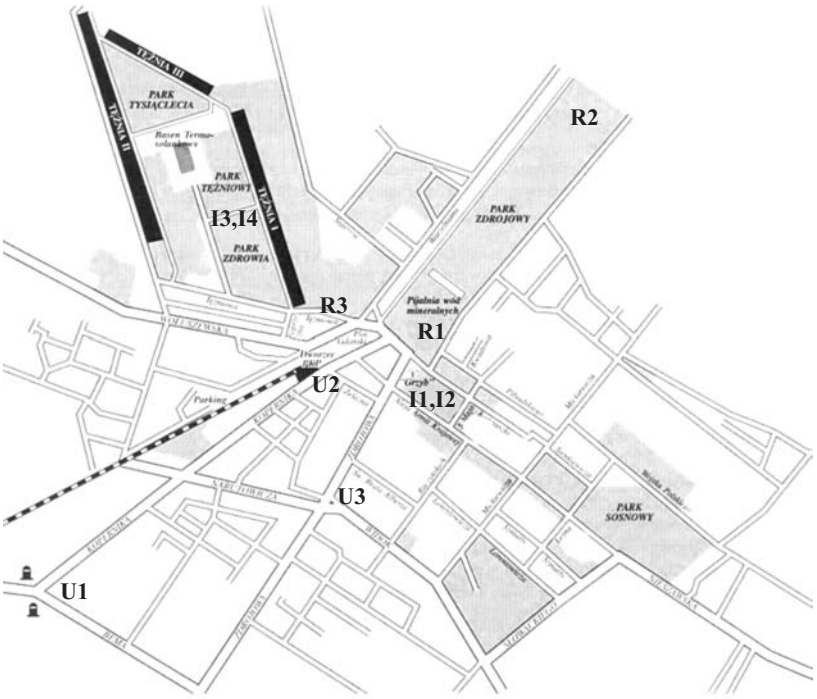

Fig. 1. The map of measurement sites in Ciechocinek health resort

graduation tower no. I, on the side without the brine flow (I4). Stations located within the recreation area were situated in the Park Zdrojowy in front of Pijalnia Wód (Pump Room) (R1), by the restaurant "Oaza" (R2), and on the promenade by Tężniowa street (R3). Stations located in the town were situated at the entrance to Ciechocinek from the Toruń side, next to the intersection of Kopernik and Bem streets (U1), on Kopernik street, across from the railway station (U2), and on Widok street (U3).

Sample collection

The air samples were collected monthly according to the Polish Standard (*Ochrona czystości powietrza...* PN-89/Z-04008/08) 1.3 m above the ground level between May, 2005 and April, 2006. The samples were obtained by the impaction method in the microbial airsampler Merck Mas-100. The air flow velocity equalled 11 m s^{-1}. This enabled detection of particles larger than 1 μm, which was important for translocation of microorganisms.

The following meteorological parameters were measured during the sample collection: air temperature, relative humidity, and wind velocity. These measurements were carried out using a Nielsen-Kellerman anemometer, Kestrel 3500 (Table 1).

Table 1

Meteorological parameters during the sample collection

	Temperature (°C)	Humidity (%)	Wind speed (m s^{-1})
12.05.2005	11.6	66.3	0.7
14.06.2005	20.7	59.5	1.1
04.07.2005	27.3	51.2	1.2
05.08.2005	24.9	52.2	1.1
28.09.2005	20.4	64.8	1.0
21.10.2005	12.6	60.7	1.2
25.11.2005	1.7	73.0	1.3
07.12.2005	1.5	79.3	0.7
19.01.2006	–6.8	74.6	1.9
27.02.2006	–2.8	72.6	3.3
23.03.2006	7.7	57.9	0.5
10.04.2006	10.2	54.8	0.9

Determination of mold abundance

The abundance of molds in the air was determined in accordance wit the Polish Standard (*Ochrona czystości powietrza...* PN89/Z-04111/03). Molds were cultured in two ways: on Czapek-Dox agar for 7 days and on wort agar medium at 26°C for 5 days. The mold colonies on all plates were enumerated everyday from the second day onwards. If the numbers of colonies on both mediums were similar, the abundance was calculated based on the average from all six plates, according to Polish Standard (*Ochrona czystości powietrza...* PN89/Z-04111/03). If the number of colonies on wort agar medium significantly exceeded the number of colonies on Czapek-Dox medium, calculations were based exclusively on the average from the wort agar plates.

All results were converted to the number of CFU in 1 m^3 of air. The results were analyzed in STATISTICA 6.0.

Identification of molds

Systematic affiliation of randomly chosen, isolated pure mold cultures was determined based on macro- and microscopic features using the FASSATIOVA key (1983).

Results

The results of enumeration of molds that occur in the air of Ciechocinek spa were presented in the Figure 2. The lowest mold content was observed from January to March – the mold abundance did not exceed 70 CFU m^{-3} in any of the measurement sites. In all measurement sites, the highest abundances of molds were observed in September. At that time, in urban areas, the mold abundance reached 4800 CFU m^{-3} and this number was the highest value in the entire research period. An exceptionally high abundance of molds was also observed in June in urban areas – 3600 CFU m^{-3}. Low abundances of molds were observed in all stations in July at very high temperature and low humidity levels (Figure 2, Table 1). After the data from the entire year was analyzed, it was concluded that the highest abundances of molds occurred in stations located in the urban section of Ciechocinek, while the lowest, around the open inhalators.

Based to the obtained data, the air in Ciechocinek can be classified according to the (*Ochrona czystości powietrza...* PN89/Z-04111/03) as clean (89% of samples) or relatively clean (8% of samples). Only 3% of samples were contaminated with molds that may have a negative impact on the quality of the environment inhabited by people (Figure 3).

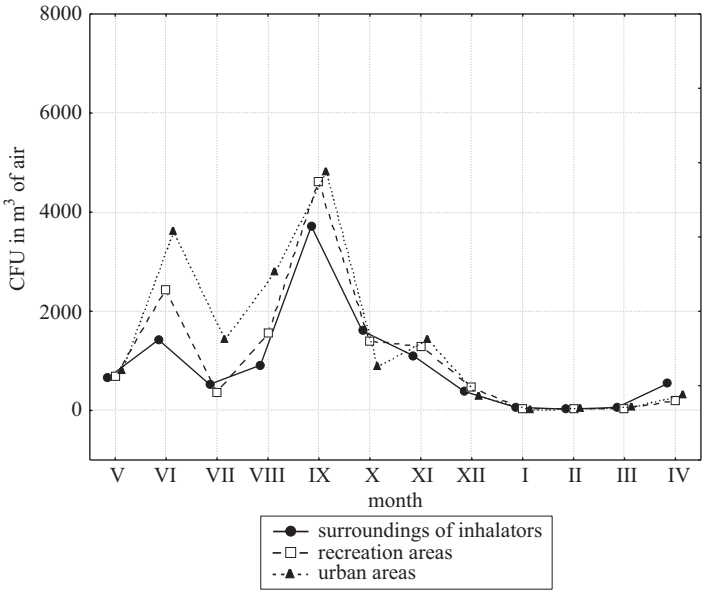

Fig. 2. Number of molds in air of Ciechocinek

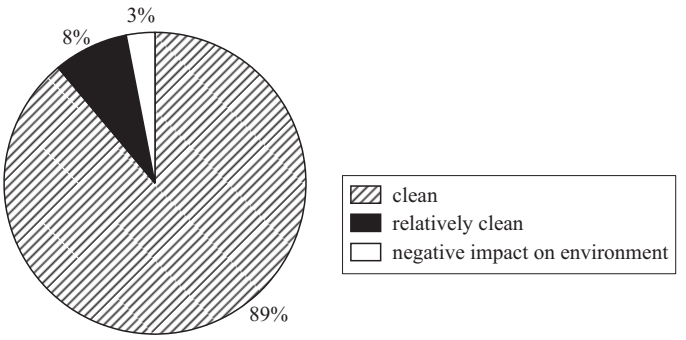

Fig. 3. The evaluation of microbiological pollution of air in Ciechocinek (acc. to *Ochrona czystości powietrza...* PN-89/Z-04111/03)

It was also found that abundance of molds was positively correlated with air temperature (r = 0.53) and negatively, with wind speed (r = -0.21).

The air of Ciechocinek spa was contaminated by molds of various systematic affiliation, but the genera *Cladosporium, Alternaria, Aspergillus, Penicillium* and *Fusarium* were the most common (Figure 4). Diversity of species was particularly high in the summer and autumn. Analyzing the isolated molds, the authors found numerous allergenic (*Cladosporium sp.* and *Alternaria* sp., among others) and potentially toxinogenic species (Table 2).

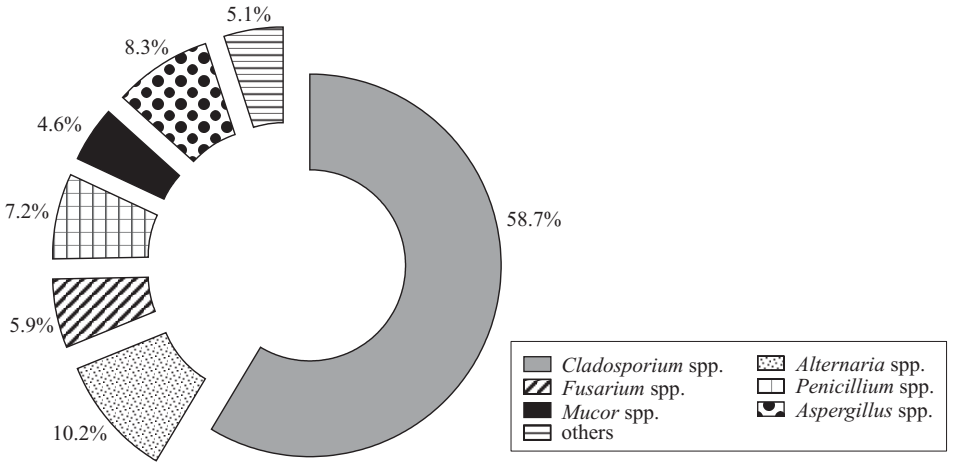

Fig. 4. Dominant genera of molds in air of Ciechocinek

Table 2

Taxonomic composition of isolated fungi

Species	Seasons			
	spring	summer	autumn	winter
1	2	3	4	5
Absidia				
A. glaca Hagem	–	+	+	–
Aletrnaria				
A. alternata Keissler	+	++	+	+
A. humicola Oudemans	+	–	+	–
Aspergillus				
A. flavus Link	–	++	–	–
A. fumigatus Fresenius	+	+	+	+
A. niger van Thieghem	+	++	++	–
A. versicolor Tiraboschi	+	++	+	–
Aureobasidium				
A. pullulans (de Bary) Arnaud	–	+	+	–
Botrytis				
B. cinerea Persoon ex Fries	+	++	++	–
Cladosporium				
C. cladosporioides de Vries	+	++	++	–
C. herbarum Link ex Fries	+	++	++	–
Fusarium				
F. nivale (Fries) Cesati	–	+	+	–
F. oxysporum Schlecht	–	+	+	–
Mucor				
M. mucedo Fresenius	–	+	++	–
M. racemosus Fresenius	–	++	–	–

cont. table 2

1	2	3	4	5
Penicilium				
P. citrinum Thom	–	–	+	+
P. expansum Link	+	–	++	+
P. notatum Westling	–	–	+	+
P. rubrum Stoll	–	+	+	–
Rhizopus				
R. nigricans Ehrenberg	–	++	+	–
Verticillum				
V. tenerum Ness	–	+	+	–

Explanations:
(–) – not present
(+) – frequent
(++) – very frequent
bold – potentially toxinogenic species

Discussion

In addition to commonly monitored chemical pollutants, the atmosphere is also polluted by organic substances, with molds being the most harmful component of bioaerosol. Abundance of molds and their species composition are also important for epidemic control.

In Ciechocinek, the highest abundance of molds was observed in September (4800 CFU m^{-3}) in research stations located in the urban section of the town. According to the (*Ochrona czystości powietrza...* PN86/Z-04111/03), the air in Ciechocinek can be classified as clean; only 3% of analyzed samples were contaminated with molds that may have a negative impact on human environment. MĘDRELA-KUDER (2004) obtained similar results in Cracow in a testing station separated from a main arterial route by a strip of vegetation. In Cracow, the mold abundance ranged from 340 CFU m^{-3} in winter to over 2000 CFU m^{-3} in summer. The results of similar analyses conducted in Poznań city center by BUGAJNY et al. (2005) indicate significantly higher air pollution with bioaerosol. The highest abundances of molds observed in Poznań reached 16 000 CFU m^{-3} and were almost four times higher than the values obtained in Ciechocinek.

In Ciechocinek, the highest abundances of molds were observed in stations located in urban areas. FILIPIAK et al. (2004) higher abundances of molds in a city center than in a location removed from the center. However, KASPRZYK and WOREK (2006) obtained different results. They found that abundances of molds were higher in the rural environment than in urban locations (Rzeszów). In Ciechocinek, in the zone affected by open inhalators,

its probable that brine aerosol has a significant impact on the abundance of molds; the brine settles cleaning the air from all pollutants (BURKOWSKA, DONDERSKI 2006).

According to numerous authors (MĘDRELA-KUDER 2000, LIPIEC 2001, BUGAJNY et al. 2004, FILIPIAK et al. 2004, STEPALSKA, WOŁEK 2005, KASPRZYK, WOREK 2006) molds appear in the air in early spring and reach their peak concentrations in late summer and early autumn. This study confirms the above pattern; in Ciechocinek, the abundances of molds in late summer and early fall were significantly higher than that in spring and winter. The abundances were correlated with temperature and wind speed (negative correlation). MĘDRELA-KUDER (2004) made similar observations in the Cracow area. This author concluded that in summer, the peak concentrations of spores in the air coincided with in periods of poor air ventilation, that is, at wind speeds below 1 m s^{-1}.

The sanitary air quality that affects people is influenced not only by concentration of molds in the air, but also by their genera and species composition. Molds of the *Cladosporium* genus were the most common in the research period, especially in the summer. Numerous studies indicate that the above genus prevail in microflora of the outdoor air and may even constitute 50–90% of all molds (MIKLASZEWSKA, GRAJEWSKI 2005, KASPRZYK, WOREK 2006). Studies conducted in other European countries confirm the above observations (NIKKELS et al. 1996, PYRRI, KAPSANAKI-GOTSI 2007). According to D'AMATO and SPIEKSMA (1995), the presence of 3000 spores of the *Cladosporium herbarum* could be responsible for a pathological condition in persons allergic to this species. In Ciechocinek, the total number of all molds in the vast majority of collected samples did not exceed this value; thus, the abundances of *Cladosporium* were certainly even lower.

The second most numerous group was *Alternaria* genus. Genera *Aspergillus* and *Penicilium* were also quite numerous in the air of Ciechocinek. According to epidemiological studies, precisely this most common component of fungi bioaerosol constitutes the most important source of mold allergens (LIPIEC 2001).

Potentially toxinogenic molds *Cladosporium cladosporioides*, *Alternaria alternata* and *Aspergillus flawus*, among others, were also found in the air of the town. Similarly, toxinogenic species of molds were observed in the air by other researchers (BIS et al. 2004, FRĄCZEK 2004, GRZYB et al. 2004). However, it should be noted that the ability to produce toxins is a species specific and not a genera specific quality (MIKLASZEWSKA, GRAJEWSKI 2005).

It is also noteworthy that the presented results are momentary and approximated values that correspond to microbiological pollution, which occurs at a given moment in a specific area. Therefore, it is necessary to

conduct long-term analyses of the air microflora, including an impact of meteorological, climatic, and topographic factors.

Conclusions

Aerosol produced in open inhalators has a positive effect on microbiological condition of air; in the areas within their operation, the concentration of molds was lower than in other sections of the resort. Due to the specific microclimate and low levels of mold pollution, Ciechocinek may well serve as an upper respiratory tract spa. According to the Polish Standard (*Ochrona czystości powietrza...* PN89/Z-04111/03), the air in Ciechocinek can be classified as clean; only 3% of analyzed samples were contaminated with molds that may have a negative impact on human environment. Strongly allergenic molds from the *Cladosporium* genus prevailed in the Ciechocinek air, but it is highly probable that their abundance did not exceed threshold concentrations responsible for allergic reactions in persons allergic to these molds.

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BIODEGRDATION OF DELTAMETHRIN BY PLANKTONIC AND BENTHIC BACTERIA OF CHEŁMŻYŃSKIE LAKE*

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K e y w o r d s: lake, water, pesticides, deltamethrin, benthic bacteria, planktonic bacteria.

A b s t r a c t

This study examined biodegradation of the insecticide deltamethrin (1 μg dm^{-3}) by homogenous cultures of planktonic (n = 25) and benthic (n = 25) bacteria as well as by heterogenous cultures (n = 1) containing a mixture of 25 bacterial strains. The bacteria were collected from subsurface water layer (25 cm below the surface) and a surface layer of bottom sediments (down to 10–15 cm) of eutrophic lake Chełmżyńskie. Results indicate that planktonic bacteria were characterized by higher average ability to biodegrade deltamethrin than benthic bacteria ($p < 0.05$). After 15-day incubation, bacteria isolated from subsurface water reduced the initial concentration of deltamethrin by 69%, while the average effectiveness of benthic bacteria equaled 23%. The level of deltamethrin biodegradation in mixed cultures of benthic and planktonic bacteria after 5, 10, and 15 days of incubation was higher than that in homogenous cultures. It was demonstrated that microorganisms from the *Sphingomonas paucimobilis* species and the *Moraxella* genus, among planktonic bacteria, as well as *Burkholderia cepacia* and *Bacillus mycoides* species, among benthic bacteria, were the most effective in reducing the concentration of this insecticide.

BIODEGRADACJA DELTAMETRYNY PRZEZ BAKTERIE PLANKTONOWE I BENTOSOWE JEZIORA CHEŁMŻYŃSKIEGO

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S ł o w a k l u c z o w e: jezioro, woda, pestycydy, deltametryna, bakterie planktonowe, bakterie bentosowe.

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A b s t r a k t

Przeprowadzono badania biodegradacji insektycydu deltametryny (1 μg dm^{-3}) przez homogenne hodowle szczepów bakterii planktonowych (n = 25) i bentosowych (n = 25), a także przez hodowle heterogenne (n = 1), zawierające mieszaninę 25 szczepów, wyizolowane z wody podpowierzchniowej (z głębokości 25 cm) oraz z powierzchniowej warstwy osadów dennych (do głębokości 10–15 cm) eutroficznego Jeziora Chełmżyńskiego. Z przeprowadzonych badań wynika, iż bakterie planktonowe charakteryzowały się średnio większą zdolnością do biodegradacji deltametryny niż bakterie bentosowe ($p < 0{,}05$). Bakterie wyizolowane z wody podpowierzchniowej rozkładały deltametrynę, redukując 69% stężenia początkowego insektycydu po 15 dniach inkubacji, natomiast bakterie bentosowe rozkłady pestycyd ze skutecznością wynoszącą 23%. Wartość biodegradacji deltametryny w mieszanych hodowlach bakterii planktonowych oraz bentosowych po 5, 10 i 15 dniach inkubacji była większa od średniej wartości biodegradacji tego związku w hodowlach jednorodnych. Wykazano, iż najskuteczniej redukowały zastosowane stężenie deltametryny bakterie planktonowe należące do gatunku *Sphingomonas paucimobilis* i bakterie z rodzaju *Moraxella*, natomiast wśród bakterii bentosowych – bakterie z gatunku *Burkholderia cepacia* i *Bacillus mycoides*.

Introduction

Deltamethrin (Figure 1) is one of the most common synthetic pyrethroid insecticide; it is widely used in agriculture, including vegetable, fruit, and ornamental plant farming, and in forestry to control gnawing and sucking pest. It is a contact and systemic neurotoxin, which strongly affects neurotransmitters in the central and peripheral nervous systems (NARAHASHI 1996). Deltamethrin belongs to an insecticide class characterized by advanced toxic properties, which means that it can be used in very small doses in comparison

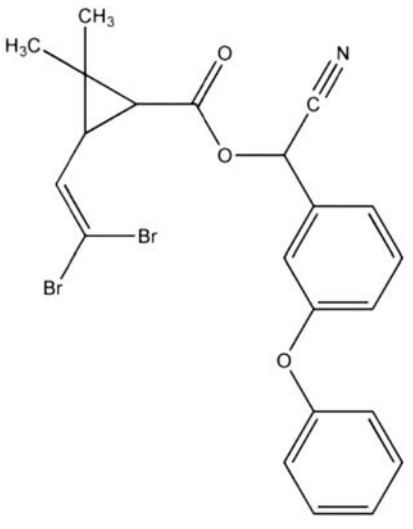

Fig. 1. Deltamethrin molecular structure

to the previously used active substances. Concentrations of this insecticide found in surface waters range from 0.04 to 24.0 μg dm^{-3} (PAWLISH et al. 1998). The half-life of deltamethrin in water ranges from 2 to 4 hours (RÓŻAŃSKI 1992). In laboratory condition, this substance shows high toxicity to fish, aquatic arthropods, and bees (*World...* 1997) In the aquatic environment, pyrethroids undergo spontaneous hydrolysis, particularly in the environment with high pH (SOGORB, VILANOVA 2002) and are subject to enzymatic degradation by enzymes produced by microorganisms (DEMOUTE 2006). Degradation of pesticides by microorganisms is an essential process in affecting the fate of these pollutants in the environment, and finds applications in bioremediation (GIANFREDA, RAO 2004). Microorganisms are highly effective in transforming organic pollutants and modifying their structure and toxic properties; even more, they can completely mineralize organic compounds to non-organic products (ZIPPER et al. 1996).

Due to the fact that the research on pesticide biodegradation has been largely limited to the soil environment, our knowledge about capability of aquatic bacteria to break down these xenobiotics is still inadequate. Thus, the purpose of this study is to determine whether any of the planktonic or benthic bacteria of lake Chełmżyńskie are capable of decomposing deltamethrin and which group, genus, and species of bacteria is the most effective in advancing this process.

Materials and Methods

Isolation of Strains

The analyzed strains of bacteria were isolated from the samples of subsurface water (planktonic bacteria) and the surface layer of bottom sediments (benthic bacteria), which were collected in a sampling station located in the extra-urban section of lake Chełmżyńskie surrounded by arable lands (Figure 2).

Lake Chełmżyńskie is located ca. 20 km from Toruń, belongs to the Fryba – Vistula river basin, and is a typical eutrophic water body. The lake has the following characteristics: surface area – ca. 271 ha, capacity – 16451.9 thousand m^3, maximum depth – 27.1 m, average depth – ca. 6 m. The watershed of the lake primarily includes arable lands, which constitutes 72% of the immediate watershed. The urbanized areas of Chełmża are located in the northwest section of the lake.

The subsurface water sample was collected from the depth of ca. 20 cm with a sterile glass pipette using an automatic pipettor Pipet-Boy (Biotechnology,

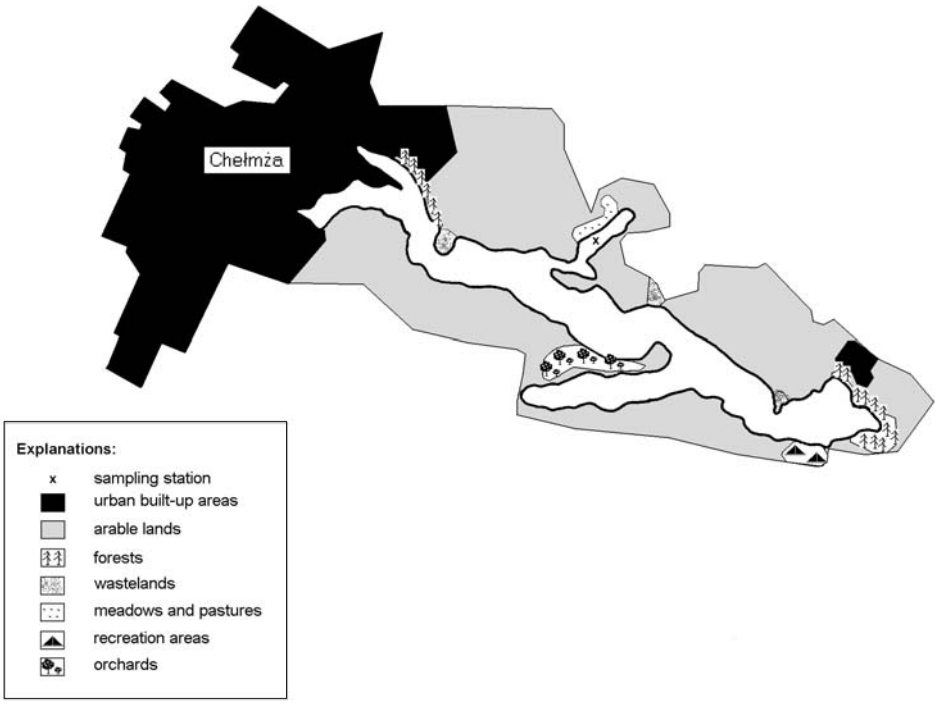

Fig. 2. Outlook of lake Chełmżyńskie

De Ville). The bottom sediment sample was collected the depth of up to 10 cm using a tube scoop (home-grown design) with a diameter of 5 cm. The samples of water and bottom sediments were transported to a laboratory in a cold container at below +4°C. The time between the sample collection and the microbiological analyses usually did not exceed 4 hours.

The samples, which had been diluted with sterile buffer water according to DAUBNER (1967), were inoculated on the peptone-iron agar (IPA) (FERRER at al. 1963) and incubated at 20°C in order to isolate bacterial colonies for further analyses. Twenty-five bacterial strains were isolated from both the water and bottom sediment samples.

Preliminary Cultures and Preparation of a Bacterial *Inoculum*

Bacterial strains isolated from individual samples and cultured on peptone-iron agar plates (IPA) were tested for purity and then used for preparation of preliminary cultures. Subsequently, bacterial inoculum for the analyses of deltamethrin degradation was obtained from these preliminary cultures.

Five cm^3 of sterile mineral medium, containing (g dm^{-3} of distilled water) KH_2PO_4 – 1, KNO_3 – 0.5, $MgSO_4 \cdot 7H_2O$ – 0.4, $CaCl_2$ – 0.2, NaCl – 0.1, $FeCl_3$ – 0.1, glucose – 1, was poured into test tubes, inoculated with pure bacterial cultures, and incubated at 20°C for 7 days on in a rotary shaker (WL – 2000, JW Electronic). Bacterial *inoculum* was retrieved from the culture with 2 inoculation loops. The medium contained deltamethrin with the final concentration of 1 μg dm^{-3}. For each bacterial strain, we also carried out control analyses, which involved culturing bacteria on a mineral medium without the pesticide.

In order to prepare the inoculum, the preliminary cultures were brought to identical optical density, $A = 0.2 \pm 0.05$. The spectrophotometric measurements of the culture absorbance was conducted with a spectrophotometer "Marcel s 330 Pro" at a wavelength of $\lambda = 560$ nm.

In addition to bacterial inocula containing homogeneous bacterial cultures, mixed bacterial cultures, containing 25 strains of pure strains, were prepared for each of the analyzed groups of lacustrine microorganisms. The cultures were brought to the appropriate optical density with the same method as the homogeneous cultures.

The Measurement of the Culture Optical Density

In order to determine the adaptability of the microorganisms to growth in the presence of a xenobiotic, we measured the optical density of preliminary cultures in a medium with and without deltamethrin after 7 days of incubation in accordance with the above method.

Proper Cultures

Five hundred mm^3 samples of bacterial inocula were poured into Erlenmeyer flasks containing 75 cm^3 of sterile mineral medium with the above composition. The final concentration of deltamethrin in cultures was 1 μg dm^{-3}. Sterile mineral medium without pesticide was used to dilute the samples. All samples were analyzed in three replicates. Simultaneously, control analyses were conducted for each bacterial strain. These analyses involved culturing bacteria in 75 cm^3 of uninoculated mineral medium with the addition of the xenobiotic.

Bacterial cultures were incubated for 15 days at 20°C in the dark in the rotary shaker (WL – 2000, JW Electronic).

Analysis of the Reduction of Deltamethrin Concentration

Degradation of deltamethrin was monitored using the gas chromatography technique (GC) – Shimazu unit with an electron capture detector (ECD). Two cm^3 culture samples after 5, 10, and 15 days of incubation were collected to determine the amount of the pesticide. In order to extract deltamethrin from bacterial culture samples, 2 cm^3 samples were treated three times with 2 cm^3 dozes of hexane (POCH). Prior to the extraction, 50 mm^3 dozes of an internal standard – 10 mm^3 solution of decachlorobiphenyl in acetone (POCH) – were added to each sample. Extracts were combined and dry-evaporated with a slow flow of ultra-pure nitrogen at ambient temperature. After the evaporation, the remains were dissolved in 50 mm^3 of methanol (POCH). The extracts were stored in dark-glass vials at -4°C in preparation for chromatographic analyses.

Analyses were conducted in a capillary column Equity-5 (30 m 0.25 mm i.d., 0.25 mm d.f.). Five mm^3 samples were used in the chromatographic analysis. Nitrogen (N_2) with the flow rate of 60 cm^3 min^{-1} was used as carrier gas. The temperature program was set to: 150°C (1 min), 35 min^{-1} to 200°C, 15°C min^{-1} to 280°C. Temperatures of a detector and an injector equaled 320°C and 260°C, respectively.

The amount of the pesticide in a sample was determined based on comparison of retention times of peaks in the sample relative to the internal standard with the relative retention times of standard reference chemicals. The calibration curve was prepared using decachlorobiphenyl as the internal standard. All plots were linear with a correlation coefficient of 0.99 or higher (least squared method).

The level of deltamethrin biodegradation (%) was calculated from the equation:

$$B = \frac{a - b}{a} \cdot 100$$

where:
B – biodegradation (%),
a – concentration of deltamethrin in a culture after t_0,
b – concentration of deltamethrin in a culture after t_5, t_{10}, t_{15}

Identification of Strains

Isolated bacterial strains capable of decomposing deltamethrin were identified with the Analytical Profile Index (API 20NE, API 50CH, API 20E, API Staph, API Strep test kit, BioMeriéux). The tests were conducted in three replicates as recommended by the producer.

Statistical Analysis

Statistical analyses were conducted in STATISTICA 6.0, 2001. T-test was used in order to determine the statistically significant differences in average ability of the analyzed bacteria to break down deltamethrin after 5, 10, and 15 days of incubation.

Results

Table 1 presents basic descriptive statistics (average and standard deviations), which characterize the optical density in planktonic and benthic bacterial cultures on medium with and without deltamethrin. The results demonstrate that both ecological groups of lacustrine bacteria were developing relatively well in the presence of the analyzed insecticide. It was observed that 1 μg dm^{-3} concentration of deltamethrin did not hinder the development of bacteria; on the contrary, the pesticide had a stimulating effect on the growth of the majority of tested bacterial strains. After 7 days of incubation, optical density of both planktonic and benthic bacterial cultures with deltamethrin was significantly higher than that without the insecticide ($p < 0.0005$ and $p < 0.05$, respectively).

Table 1

Culture optical density in the medium with (A_d) and without (A_0) deltamethrin

Group of bacteria	Statistics	A_d	A_0
		t_{168}	
Planktonic	average	0.318	0.188
	SD	0.161	0.134
Benthic	average	0.396	0.319
	SD	0.178	0.126

Explanations: * – Average (n = 26), SD – standard deviation

Data regarding the reduction of deltamethrin concentration in cultures of planktonic and benthic bacteria are presented in Table 2 and Figure 3. The results indicate that planktonic bacteria were more effective in reducing the concentration of the deltamethrin than benthic bacteria. The differences in reduction of deltamethrin concentration in bacterial cultures of different ecological groups of lacustrine microorganisms after 5, 10, and 15 days of incubation were significant ($p < 0.05$, Table 3).

Table 2

Biodegradation of deltamethrin by planktonic and benthic bacteria

Group of bacteria	Statistics	C (ng dm^{-3})	B (%)	C (ng dm^{-3})	B (%)	C (ng dm^{-3})	B (%)
		t_5		t_{10}		t_{15}	
Planktonic	**average**	608	39	438	56	306	69
	min.	244	0	128	0	62	0
	max.	1013	76	1004	87	1002	94
	SD	268	27	261	26	247	25
Benthic	**average**	967	4	877	12	770	23
	min.	376	0	310	0	252	0
	max.	1016	62	1008	69	1002	75
	SD	129	13	203	20	267	26

Explanations: C – deltamethrin concentration; B – biodegradation; t_5, t_{10}, t_{15} – incubation time 5, 10, 15 – days, SD – standard deviation

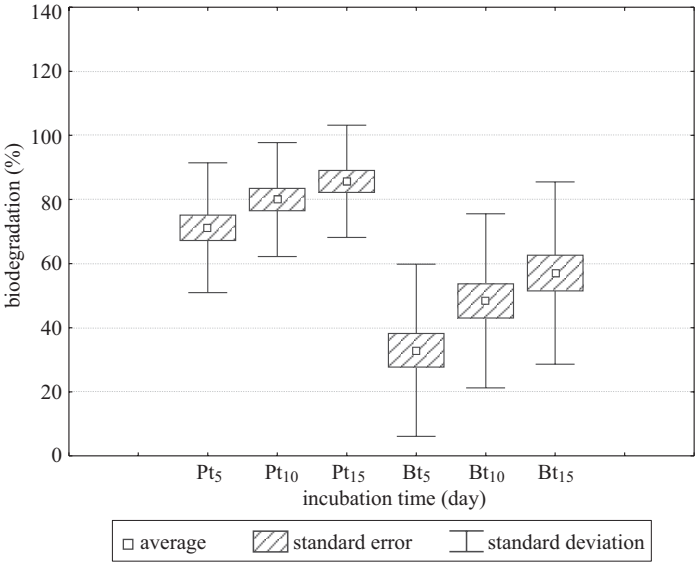

Fig. 3. Biodegradation of deltamethrin in the cultures of planktonic (P) and benthic (B) bacteria after 5, 10 i 15 days of incubation (t_5, t_{10}, t_{15})

Table 3

Differences between the rate of biodegradation of deltamethrin in 5, 10 and 15 – day cultures of planktonic (P) and benthic (B) bacteria

Variable	P 5	P 10	P 15	B 5	B 10	B 15
P 5	n.s.	*	*	*	*	*
P 10	n.s.	n.s.	*	*	*	*
P 15	n.s.	n.s.	n.s.	*	*	*
B 5	n.s.	n.s.	n.s.	n.s.	*	*
B 10	n.s.	n.s.	n.s.	n.s.	n.s.	*
B 15	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Explanations: * – difference statistically significant ($p < 0.05$), n.s. – non-significant

In 15-day cultures of planktonic bacteria (n = 26), the average concentration of deltamethrin equaled 306 ng dm^{-3}, and thus was reduced by 69%. All analyzed bacterial strains but one were capable of decomposing deltamethrin.

The average concentration of deltamethrin in 15-day cultures of benthic bacteria equaled 770 ng dm^{-3}, so, these bacteria decomposed the analyzed pesticide with an average effectiveness of 23%. Thirteen strains of benthic bacteria demonstrated capability of decomposing deltamethrin.

Table 4 presents results of analyses of deltamethrin biodegradation by pure strains of planktonic and benthic bacteria and by mixed cultures containing 25 strains. According to the data obtained after 5, 10, and 15 days of incubation, mixed cultures of bacteria were more effective in decomposing the insecticide than homogeneous cultures.

Table 4

Biodegradation of deltamethrin in mixed (M) and homogenous (H) cultures of bacteria

Group of bacteria	Type of culture	Biodegradation (%)		
		t_5	t_{10}	t_{15}
Planktonic	M	70	73	77
	H*	38	56	69
Benthic	M	62	69	75
	H*	1	10	21

Explanations: * – average (n = 25); t_5, t_{10}, t_{15} – incubation time 5, 10, 15 days

Table 5 presents results of analyses of the genus composition of microorganisms capable of decomposing deltamethrin.

Among the identified planktonic bacteria capable of degrading deltamethrin, there were 5 strains of the *Sphingomonas paucimobilis*, 5 strains of the

Aeromonas hydrophila, and the *Pseudomonas* genus (*P. fluorescens* – 2 strains, *P. luteola* – 2 strains). *Burkholderia cepacia* (5 strains), *Aeromonas hydrophila* (3 strains), and *Bacillus megaterium* (3 strains) were the most abundant among benthic bacteria capable of decomposing the pesticide. The remaining species of bacteria degrading the insecticide were represented by single strains.

It was demonstrated that microorganisms from the *Sphingomonas paucimobilis* and the *Moraxella* genus, among planktonic bacteria, and *Burkholderia cepacia* and *Bacillus mycoides*, among benthic bacteria, were the most effective in reducing the concentration of this insecticide.

Table 5

Generic composition of planktonic and benthic bacteria able to degrading deltamethrin

Planktonic bacteria Species	$B(t_{15})$ (%)	Benthic bacteria Gender/Species	$B(t_{15})$ (%)
u.s.	54	*Aeromonas hydrophila*	28
Pseudomonas luteola	76	*Bacillus mycoides*	64
Aneuribacillus sp.	89	***Burkholderia cepacia***	**74**
Geobacillus sp.	59	*Pseudomonas luteola*	47
Aeromonas hydrophila	22	u.s.	46
Moraxella sp.	93	*Burkholderia cepacia*	40
Pseudomonas fluorescens	81	*Burkholderia cepacia*	4
Sphingomonas paucimobilis	8	*Burkholderia cepacia*	39
u.s.	93	u.s.	44
Pseudomonas fluorescens	82	*Aeromonas hydrophila*	6
Aeromonas hydrophila	82	*Bacillus mycoides*	42
Aeromonas hydrophila	80	*Aeromonas hydrophila*	31
Sphingomonas paucimobilis	8	*Burkholderia cepacia*	60
Pseudomonas luteola	78		
Aeromonas hydrophila	80		
Staphylococcus intermedius	83		
Vibrio metchnikovii	65		
Sphingomonas paucimobilis	**94**		
Staphylococcus intermedius	77		
Sphingomonas paucimobilis	55		
Sphingomonas paucimobilis	71		
Staphylococcus intermedius	82		
Aeromonas hydrophila	87		
Brevundimonas vescicularis	55		

Explanations: No – number of strain, $B(t_{15})$ – biodegradation after 15 days of incubation, u.s. – unidentified strain

Discussion

Deltamethrin is among those pyrethroid insecticides that are decomposed in the natural environment relatively easily as a result of photodegradation and biodegradation; the latter is thought to be the primary process responsible

for breakdown of this compound in alkaline or neutral pH waters with limited amount of light. The principal mode of pyrethroid decomposition includes hydrolysis of ester bonds and oxidation of acid and alcohol functional groups (DEMOUTE 2006).

This study demonstrated that as early as 7 days after the xenobiotic was added, both pure and mixed cultures of analyzed groups of lacustrine bacteria were developing relatively well in the presence of deltamethrin with the concentration of 1 μg dm^{-3}. The 1 μg dm^{-3} concentration of deltamethrin was found to have a stimulating effect on the majority of bacterial strains. ZHANG et al. (1984) observed an increase in bacterial and actinomycetes populations in soil containing deltamethrin during a 180-day experiment on degradation of this compound in organic soil. However, certain organic micropollutants may have a toxic effect on populations of microorganisms, inhibiting their metabolism; as a result, degradation of these compounds is possible only to a small degree (WARREN et al. 2003).

According to available literature data regarding decomposition of deltamethrin in soil, this insecticide undergoes microbiological degradation in 1-2 weeks (KIDD, JAMES 1991). In order to confirm the priority of biodegradation over abiotic modes of transformation in decomposing pyrethroids, CHAPMAN et al. (1981) investigated stability of permethrin, cypermethrin, deltamethrin, fenpropathrin, and fenvelaret in sterile and non-sterile soil samples. The initial concentration of pesticides equaled 1 ppm. The remaining percentages of pesticides after 8-week incubation were as follows: fenpropathrin – 2%, permethrin – 6%, cypermethrin – 4%, fenvelaret – 12%, and deltamethrin – 52%. In sterile soil samples, the content of pesticides exceeded 90%, which suggests that biodegradation plays a key role in decomposing these compounds in soil. In biodegradation tests with deltamethrin as the only source of carbon and energy conducted on bacterial strains isolated from soils, the initial concentration of the insecticide was reduced by 35.7–44.4% within a week and by 59.7–72.5%, within two weeks. In samples without microorganism, the initial concentration of this compound was reduced by 3–10% (KHAN et al. 1988). Research on degradation of the following pyrethroids: biosmerin, permethrin, cypermethrin, deltamethrin, fevelaret, chlorpyrifos, and pesticide Nurelle D 550 EC (mixture of 50 g cypemethrin and 500 g of chlopyrifos in 1 dm^3) in a model of an aquatic ecosystem show that, among the analyzed compounds, deltamethrin is characterized by the highest rate of degradation with decomposition time up to 21 days (LUTNICKA et al. 1999).

Microorganisms inhabiting various ecological niches of a water body are characterized by different metabolic activity levels and, in consequence, a different response to xenobiotics introduced to the environment. The results indicate that planktonic bacteria are characterized by higher average capacity

to decompose deltamethrin than benthic bacteria. Among 25 analyzed strains of planktonic bacteria, all but one strain were capable of decomposing deltamethrin. In the case of bacteria isolated from bottom sediments, 13 strains degraded the insecticide, while 12 showed no such ability. The half-life of deltamethrin in cultures of planktonic bacteria equaled on average ca. 10 days (Table 4). Benthic bacteria, on average, needed 15 days to reduce the concentration of fungicide by 23%. According to numerous studies related to the metabolic activity of heterotrophic bacteria that occur in the water column and bottom sediments, bacterioplankton is characterized by a higher metabolic and biochemical activity than benthic bacteria (FISHER et al. 2002, HAGLUND et al. 2003). In spite of relatively high abundance of the latter microorganisms (NIEWOLAK 1968, KALWASIŃSKA, DONDERSKI 2005) and the fact that cells of bacteria from deep bottom sediments are capable of growing on solid mediums in laboratory conditions (viable bacteria) (MISKIN et al. 1988, PARKES et al. 1994), their activity is often low, which indicates that these bacteria are characterized by a low cell-specific activity. It is probable that precisely this low cell-specific activity of bacteria inhabiting bottom sediments is responsible for their limited ability to decompose deltamethrin.

Comparing pure strains and mixed cultures of bacteria, it was concluded that during the entire period of the experiment, heterogeneous cultures were, on average, more effective in decomposing deltamethrin than pure strains. More effective biodegradation of deltamethrin in mixed cultures could be attributed to the prevalence of stimulating interaction over antagonistic interactions between organisms belonging to different genera and species. CHODYNIECKI (1968), when analyzing various combinations of bacteria from the *Pseudomonas, Aeromonas, Vibrio,* and *Flavobaterium* genera, found that 12% of bacterial interactions were of antagonistic character, and 8% of stimulating. This latter type of interactions and, most probably, also bacterial synergism that occurred in heterogenous mixtures were responsible for the fact that these cultures decomposed deltamethrin more effectively than single strains of bacteria.

Screening microorganisms with particularly high capacities for decomposing deltamethrin is very important in bioremediation of this type of pollutants. In spite of this, reports regarding isolation and identification of pure bacterial cultures capable of degrading deltamethrin are scarce and primarily pertain to soil environments.

According to MALONEY et al. (1988), pure cultures of the *Bacillus cereus* and *Pseudomons fluorescens* species and bacteria from the *Achromobacter* genus are capable of decomposing delthametring with the concentration of 50 μg dm^{-3} in the presence of Tween 80. The half-time of deltamethrin in oxygen cultures of these microorganisms ranged from 21 to 28 days. GRANT et al. (2002)

discovered that *Pseudomonas* sp. and *Serratia sp.* isolated from soil are useful in breaking down synthetic pyrethroids. LEE et al. (2004) isolated 56 bacterial strains capable of decomposing synthetic pyrethroids from bottom sediments polluted with pesticides. According to TALLUR et al. (2007), deltamethrin is degradable by bacteria from the *Microccocus sp*.

This study demonstrated that among the planktonic bacteria, species *Sphingomonas paucimobilis* and microorganisms from the *Moraxella* genus were characterized by the highest ability to decompose the analyzed insecticide, while *Burkholderia cepacia* and *Bacillus mycoides* were the most effective among benthic bacteria.

The bacterial species *Sphingomonas paucimobilis* and *Burkholderia cepacia,* which are characterized by an ability to decompose a wide spectrum of organic pollutants, including pesticides, are useful in bioremediation. *Sphingomonas paucimobilis* is, for example, capable of decomposing hexachlorocyclohexane and biosorption of cadmium (PAL et al. 2005, TANGAROMSUK et al. 2002) and shows ability to biodegrade phenyl-methyl-ethers (NISHIKAWA et al. 1998).

The *Burkholderia cepacia* species demonstrates exceptional capability to decompose many structurally complex organic compounds. The abilities of this microorganism to decompose 2,4,5-trichloroacetic acid (DAUBRAS et al. 1996), benzo(a)pyrene, dibenz(a,h)anthracene, coronene (JUHASZ et al. 1997), p-nitrophenol (BHUSHAN et al. 2000), and other polyaromatic hydrocarbons (KIM et al. 2003) have been confirmed.

Conclusions

1. Results indicate that planktonic bacteria were characterized by higher average ability to biodegrade deltamethrin than benthic bacteria ($p < 0.05$).

2. The level of deltamethrin biodegradation in mixed cultures of benthic and planktonic bacteria after 5, 10, and 15 days of incubation was higher than that in homogenous cultures.

3. Sphingomonas paucimobilis and the *Moraxella sp*., among planktonic bacteria, as well as *Burkholderia cepacia* and *Bacillus mycoides*, among benthic bacteria, were the most effective microorganisms in reducing the concentration of this insecticide.

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EFFECT OF β RADIATION ON BACTERIOLOGICAL AND PARASITAL DECONTAMINATION OF SEWAGE SLUDGE

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K e y w o r d s: disinfection, β radiation, sewage sludge, bacteria, larvae *Ascaris suum*.

A b s t r a c t

The aim of the study was to estimate the efficacy of sewage sludge disinfection by radiation using an electron beam accelerator. The samples of sludge from the aeration chamber as well as raw and dewatered sludge were exposed to the doses of 1, 3, 4, 5, 6, 8, 10 and 12 kGy of β radiation. The number of *Salmonella senftenberg* W775*, Escherichia coli*, D-group streptococci, anaerobic bacteria reducing sulfites, and the vitality of *Ascaris suum* larvae were determined in the sludge. It was observed that the reduction of investigated bacteria depends on the kind of pathogens and applied doses of radiation. The ova of the enteric parasite *A. suum* and *Salmonella* were more sensitive to radiation in comparison with other investigated microorganisms. The highest lethal radiation doses were necessary to eliminate streptococci and anaerobic bacteria. It seems that sludge after β radiation could be used for agricultural practice without hazard for environment.

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WPŁYW PROMIENIOWANIA *β* NA ZANIECZYSZCZENIE BAKTERIOLOGICZNE I PASOŻYTNICZE OSADÓW POŚCIEKOWYCH

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S ł o w a k l u c z o w e: dezynfekcja, promienie *β*, osady pościekowe, bakterie, larwy *Ascaris suum*.

A b s t r a k t

Celem badań było określenie skuteczności dezynfekcji osadów pościekowych za pomocą wiązki szybkich elektronów. Próbki osadu z komory napowietrzania, osadu surowego i odwodnionego poddano oddziaływaniu dawek: 1, 3, 4, 5, 6, 8, 10, 12 kGy. W osadach oznaczano zawartość pałeczek *Salmonella senftenberg* W775, *Escherichia coli*, paciorkowców grupy D, bakterii beztlenowych redukujących siarczyny oraz żywotność larw *Ascaris suum*. Zaobserwowano, że redukcja mikroorganizmów zależała od rodzaju drobnoustrojów oraz zastosowanych dawek promieniowania. Najbardziej wrażliwe okazały się larwy pasożytów jelitowych *A. suum* i pałeczki *Salmonella*. Do likwidacji paciorkowców kałowych i bakterii beztlenowych były niezbędne najwyższe dawki letalne promieniowania. Z przeprowadzonych badań wynika, że ścieki poddane procesom radiacji nie stanowią zagrożenia dla środowiska i mogą być użyte do celów rolniczych.

Introduction

The application of radiating methods is an alternative method for disinfection of sewages and sewage sludge which normally contain *E. coli, Klebsiella* sp. *Salmonella* sp. and many others (DUDLEY et al. 1980, WARD et al. 1981). The method is based on the action of γ radiation generated by radioactive sources (Co^{60} and Cs^{137}) or β radiation generated in electron beam accelerators, commonly referred to as the fast electron beam (CAPIZZI et al. 1999, CAPIZZI-BANAS et al. 2001). Low doses of ionizing radiation do not induce radioactivity in irradiated sewage, while they result in favourable changes in the structure of organic compounds and efficiently eliminate bacteria, viruses and enteric parasite ova in sludge. Thus these methods can be implemented in the process of organic waste hygienization on a large scale in the near future (BORRELY et al. 1998, SAMPA et al. 1995). In Poland sewage sludge is most often subjected to stabilization by composting or liming with highly reactive calcium oxide. Radiating methods, however, are hardly used for this purpose (PALUSZAK et al. 2003, 2003, 2006). Technologies based on a radiation found a practical use

in Germany and India, while those using a beam – in Japan, Austria and USA (BORRELY et al. 1998). The aim of the study was to evaluate the effect of ionizing radiation on sewage sludge contaminated with *Salmonella senftenberg* W775, *E. coli,* D group streptococci and parasite *A. suum* eggs.

Materials and Methods

The study was conducted on sewage sludge coming from a waste water treatment plant gathering municipal and abattoir sewage. Before starting the research 4.5 dm^3 of sludge with different content of dry matter was contaminated with 100 cm^3 suspension of *Salmonella senftenberg* W775, *E. coli* and D group streptococci with a concentration of 10^8–10^9 MPN g^{-1}. Dry matter content was 0.003% in active sludge from the aeration chamber, 4% in raw sludge and 18–20% in dewatered sludge. After thorough mixing and storing the sludge for 12 h, which was needed for microorganisms to absorb to solid particles contaminated sludge was placed in plastic bags in 200-gram portions. Carriers made of perlon with 20 μm pores containing suspension of *Ascaris suum* eggs obtained from uteruses of mature females were added to the plastic bags. So prepared samples were placed in metal cases, paying attention that the layer of sludge exposed to radiation was not thicker than 2 cm. Then the samples were subjected to the doses of 1, 3, 4, 5, 6, 8, 10 and 12 kGy of β radiation in the Institute of Nuclear Chemistry in Warsaw. Before and after irradiation the total number of bacteria, the number of *E. coli,* D-group streptococci, *Salmonella senftenberg* W775, sulphite-reducing anaerobic bacteria and the percentage of *Ascaris suum* eggs containing live larvae were determined in sludges. The total number of microorganisms was determined with the plate method, using Standard I Agar (37°C, 24–48 h). Liquid proliferating broth LPB and selective solid medium Endo were used for determination of *E. coli* number. The colony were verified with API 20E test. The ability to lactose decomposition into acid with deliberation of gas (44°C, 48 h) was also tested. Quantitative determination of D-group streptococci was made using the liquid medium with glucose and natrium azide (37°C, 48 h), and then solid medium based on agar containing natrium azide and esculine (37°C, 48 h). At the final stage, the cultivated colonies were subjected to the serological test (Phadebact-Test). 1% peptonic water (37°C, 24 h), the liquid medium according to Rappaport-Vassiliadis (43°C, 24 h) and the solid agar medium (BPLA) according to Kaufmann (37°C, 24 h) were used in the process of *Salmonella* isolation. Since the sludges intended to the research did not contain other *Salmonella* rods except for the added strain *Salmonella senftenberg* W775 the final identification consisted in applying the polyvalent serum HM. Determina-

tion of anaerobic sporing bacteria reducing sulphites was made on the liquid medium according to Polish Standard (*Woda i ścieki...* PN-79/C-04615.12. using differentiating broth DRCM. MPN of the tested bacteria was determined on the basis of 3-test tube sets using the Mc Crady tables. The percentage of live *A. suum* ova was determined on the basis of the method which was described before (KELLER 1983, RAWAT et al. 1998). All investigations were made triplicate. The results obtained were verified and analysed statistically with variance analysis in the program Statistica.

Results

The inactivation of the tested microorganisms and *Ascaris suum* larvae in sewage sludge were illustrated in Figures 1–5 and Table 1. As no changes were observed in control test tubes in relation to the initial samples during the investigation time, the results were not shown in figures. Application of particular doses of β radiation had different effect on individual microorganisms. A dose of 5 kGy caused the reduction of the total number of microorganisms by 7 log in raw and active sludge and by 5 log in dewatered sludge. Increasing the dose of β radiation to 12 kGy did not result in further reduction in number of the tested microorganisms in sewage sludge. The number of microorganisms in all the tested sludges did not fall below 10^3 cfu g^{-1} (Figure 1). Figure 2 shows the decrease in *E. coli* number in sludges exposed to β radiation. Applying a dose of 5 kGy eliminated *E. coli* rods in raw and active sludge. Dewatered sludge, being a kind of protective barrier for bacteria, did not show a presence of *E. coli* rods only after using a dose of 8 kGy. D-group streptococci proved to be definitely more resistant to β radiation (Figure 3). A dose of 8 kGy caused a decrease in their number by 7 log in raw sludge and by 5 log in watered sludge. No fecal streptococci were isolated from sludges exposed to β radiation with a dose of 10 kGy. A high susceptibility of *Salmonella* rods on β radiation (Figure 4) is worthy of note. Even a dose of 1 kGy induced the reduction in number of *Salmonella* rods by 3 log in watered sludge and by 2 log in dewatered sludge. The total elimination of *Salmonella* was obtained after applying a dose of 3 kGy in raw and active sludge and 4 kGy in dewatered sludge. Of all the microorganisms observed, anaerobic sulphite-reducing bacteria proved to be the most resistant to β radiation (Figure 5). Despite high doses of radiation they failed to be fully eliminated from sewage sludge. After applying a dose of 12 kGy their number decreased by 3 log in raw and active sludge and by 4 log in active and dewatered sludge. A number of anaerobic bacteria isolated in raw and active sludge was $5.0 \cdot 10$ MPN g^{-1}, and in dewatered sludge it was $1.1 \cdot 10^3$ MPN g^{-1}.

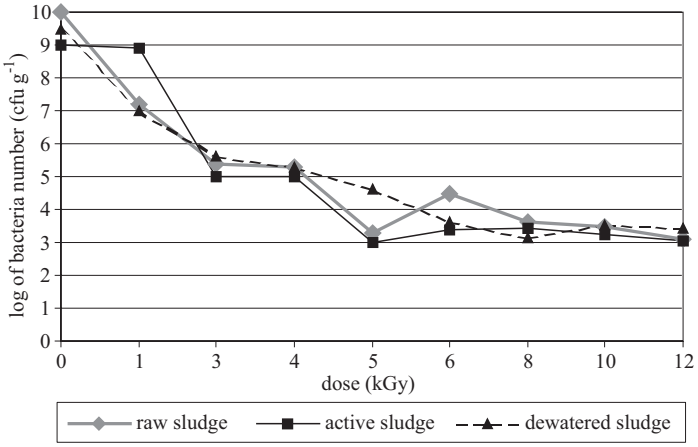

Fig. 1. The influence of β radiation on the total number of bacteria (average of three replications)

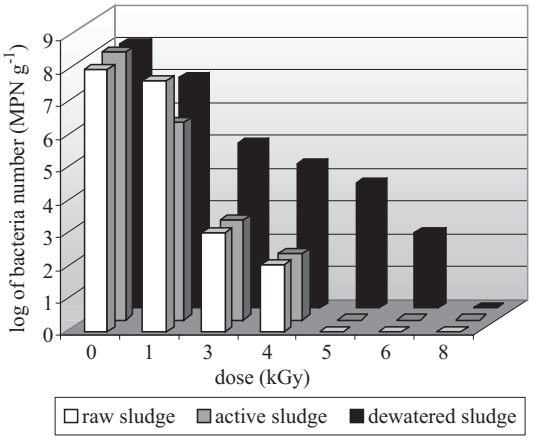

Fig. 2. The influence of β radiation on *Escherichia coli* number (average of three replications)

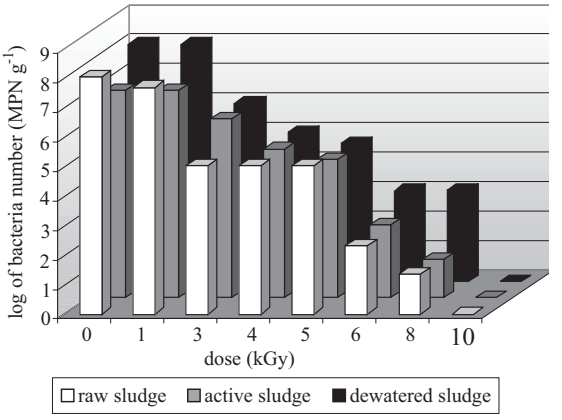

Fig. 3. The influence of β radiation on faecal streptococci number (average of three replications)

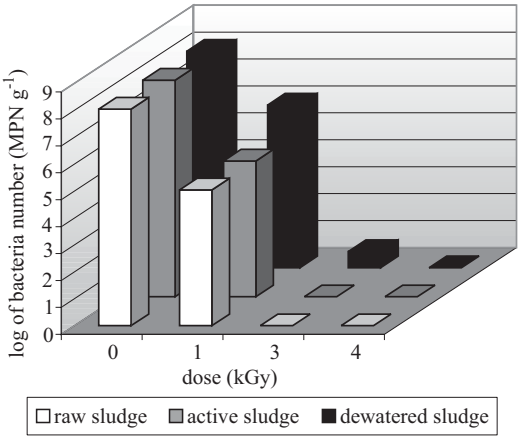

Fig. 4. The influence of β radiation on *Salmonella senftenberg* W 775 number (average of three replications)

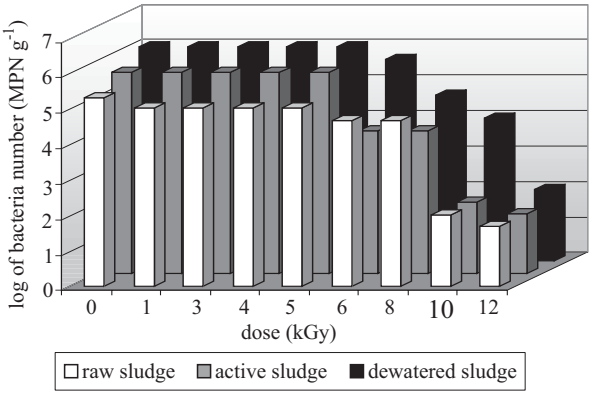

Fig. 5. The influence of β radiation on anaerobic bacteria number (average of three replications)

Table 1
Percentage of live *Ascaris suum* larvae after application of β radiation (average of three replications)

Sludge	Dose (kGy)			
	0	1	3	≥ 4
Raw	94.8 ± 2.76	12.2 ± 3.15*	0.8 ± 0.79*	0.0*
Active	93.4 ± 2.78	8.3 ± 2.35*	0.0*	0.0*
Dewatered	92.9 ± 3.06	16.1 ± 4.20*	0.7 ± 0.97*	0.0*

* variance in ratio of live *A. suum* eggs between control and experimental group significant in the level $P < 0.001$

The research carried out proved a high effectiveness of the radiation method for the inactivation of gastrointestinal parasite larvae. Even a dose of 1 kGy caused a radical fall in the percentage of live eggs in all kinds of sludges (Table 1). Live larvae of *Ascaris suum* were not observed in sludges exposed to a dose of 4 kGy.

Discussion

It should be noted that the effect of β radiation on microorganisms contained in sewage sludge depends on the water content, types of microorganisms, chemical composition and many others (AL-BACHIR et al. 2003, CHMIELEWSKI et al. 1995). From the literature available it can be concluded that aerobic bacteria are more susceptible to ionizing radiation than anaerobic and sporing bacteria (PRINCE 1978). A dose of 5 kGy is needed to decrease a population of pathogens by 1 log in dewatered sludge, while a similar effect in watered sludge could be obtained using a five times less dose (AL-BACHIR et al. 2003). A similar tendency was observed in the present study. The total elimination of *E. coli* in watered sludges was obtained under the influence of a dose of 5 kGy, while a dose of 8 kGy of β radiation was needed in dewatered sludge.

It is interesting to note that the D_{10} value for microorganisms contained in sludge stored for 12 months decreases from 1.55 to 1.02 kGy along with the increase in its humidity from 2 to 80% (AL-BACHIR et al. 2003). A modified effect of sludge humidity level on the bactericidal effect of ionizing radiation was also observed by WARD et al. (1981). No significant differences concerning the effect of β radiation on *Salmonella* rods in watered and dewatered sludges were found in the present study, since the lethal doses were 3 and 4 kGy respectively.

From the data in the literature it can be concluded that viruses and enzymes are less susceptible to the action of ionizing radiation than bacterial cells. The D_{10} value calculated for buffer suspensions of *E. coli* is 0.34, for *Vibrio cholerae* 0.48, for *Shigiella dysenteriae* 0.60 and for Picornaviruses 1.84 kGy (BORRELY et al. 1998). A susceptibility of pathogenic microorganisms present in sewage sludges exposed to ionizing radiation is also diverse. The lethal dose for *E. coli* in dried sludge containing 2% of dry matter is 2 kGy, for *Salmonella* 3 kGy, for *Enterobacteriaceae* 4 kGy, while for *Klebsiella* sp. 5 kGy (AL-BACHIR et al. 2003). This was also used in the present study. Anaerobic bacteria proved to be the most resistant to β radiation. A beam with an energy of 12 kGy caused a reduction in their number by 4 log in raw and active sludge, and by 3 log in dewatered sludge. Beside anaerobic microorganisms fecal

streptococci showed a remarkable resistance to ionizing radiation, since their total elimination in the tested sludges was obtained only under the influence of a dose of 10 kGy. The research carried out indicated a high effectiveness of the radiating method for the inactivation of *Ascaris suum* ova. It was found that even a dose of 1 kGy resulted in inhibiting the growth of gastrointestinal nematode larvae, while a dose of 3–4 kGy eliminated them totally. A high susceptibility of gastrointestinal parasite eggs on ionizing radiation was supported by KELLER (1983) and CHMIELEWSKI et al. (1995). BREWE and OWEN (1983), however, observed less favourable effects. The results obtained indicate the ability to apply β radiation for sewage sludge hygienization, which could be performed on a large scale. It is safer for the environment, and its generation is cheaper in relation to γ radiation.

Conclusions

1. The high effectiveness of electron beam in sewage sludge desinfection was observed. *Ascaris suum* ova and *Salmonella senftenberg* W775 were the most sensitive to β radiation.

2. Anaerobic bacteria and streptococci deeded the highest doses of β radiation to be eliminated.

3. The sewage sludges treated by β radiation are suitable for agricultural use.

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THE VIEW OF USEFULNESS THE HYDROGEN PEROXIDE (H_2O_2) AND SOLID MAGNETIC FIELD (SMF) IN THE COD REDUCTION VALUE IN MEAT INDUSTRY WASTEWATER

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K e y w o r d s: the hydrogen peroxide (H_2O_2), the solid magnetic field (SMF), the hydroxyl radicals (OH*), the advanced oxidation process (AOP), wastewater coming from meat industry.

A b s t r a c t

The possibility of the use of the hydrogen peroxide to the sewage treatment coming from meat industry in experiment was analysed. The experiment was realized in two stages, in laboratory scale, by the use of model through construction. The first part of the experiment concerned the description of influence of hydrogen peroxide for final treatment effects. Simultaneous use of the advanced oxidation process (AOP) and the solid magnetic field (SMF) in carbon substances degradation process was studied in the second stage. Magnetic field with the strain amounted 12,000 Gausses was generated by magnetic activator of liquids (MLA). The got effect of the technological removal COD is the principle ascertainable that the hydrogen peroxide influences efficiently on studied wastewater treatment. It was affirmed additionally, that the use of the SMF as a helping factor of the AOP increased to get of higher technological effect of The COD reduction value from wastewaters.

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OCENA PRZYDATNOŚCI NADTLENKU WODORU I STAŁEGO POLA MAGNETYCZNEGO W REDUKCJI WARTOŚCI CHZT W ŚCIEKACH POCHODZĄCYCH Z PRZEMYSŁU MIĘSNEGO

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S ł o w a k l u c z o w e: nadtlenek wodoru (H_2O_2), stałe pole magnetyczne (SMF), rodniki hydroksylowe (OH*), procesy pogłębionego utleniania (AOP), ścieki z przemysłu mięsnego.

A b s t r a k t

W eksperymencie przeanalizowano możliwość zastosowania nadtlenku wodoru do oczyszczania ścieków pochodzących z zakładu mięsnego. Eksperyment prowadzono w dwóch etapach, w skali laboratoryjnej, z zastosowaniem modelowego układu przepływowego. Pierwsza jego część dotyczyła określenia wpływu nadtlenku wodoru na końcowe efekty oczyszczania. W etapie drugim zbadano jednoczesne wykorzystanie metody pogłębionego utleniania (AOP) i stałego pola magnetycznego (SMF) w procesie degradacji substancji węglowych. Pole magnetyczne o natężeniu 12 000 Gaussów generowane było przez magnetyczne aktywatory płynów (MLA). Uzyskany efekt technologicznego usunięcia ChZT jest podstawą do stwierdzenia, że nadtlenek wodoru wydajnie wpływa na oczyszczanie badanych ścieków. Wykazano również, że zastosowanie SMF jako czynnika wspomagającego AOP przyczyniło się do uzyskania wyższego efektu technologicznego obniżki wartości ChZT ze ścieków.

Introduction

The wastewater from meat industry is treated as difficult sewages on large load of organic pollutions, mainly proteins and fats. It is in them a lot of blood coming from slaughter and disemboweling additionally. These sewages are characterized by the lability of composition, large content of organic compounds and the large concentration the solvable substance for example: chemical elements of nutrients and chlorides.

The wastewater composition from processing of meat decides about classifying of them to the category of sewages to specific proprieties. It causes, that they require the study of special, alternative treatment technologies. During last years the large interest among techniques of water and wastewater treatment, inspire the AOP.

The AOP have the task to generate high reactive hydroxyl radicals (OH*) having an oxidative potential 2.8V, which is count among the oxidants in the first place. This radical distinguishes itself with non-selective and very quick ingress in chemical reactions with a lot of organic compounds, hard susceptible on biodegradation and oxygenation. The high effectivity, universality and

quick pace of decomposition of pollutions contribute to more and more common use of AOP to the sewage treatment and the alteration of sewer precipitates (HUANG et al. 1993, INCE et al. 1999, BRILLAS et al. 2000, KRZEMIENIEWSKI et al. 2004). Numerous literature positions confirm the usefulness of the use of AOP to removing the colours, the odour, the reduction of organic substances and the degradation of toxic substances (PARK et al. 1999, RIVAS et al. 2001, CHAMARRO et al. 2001). Moreover, the researchers (BARBUSIŃSKI et al. 1999, BIGDA 1995) show the possibility of treatment of toxic and hard-decomposable sewages by the help of these techniques. It is mentioned here that the sewages from the production of polymers including the phenol or the formaldehyde, chemicals, medicine, insecticides, dye, explosive material, waste from refinery and the station of fuels, and also from the specialist uses of the chemicals, how e.g.: the impregnation of the wood, the production of artificial materials and adhesive means.

Among factors which are responsible for generating the free radicals OH*, the hydrogen peroxide is the most widespread. The hydrogen peroxide is strongly endothermical compound, which dissolves in every proportion of water. In room temperature it decomposes into water and oxygen. The use of the hydrogen peroxide as an oxidant is connected with a lot of benefits such as: the accessibility in trade, the thermal stability, possibility of stockpiling in the place of application, very good dissolubility in the water and the effective source of the hydroxyl radicals OH*. These adventages make the hydrogen peroxide to be often taken into consideration as a promising, cheep and and effective oxidative factor (WĄSOWSKI et al. 2002).

The SMF is recognized as an expediter process element of sewage treatment. The researches relating definitions of influence SMF on improvement of the effectiveness and intensification of radical-process, do confirm this. The growth of oxidative power of the peroxide hydrogen and in consequence the improvement the final effect of degradation pollutions was the result of the introduction of SMF to technological construction (KRZEMIENIEWSKI et al. 2002, KRZEMIENIEWSKI et al. 2003, KRZEMIENIEWSKI et al. 2004).

The definition of possible use of SMF as a helping factor of the AOP with use of hydrogen peroxide in degradation processes of organic compounds, coming from trade meat sewages, was the aim of the test.

Materials and Methods

For the realization of the experiment was used the raw sewages coming from meat industry institution (Table 1). Sewages were characterized by the red dye and high indicator dry residue in which over 70% of organic compounds took part in.

Table 1

The characteristic of raw sewages which were used in the experiment

The parameter	The unit	The average value	The minimum value	The maximum value
BOD_5	mg O_2 dm^{-3}	612	590	634
COD	mg O_2 dm^{-3}	926.45	843.8	983.9
Suspension solids	Mg dm^{-3}	410	384	436
Fats	Mg dm^{-3}	193	180	206
Nitrogen organic	Mg dm^{-3}	34	30	38
Phosphates	Mg dm^{-3}	7	6.6	7.4
Dissolved substance	Mg dm^{-3}	850	831	869
Reaction	PH	7.1	7.0	7.2

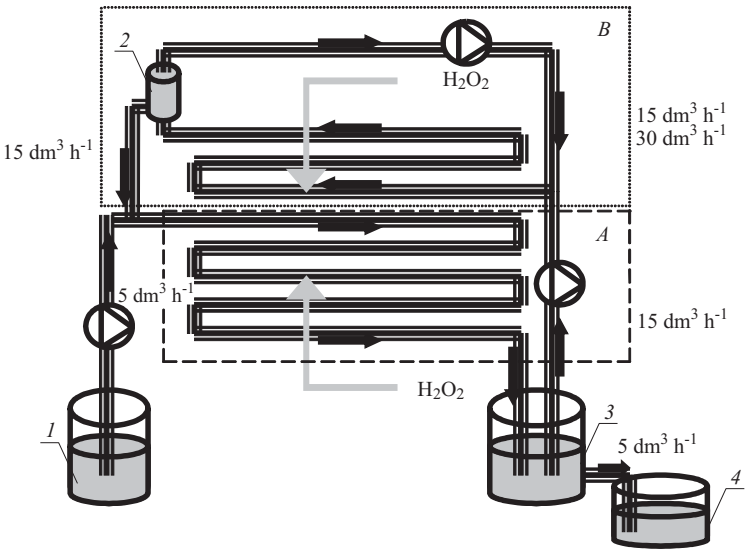

Fig. 1. The schema of flow arrengment of used in the first stage of researches: *A* – the first zone, *B* – the second zone, *1* – the tank with raw sewages, *2* – the tank of separation, *3* – the sediment tank, *4* – the tank with purified sewages

The experiment was realized in two stages in laboratory scale in temperature surroundings of 15–18°C. The influence of the hydrogen peroxide on the process of the sewage treatment was defined in the first stage. The simultaneous application of the process of advanced oxidation process and solid magnetic field were analysed in the second stage of the experiment.

In the first stage of researches the reactor consisted of two the functionally separated zones (Figure 1). The principle process of the sewage treatment

which was based on the oxygenation of the pollutions by the use of the hydrogen peroxide and hydroxyl radicals were in the first zone (A). The sewages treatment and probable generating hydroxyl radicals OH* were in second zone (B). The raw sewages flew into the first zone, in which the hydrogen peroxide was dosed to oxidate the pollutions and initiate the reaction of formation of free radicals OH*. Purified sewages flew into the sediment trap.

Sewages from the settling tank flew into the second zone, in which the hydrogen peroxide was also dosed. The sewages flew then into the tank of separation, the part of which returned to the second zone. The regular circulation and generating of the free radicals OH* in the second zone are the effects of the operation. The part of sewages from tank separation was directed to the place of flow the raw sewages to intensify the transformations in the first zone.

In the experiment the 30% of liquor of hydrogen peroxide was given to the construction. It was dosed to the construction throughout the duration of the cycle research. The series of researches differed from the efficiency of the flow in the second zone (Table 2).

Table 2

The hydraulic parameters which were applied in the first stage of researches

Series	The efficiency of flow in the first zone ($dm^3\ h^{-1}$)	Total dose of hydrogen peroxide ($cm^3\ dm^{-3}$ of sewages)	The efficiency of flow in second zone (closed cycle) ($dm^3\ h^{-1}$)	The efficiency of flow to second zone ($dm^3\ h^{-1}$)
A	5	1	15	15
B	5	1	30	15

Having aim at realizations of the second stage of the researches the model flow construction was equipped in physical factor in the aspect of SMF. In second stage there were applied identical hydraulic parameters like in the first stage. In order to generate SMF installation to magnetic activation of liquids (MLA) was used. Two magnetic installations of RWE-S type were situated in the second zone, so that the sewages crossed the line of magnetic field. The first magnetic installation was placed in the distance of 0.2 m from the place of dosage of the hydrogen peroxide, whereas the second was placed before the separation tank (Figure 2).

The applied magnetic installation consist of the suitable number of the rectangular prism of magnetic ceramic stakes, located radially to axle of the line of the matmonomial field poles S, opposite N directed outside (Table 3). Thanks to this kind of construction flowing through the pipe of medium it affects only the South magnetic pole, the field of which is concentrated on the axled of the flow.

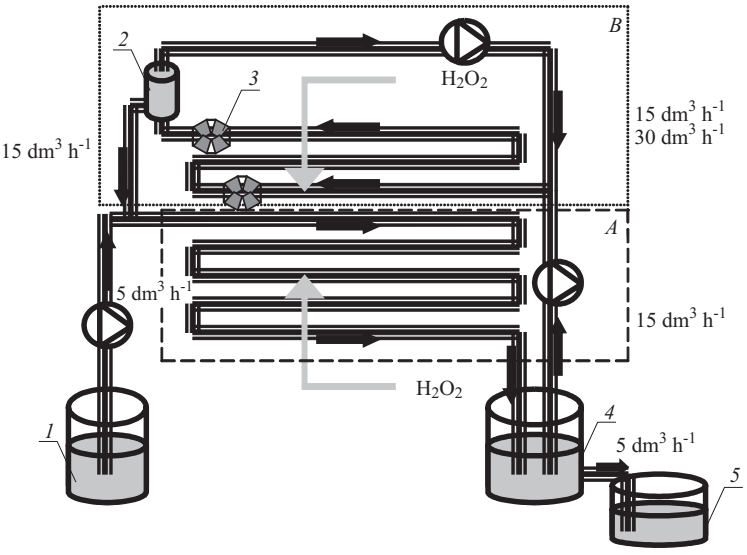

Fig. 2. The schema of flow arrengment of used in the second stage researches: *A* – the first zone, *B* – the second zone, *1* – the tank with raw sewages, *2* – the tank of separation, *3* – the magnetic liquid activator, *4* – the sediment tank, *5* – the tank with purified sewages

Table 3

The technical data of MAL used in the presented researches

The parameter	The value
Width of halo	45 mm
Height of single ceramic magnet	45 mm
Mass of single halo	1.25 kg
Range of nominal diameters	2 1/2"–3 1/2"
Range of outside diameter of pipeline	75–95 mm
Magnetic induction	6000 Gausses

Every series in research stage went on 120 minutes. The materials to chemical analysis were taken from sediment trap after 0 min., 30 min., 60 min., 90 min., 120 min., the continuance of research series. The folowing marks were made: the reaction, content of organic substance expressed as COD (the method bichromate) and the content of residual hydrogen peroxide (the method iodinemetrical).

It is claimed analytically that the value of COD was corrected in the case of affirmation of the presence of residual hydrogen peroxide on the ground the relativeness:

$$COD\ (mg\ O_2\ dm^{-3}) = COD_M - d \cdot f$$

where:

COD_M – the value of COD defined on the base of bichromate method (mg O_2 dm^{-3}),

d – concentration residual H_2O_2 in the sample defined by the iodinemetrical method (mg H_2O_2 dm^{-3}),

f – coefficient corrective 0.25 (for range 20–1000 mg H_2O_2 dm^{-3}).

Results

Received effect of purification in this stages of research was dependent from applied efficiency of flow in the second zone. The highest efficiency of purification was affirmed at efficiency of flow Q = 30 dm^3 h^{-1} (Figure 3). The COD reduction value was obtained in the rate 42.8% and in sewages purified value COD totaled 530.1 mg O_2 dm^{-3}. The speed of removal the carbon substances from sewages (r) totalled 13.6 mg O_2 dm^{-3} min^{-1}. But at the flow totalling 15 dm^3 h^{-1} the lowest efficiency was obtained the reduction of carbon compounds concentration expressed COD in the rate 41.8% (Figure 4). In purified sewages has noted down value COD in height 539.0 mg O_2 dm^{-3}. The speed of removal the carbon substances from sewages (r) totalled 12.3 mg O_2 dm^{-3} min^{-1}.

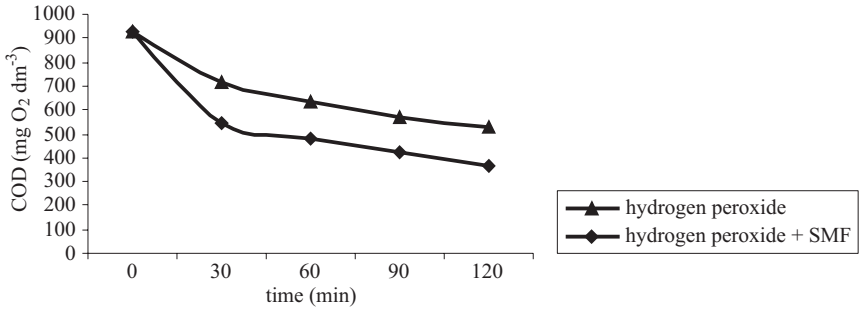

Fig. 3. The change of COD value in the first and second stage of researches, by the use of efficiency of flow totalling 30 dm^3 h^{-1} in the second zone

The visible improvement of results the sewage treatment was affirmed in second stage researches. In every research series was observed the clear influence of physical factor (SMF) on efficiency of reduction the value COD. Like in the first stage the highest result of purification was obtained by the use

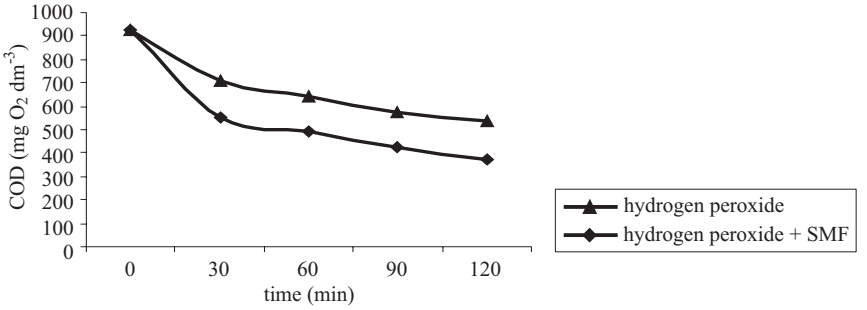

Fig. 4. The change of COD value in the first and second stage of researches, by the use of efficiency of flow totalling 15 dm^3 h^{-1} in the second zone

the efficiency of flow 30 dm^3 h^{-1}. It was higher about 17.5% than in the first stage. The speed of reaction (r) in this technological variant totalled 14.7 mg O_2 dm^{-3} min^{-1}. The value in purified sewages has noted down COD in the level of 367.3 mg O_2 dm^{-3}. In the second stage by the use of flow efficiency 15 dm^3 h^{-1} was obtained the reduction of the value COD in rate 59.5% which totalled 375.2 mg O_2 dm^{-3}. The speed of removal of carbon substances from sewages totalled 12.5 mg O_2 dm^{-3} min^{-1}.

It was affirmed, that the quickest and the most effective reduction of the concentration of carbon substances, took place within the first 30 minutes after staying the sewages in construction. In the first stage of research of the efficiency of the purification after 0.5 h totalled in both series over 23%. But in the second stage, where the action of the hydrogen peroxide was supported by SMF the efficiency of purification after 30 minutes totalled over 40%.

The statistical analysis of given results was conducted with the use of the monofactor of variantional analysis, at established level of significance ($p < 0.05$). The normality of expansion was confirmed by the use of the test Szapiro-Wilk, however hypothesis about variantional homogeneity in groups was verified on the base of Leveney's test. The investigation differences between average from individual groups was conducted applying the test RID (the reasonable intrinsic differences).

The permanent speeds of reaction is marked on the base of experimental data by the method of regress. Iterative method in the program was used. In this method in every iterative step the function was replaced according to linear differential appointed parameters. As a measure of adjustment the bow line (at appointed parameters) to experimental data was accepted as a coefficient of compatibility φ^2. The adjustment of the model to the experimental points was accepted in order not to cross the value 0.2 of the coefficient of compatibility φ^2.

Discussion

Wastewater used in the experiment have predispositions to treatment them by the use of the Fenton's reaction. It is based on the catalytic degradation H_2O_2 by the use of the ions Fe^{2+} or Fe^{3+} (BALCIOGLU et al. 2001, SIGGE et al. 2001). There is blood in the wastewater, mainly coming from the process of the slaughter and disemboweling. It might be used as the source of the catalyst – the Fe^{2+} ion exists in hemoglobin. Supposedly this property was used in the presented investigations.

Among the numerous literature we can find the successful tests of wastewater treatment by using of the hydrogen peroxide and/or Fenton's reagent. The author (KOS et al. 2001) informs us that the value of the COD was lowered about 90% by using the hydrogen peroxide in textile wastewaters, coming from the process of dying the cotton, polyester and polyurethane. The other author (KUO 1992) got such proportional of removal efficiency of carbon substances with using the Fenton's reagent by wastewater treatment coming from dyehouse. In both cases such a high reduction of the value of the COD occurs in very drastic conditions of the reaction. For the proper course of the process of the oxidation reaction required wastewaters acidification to pH ~ 3.0. In the similar conditions of the reaction (pH ~ 3.0) concentrated industrial wastewater were treated characterizing of COD within limits from 1700 to 11,000 mg O_2 dm^{-3}. The reduction of the value of COD of wastewater came to 98%. However the growth of the wastewater salinity was the shortcoming of the process as a result of acidifiacation and neutralization (GILBERT 1984). The reduction of the value COD from 41 to 60% was received in presented researches. The lower efficiency of the process results from the conditions of carrying out the experiment. Only one chemical reagent (H_2O_2) was dosed to the technological arrangement. The reaction of the oxidation took place in the neutral reaction conditions pH ~ 7.0. The aim of the achievement of the high removal efficiency of organic effluents (above 90%), is preferred to be the range pH of 3.0–4.0 and the introduction of additional reagents e.g. the ions Fe^{2+} or Fe^{3+}.

The qualification of the possibility of the use of SMF as the factor helping advanced oxidation reactions was also aim of authors in the presented experiment. Earlier experiences present the proofs of the intensification of the advanced oxidation reaction by the use of SMF (KRZEMIENIEWSKI et al. 2004).

The most widespread association of methods is the processes with the use: UV/H_2O_2, UV/O_3, $UV/H_2O_2/O_3$. By associating methods, on the one hand we get the better degree of the effluents degradation,on the other hand we can reduce the doses of oxidizing factors and to lower the costs of the wastewater treatment (KOS et al. 2001).

Wastewaters coming from the meat industry belong to turbid and colourful solutions which makes the use impossible of the UV radiation as the factor catalyzing Fenton's reactions. Because of this in the presented experiment the SMF was also used as a helping factor in the effective course of the advanced oxidation process.

Hypotheses introduced by MCLAUCHLAN et al. (1991) and JAJTE et al. (2002) in the medical literature were basis for the study of this technology. Authors concentrated in their investigations on the qualification of influence electromagnetic fields (PEM) on the behaviour of reactive oxygenic forms.

The SMF can influence on the parameters of treated solutions and modify them (KRZEMIENIEWSKI et al. 2003). The cause of the changes of the flowing liquid property through the magnetical field can explain as follows: the concentrated magnetical field about the suitably well-chosen height of the induction changes the molecular structure of liquids. This results in the function zone of the external magnetical field appear the following: selective ionization, circulatory eddy currents, the electric fields and internal magnetical fields, the change in the profile of speed and pressure, the electric conductivity of liquids and also additional magnetical moments. The polarization, the settlement of molecules and addition them of the positive load also follows. As a result in the zone of the contact of liquid magnetization with the air follows the process of the ionic and magnetical attraction of oxygen (SZCZYPIOROWSKI et al. 1995).

The A.R. Davis' scientific discoveries and the continuation of reserch by P. Kulisha prove that different it is the influence of individual poles N and S of the magnet on liquids and alive organisms. In the monopole magnetical field (to that of similar polarity) the change of the property of liquids are selective and more clear (SZCZYPIOROWSKI et al. 1995). The change of the liquid property takes place first of all under the influence of the south pole (S). This phenomenon is used in the new generation of the magnetizers of the type RWE-S, which was used in the presented experiment.

KRZEMIENIEWSKI et al. 2002 proved on the basis of the changes of the sodium sulphite concentration in the deoxidized solution the way the magnetical field essentially influences on the quantity of generated hydroxyl radicals in the Fenton's reaction. The effects of dairy wastewaters pretreatment received by the author (KRZEMIENIEWSKI et al. 2002) also testify about the profitable combination of the Fenton's reaction and magnetical field. He affirmed about the effective way of oxidation of the organic substance use which took place by the imediate of the hydrogen peroxide and hydroxyl radicals. The considerable influence on the final effect of the treatment had also an impact of the magnetical field which improved the process of making radicals and could modify the parameters of wastewaters treatment in the spontaneous way. The magnetization wastewaters are characterized with the reduced surface tension

and in the contact with the atmosphere they absorb the paramagnetic particles of oxygen, as a result of it concentration in solutions increase.

The profitable effect of the combination of the hypothetic Fenton's reaction and magnetical field was noticed in the represented experiment, as well. The impact of the magnetical field influenced on the speed and quantity of generated hydroxyl radicals which was manifested by the higher reduction of the organic substance. The differences were significant and in the case of the reduction COD was 17.5%. Achieved results induce to continuation of the research concering the uses of the Fenton's reagent helped by the magnetical field to the wastewater treatment coming from the meat industry.

Conclusions

The efficiency of the degradation of organic substances depended on the applied efficiency of the flow in the second zone in the presented experiment. In the first stage of the received reduction of the organic matter was expressed, according to COD, having its place in all investigative series. The largest percentage of the organic effluent removal was observed by the flow efficiency of 30 dm^3 h^{-1}.

It was also affirmed that the intensive degradation of the organic substances took place in the first 30 minutes of the oxydation process.

The use in the II stage of SMF influenced on the intensification of the advanced oxidation process. Utilization of SMF improved in all analysed series wastewater treatment efficiency above 17.5%. The influence of SMF the most clearly visible is in the investigations series in which the flow efficiency of 30 dm^3 h^{-1} is applied. Efficiency decrease of the value of COD in this technological variant is equal to 60.3%. The experiment showed that there is existed the possibility of behaviour the treatment effect in the case of the use of MLA by the use of considerably smaller doses of chemical reagent.

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THE INFLUENCE OF SOLID MAGNETIC FIELD (SMF) ON PSEUDO-FENTON'S REACTION OF EFFICIENCY IN MEAT INDUSTRY SEWAGES TREATMENT

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K e y w o r d s: pseudo-Fenton's reaction, solid magnetic field (SMF), advanced oxydation process (AOPs), meat industry sewages.

A b s t r a c t

The possibility of solid magnetic field (SMF) used as a catalysator of radical process in pseudo--Fenton's (Fe^{3+}/H_2O_2) reaction was invesigated in this experiment. Physical factor influence was qualified indirectly on the technological effect basis of received of reduce of COD value in sewages coming from the meat industry. The research was divided into three stages on laboratory scale. The influence of Fe^{3+}/H_2O_2 was defined in first stage, whereas the influence of SMF was qualified in the second stage. The effect of simultaneous use of the advanced oxydation process (AOPs) and SMF in carbon substances degradation was researched in the third part of the experiment. The physical factor was generated by the magnetic heaps, located in magnetic liquids activator (MLA). SMF intensity applied in the experiment, comprised in levels of 0.4–0.6 T.It was affirmed that the SMF use as a helping element of AOPs allowed to get higher technological effect in the range of all tested doses of pseudo-Fenton's reagent.

WPŁYW STAŁEGO POLA MAGNETYCZNEGO NA EFEKTYWNOŚĆ REAKCJI PSEUDO--FENTONA W PROCESIE OCZYSZCZANIA ŚCIEKÓW Z PRZEMYSŁU MIĘSNEGO

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S ł o w a k l u c z o w e: reakcja pseudo-Fentona, stałe pole magnetyczne (SPM), pogłębione utlenianie (AOP), ścieki z przemysłu mięsnego.

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Abstrakt

W eksperymencie przebadano możliwość wykorzystania stałego pola magnetycznego (SPM) jako katalizatora procesu rodnikowania w reakcji pseudo-Fentona (Fe^{3+}/H_2O_2). Wpływ czynnika fizycznego określono na podstawie uzyskanego efektu redukcji wartości ChZT w ściekach pochodzących z zakładu przemysłowego z branży mięsnej. Badania w skali laboratoryjnej podzielono na trzy etapy. W pierwszym etapie określono wpływ reagentów chemicznych Fe^{3+}/H_2O_2, w drugim – SPM. W trzeciej części eksperymentu analizowano efekt jednoczesnego wykorzystania procesu pogłębionego utleniania (AOP) oraz SPM w procesie degradacji substancji węglowych. Czynnik fizyczny generowany był przez stosy magnetyczne magnetycznego aktywatora płynów (MAP). Natężenie SPM mieściło się w granicach 0,4-0,6 T. Stwierdzono, że zastosowanie SPM jako elementu wspomagającego AOP pozwoliło uzyskać wyższy efekt technologiczny w zakresie wszystkich testowych dawek odczynnika pseudo-Fentona.

Introduction

In last years, a lot of attention is paid to the research implementation of investigations and initiation advanced oxygenation methods (AOPs). Generating of high reactive hydroxide radicals ($OH^{\bullet}$) having 2.8 V oxidizing potential, which come in reactions with almost all organic compounds is the common feature of this kind of techniques. Effluence decomposition of quick pace, generality and high efficiency, inflict that AOPs are often taken into the consideration as a promising, alternative, in the relation to conventional methods, sewage and sewage sludge treatment techniques (HUANG et al. 1993, INCE, TEZCANLI 1999, BRILLAS et al. 2000, KRZEMIENIEWSKI et al. 2004).

The hydroxide radicals are generated among others, under the influence of simultaneous use of ozone and hydrogen peroxide (O_3/H_2O_2 – *Peroxone*) or ozone in the alkaline medium (O_3/OH^-). Fenton's reaction arouses largest interest among investigators. It is base on catalytic degradation H_2O_2 by the use of ions Fe^{2+} or Fe^{3+} (BALCIOGLU, ARSLAN 2001, SIGGE et al. 2001). This fact is connected directly with technological effectiveness, applying easiness, chemical reagents accessibility and economic regards.

Having an aim to improve the radicals $OH^{\bullet}$ creating efficiency have been worked out the modifications and improvements of the classical Fenton's reaction. They are often based on physical factors introducing to the technological arrangements. We can classify to them UV-Fenton's reaction with the use of UV radiation and electro-Fenton's, by the co-operation of electrochemical processes. The introduction of UV radiation to the technological arrangement let us perform the direct photo-reduction ions Fe^{3+} to Fe^{2+} in simultaneous generating of radicals $OH^{\bullet}$ (SUN, PIGNATELLO 1993, PIGNATELLO, SUN 1995, MCGINNIS et al. 2000).

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + OH^{\bullet} + H^+$$

Fe^{2+} ions can react with hydrogen peroxide according to the classical Fenton's reaction, generating new radicals $OH^{\bullet}$, which leads to increase of efficiency of effluents degradation process (ENGWALL et al. 1999, ACERO et al. 2001).

In the last years there was also conduced researches concerning the usage of electrochemical processes in the Fenton's reaction (electro-Fenton's method; EF). Generally we can divide these investigations into three groups. In the first group (EF-H_2O_2) we use the ions Fe^{2+} and electrochemically generated hydrogen peroxide (BRILLAS et al. 2000). The second group (EF--Feox) uses the hydrogen peroxide and electrochemically generated ions Fe^{2+} by the oxydation of iron anode (HUANG et al. 1999, ARIENZO et al. 2001). The modification about the name EF-Fere consists in the use of the titanic anode which is covered with the oxide shell (RuO_2/lrO_2) (CHOU et al. 1999). The third group (FSR – Fenton's sludge recycling system) contains the Fenton's reactor with electrolyser to iron hydroxide reduction to ferrous ions (HUANG et al. 1999, CHOU et al. 1999).

The factor, which is more and more taken under the consideration as the an influent element on an intensification and improvement of the effectiveness of the radical process, is the solid magnetic field (SMF). Mechanisms and responsible dependences for improvement of AOPs with the use of SMF as a catalyst of the process have not been explained and described so far. Many hypotheses occure to speak about the possible influence of this physical factor for more intensive way of creating and providing for concentration $OH^{\bullet}$ in biological arrangements, mainly (MCLAUCHLAN, STEINER 1991, JAJTE et al. 2002, KRZEMIENIEWSKI et al. 2004).

There is a real need for carrying out researches aiming to explain the chemical and physical factor co-operation mechanism and the definition of conditions allowing to get effective final results. The aim of the experiment was definition of possibility of the use of SMF as an intensifying AOPs factor with using pseudo-Fenton Fe^{3+}/H_2O_2 reagent in organic compounds degradation processes (COD) in sewages coming from the meat industry.

Materials and Methods

Raw sewages coming from meat industry (Table 1) were used in investigations.

The experiences were conducted in three stages, in surroundings temperature above 20°C, by the use of different investigative positions.

The influence of chemical reagents (Fe^{3+}/H_2O_2) on the sewage treatment process was definied in the first stage. The model laboratory reactor, which

Table 1

The characteristic of sewages which were used in the experiment

The parameter	The individual	The average value	The value min.	The value max.	The deviation stand.
BOD_5	mg O_2 dm^{-3}	612	590	634	31.11
COD	mg O_2 dm^{-3}	880	858	902	31.11
Total suspended solids	mg dm^{-3}	410	384	436	36.77
Fats	mg dm^{-3}	193	180	206	18.38
Organic nitrogen	mg dm^{-3}	34	30	38	5.66
Phosphates	mg dm^{-3}	7	6.6	7.4	0.56
Dissolved substances	mg dm^{-3}	850	831	869	26.87
The reaction	pH	7.1	7.0	7.2	0.14

is characterized by the active volume 1.0 dm^3, equipped in magnetic stirrer was used for this aim (Figure 1). The chemical reagents were dosed directly to the reactor only once at the beginning of the experimental cycle, preserving solid proportion of Fe/H_2O_2 which was 1:6. The source of Fe^{3+} ions was 40% solution $Fe_2(SO_4)_3 \cdot 14\ H_2O$ (PIX), and H_2O_2 was as 30% solution (perhydrol). At first Fe^{3+} ions were dosed to the raw sewages and after 10 minute time of stirring, H_2O_2 was added to sewages in order to initiate the Fenton's reaction. Sewage retention time in the technological arrangement lasted 2 h. During this part of experience sewages were mixed intensively.

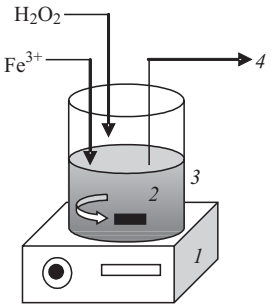

Fig. 1. The scheme of research arrangement in the first stage of investigations: *1* – magnetic stirrer, 2 – raw sewage, *3* – reactor, *4* – taking sewage samples

The next series of this investigation stage differed from the used of the pseudo-Fenton's reagent doses. Investigations were conducted for six increasing reagents' portions (Table 2). The reagents' portions were established on the basis of preliminary investigations and literature data (KRZEMIENIEWSKI et al. 2004). During of this part of the experiment sewages were intensively mixed.

Table 2

The chemical reagents doses used in the experiment

Series	The dose Fe^{3+} (g dm^{-3})	The dose H_2O_2 (g dm^{-3})
1	0.05	0.3
2	0.10	0.6
3	0.15	0.9
4	0.20	1.2
5	0.25	1.5
6	0.30	1.8

Stage II related to the definition of SMF by direct influence on the effectiveness of carbon substances degradation. In this part of the experiment the installation to magnetic liquid activation (MLA) was applied (Table 3). MLA consisted of steel, cylindrical trunk and magnetic heap. Magnetic heap made from the durable magnets generated SMF with solid magnetic induction with intensity on levels 0.4–0.6 T.

Table 3

The technical dates of MLA applied in the experiment

Parameters	The series I
Total length	240 mm
Nominal pipe diameter	30 mm
Volume	0.7 dm^3
Magnetic induction level	0.4–0.6 T
Weight	2.0 kg

Sewages were in the reservoir with a capacity of 1.0 dm^3 and were led to the magnetizer with the help of peristaltic pump with efficiency of 0,5 dm^3 min^{-1} (Figure 2) which assured hydraulic mixing of sewages.

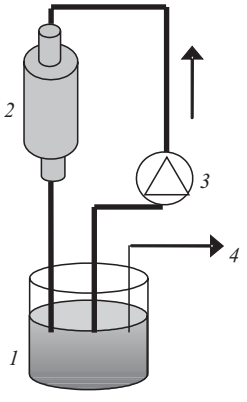

Fig. 2. The scheme of research arrangement in the second stage of investigations: *1* – reactor, *2* – magnetic liquid activator (MLA), *3* – peristaltic pump, *4* – taking sewage samples

The chemical (AOPs) and physical SMF factor was connected in the third stage. For this aim to the reactor with sewages was introduced $Fe_2(SO_4)_3$ and H_2O_2, in identical quantities like in the first stage, and then the medium was introduced to the influence area of SMF (Figure 2). In the next series of the third stage differed chemical reagents doses (Table 1).

Sewage retention time in technological arrangement lasted 120 minutes in all experience stages. Sewage samples were taken after 0, 30, 60, 90 and 120 minutes, from reactors directly. Sewages before executing chemical analyses were subjected 30 minutes of sedimentation. The content of the organic compounds expressed according to COD (dichromate method) and the content of the residue hydrogen peroxide (iodometric method) were marked in clear sewages. It is claimed analytically that the value of COD was corrected in the case of affirmation of the presence of residual hydrogen peroxide on the ground the relativeness:

$$\text{COD (mg } O_2 \text{ dm}^{-3}) = \text{COD}_M - d \cdot f$$

where:

COD_M – the value of COD defined by the dichromate method (mg O_2 dm^{-3}),
d – concentration residual H_2O_2 in the sample defined by the iodometric method (mg H_2O_2 dm^{-3}),
f – coefficient corrective 0.25 (for range 20–1000 mg H_2O_2 dm^{-3}).

Results

It was affirmed in the first stage in all analyzed investigative series, that the biggest changes of the COD value received after first 60 minutes of sewages retention in the arrangement. After this time, in dependence on the applied chemical reagents dose, the efficiency of treatment was recived on the level from 22.41% to 49.18%. The insignificant of COD value changes in treatment sewages were in the later periods of time.

Treatment efficiency, recived in the first stage, directly depended on the applied doses of chemical reagents. The highest, final technological effect was affirmed in the third series in which 0.15 g Fe^{3+} dm^{-3} and 0.9 g H_2O_2 dm^{-3} was used and recived 52.99% of COD value efficiency of decrease and concentration on the level of 324.6 mg O_2 dm^{-3} (Figure 3). The speed of removing of carbon substances from sewages (r) was formed on the level of 32.55 mg O_2 dm^{-3} min^{-1} (Table 4). The use of Fe^{3+}/H_2O_2 doses in quantities 0.10 g Fe^{3+} dm^{-3}; 0.6 g H_2O_2 dm^{-3} and 0.20 g Fe3 + dm^{-3}; 1.2 g H_2O_2 dm^{-3} allowed to 46.0% limitations of carbon substances concentration. Final technological effect received in the

second serie was 475.1 mg O_2 dm^{-3} (r = 28.20 mg O_2 dm^{-3} min^{-1}), in the fourth serie was 475.9 mg O_2 dm^{-3} (r = 20.25 mg O_2 dm^{-3} min^{-1}). The lowest technological efficiency was noted down in the serie VI, when the highest, tested chemical reagents doses were introduced to the reactor. After 120 minutes the retention of sewages in the arrangement on the outflow affirmed 653.4 mg O_2 dm^{-3}, which was 25.75% of COD removal efficiency. The noted down reaction speed, in this technological variant was r = 8.90 mg O_2 dm^{-3} min^{-1} (Table 4).

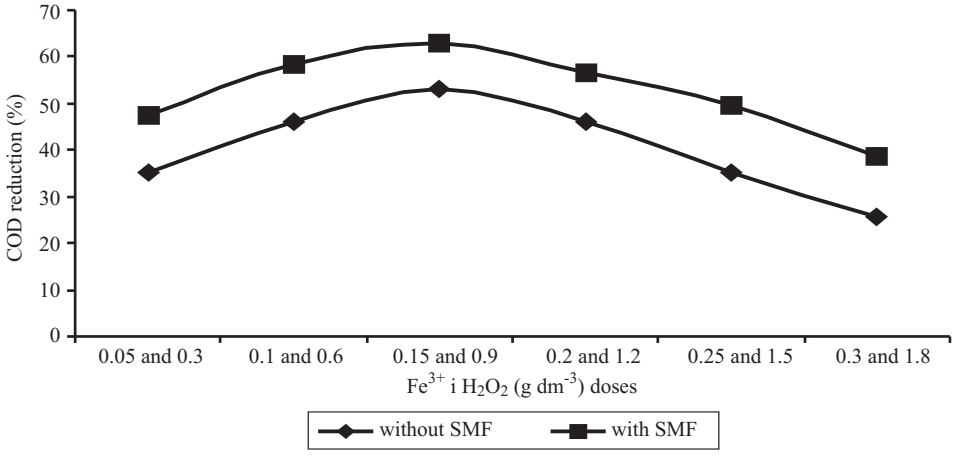

Fig. 3. Comparison of COD reduction with or without SMF using

Table 4

The value of speed constant k and reaction speed r which was received in the series of experiment

Series	Reagents dose (g dm^{-3})		Speed constant k (1 min^{-1})		Reaction speed r (mg O_2 dm^{-3} min^{-1})	
	Fe^{3+}	H_2O_2	Fenton's reagent	Fenton's reagent + SMF	Fenton's reagent	Fenton's reagent + SMF
1	0.05	0.3	0.05	0.03	15.9	12.3
2	0.10	0.6	0.07	0.06	28.2	30.7
3	0.15	0.9	0.07	0.07	32.55	38.92
5	0.20	1.2	0.05	0.07	20.25	35
5	0.25	1.5	0.04	0.05	12.5	21.9
6	0.30	1.8	0.04	0.04	8.9	13.5

The SMF introduction to the technological arrangement allowed to an intensification of advanced oxydation reaction and essential improvement received of the technological effects. This dependence was observed in all analyzed experimental variants. The clearest physical factor influence was noted down in the series V, when 0.25 g Fe^{3+} dm^{-3} and 1.50 g H_2O_2 dm^{-3} was dosed to the technological arrangement. The removal efficiency of COD from sewages in the arrangement of SMF was about 14.46% higher, than in the variant testing only chemical reagents. The physical factor stimulating influence was also confirmed defining by COD removal reaction speed from the arrangement. In the third stage the value of r was 21.90 mg O_2 dm^{-3} min^{-1}, while in the variant without the use of SMF – 12.50 mg O_2 dm^{-3} min^{-1}. In all analyzed experiment variants, the efficiency of arrangement equipped in the MLA was over 10.0% higher than systems Fenton's reaction only (Table 5).

Table 5

Comparison of COD reduction received during the experiment

Fe^{3+} i H_2O_2 dose (g dm^{-3})	COD reduction (%) without SMF	COD reduction (%) with SMF
0.05 and 0.3	35.07	47.48
0.1 and 0.6	46.01	58.52
0.15 and 0.9	52.99	63.11
0.2 and 1.2	45.92	56.78
0.25 and 1.5	35.17	49.69
0.3 and 1.8	25.79	38.58

Only in the lowest chemical reagents doses use, in the quantity 0.05 g Fe^{3+} dm^{-3} and 0.3 g H_2O_2 dm^{-3} noted down COD reduction speed from the technological arrangement was higher in the first stage of research and was r = 15.90 mg O_2 dm^{-3} min^{-1}. In the third stage the use of analogical Fenton's reagent dose and SMF let to obtain the speed of reaction of 12.30 mg O_2 dm^{-3} min^{-1}.

Statistically essential final effects were not affirmed after the use of SMF in the experiment as the only factor influencing on the change of organic substances concentration expressed by COD coefficient. The statistical analysis of given results was conducted with the use of the monofactor of variantional analysis, at established level of significance ($p < 0.05$). The normality of expansion was confirmed by the use of the test Szapiro-Wilk, however hypothesis about variantional homogeneity in groups was verified on the base of Leveney's test. The investigation differences between average from individual groups was conducted applying the test RID (the reasonable intrinsic differences). The permanent speeds of reaction is marked on the base

of experimental data by the method of regress. Iterative method in the program was used. In this method in every iterative step the function was replaced according to linear differential appointed parameters. As a measure of adjustment the bow line (at appointed parameters) to experimental data was accepted as a coefficient of compatibility φ^2. The adjustment of the model to the experimental points was accepted in order not to cross the value 0.2 of the coefficient of compatibility φ^2.

Discussion

To the factors which help effectively generating of $OH^{\cdot}$ in the Fenton's reaction, except Fe^{3+} ions there are also lots of physical catalyzing elements (LU et al. 1994, CHOU et al. 1999, BRILLAS et al. 2000). In order to obtain the better effect of wastewater treatment the working of H_2O_2 is connected with O_3, radiation of UV or gamma (PIGNATELLO, SUN 1995, MCGINNIS et al. 2000, MARTI et al. 2001). Applying of these types of catalysts encounter with on large difficulties very often. It was affirmed, that in some cases of applying of UV radiation there comes a necessity of dosing of larger portions of H_2O_2. However the use of gamma radiation creates some large exploational problems and requires applying of the highly specialized apparatus.

Because of this the attention was paid to a replacement of these factors by SMF (KRZEMIENIEWSKI et al. 2002, KRZEMIENIEWSKI et al. 2003, KRZEMIENIEWSKI et al. 2004). This is a new, original technology the bases of which were mentions and hypothesis in the medical literature relating to the influence of electromagnetic fields (PEM) for the preservation of the reactive oxygenic forms (JAJTE et al. 2002, MCLAUCHLAN, STEINER 1991). The use of SMF as a catalyzing factor in the Fenton's reaction is particularly justified in the case of turbid and colorful solution treatments to which one can classify the meat industry sewages with the high blood concentration. Such kind of properties are limited, the possibility of using the different types process helping factors e.g.: UV radiation. Moreover, the SMF can modify an influenceon the parameters of the treated solutions spontaneously (KRZEMIENIEWSKI et al. 2003).

It was affirmed, that free radicals are created in ripping bonds of homolytic processes or in the result of electrons transfering among the particles of chemical substances. Free radicals possess one or more non-paired electrons the spin quantum number of which can rise the value either -1/2 or +1/2. When the two free radicals paired to each other their non-paired electrons can have the same spin which we define as a triplet configuration or an opposed spin of the singlet configuration. Free radicals which possess the triplet configuration

cannot create bonds. They can be formed by the help of intersystem crossing to the singlet configuration which leads to the creation of the bond among their radicals. Such an ability also can show free radicals possessing non-paired electrons of having to that of opposed spins. The intersystem crossing can be inhibited by the electromagnetic field relatively weak. It causes to lowering of the efficiency of the intersystem crossing which leads to reducing of quantity of radicals of which in turn transform to the singlet configuration with simultaneous increase of their general quantity. Hence, the SMF is regarded as a factor which is able to generate free radicals (JAJTE et al. 2002, MCLAUCHLAN, STEINER 1991, KRZEMIENIEWSKI et al. 2004).

The influence of the SMF on rodical process genesis has been studied only in the medicine so far. Three hours; time lymphocytes exposition on SMF with an intensity of 7 mT in the presence Fe^{2+} ($FeCl_2$, 10 μg cm^{-3}) has clearly increased mortality cells. This effect was not observed in the case, when the SMF and ions Fe^{2+} were tested separately. These experience conclusions show the possibility of the physical factor stimulating influence on free oxide radicals generating in the case presence of Fe^{2+}. These highly reactive chemical substances with high strength oxidizing had direct influence on cellular lipids peroxidation, which led to the increased mortality of lymphocytes (JAJTE et al. 2002). It seems to be purposeful and well justified that the undertaken attempts of using of positive values of these experiences in sewage treatments, sewage sludge treatments and in different fields of environmental protection.

The SMF influence on the speed and quantity of hydroxide radicals generated in the Fenton's reaction, was qualified for the first time, on the base of sodium sulphite concentration changes in the deoxidized solution (KRZEMIENIEWSKI et al. 2002). In the first stage of the experiment the only Fenton's reagent is added to the sodium sulphite solution, whereas in the next part the whole of the solution was subjected to the SMF working. After 120 minute time of the reaction there was 74% of sulphite oxidation in the arrangement with SMF, whereas without any physical factor uses the received reduction of sulphites was about 25%. In the arrangement modified by the SMF, the quickness of the oxydation reaction was equal to r = -7.94 mg dm^{-3} min^{-1}. In case of using only chemical reagents the quickness of the oxidation reaction was hardly equal to r = -0.74 mg dm^{-3} min^{-1}. It turned out, that the SMF use enlarged oxydation speed over ten times, in relation to the arrangement with chemical reagents only (KRZEMIENIEWSKI et al. 2002).

About the profitable association of Fenton's reaction and SMF also testify the effects of various kind of sewages pretreatment. During performing of the advanced oxidation process, helped by the SMF was noticed to have a tendency of triple reduction of the chemical reagent use in case of unchanged effect of effluent reduction containing in the dairy sewages. Worse results were

received for sewages made from powdered whey (KRZEMIENIEWSKI et al. 2000). Actually the whole process was fast, and the SMF influence came out after a dozen of minutes of sewage exposition.

The use of the physical factor as an element influencing on creating free radicals process intensification, it also turned out to be lees efficient way of treatment such sewages as: domestic, brewery, sewages coming from wood industry and artifical sewage coming from food boullion (KRZEMIENIEWSKI et al. 2004). The highest value of speed reaction was noted down in the case of brewery (r = 52.20 mg dm^{-3} min^{-1}) and domestic (r = 51.30 mg dm^{-3} min^{-1}) sewage. The treatment process ran in the case of food bouillon sewage very slowly (r = 17.15 mg dm^{-3} min^{-1}). However, the use of this kind of catalyst caused to improvement of the sewage treatment efficiency in all analyzed cases. The experience shows the possibility of applying considerably smaller chemical reagent doses in the case of unchanged treatment efficiency, during the use of MLA (KRZEMIENIEWSKI et al. 2004).

The potential possibilities of SMF use as a formative factor was tested on the example of domestic and dairy sewage (KRZEMIENIEWSKI et al. 2004). In this experiment was analized the SMF direct influence on properties of sewage after 24 h and 48 h retention in the arrangement. The clear systematic organic substance, ammonium nitrogen and phosphates reduction was affirmed (KRZEMIENIEWSKI et al. 2004).

In the experiment relating to the influence of SMF as the only factor of influencing on the properties change of meat industry sewage wasn't confirmed this type of observation. Relatively short time of sewage retention in the arrangement, which exacltly lasted 120 minutes, was problably an influencing factor of limitation of SMF influence on technological effects. The assumption of the experiment was however the definition of the influence of the SMF of efectivness of advanced oxydation reaction, which in accordance with literature data runs efficiently with the period of cauple of minutes until a dozen of hours.

Conclusions

The efficiency of organic compounds degradation in the carried out experiment depended mainly on the applied reagents dose. Together with increasing doses of H_2O_2 and Fe^{3+} in the range from 0.05 g Fe^{3+} dm^{-3}; 0.30 g H_2O_2 dm^{-3} to 0.15 g Fe^{3+} dm^{-3}; 0.90 g H_2O_2 dm^{-3} higher percentage of the organic matter removal from sewages was observed. Testing higher Fenton's reagent doses did not influence on the improvement of the final technological effect.

The use of the SMF as an influencing factor on the advanced oxidation process intensification was effective in the whole range of applied doses

of chemical reagents. The use of this kind of physical catalyst improved the sewage treatment efficiency in all analyzed technological variants above 10%. The greatest SMF influence was affirmed, when 0.25 g Fe^{3+} dm^{-3} and 1.50 g H_2O_2 dm^{-3} was dosed to the technological arrangement. The efficiency COD removal from sewages in the arrangement was helped by strengths of SMF was about 14.46% higher, than in the variant testing only chemical reagent. The experience shows a possibility of applying considerably smaller chemical reagent doses preserving unchanged treatment efficiency, in the case of applying the magnetic liquid activators. The SMF introduction to the arrangement also influenced on the acceleration of organic effluents degradation process.

It was affirmed, that in all research stages the oxydation process was very quick. In analyzed sewages the intensive oxydation process was only in the first 60 minutes of the process. The longer reaction time did not cause COD reduction value in the applied experimental variants any more.

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EFFECT OF STOCKING DENSITY AND THREE VARIOUS DIETS ON GROWTH AND SURVIVAL OF EUROPEAN CATFISH (*SILURUS GLANIS* L.) LARVAE UNDER INTENSIVE REARING CONDITION

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K e y w o r d s: European catfish (*Silurus glanis* L.) larvae, stocking density, body weight, survival.

A b s t r a c t

Catfish frays were distribute on three groups: *A* – fish fed artificial diet, *B* – fish fed combined diet, *C* – fish fed natural food, during three weeks stocking under controlled condition. Two variants of density were applied in each experimental group – 7.5 (1) and 15 (2) ind dm^{-3}. Combine diet (natural food and trout starter) appeared the most effective. The mean body weight on termination of the experiment in these groups was 1203.5 and 815 mg (respectively in B_1 and B_2). Commercial trout starter was utilized readily and enough to obtain by larvae the mean body weight 848 mg and 966 mg in A_1 and A_2 respectively. Cumulative mortality was lowest in groups fed natural food, but poor growth ratio made impossible efficient intensive rearing. The experiment proved that 1) preliminary phase of rearing of European catfish larvae could relay on artificial food only, 2) natural food addition elevates survival and growth ratio, 3) Low initial stocking density limits cannibalism.

WPŁYW GĘSTOŚCI OBSADY I RODZAJU OFEROWANEGO POKARMU NA WZROST I PRZEŻYWALNOŚĆ LARW SUMA EUROPEJSKIEGO (*SILURUS GLANIS* L.) W WARUNKACH INTENSYWNEGO WYCHOWU

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S ł o w a k l u c z o w e: larwy suma europejskiego (*Silurus glanis* L.), gęstość obsady, masa ciała, przeżywalność.

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A b s t r a k t

Podczas 3-tygodniowego podchowu w warunkach kontrolowanych w temperaturze 28°C larwy suma europejskiego podzielono w zależności od oferowanego pokarmu na trzy grupy: *A* – karmione paszą komercyjną, *B* – karmione pokarmem mieszanym, *C* – karmione pokarmem naturalnym. W każdej z nich zastosowano dwa warianty gęstości obsady – 7,5 i 15 osobn. dm^{-3}. Najefektywniejsza okazała się dieta mieszana. Średnia masa osobników po zakończeniu podchowu wynosiła 1203,5 i 815 mg (odpowiednio B_1 i B_2). Stosowany w doświadczeniu starter pstrągowy okazał się wystarczający do osiągnięcia przez ryby średniej końcowej masy w gr. A_1 – 848 mg, a w grupie A_2 – 966 mg. Śmiertelność skumulowana była najniższa w grupach karmionych pokarmem naturalnym, ale osiągnięte przez ryby tempo wzrostu nie pozwoliło na efektywny intensywny podchów. Wyniki doświadczenia dowodzą, że we wstępnej fazie kontrolowanego podchowu karmienie może opierać się na pokarmie komercyjnym. Dodatkowe stosowanie pokarmu naturalnego poprawia przeżywalność i przyspiesza tempo wzrostu. Stosowanie rzadszych obsad początkowych pozwala natomiast uniknąć strat spowodowanych kanibalizmem.

Introduction

European catfish become popular commercially fish in Poland during through last years. As a valued consumption fish catfishes are farmed in policulture with carp or in monoculture in warm – water facilities. To support its occurrence in rivers and lakes catfishes are also farmed at the age 1–3 in fish farms and then restocked.

Great requirement for stocking material for further culture caused necessity of getting in hand artificial reproduction and production of good quality fry. First efforts of generation based on natural spawning of catfish in carp ponds. At present artificial reproduction based on hormonal stimulation with CPE or Ovopel injection and the procedures are similar to hormonal stimulation using in cyprinid species.

Production of larvae was tilled recently limited by feeding. For many years catfish larvae were rearing in policulture with a carp or in monoculture in simple facilities. To elevate growth ratio larvae were fed zooplankton (HOROSZEWICZ 1971). Occurrancy of good quality starters has brought new and satisfactory possibilities of feeding the catfish larvae HAMÁČKOVÁ, KOUŘIl 1996, SCHLUMBERGER et al. 1995, WOLNICKI, KAMIŃSKI 1998 , WOLNICKI et al. 1998). It has also enabled intensify the production of stocking material by increased of stocking density.

The aim of this study was to rear the European catfish larvae in controlled condition with particular including stocking density and sort of offering food and their effect on growth and survival of the larvae.

Materials and Methods

Materials and condition of culture

European catfish larvae in number of about 10 000 individuals were obtained from Experimental Fish Hatchery "Dgał" in Pieczarki Inland Fisheries Institute in Olsztyn, 5 days after hatching with the yolk sac still present.

Fish were reared under controlled condition in twelve 40 dm^3 tanks with closed and purification system. The temperature of water at the first day was 23°C. In the course of the 36 hours she was elevated to 28?o?C and keep till the termination of the experiment. The oxygen level did not drop below 6 mg dm^{-3} and ammonium did not cross 0.1 mg dm^{-3}. The fish were kept in darkness.

Feeding

Larvae were distributed into 3 groups according to offering food: *A* – artificial diet (Aller Möller commercial trout starter) (Table 1, Table 2), *B* – mixed diet (trout starter and natural food), *C* – natural food (Artemia salina, frozen zooplankton, frozen Chironomidae). Each group involved 2 variants of density – 7.5 (1) and 15 (2) ind. dm^{-3}. Fish were fed 3 times a day during the first and 4 times during last two weeks of experiments.

Table 1
Composition of trout starter

Ingredient	Contents (%)
Protein	53
Fat	14
Carbohydrates	14
Cellulose	1
Ash	10

Table 2
Composition of trout starter

Ingredient	Contents per kilo
Vitamin A	10 000[a]
Vitamin D	800[a]
Vitamin E	300[b]
Cu	5[b]
Energy	16.5 MJ

a – IU per kilo; *b* – mg

Sample collection

The experiment lasted 21 days. Before the beginning of feeding a sample of 10 fish was taken and preserved in 4% formaldehyde solution. Fish were also sampled after 7, 14 and 21 days of rearing (20 ind. from each tank) and they were weighted and measured with 0.1 mg and 0.1 mm accuracy (anaesthetized using 2 – phenoxyethanol).

The tanks were cleaned once a day and died fish were counted. Every week fish were bathed in FMC solution (1 cm^3 100 dm^{-3}).

The differences among the groups were tested using Duncan's test at $p < 0.05$.

Results

Growth

Initial fray weight before feeding was 6.4 mg. From the onset of exogenous feeding significant higher ratio growth was observed in group B_1 and it was generally higher during the experiment. On the 14 day of the trial differences among the groups were much more visible (Figure 1). At the termination of rearing the highest average weight 1203.5 mg was achieved in group fed mixed diet with rare stocking density – B_1. Considerably lower final body weight was noticed in groups fed natural food either

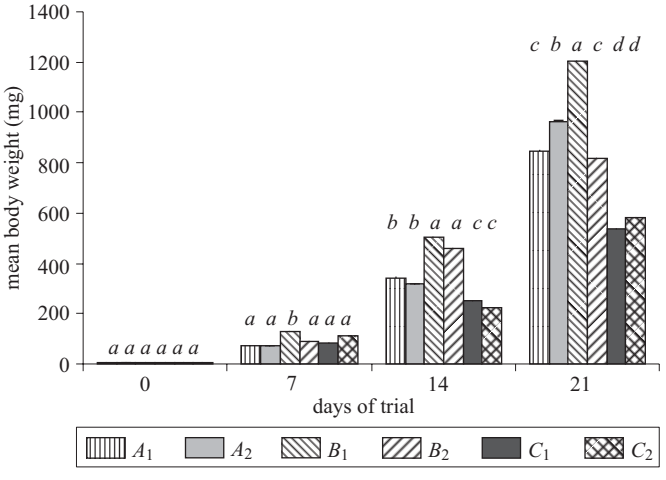

Fig. 1. The mean body weight (± SD) of catfish larvae reared at different densities and feeding regimes at the end of the experiment. Groups marked with the same letter did not differ statistically ($P = 0.05$)

in first and second variant of density and did not go beyond 600 mg. Groups A_1 and A_2, fed commercially trout starter obtained individual final weight 848 and 966 mg (respectively for A_1 and A_2).

Average body length and differences among the groups during next weeks are shown at Figure 2.

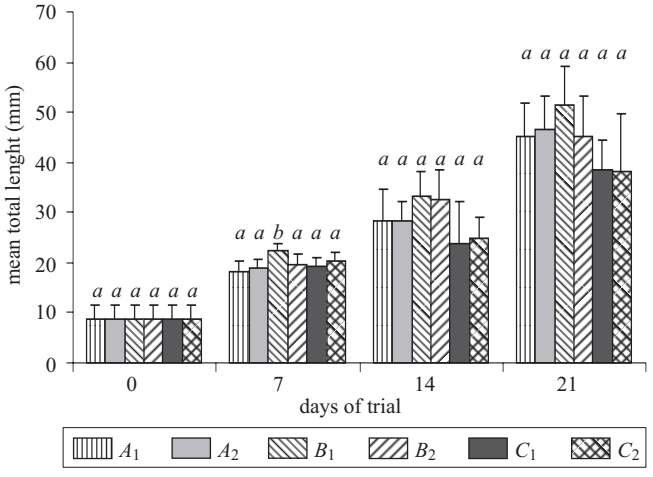

Fig. 2. The mean body length (± SD) of catfish larvae reared at different densities and feeding regimes at the end of the experiment. Groups marked with the same letter did not differ statistically ($P = 0.05$)

Fish behavior

Trout starters as well as natural food were eaten readily by larvae. At onset of feeding the fish grouped together and feeding only below the water surface. The unfed particles lying on the tanks bottom were ignored by the fish. During next weeks of feeding fish in all experimental groups were dispersed over the tanks and active during and between feeding.

Mortality

At the termination of the trial the highest cumulated mortality was found in groups fed trout starter 16.33 and 15.08%, A_1 and A_2 respectively (Figure 3). In those groups survival rates was lowest from the onset of the experiment. The best final survival rates were achieved in groups fed natural food – C_1 and C_2. It did not go below 10%.

From the beginning of the second week increase of mortality and injuring fish was observed in all of the groups. The losses were caused by cannibalism. Figure 4 shows the balance of stocks at the end of the trial. The highest per cent of losses was found in groups fed natural food in each variants of stocking density.

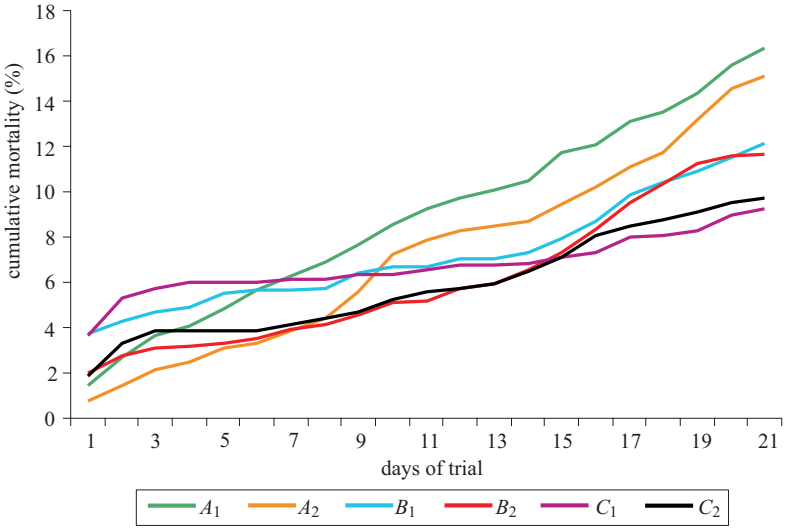

Fig. 3. Cumulative mortality (%) of catfish larvae in each experimental group under intensive rearing

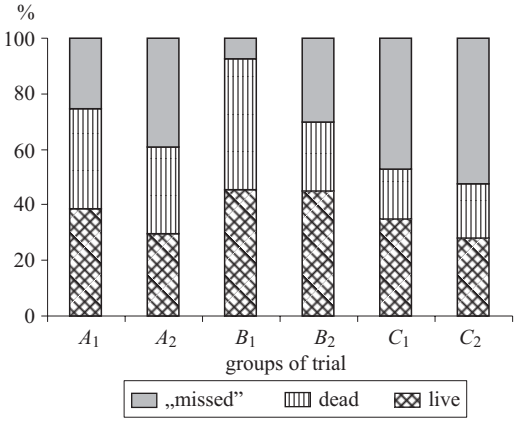

Fig. 4. The percentage of live, dead and “missed” fish on the end of the experiment

Discussion

There are three most popular methods that intensive rearing of European catfish stocking material may be carrying out. The first and the easiest one are placing in plastic or concrete tanks with recylculating and purification system. Fish are fed artificial food only. In second way of rearing fish are farming in tanks at first and then they are carrying over into small ponds with natural thermal conditions. First step of the last method is the same as in second but then fish are cultured in warm – water facilities ULIKOWSKI 2003. In spite of good tank stocking results farming in ponds with natural feeding is still prevailing method of rearing of European catfish larvae in Poland. It is caused by lower costs of rearing and aversion of farmers to use commercial feeding staffs because of many unsuccessful efforts of farming.

Our results with artificial feeding are comparable with data published by HAMÁČKOWÁ, KOUŘIl 1996, HAMÁČKOWÁ et al. 1997, WOLNICKI, MYSZKOWSKI 1998, WOLNICKI et al. 1998. According to data presented by WOLNICKI, KAMIŃSKI 1998 short – term (10–15 days), intensive rearing with trout feeding allow to obtain fray with average individual body weight equal 0.2 g. However, further reports of WOLNICKI, MYSZKOWSKI 1998, showed that average body weight of larvae might be lower and the highest ratio growth in their studies was achieved in control groups fed Artemia naupli – 202 mg. In our studies during three weeks rearing larvae fed trout starter obtained average body weight equal 848 and 966 mg (resp. A_1 and A_2).

There is no confirmation of data's published by HAMÁČKOWĂ, KOURIL 1996, WOLNICKI, MYSZKOWSKI 1998, that better ratio growth were achieved on natural food, in our studies. Both groups (C_1 and C_2) in our trial represented considerably lower ratio growth and the balance of stock at the termination of experiment was the worst. It seems that in case of mass rearing natural food is not enough diet.

The best ratio growth was found in groups fed mixed diet. The weight and length of the larvae from this group was greater after first week of our trial.

Conclusions

1. Preliminary phase of European catfish larvae rearing in controlled condition could relay on artificial diet only. Growth ratio and survival are satisfactory.

2. Addition of live food improves growth ratio and survival of larvae.

3. Effective intensive rearing is not possible with only using the natural food because of poor survival.

4. Low initial stocking density limits cannibalism.

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REPRODUCTION OF BUENOS AIRES TETRA (*HEMIGRAMMUS CAUDOVITTATUS*) UNDER CONTROLLED CONDITIONS

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K e y w o r d s: Buenos Aires tetra, controlled reproduction, hatch, aquaristics.

A b s t r a c t

Studies on reproduction of Buenos Aires tetra were carried under controlled conditions. Buenos Aires tetra is a species popular for keeping in aquaria. It was established that spawners of this species produce viable gametes during a few (5–6) spawning periods only; later, although they get involved in reproduction viable issue cannot be obtained from them. From the breeding perspective fish of that species should be reproduced again shortly after the completed spawning and that time should be from 5 to 15 days. Excessively long keeping the fish between spawning periods results in a significant deterioration in quality of gametes expressed by the decreased number in obtained issue. It was shown that before spawning spawners should be kept in water at 22°C. The negative effect of keeping the reproducers in water at 25°C accumulated with time.

ROZRÓD ZWINNIKA OGONOPRĘGIEGO (*HEMIGRAMMUS CAUDOVITTATUS*) W WARUNKACH KONTROLOWANYCH

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Abstrakt

Przeprowadzono badania nad rozrodem zwinnika ogonopręgiego w warunkach kontrolowanych. Zwinnik jest rybą popularnie hodowaną w akwariach. Stwierdzono, że tarlaki tego gatunku oddają żywotne gamety tylko przez kilka (5–6) tareł. Później, pomimo tego że przystępują do rozrodu, nie można uzyskać od nich żywotnego potomstwa. Z hodowlanego punktu widzenia ryby tego gatunku powinny być rozradzane ponownie w niedługim czasie po odbytym tarle (5–15 dni). Zbyt długie przetrzymywanie zwinnika ogonopręgiego w okresie pomiędzy kolejnymi tarłami powoduje znaczny spadek jakości otrzymanych gamet, wyrażający się obniżoną ilością otrzymanego wylęgu. Wykazano, że przed tarłem tarlaki powinny być przetrzymywane w wodzie o temperaturze 22°C. Negatywny efekt przetrzymywania reproduktorów w wodzie o temperaturze 25°C kumulował się w czasie.

Introduction

Aquaculture is not only breeding fish for consumption but also breeding ornamental species (TLUSTY 2002, CEK, GOKCE 2005, CHELAPPA et al. 2005). Breeding of aquarium fish develops particularly dynamically in Asia although recently extensive development has also been observed in some European countries, e.g. Czech Republic. *Characidae* living mainly in the tropical waters of South America are one of the groups of ornamental fish commonly bread worldwide. The best-known representatives of that group are Neon tetras. That group also includes other tetras, including the Buenos Aires tetra (*Hemigrammus caudovittatus*). This is a small fish in captivity reaching ca. 7–9 cm in total length. In the natural environment it is most numerous in the La Plata basin. The species was imported to Europe in 1922. Considering its size, it is a relatively fertile fish. During a single spawning act that is extended over time and usually takes from 2 to 4 hours, the female lays even over 2000 grains of eggs (KUJAWA 2000). The larvae hatch in most cases within 24 hours from fertilization of the eggs. That species is found in two colors: natural and albino. The natural (wild) form has silvery-gray, shiny body. A horizontal blue belt runs along the body. Tail base is decorated with a black spot from which a black line continues as far as the edge of tail fin. The tail fin and anal fin are bright red. The eye iris is silvery-gray and the pupil is black. In case of albino form the body is yellowish-orange and the odd fins are intensely red. A white horizontal belt runs along the body. The eye pupil is red and the iris is white.

Very limited number of reproductive acts during which the fish produce viable gametes is one of the characteristics of small body size fish belonging to the *Characidae* family. In case of some species such as cardinal tetra (*Paracheirodon axelrodi*) or neon tetra (*Paracheirodon innesi*) the number of such acts can be just two or three. The quality of gametes and number of offspring obtained can also be influenced by other factors such as feeding, water temperature, light conditions or length of interval between consecutive reproductive acts (BROOKS et al. 1997, TARGOŃSKA 2007).

Determining the influence of the temperature of water used for keeping the spawners on the number of offspring obtained was the primary goal of this study. Checking the number of reproductive acts during which Buenos Aires tetra produce viable gametes and the length of intervals between individual spawning acts on the number of hatched fish obtained was the secondary goal.

Material and Methods

Around 1000 larvae of Buenos Aires tetra were obtained from one of the private aquarium breeders in Olsztyn. The larvae were initially reared in 1 dm^3 tanks at the concentration of 100 larvae per 1 dm^3 and after 4 weeks in four tanks of 50 dm^3, working in a closed system (KUJAWA et al. 2000). During the initial rearing taking 4 months the fish were fed 3 times per day with aquarium feeds: flakes by Tropical as well as frozen *Chironomus* and trout granulate. The temperature during initial rearing was within 22–23°C. After reaching sexual maturity by the fish the mass spawning was conducted. For that purpose in each of twenty 10 dm^3 spawning tanks five pairs of spawners were placed. Spawn grilles were placed on the bottom of each tank (TARGOŃSKA 2007). Water temperature was increased to 25–26°C. Generally, spawning occurred after a few days, most frequently between day 2 and day 4 as of placing the fish in the tanks. After 24 hours from spawning the hatch was counted (hatching occurred 18–22 hours after spawning) on the basis of five return samples, according to the methodology described by TARGOŃSKA (2007) for rosy barb (*Puntius conchonius*). After the first (controlled) spawning the fish that reproduced were split into three groups and three separate experiments were conducted.

Experiment 1. The first experiment investigated the influence of consecutive spawning acts on the number of obtained eggs. For that purpose 10 reproductive pairs (1 male and 1 female) were used. Every 15 days they were moved to spawning tanks. The time between individual spawning acts was 11–12 days. After each completed spawning the fish were caught and 24 hours after spawn the number of fry was counted.

Experiment 2. In the second experiment the investigation concerned the duration of the most appropriate period between spawning from breeding perspective. For that purpose 10 reproductive pairs that completed the first spawning were moved every 5 days to spawning tanks. After completed spawn the procedure was the same as in case of experiment 1.

Experiment 3. The third experiment investigated the influence of the temperature (22 and 25°C) of water in which fish were kept before spawning and possible effect of accumulation. For that purpose two sets of fish

(10 reproductive pairs each) were used. Three times, at 15 days intervals they were moved to the spawning tanks. After completed spawn the procedure was the same as in case of experiment 1.

Physicochemical parameters of water used for Buenos Aires tetra spawning were constant. For that purpose tap water was mixed with water obtained from the process of reversed osmosis. As a result water with carbon hardness below 2°n and total hardness at 3°n was obtained. The temperature of water in spawning tanks was 25–26°C. On tank bottom spawn grilles with mesh size of 5 mm were placed. The tanks were aerated. The spawning tanks were placed in dark, as Buenos Aires tetra spawn is sensitive to light. Three times a day the tanks were checked whether spawning took place. After completed spawning the fish were caught. The hatch was counted 24 hours after completed spawning on the basis of 5 return samples (TARGOŃSKA 2007).

The results obtained were analyzed statistically. The differences in the number of hatch between groups in individual experiments were processed by variance analysis and Tuckey's *post-hoc* test at significance level of 5%. The correlation between the hatch number and the consecutive spawning as well as intervals between individual spawning acts were subjected to regression analysis. The correlation between the hatch number obtained and the consecutive spawning acts for fish maintained at different temperatures was also determined.

Results

Indifferent of the type of experiment conducted the percentage of ovulating females was high and ranging from 90% to 100%.

In analyzing the hatch numbers from consecutive Buenos Aires tetra spawning acts it was determined that for the reproductive acts 1 to 5 the numbers of obtained fry were at similar level of around 1000 individuals (Figure 1). A significant decrease of the value investigated was found during further reproduction acts. From spawning 8 and two later ones no single hatched individual was obtained.

Figure 2 presents the numbers per hatch obtained from spawning acts performed at fixed time intervals, i.e. after 5, 10, 15, 20, 25 and 30 days from the controlled spawning. It was established that the interval between individual reproductive acts in case of Buenos Aires tetra should be from 5 and 15 days. Longer than 15 days keeping of the fish before consecutive spawning resulted in a significant decrease of the hatch numbers obtained, for example, when comparing the number of larvae obtained in case of 10 and 30 days interval between spawning acts an almost 60-fold decrease in the number of larvae obtained was recorded.

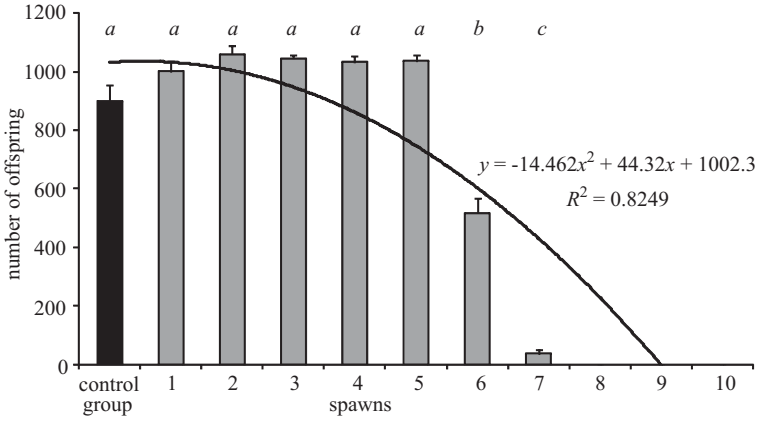

Fig. 1. The influence of the number of spawning acts completed on the average hatch numbers obtained from one pair of the Buenos Aires tetra spawners. The data marked by the same letter index within individual spawning acts did not differ statistically

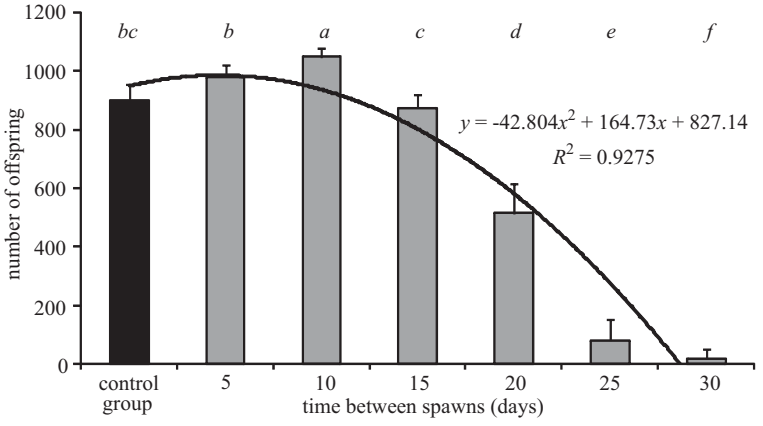

Fig. 2. Influence of the length of interval between spawning acts on the average hatch number obtained from one spawning pair of Buenos Aires tetra. The data marked by the same letter index within individual spawning acts did not differ statistically

It was also established that the level of temperature is significant for the numbers of hatch obtained (Figure 3). Longer keeping of fish at the higher of the tested temperatures (25°C) had a significant influence on decreasing the numbers of offspring obtained as compared to the results obtained when the fish before spawning were kept in water at 22°C. Accumulation of the

negative effect of the temperature influence on the results of reproduction in case of longer keeping the spawners at 25°C was also determined.

During the experiments conducted no cases of spawners death were recorded.

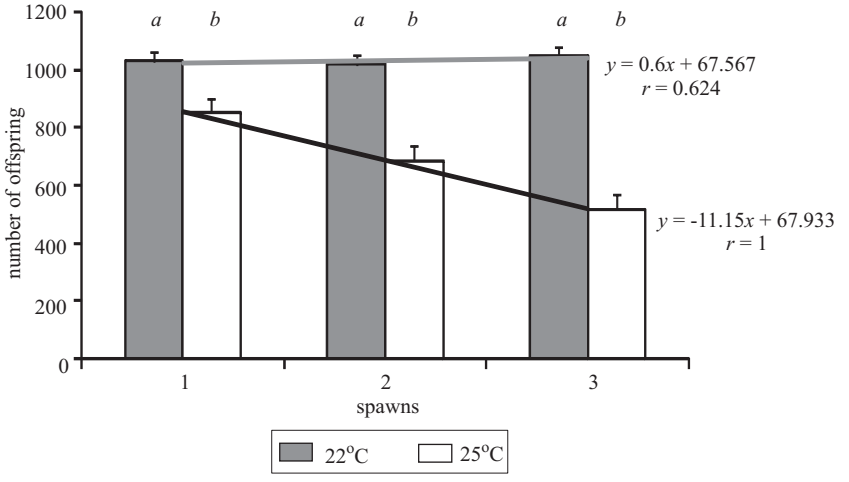

Fig. 3. Influence of the temperature of water used for keeping the Buenos Aires tetra spawners on the average number of hatch obtained from a single spawning pair. The data marked by the same letter index within individual spawning acts did not differ statistically

Discussion

In modern aquaculture obtaining high quality gametes, which allows both obtaining the required number of larvae for initial rearing and planning of production, is one of the most important problems to be solved. It is also highly important from economic perspective. This applies not only to fish reared for consumption but also other fish including the decorative and aquarium species.

Numerous factors influence the effects of reproduction, in this study understood as spawning and obtaining a specific number of larvae from the hatch. They include, among others, the fish diet and environmental conditions. According to BROOKS et al. (1997) the differences between species in the influence of environmental conditions on effectiveness of fish reproduction and quality of gametes produced by them were determined. In some cases the environmental conditions to which spawners are subjected can also influence such characteristics of the offspring as the survival rate, growth rate and resistance to stress (BROOKS et al. 1997, TARGOŃSKA 2007).

The fact that viable offspring of Buenos Aires tetra can be obtained during a few initial reproductive acts only (counting from the first, controlled spawning) and later, although the fish performed the reproduction and laid spawn obtaining viable offspring is not possible should be considered very interesting from both research and application perspective. On the basis of the data obtained from the presented experiments it can be concluded that during further reproductive acts spawn was laid but hatched larvae were not obtained because developing spawn died during the initial hours of incubation. That observation is confirmed by numerous observations presented by aquarium owners and it is a characteristic of *Characidae* as well as some *Cichlidae* fish. The genus Xenotilapia *Cichlidae* living in Lake Tanganyika can effectively reproduce just once in the lifetime. Sometimes, under aquarium conditions, another spawning can be induced by placing a group of young females in the tank (PRUSIŃSKA – unpublished data). The reasons for that situation have not been explained sufficiently so far. During these studies not a single viable hatch was obtained from spawning 8 on. Considering the number of spawning pairs and the data published by KUJAWA (2000) on fertility of that species it should be estimated that not a single larvae hatched from around 20 000 grains of spawn. Dying of spawn in this case could be caused by so-called internal factors only as similar environmental conditions were maintained during all the experiments. Internal factors such as quality of the gametes and fitness of the spawners are considered very important for survival of embryos and the development of early ontogenesis although so far few studies have been published on that subject (BUNN et al. 2000).

The influence of some factors (positive or negative) on the effect of fish reproduction can accumulate over time. That was confirmed, among others, by TARGOŃSKA (2007) during studies on the influence of feeding of reproducers on the effects of reproduction of *Symphysodon discus* fish. The accumulation of negative influence of high water temperature in which spawners were kept was also found in case of the studied species of Buenos Aires tetra. Extended keeping of fish in water at 25°C caused a decrease in the number of offspring. The influence of water temperature on reproductive capacity has been described for many fish species (KRAAK, PANKHURST 1996). A similar relation to that in the current study between the temperature at which the spawners were kept before reproduction and survival of the hatch was observed in case of *Menidia audens*, when the fish were kept under controlled conditions (HUBBS, BRYAN 1974).

The results of studies obtained indicate that keeping spawners of Buenos Aires tetra in excessively high temperature and excessively long intervals between consecutive spawning actions can have a significant influence on effects of reproduction. It is known that during selection of reproducers to the

spawning stock the age and number of spawning actions completed should also be considered. The results presented in this paper close the information gap concerning biotechnology of aquarium fish reproduction, which is quite significant even for species highly popular in aquaculture.

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DETERMINATION OF EUROPEAN CATFISH (*SILURUS GLANIS* L.) LARVAE RESISTANCE TO TEMPORARY LACK OF FOOD UNDER CONTROLLED CONDITIONS

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K e y w o r d s: *Silurus glanis,* larvae, water temperature, PNR, controlled rearing, natural food.

A b s t r a c t

This paper describes the resistance of european catfish (*Silurus glanis* L.) larvae to temporary lack of food. Then PNR (point of no return) for european catfish larvae kept in water at 15, 20 and 25°C (±0.5°C) was determined. As a result of the conducted experiments it was observed that european catfish larvae reach the PNR_{50} in the water at 15, 20 and 25°C after 11.0, 9.2 and 4.4 days respectively. The conducted experiment confirmed that water temperature plays the key role in determining the period of resistance to lack of food. The observed, at least one week long period of european catfish larvae kept in water at 20°C resistance to shortage of food allows freedom of manipulation and wide safety margin in case of controlled rearing of them.

OKREŚLENIE ODPORNOŚCI LARW SUMA (*SILURUS GLANIS* L.) NA OKRESOWY BRAK POKARMU W WARUNKACH KONTROLOWANYCH

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S ł o w a k l u c z o w e: *Silurus glanis,* larwy, temperatura wody, PNR, chów w warunkach kontrolowanych, pokarm naturalny.

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Abstrakt

W pracy opisano wytrzymałość larw suma (*Silurus glanis* L.) na okresowy brak pokarmu. Określono PNR (point of no return) dla larw suma przetrzymywanych w wodzie o temperaturze: 15, 20 i 25°C (±0,5°C). Zaobserwowano, że larwy suma osiągają PNR_{50} w wodzie o temperaturze: 15, 20 i 25°C odpowiednio po 11.0 dniach, 9.2, 4.4 dnia. Przeprowadzone doświadczenie potwierdziło, że temperatura wody odgrywa podstawową rolę w determinowaniu okresu odporności na brak pokarmu. Zaobserwowany, co najmniej tygodniowy, okres odporności larw suma na niedostatek pokarmu, przetrzymywanych w wodzie o temperaturze 20°C, pozwala na swobodę manipulacji i duży margines bezpieczeństwa w przypadku ich wychowu w warunkach kontrolowanych.

Introduction

Fish larvae are very sensitive to various factors that influence their physiological and health condition (MARR 1956, DĄBROWSKI 1975, KORWIN-KOSSAKOWSKI 1992). They are particularly sensitive to shortage of food at the time of transition from endogenous (reserves of nutrients from yolk sack) to exogenous (zooplankton) feeding (VLADIMIROV 1964). In the natural environment the situation of total lack of food is observed very rarely. Much more frequently the shortages of food of appropriate size or in appropriate quantities are encountered caused mainly by temperature fluctuations. They cause malnutrition of the majority of individuals and even fasting of some of them, which can lead to death in a short time (HJORT 1914, DEHNIK et al. 1970). Mortality resulting from that can be significant reaching the values as high as even 60–90%.

The larvae, after completing resorption of the yolk sack are able to survive a certain time without intake of exogenous food (HJORT 1914), although, to survive, they must receive food within time not exceeding a certain critical deadline referred to in literature as the "point of no return" (PNR) (BLAXTER, HEMPEL 1963). It represents the number of days before expiration of which the larvae should receive food. After exceeding that deadline the larvae die even though the food can be available in the environment.

Determining the PNR in fishery practice is quite troublesome. Intake of food by the larvae does not offer certainty that it would be digested and assimilated by the body (WOLNICKI, OPUSZYŃSKI 1988). It is much easier to notice the death of an individual than to determine the moment, when it reached the PNR. That is why IVLEV (1955) assumed in his studies the death of 50% of the stock to represent the PNR. Determining the resistance of larvae of individual species to lack of food during the initial period of life is very important in case of both initial rearing under controlled conditions (FROLENKO 1959, KOSTOMAROVA 1962), and, first of all, in case of stocking open waters (HOROSZEWICZ 1974, GRUDNIEWSKI 1980, WOLNICKI, OPUSZYŃSKI 1988, KUJAWA 2004).

Absence of detailed studies on the above issues for the european catfish larvae was the inspiration for initiating the studies aiming at determining their resistance to periodic lack of food depending on water temperature. The experiment involved delaying access of european catfish larvae to food until they reach the point after exceeding which they would not be able to assimilate already available food as a consequence of far-reaching changes related to partial self-digestion of the alimentary system. Knowledge on the PNR of european catfish larvae represents very important information during initial rearing under controlled conditions. Breeders knowing the larvae resistance time for temporary food shortage at a given temperature could change it safely without exposing themselves to losses in initially reared material. Such data could also help in understanding some aspects of biology and ecology of European european catfish that have not been studied extensively so far.

Material and Methods

European catfish spawners originated from pond breeding of Gospodarstwo Stawowe (Pond Farm) in Samoklęski. Following the first stimulating injection, water temperature was increased to 21°C. After the second releasing injection water temperature was increased to 24°C. The injections were administered intramuscularly from pituitary gland at two doses (0.3 and 2.7 mg kg^{-1}) at 12-hour interval (BRZUSKA 2000, ULIKOWSKI 2003). The spawn was obtained after around 10–12 hours after the second injection. Next the fertilized spawn was deprived of viscosity by placing it alternately in 0.3% NaCl and tannin (5–7 g per 10 dm^3 water) solutions. Following that the spawn at 0.5 kg was incubated Weiss apparatuses. The water flow was set at the level assuring steady flow of spawn within the entire volume of the apparatus. Initially the incubation temperature was 20–21°C; after one day it was increased to 22–23°C. Spawn incubation time in water at that temperature was 3 days. The larvae hatched within one day. After hatching the larvae remained in the receiver where they went through resorption of the yolk sack.

Experiment protocol

Larvae were placed in specially designed for the purpose two-litre flow-through aquaria connected to a closed water circuit (KUJAWA et al. 2000). In every aquarium 30 individuals starting feeding were placed. The major part of the yolk sack was already resorbed in case of the majority of the larvae. The experiments were conducted in three separate closed circuit systems. Water

temperature in the systems was set at 15, 20 and 25°C respectively. The choice of those temperatures was not random. During stocking with larvae or minor individuals the water temperature in rivers is around 15–20°C (HOROSZEWICZ 1971) while 25°C is the optimum temperature recommended during the initial rearing of european catfish under controlled conditions (WOLNICKI 1995, WOLNICKI, KAMIŃSKI 1998, ULIKOWSKI 1999, ULIKOWSKI, BORKOWSKA 1999). After placing the larvae in the aquaria the water in the systems was either cooled down or heated up (1°C h^{-1}) until reaching the required temperature according to the procedure described by HOROSZEWICZ (1974). The thermal control devices maintained the temperature later at the level set with the accuracy of 0.5°C. Aquaria were shaded from direct influence of discharge tube light, which slightly lit the aquaria with fish for 12 hours during the day.

The larvae were fed *ad libitum* with natural food consisting of live nauplius larvae of *Artemia* sp. prepared according to the procedure described by SORGELOOS et al. (1977). Aiming at determining the influence of lack of food on survival of the larvae, feeding the fish in individual experimental groups was started with fixed one-day delay. The experiments were carried out in duplicate.

During the experiment, every day in the morning, before administration of food, the dead fish were counted and removed. Daily recording of the number of dead fish allowed determining the cumulated mortality, i.e. the number of dead larvae in relation to the initial stocking in (%). In determining the resistance of larvae to lack of food the authors based their work on studies by IVLEV (1955) and KUJAWA (2004). The "critical point" was set at death of 50% of the initial stock. Particular attention was also paid to appearance of the first deaths because, as indicated by the literature, their appearance informs about the resistance of the larvae to lack of food and not the mortality at 50% as is commonly assumed in the methodology of studies on the lethal factors (HOROSZEWICZ 1974).

When in the aquaria in which administration of food started the latest the mortality of the larvae reached 100%, 10-day rearing was started, which involved administering food to all the other fishes. This aimed at verification whether all the larvae that were earlier subjected to fasting and survived the experiment would not have problems with digestion and assimilation of food (KOSTOMAROVA 1962, CUNIKOVA 1972, SZLAMIŃSKA 1982, WOLNICKI, OPUSZYŃSKI 1988). On those bases the allowed and safe period for keeping the larvae without food supply without negative influence on their later development and fitness was determined and the samples of larvae collected during the experiment allowed calculation of average mass and length increases during rearing preceded by temporary shortage of food.

Measurements and data analysis

During the experiment, every morning before administration of food, the dead fishes were counted and waste was removed. Aiming at determining the diversities in mass and length increases the following samples were collected:
– every 2 days from the aquaria with water temperature at 25°C,
– every 3 days from the aquaria with water temperature at 20°C,
– every 4 days from the aquaria with water temperature at 15°C.

Sampling took place just before switching on the lighting and before start of feeding. Before measuring and weighting, the fishes caught were subjected to short anaesthesia in the solution of 2-phenoxymethanol according to the methodology described by WEYL et al. (1996). The appropriate concentration of 2-phenoxymethanol (0.3 cm^3 dm^{-3}) was determined on the basis of the results of own earlier experiments. The larvae were weighted with the accuracy of 1 mg and their total length (*longitudo totalis* – l. t.) was measured with the accuracy of 0.1 mm. After measurements the fishes were returned to appropriate aquaria.

During the final step of the experiment, when in the last aquaria the mortality reached 100%, rearing started by administering food for all the fishes. The rearing time was 10 days. After that period samples were also collected to determine the conditions of initial rearing combined with initial fasting. After anesthetising the larvae were measured and weighted. During the experiment observations of activity of larvae taking the food were carried out. The differences between experimental groups were determined using Duncan's test (1955) at the significance level $\alpha = 0.05$. Statistic processing of the results was done using Excel 9.0 and Statistica 6.0 for Windows computer software.

The mortality curves for larvae deprived of food had the typical S-shape. Aiming at precise determination of the moment of appearance of 5, 50 and 95% mortality of the larvae the data transformation was performed (BLISS 1937, KAMLER 2002). The mortality tome was presented in the format of the logarithm (x), and the cumulated mortality (H) in the format of logit (y) computed according to the formula:

$$y = \ln \cdot \frac{0.01 \cdot H}{1 - (0.01 \cdot H)}$$

The results obtained were described using straight-line regressions $y = a + bx$, from which the time of appearance of 5, 50 and 95% mortality was computed (Table 1).

Table 1

Parameters of linear regression ($y = a + bx$), and time of mortality 5, 50 and 95% of european catfish (*Silurus glanis* L.) larvae deprived of food at different water temperatures

Temperature (°C)	Regression			Time (days)			Period
	a	b	R^2	5%	50%	95%	
15	–36.899	35.475	0.7908	9.1	11.0	13.3	4.2
20	–45.716	47.403	0.8675	8.0	9.2	10.6	2.6
25	–26.71	41.569	0.9128	3.7	4.4	5.2	1.5

The mortality time (days) of 5, 50 and 95% of european catfish (*Silurus glanis* L.) larvae deprived of food at different water temperatures was derived from regressions: $y = a + bx$, where y is the cumulative percentage of individuals (converted to logits) and x is the logarithm of the time. The period values (days) are calculated by subtracting the 95% value from 5% value ($P < 0.01$) ($n = 30$)

Results

In water where the temperature was the lowest (15°C), european catfish larvae could be kept without food for 8 days. After the next three days without food PNR_{50} was recorded. Longer keeping of european catfish larvae (15 days) without food resulted in loss of the entire stock.

On the other hand in water at the temperature of 20°C, 50% mortality of european catfish larvae maintained without food occurred on day 9.2. After 11 days all the larvae were dead. The european catfish larvae could be maintained without loss in water of that temperature for 7 days.

Observations of european catfish larvae maintained in water at the temperature of 25°C showed that they could survive without food for 3 days counting from the time of yolk sack resorption and commencement of active floating. After around another two days (4.4 days) the fish reached the moment defined as PNR_{50}, which represents mortality of 50% of the stock. One more day without food administration resulted in loss of the entire stock of fish.

During the experiments a change in mobility of fish with time was observed. After reaching the PNR only few fishes attempted to catch food while the majority of them rested on the bottom and only sporadically performed slow movements. Attempts at biting were observed among the larvae during the experiment. This applied to both the fishes that were fasting and those that received their food. Attacks frequently ended in severe skin damage. In case of large difference in size between individuals, attempts at swallowing were observed. During further 10-day rearing of european catfish larvae that remained alive no deaths were observed and the differences in body length and weight increases are presented in Figures 1–7. The figures show that even 2–3 days delay in administration of food resulted in major decreases in body mass of fishes at the end of rearing period, which in case of stocking material

production for stocking is an important problem. Size diversity of european catfish larvae is supportive for cannibalism. Lower unit weight of fishes frequently translates to poor survival rates in the target reservoirs. The relation between the final mass and length of larvae that received the food the fastest and those that received the food the latest in a specific water temperature is presented in Figure 8.

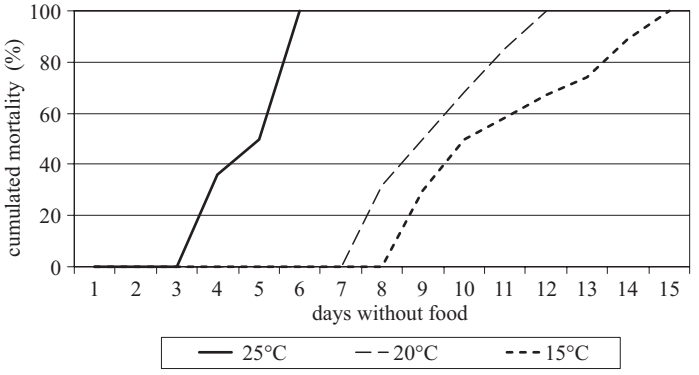

Fig.1 Mortality of european catfish (*Silurus glanis* L.) larvae caused by lack of food

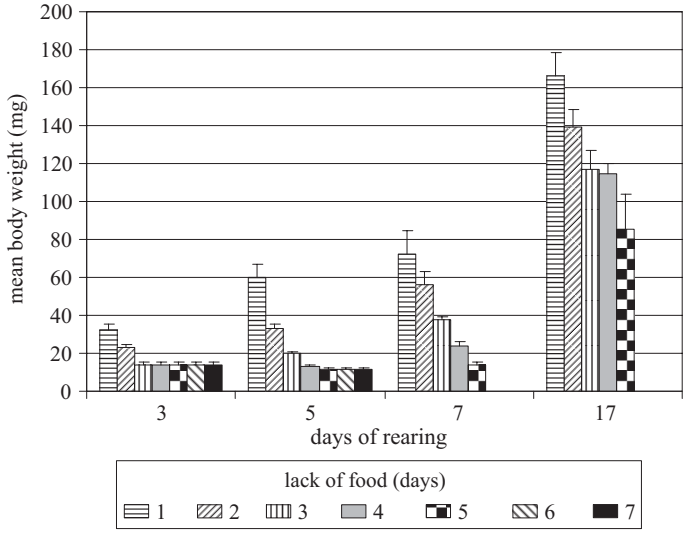

Fig. 2. Weight increase of european catfish (*Silurus glanis* L.) larvae during rearing in water at the temperature of 25°C after a period without food

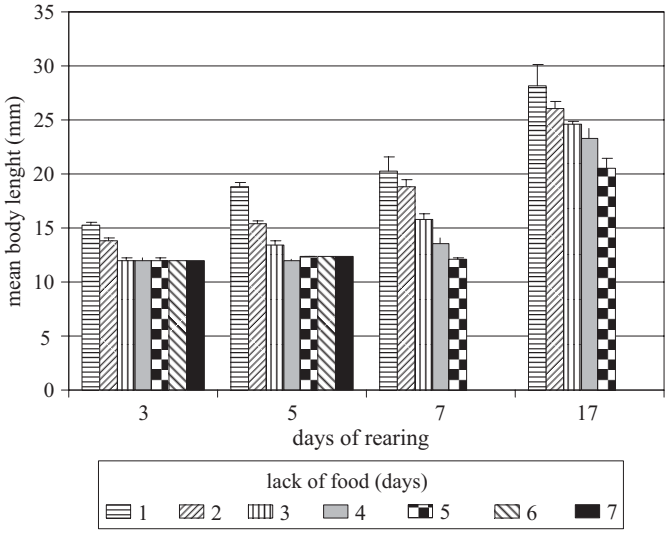

Fig. 3. Length increase of european catfish (*Silurus glanis* L.) larvae during rearing in water at the temperature of 25°C after a period without food

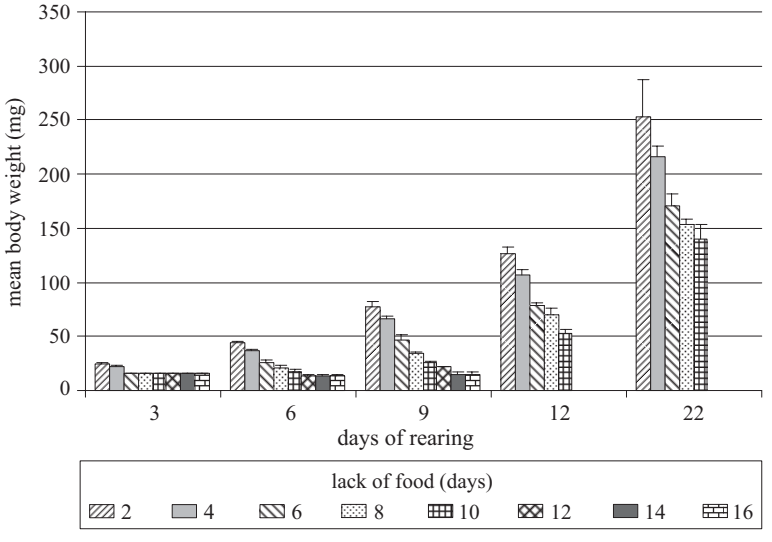

Fig. 4. Weight increase of european catfish (*Silurus glanis* L.) larvae during rearing in water at the temperature of 20°C after a period without food

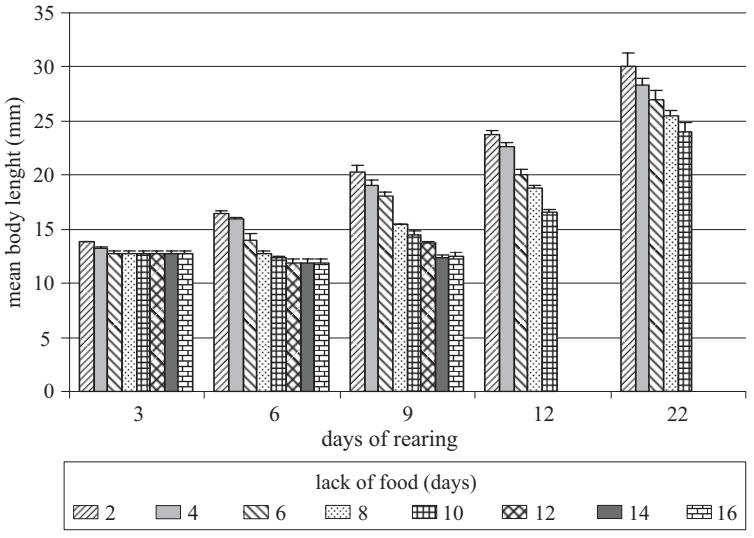

Fig. 5. Length increase of european catfish (*Silurus glanis* L.) larvae during rearing in water at the temperature of 20°C after a period without food

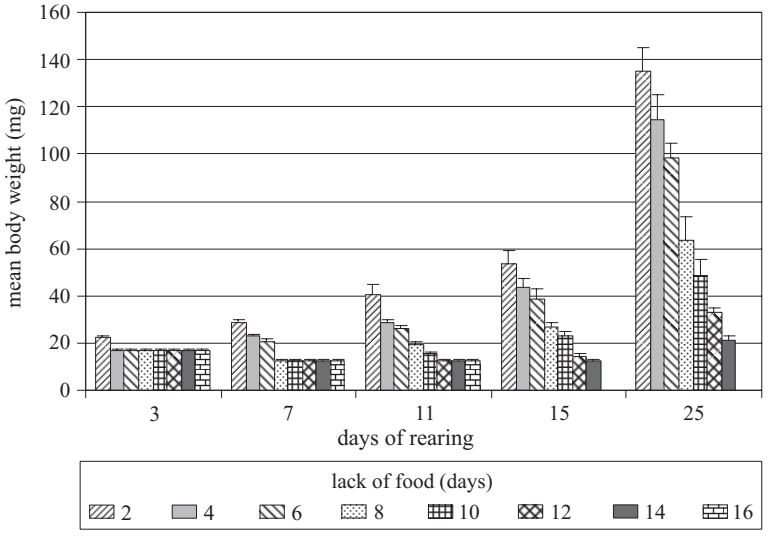

Fig. 6. Weight increase of european catfish (*Silurus glanis* L.) larvae during rearing in water at the temperature of 15°C after a period without food

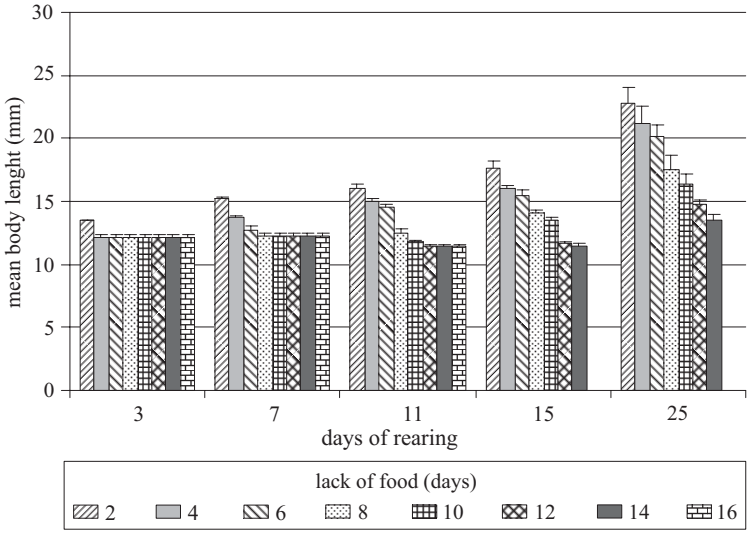

Fig. 7. Length increase of european catfish (*Silurus glanis* L.) larvae during rearing in water at the temperature of 15°C after a period without food

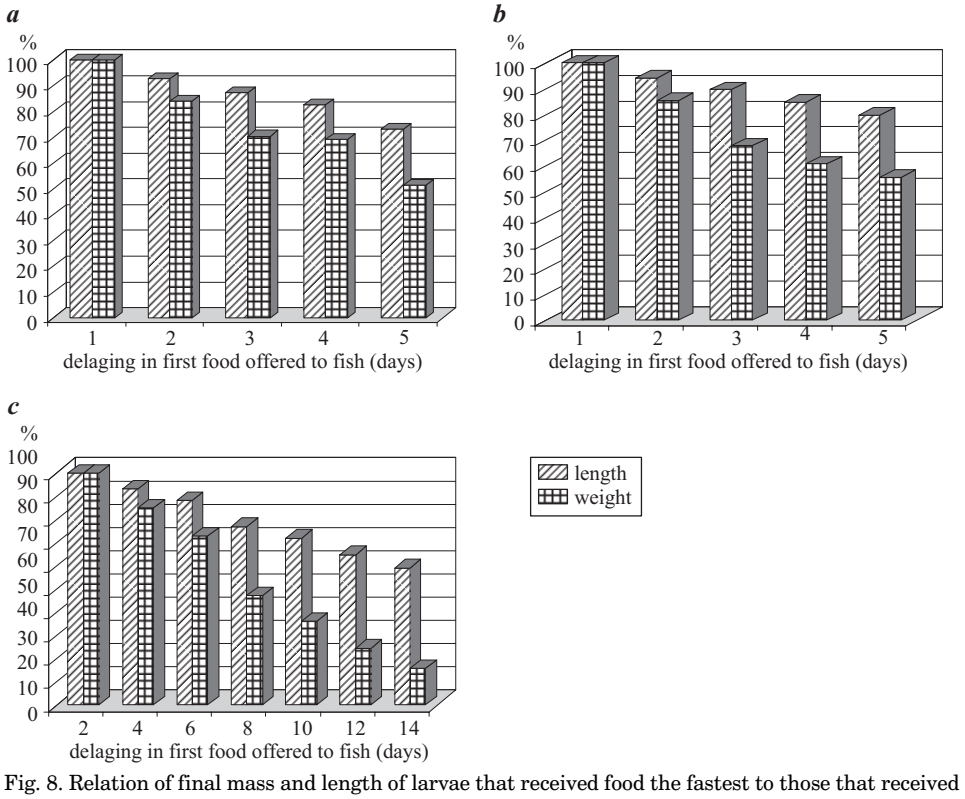

Fig. 8. Relation of final mass and length of larvae that received food the fastest to those that received the food the latest as a specific temperature: *a* – 25°C, *b* – 20°C, *c* – 15°C

Discussion

European catfish belongs to the group of species for which the PNR has been determined for the first time. As a consequence any conclusions or comparisons will refer to studies carried out on other species. The term "Point of no return" – PNR – currently used in literature was described in detail by BLAXTER and HAMPEL (1963). They proved that the hatch, after completing resorption of the yolk sack, was able to survive a certain period of time without exogenous food. However, to survive, it must receive food at the time not exceeding a specific critical point. In every case, after exceeding that critical moment the hatch dies, even though the food can already be available in the environment. They also showed that the period depends on the origin of spawners (fish population), size of eggs, period and temperature of incubation and hatch keeping temperature.

Numerous later works also reported that among many environmental factors influencing survival of the larvae, shortage of food as concerns both quantity and quality played the decisive role. Similar reasons for high mortality of larvae were also presented by other researchers such as: SMITH (1935), SETTE (1943), IVLEV (1955), KRYZHANOVSKI (1956), BEVERTON, HOLT (1957), FROLIENKO (1959), BALON (1960) and WOYNAROVICH (1960). A large role in obtaining knowledge on the issue was played by experiments by BOGDANOVA (1965), PANOV (1968), ZAWISZA, BACKIEL (1970), DABROWSKI (1976a,b), DĄBROWSKI (1975). The study described by Ivlev in 1955 concerned resistance to fasting and whether, and to what extent the biotic and abiotic factors influenced that phenomenon in the hatch and stock of numerous fish species. It was established that lack of food had a particularly negative influence among the youngest fishes. It decreased the speed of their adaptation reactions to unfavorable environment conditions and, when it continued long enough, it caused death, even if the food conditions improved.

During the last years of the 20th c., a number of studies on resistance of fish hatch to shortage of food were also carried out in Poland (Table 2). In all those works the authors notice that with the passage of time the mobility of fishes decreases. The hatch spends most of the time laying on the bottom and its sensitivity to the administered food decreases. Detailed mobility measurements in cyprinids were conducted by WOLNICKI and OPUSZYŃSKI (1998). They showed diversity in mobility of individual species. The hatch of carp and amur showed larger activity than the hatch of bighead carp and silver carp.

Table 2
Time of occurrence of 50% (PNR_{50}) mortality in fish larvae deprived ot feed in relation to temperature according to different authors

Species	Temperature (°C)	PNR_{50} (days)	Authors
European whitefish *Coregonus lavaretus* (L.)	7.8 9.4	16 15	GRUDNIEWSKI (1980)
Peled *Coregonus peled* (Gmel.)	7.5 10.2	23 22	
European cisco *Coregonus albula* (L.)	7.3 9.9	18 16	
	10.4	12	DOSTATNI, ŁUCZYŃSKI (1992)
Asp *Aspius aspius* (L.)	10 15 25	22 16 10	KUJAWA (1998)
Vimba *Vimba vimba* (L.)	15 20 25	12.6 9.2 7.6	KUJAWA (2004)
Chub *Leuciscus cephalus* (L.)	15 20 25	14.4 10.9 8.6	
Dace *Leuciscus leuciscus* (L.)	15 20 25	14.8 11.1 9.0	
Ide *Leuciscus idus* (L.)	15 20 25	15.4 11.4 9.5	
Barbel *Barbus barbus* (L.)	15 20 25	16.5 12.1 10.0	
Nase *Chodrostoma nasus* (L.)	15 20 25	19.4 14.4 11.9	

After reaching the "critical moment" only few fishes made attempts to catch food. That activity nevertheless did not influence their survival because at that time, as reported in literature, internal changes had gone so far that the fishes were unable to digest the food. KOSTOMAROVA (1962) showed that in case of pike the process of self-digestion of the alimentary system started already with resorption of the yolk sack. In carp that process occurs later; it starts 4 days after resorption. Those differences are probably consequential to different fat content in yolk in case of different fish species (KRYZHANOVSKI 1960). Already 30 years earlier NEEDHAM (1931) determined that quantity at 6.2% in case of carp and 1.4% in case of pike. Irreversible changes in the internal organs were also found in powan during the studies carried out by KOROVIN et al. in 1972. On the basis of the above examples it can be concluded

that the time for reaching the critical point and the rate of internal changes depend on the species. Water temperature, however, has the major influence. The lower it is the slower the rate of the changes and the longer the time of resistance to food shortage.

Attempting a comparison of the PNR results obtained for the european catfish with other species, an appropriate group of fish species with similar environmental requirements should be selected. A comparison between the hatch of european catfish with Coregoninae fish hatch for which the natural reproduction occurs at water temperature below 10°C would be inappropriate. Cyprinid fish species, mainly thermophilous species, e.g.: carp, white amur, silver carp and bighead carp are closer to european catfish. Comparing PNR_{50} for the hatch of those species and european catfish at the temperature of 25°C, it can be easily noticed that dying of european catfish occurred after the twice shorter period.

The situation is similar in case of comparison of PNR for the european catfish larvae and asp larvae. Reviewing the results obtained in water at the temperature of 15°C, 50% mortality of european catfish larvae is exactly 1.5 times faster than that of the asp. Analysing all the results we should consider the size of spawn and hatch because those characteristics could have the major influence on extending the critical periods, particularly in low temperatures. WOLNICKI, OPUSZYŃSKI (1988) studied the resistance of young development forms of carp, amur, silver carp and bighead carp to temporary shortage of food Water temperature during the experiment was maintained at 25°C. For the hatch of carp and silver carp the "point of no return" was determined for day 11 of life without food while for amur and bighead carp hatch for day 13.

Considering the experiments carried out at the highest temperature of 25°C it is not difficult to draw the conclusion that the survival of european catfish is relatively low as compared to other fish species. Maybe the cause should be searched for in the differences in fat content in yolk as european catfish, similar to pike, is a predator. KOSTOMAROVA (1962) conducted a series of experiments comparing hungry hatch of carp with that of pike. Studying the hatch of carp he noticed deaths after 8 days, in case of pike after 4 days. In this case the information by IVLEV (1955) concerning supposedly higher resistance of predator fish to shortage of food was not confirmed in that case.

Conclusion

1. Resistance of european catfish larvae to shortage of food, although different as compared to other fish species, does not differ from the expected results assumed on the basis of literature data concerning predator fish species.

2. The conducted experiment confirmed that temperature plays the basic role in determining the period of resistance to food shortage.

3. Shown at least one week period of european catfish larvae resistance to food shortage in water at the temperature of 20°C offers the breeder a wider safety margin in case of controlled rearing.

4. Short-term keeping of european catfish larvae without food has no negative influence on its development and fitness. At higher or lower temperatures the resistance of european catfish larvae to temporary food shortage decreases or increases respectively.

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THE INFLUENCE OF STOCKING DENSITY ON SURVIVAL AND GROWTH OF BUENOS AIRES TETRA (*HEMIGRAMMUS CAUDOVITTATUS*) LARVAE REARED UNDER CONTROLLED CONDITIONS

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K e y w o r d s: *Hemigrammus caudovittatus*, initial rearing, survival, controlled conditions.

A b s t r a c t

Studies were conducted on the influence of stocking densities: 50, 100, 150 and 200 individuals dm^{-3} on survival and growth rates of Buenos Aires tetra larvae during 25 days of initial rearing under controlled conditions. The larvae were fed *ad libitum* with live nauplial stages of *Artemia* sp. The results of initial rearing obtained indicate that in case of tested appropriate environmental conditions of rearing larvae the densities up to 200 individuals dm^{-3} could be applied without clear negative influence on their survival and growth rate.

WPŁYW ZAGĘSZCZENIA OBSAD NA PRZEŻYWALNOŚĆ I WZROST LARW ZWINNIKA OGONOPRĘGIEGO (*HEMIGRAMMUS CAUDOVITTATUS*) PODCHOWYWANEGO W WARUNKACH KONTROLOWANYCH

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S ł o w a k l u c z o w e: *Hemigrammus caudovittatus*, podchów, przeżywalność, warunki kontrolowane.

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Abstrakt

Przeprowadzono badania nad wpływem zagęszczenia obsad (50, 100, 150 i 200 osobn. dm^{-3}) na przeżywalność i tempo wzrostu larw zwinnika ogonopręgiego podczas trwającego 25 dni podchowu w warunkach kontrolowanych. Larwy karmiono *ad libitum* żywymi stadiami nauplialnymi *Artemia* sp. Otrzymane wyniki podchowu wskazują, że w hodowli tego gatunku przy zapewnieniu odpowiednich warunków środowiskowych, można stosować zagęszczenia sięgające 200 osobn. dm^{-3} bez wyraźnego negatywnego wpływu na ich przeżywalność oraz tempo wzrostu.

Introduction

Buenos Aires tetra, *Hemigrammus caudovittatus,* belongs to the *Characidae* family and similar to neon tetra, *Paracheirodon innesi,* or yellow tetra, *Hyphessobrycon bifasciatus,* is among the most popular species of that family in aquarium breeding. In the natural environment they are found mainly in tropical waters of South America although they are also highly numerous outside their natural geographic location being objects of interest in high demand among aquarium keepers worldwide. Their high popularity results from both attractive coloring and ease of breading. Buenos Aires tetra is a small fish reaching 7–9 cm in length. In the natural environment it is found most numerously in the basin of the La Plata. The species was imported to Europe during early 1920's. It is relatively fecund for its size. During one spawning act the female lays even more than 2000 of eggs (KUJAWA 2000). During reproduction under controlled conditions the satisfactory number of live gametes can be obtained during 5, maximum 6 spawning acts. Later, despite attempts at reproduction no live offspring is obtained. Young fish hatch within 24 hours from fertilization of the eggs and after several days they start independent feeding (KUCHARCZYK et al. 2008).

High demand of the market for decorative species of *Characidae* fish that is accompanied by increasingly fierce competition between producers aiming for limiting the costs of breeding at any effort forces development of new production methods based on new technologies. Use of recirculation systems offers one of the possibilities of production intensification (BUCKLIN et al. 1993, HALACHMI 2006). Obtaining knowledge on the environmental requirements and adaptation potential of the studied species should be prerequisite to applying them (SZLAMIŃSKA 1988).

For several years, studies concerning conditions for initial rearing of decorative fish have been subject to extensive development covering all the time new species. During the recent years aspects related to application of different feeds, both live and artificial have been subject to particular interest of researchers (LIM 1997, 2002, 2003, SALES, JANSENNS 2003, TARGOŃSKA 2007). Increasing of the stocking density is, next to diversified

feeding, another method allowing intensification of production of fish. The issues of selection of the appropriate initial density have been the subjects of detailed studies conducted on numerous species. The optimum values of those densities differ not only for individual species but also depend on the initial rearing method (TRUCKER 1998). Achievement of the highest density values reaching 200 individuals dm^{-3} is allowed by closed recirculation systems most often used in initial rearing of sea fish (ALVAREZ-GONZALES et al. 2001) In case of freshwater fish the densities suggested by researchers are much lower and usually do not exceed 100 individuals dm^{-3} (ALBRECHT et al. 1977, WOLNICKI 1997, WOLNICKI, MYSZKOWSKI 1999, KUJAWA 2004, SZKUDLAREK, ZAKĘŚ 2007).

This study aimed at determining the effect of the stocking density on the growth rate and survival of Buenos Aires tetra during initial rearing under controlled conditions.

Materials and Methods

Initial rearing of the larvae was carried out in the device designed for that purpose. It consisted of one large glass tank with 50 dm^3 capacity functioning as a water bath and 16 smaller tanks with water capacity of 1 dm^3. The bath was equipped with a aquarium heater connected with a microprocessing thermoregulator with accuracy of 0.1°C, internal turbine filter and gas-discharge tube lighting. In each of the small tanks a half of one wall was replaced with mill gauze, mesh side length 200 μm, which allowed continuous water circulation.

The initial rearing was conducted at four densities of 50, 100, 150 and 200 individuals dm^{-3}. The material for initial rearing consisted of larvae originating from four spawner pairs the reproduction of which was conducted according to the methodology applied by KUCHARCZYK et al. (2008).

The experiment started with exogenous feeding and ended after 25 days of initial rearing. Water temperature during the initial rearing was 26°C. Oxygen saturation ranged from 65.0 to 82.0%, ammonia concentration from 0.01 to 0.05 mg dm^{-3}, and pH from 8.25 to 8.50. The photoperiod during the entire initial rearing period was constant at 12 hours of light and 12 hours of darkness.

During the entire period of initial rearing the larvae were fed 3 times per day (8.00, 12.00 and 16.00) *ad libitum* with live nauplial stages of *Artemia* sp. The feed doses were proportional to the number of larvae in individual tanks. Every day in the morning, before feeding, the aquaria were cleaned from remains of food and fish wastes and 30% of water was replaced with fresh water at the same temperature. The initial rearing for each density variant was conducted in four replications.

Sampling and processing of samples

The control sample (4 times ten individuals) was collected on the first and the last day of rearing. Before length measurement the fish caught were subjected to a few minutes of anesthesia in 2-phenoxylene solution. The density of the solution set at 0.3–0.4 cm^3 dm^{-3} was established on the basis of own earlier experiments. The length was measured with the accuracy of up to 0.1 mm. After measurement the fish were returned to the appropriate tanks.

On the basis of the data obtained the coefficient of total length increase per time unit (ITL) was computed according to the formula (PENAZ et al. 1989):

$$\mathrm{ITL} = \frac{\mathrm{TL}(n_1) - \mathrm{TL}(n_0)}{\Delta t}$$

TL – average length of the individual
n_1 – beginning of the period
n_0 – end of the period
Δt – duration of initial rearing (days)

Statistical analysis

The average values and the standard deviation were computed for survival and length of individuals at the end of the experiment. The statistical differences between groups in survival and total lengths achieved at the end of the initial rearing period were analyzed by one-way analysis of variance (ANOVA) and Tuckey's *post-hoc* test. Statistical significance was assumed at $P < 0.05$.

Results

The survival of Buenos Aires tetra larvae after 25 days of initial rearing at different densities was similar and ranged from 89.0 to 95.7% (Table 1). The values of that parameter for the two lowest densities (50 and 100 individuals dm^{-3}) were higher than in the group with the largest number of fish per 1 liter of water (200 individuals dm^{-3}). The differences observed in the individual experimental groups were statistically significant. The average length of larvae recorded at the end of the experiment was the highest in the groups where initial rearing was conducted at the lowest density of fish (18.67 mm). In the other cases the values of that parameter were lower (range: 16.64–17.09 mm)

and did not differ statistically (Table 1). Different values of ITL of the larvae were the result of the differences observed in the length of fish at the end of the initial rearing while there were no such differences at the beginning of the experiment and the duration of the initial rearing was the same for all the fish. The values of that parameter were very similar for three groups (ca. 0.5 mm/day), where the highest densities were applied. In the aquaria in which the number of fish per 1 liter of water was the lowest the ITL coefficient value was evidently higher (0.58 mm/day) – Table 1.

Table 1
Results of initial rearing of Buenos Aires tetra, *Hemigrammus caudovittatus,* at different densities

Parameter	Density (individuals dm^{-3})			
	50	100	150	200
Survival (%)	94.5^{a} (±5.36)	95.7^{a} (±3.03)	89.0^{ab} (±7.56)	90.7^{b} (±3.17)
Average length of larvae at the beginning of rearing (mm)	4.25^{a} (±0.19)	4.25^{a} (±0.19)	4.25^{a} (±0.19)	4.25^{a} (±0.19)
Average length of larvae after rearing (mm)	18.67^{a} (±2.07)	17.09^{b} (±0.97)	16.80^{b} (±1.18)	16.64^{b} (±1.56)
ITL coefficient (mm day^{-1})	0.58	0.51	0.50	0.50

In brackets the value of standard deviation is given. The same letters mean absence of statistically significant differences in rows.

Discussion

Providing appropriate environmental conditions is one of the major problems during initial rearing of fish larvae under controlled conditions (OPUSZYŃSKI 1983). This is even more important if increasing the density of stocking is to be the method for intensification of production. Undoubtedly high and very similar level of survival of the larvae in all groups offers confirmation of favorable environmental conditions in the tanks during the initial rearing of Buenos Aires tetra. The mortality rate at a few percent level recorded at the end of the experiment resulted most probably from developmental defects and deterioration of initial rearing condition with growth of the fish. Similar conclusions were reached by KUJAWA (2004) during three weeks initial rearing of selected reophile fish species. The results of those experiments gave even higher and balanced survival rate (ca. 99%) of the larvae in individual experimental groups. Achievement of such a good result at the end of the experiment could result from slightly shorter and conducted at a lower density (25, 50 and 75 individuals dm^{-3}) initial rearing.

The results presented in this paper also confirm the observation that the larger the volume of water per one fish, the faster is the growth (BROWN 1957). It was no surprise then that also in case of Buenos Aires tetra the definitely highest coefficient of day length increase was recorded in the tanks with the lowest stock of fish. What is interesting, however, in the other experimental groups where the density of individuals was at least twice higher, the values of that parameter were almost identical. For comparison: in case of reophile Cyprinid fish such as barbel *Barbus barbus* (L.) or nase *Chondrostoma nasus* (L.) the increase in stocking density from 50 to 75 individuals dm^{-3} resulted in evident decrease in length increases per time unit (KUJAWA 2004).

The observed results of initial rearing of Buenos Aires tetra show that if optimal environmental conditions was prepare and satisfactory availability of food the rearing of that species can be carried out at a density reaching even 200 individuals dm^{-3} successfully. High densities had no significant influence on survival and obtaining a larger number of larvae from one volume unit compensates for a slightly slower growth rate.

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EARLY ONTOGENY OF *TROPHEUS MOORII* BOULENGER 1898 (PISCES, *CICHLIDAE*, LAKE TANGANYIKA) IN LABORATORY CONDITIONS

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K e y w o r d s: Great African Lakes, cichlid, mouthbrooder, larva, development, embryo.

A b s t r a c t

In this work were shown some crucial moments in early *Troheus moorii* development (for example: begin of exogenous feeding period, end of endogenous feeding period, time of finish fins development and others). Fertilized eggs, obtained after natural spawn from females of *Tropheus* species kept in tank, were incubated in laboratory conditions. Hatched embryos were reared in incubation tank, in constance temperature, until resorption of the yolk sac and finish fins development. Embryos were fed *ad libitum* with live *Artemia* nauplii. In *Tropheus*, the fins became fully developed and the yolk sac supplies depleted on the same day, 25th since the mating. Since this species keeps its offspring in the mouth for 30–35 days (YANAGISAWA, SATO 1990), the young stay in they shelter for another one to two weeks. When they are let out for the first time, they are in an advanced stage of their development, mature enough to live like the adults of their species. Since their yolk sack supplies are depleted before the termination of parental care period, it is obvious that they must be intrabucally fed in this time. It is confirmed by the fact that in aquarium conditions incubating females pick the food particles as the non-incubating females do. The food had been found on a regular basis in incubating females mouths when acquiring eggs and embryos for observations. Since the young *Tropheus* already can ingest exogenous food while being still in mothers mouth, it is on purpose to feed crushed food to the incubating females in aquarium conditions.

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WCZESNY ROZWÓJ GĘBACZA Z GATUNKU *TROPHEUS MOORII* BOULENGER 1898 (PISCES, *CICHLIDAE*, JEZIORO TANGANIKA) W WARUNKACH LABORATORYJNYCH

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S ł o w a k l u c z o w e: Wielkie Jeziora Afrykańskie, ryby pielęgnicowate, gębacz, larwa, rozwój, zarodek.

A b s t r a k t

W pracy opisano niektóre kluczowe momenty we wczesnym rozwoju *Tropheus moorii* (np. rozpoczęcie odżywiania egzogennego, zakończenie odżywiania endogennego, zakończenie rozwoju płetw i inne). Zapłodnione jaja tego gatunku, otrzymane w wyniku naturalnego tarła od tarlaków przetrzymywanych w akwariach, inkubowano w warunkach laboratoryjnych. Po wykluciu zarodki umieszczano w specjalnym akwarium, w stałych warunkach środowiskowych, aż do pełnej resorpcji woreczków żółtkowych oraz zakończenia rozwoju płetw. Karmiono je *ad libitum* żywymi stadiami nauplialnymi solowca. Zaobserwowano równoczesne zakończenie rozwoju płetw i wyczerpanie woreczka żółtkowego w 25. dniu od tarła. W związku z faktem, iż młode *Tropheus* przebywają w jamie gębowej samicy przez 30–35 dni (YANAGISAWA, SATO 1990), znajdują one tam schronienie jeszcze przez tydzień lub nawet dwa od momentu wkroczenia w stadium juwenilne. Kiedy samica wypuszcza je po raz pierwszy są rozwinięte na tyle, że mogą pędzić życie podobne do osobników dorosłych. Można więc stwierdzić, że młode tego gatunku muszą pobierać pokarm egzogenny, jeszcze w czasie gdy przebywają w jamie gębowej matki. Potwierdzeniem tej tezy jest także fakt, że wielokrotnie obserwowano inkubujące samice, które aktywnie poszukiwały pokarmu i pobierały go, zupełnie tak samo, jak czynią to samice nieinkubujące. Podczas pobierania młodych znajdowano również pokarm we wnętrzu jam gębowych samic. Skoro młode trofeusy mogą połykać pokarm, znajdując się jeszcze w jamie gębowej samicy, to podawanie go samicom inkubującym może wpłynąć na lepsze wyniki w hodowli tego gatunku.

Introduction

Cichlids family (*Cichlidae*) is a big group of tropical fishes belonging to Perciformes order. Estimated number of currently living cichlid species varies from1300 (NELSON 1994) to 2000 (FARIAS et al. 2000), with most of them living in Great African Lakes. About 66% of Tanganyikan cichlids are mouth-brooders.

Unfortunately, there is not much information about early ontogeny of African mouthbrooders. Most studies were based on underwater, not laboratory observations, therefore the information is incomplete (YANAGISAWA, NSHOMBO 1983, YANAGISAWA 1985, MIHIGO 1986, YANAGISAWA 1986, KUWAMURA 1988, KUWAMURA, MIHIGO 1988, YANAGISAWA, SATO 1990, YANAGISAWA, OCHI 1991). The researches concentrated on breeding strategy rather than on early ontogeny of individual species.

One of the most precise descriptions of the early ontogeny can be found in only a few publications (BALON 1977, HOLDEN, BRUTON 1992, HOLDEN, BRUTON 1994).

The Tanganyikan mouthbrooder, *Tropheus moorii* Boulenger 1898, occurs only in Tanganyika Lake, in the rocky lithoral (YAMAOKA 1983). *Tropheus* lives near rock shore, from 1 m to 15 m deep and feeds on peryphiton. Strong relation with rocky substrate is a cause of almost no migration between the rocky aggregates, which is likely to be an origin of isolated populations and the existence of over 100 geographical variations.

Fig. 1. The pack consisting of adult specimens *Tropheus moorii* Chaitika

Tropheus moorii is a polygamic species displaying minor exterior sex differentiation (Figure 1). Maximal total length of an adult specimen is 14.5 cm. *Tropheus moorii* lives in strongly hierarchized packs. In the aquariums, the fishes manifest strong intraspecies aggression, which is one of the causes of troubles in breeding and raising the species in the artificial environment.

The spawning takes place on an unprepared rock surface. Fertilization takes place in female's mouth. In one spawning she can lay up to 20 eggs. The incubation continues for about 30 days (26°C), after this period the female takes care of the swimming offspring for about 14 days. In the mouth incubation period it is possible to observe females pick up particles of food to feed herself and/or the offspring (YANAGISAWA, SATO 1990).

Materials and Methods

Parent fishes were obtained from wild population of *Tropheus moorii* Chaitika = Blue Rainbow (24 specimens) from Lake Tanganyika. Their offspring was obtained in the natural way, without using hormonal, or any other kind of artificial induction. Adult fishes were kept in aquariums, fulfilling their environmental needs. *Tropheus* in aquarium culture are rather sensitive, because of their strong territorial aggression and problems with settlement and sustaining their social hierarchy without killing each other. Adult fishes were kept in 360 dm^3 aquariums with automatically heater (26°C ± 0.5) and effective filtration system (internal – mechanical filter Aquael and external – biological and mechanical filter Eheim 2250). Photoperiod was 12/24. Fishes were fed daily with artificial food designed for herbivorous fishes (Spirulina Tropical, Sera Granu Green) and occasionally with frozen *Chaoborus* larvae, *Daphnia* or *Cyclops*, as a diversification of diet. Two times a week, 25% of water capacity was changed. Two artificial, hollow rocks were put into each tank, used by the fish for the hiding places, in order to diminish the aggression.

Observations of the development were carried out on eggs and embryos from natural mating. Eggs or embryos were collected by catching the incubating females. The offspring was removed from her snout to a separate tank. Time of spawning was noted every time. Eggs were removed to 100 dm^3 incubating tank with automatically heater and internal filter (Aquael). Water conditions in the incubation tank were matched with the main tanks. Temperature oscillations can change tempo of early ontogeny, therefore in incubating aquarium temperature was kept constant (26°C ± 0.5) (ŁUCZYŃSKI, KIRKLEWSKA 1984). Low oxygen concentrations can slow down development process, and to avoid this the tank's internal filter had been set up in the way

that ensured good water surface circulation (CARLSON, SEIFERT 1974). Each group of eggs was kept in separate basket hanged in incubation aquarium.

Embryos were fed 2–3 times daily, *ad lib*., with *Artemia* nauplius and powdered artificial food designed for herbivorous fishes (Spirulina Tropical). Every day 10 to 20% of water was changed to fresh and the sediment or dead specimens were removed from the tank.

5–10 specimens were taken randomly from each spawning to exam in certain time intervals (see below), examinations were repeated 6 times on different spawns. Examination manipulations were increasing the death rate, so the observation was divided into 2 stages: from fertilization to hatching, and from hatching to the end of the observation period. Thus, all specimens damaged by manipulations were removed from further examinations.

Examinations were carried out in following time intervals:
– from the fertilization to the end of cleavage phase – every 2 hours,
– from the end of cleavage phase to hatching – every 12 hours,
– in the free embryo phase – every 24 hours.

Examinations were discontinued when specimens had no more visible yolk sac and their fins were fully developed (all the fin rays present).

The specimens were placed under the stereomicroscope (magnification 16–32 times). They were photographed using a digital camera attached to the microscope. All the measurements were taken from the photographs. In order to avoid overheating of the specimens, an optical fibre light source was used were lighted up using for microscope observations. No anesthetics were used to avoid change the development rate of the specimens (MASSEE et al. 1995).

Examined groups of specimens were attributed to specific development stage if at least 50% specimens from the group had reached the stage criteria (GADOMSKI 1995).

Activation was considered to be the midway point between the time of first egg deposition and the last sperm uptake by female (HOLDEN, BRUTON 1994). The time difference between fertilization and activation was considered irrelevant regarding the general time span of the research (ARAUJO-LIMA 1994).

Chosen terminology of ontogenetic events and intervals was following BALON (1975, 1990) and HOLDEN, BRUTON (1992, 1994):

– the cleavage phase begins with activation and ends once organogenesis begins,

– hatching of more than 50% specimens marks the beginning of the free embryo phase,

– juvenile period begins with yolk sac supplies completely absorbed and fins fully developed.

Examinations were finished once the juvenile period had begun.

For calculating the volume of yolk sac, mathematic formula was chosen (BLAXTER, HEMPEL 1963):

$$V = 0.5236 \cdot l \cdot h^2$$

where:
V – yolk sac volume,
l – yolk sac length,
h – yolk sac height.

The formula is appropriate for yolk sac shape similar to *Tropheus*'.

Mobility of the young fish is strongly related with their ability to avoid the predators and to catch food, therefore development of the fins was examined (KOUMOUNDOUROS et al. 2001). Rays of all fins were counted everyday.

Photographic documentation was made using the digital camera attached to the stereomicroscope. The specimens chosen for documentation specimens were demonstrating most of the characteristics typical for given development stadium. Results of all live specimens' measurements were recorded, following the above – given methodology.

Data collection and processing has been made with the use of Microsoft Excel and Statistica.

Results

Eggs

Eggs were obtained from natural spawning, were laid and fertilized in water. Therefore all the measurements were made on bulged and fertilized eggs. Mouthbrooders' eggs in ovaries have irregular spherical shape and change the shape to oval because of their contact with water and squeezing in the oviduct (BALON 1977). All eggs were measured in two axes: longest axis longitudinal (L) and shorter axis transverse (H).

Tropheus moorii lays very big eggs: L = 5.78 mm (± 0.22), H = 4.11 mm (± 0.08), N = 60.

The eggs are intensely orange. Yolk supplies are not transparent, but the observation was possible because of transparent egg membrane. There were no fat globules in yolk. Average number of eggs in one spawning was 12 (± 3.54), average calculated from 6 spawnings from 6 different females, ranging between 7–16 eggs.

The cleavage phase

During two first hours, perivitalline space became visible at the animal pole, a little yellowish translucent blastodisc was formed and the egg membrane hardened. After 2 hours, the first division took place and two cells become visible on the animal pole. Perivitalline space was strongly cramped by olk supplies and egg membrane (Figure 2). Because of big mass of yolk supplies, the cleavage process was partial (GRODZIŃSKI 1971). The epiboly begun after 24 hours (Figure 3).

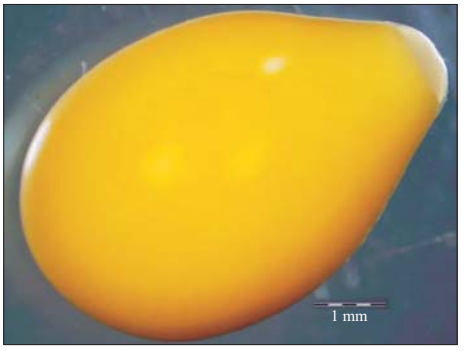

Fig. 2. *Tropheus moorii* Chaitika. The cleavage phase

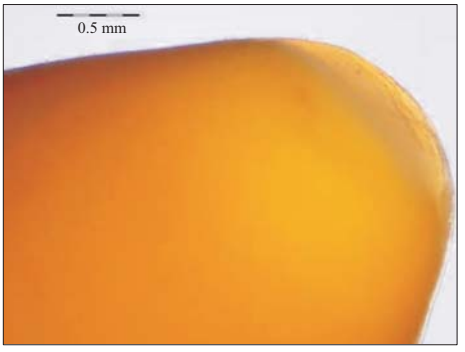

Fig. 3. *Tropheus moorii* Chaitika. Beginning of the epyboly

The embryonic phase

After 48 hours from fertilisation, the organogenesis begun (Figure 4), aggregation of transparent cells located on the narrow pole of the yolk was relatively poorly visible.

Fig. 4. *Tropheus moorii*. End of the epyboly

Fig. 5. *Tropheus moorii*. Embryo until hatching

First melanophores on the yolk sac became visible after 48 hours from spawning. After 72 hours from spawning haemoglobine appeared visible in blood cells (the blood had been transparent earlier, after 72 hours became little pinkish). Heartbeating and first embryo's movements were observed after 96 hours from spawning. Melanine in eyes of embryos became visible after 168 hours from spawning, and after 192 melanophores became visible on their heads.

During all periods of the egg membrane development embryos were shorter than longer axis of egg, shortly before the hatching their tail could attain the vegetal poles of eggs. (Figure 5).

The free-embryo phase

Embryos hatched about 192 hours after spawning (50% hatching) – Figure 6. Before hatching, the embryos were demonstrating strong tail movements, which probably conducts discerption of the egg membrane. In this process, the fluid in the egg membrane became little cloudy, probably as a result of hatching enzymes secreted by the embryos. Free embryos were demonstrating a very strong photophobic reaction. There were no visible cement glands on the heads (Figure 7). Eyes were fully pigmented, mouth was open and functional (mouth movements visible).

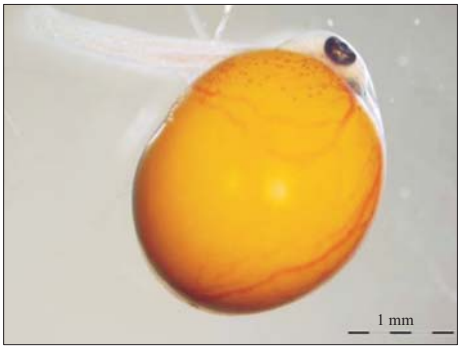

Fig. 6. *Tropheus moorii*. Hatching embryo

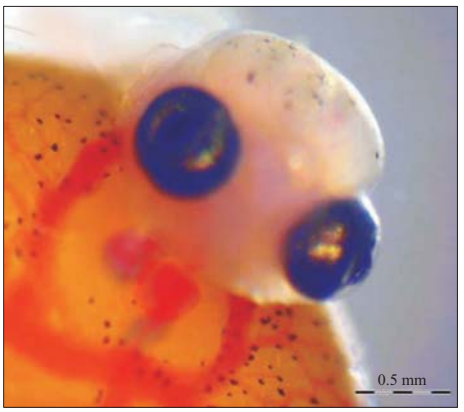

Fig. 7. *Tropheus moorii*. Free embryo. Lack of the cement glands on the head

Embryonal respiratory glands – Cuvier's ducts – were very well visible on the surface of the yolk sac of hatched embryos, pectoral fins were also developed well. Primary fold was not very well developed, being rather small.

Measured volume of the yolk sac of fresh hatched *Tropheus* embryos reached average 61.66 mm^3, SD = 6.03. *Tropheus* has a very long period of endogenous feeding (25 days from spawning) – Figure 8. Exogenous feeding begun on the 14th day post spawn, therefore mixing (endo- and exogenous) feeding period was prolonged from 14th to 25th day after fertilization (11 days).

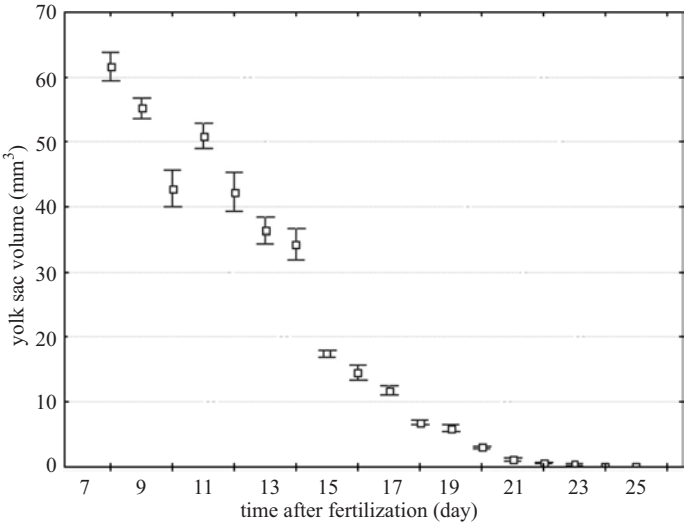

Fig. 8. Changes of the yolk sac volume in the *Tropheus moorii* embryos. Vertical bars indicate SD

Fins' development of *Tropheus'* embryos continue from 6th to 25th day from spawning. Caudal fin developed first, first caudal fin rays were visible before hatching. The development of caudal fin was longer than other fins', being as long as 16 days. In the day of hatching, embryos had fully functional pectoral fins, but with no visible rays yet. First rays became visible in caudal fin (before hatching), later in dorsal fin (on the 11th day from spawning), followed by the rays in anal and pectoral fins (12th day after spawning), with the pelvis fin rays being the last (18th day after spawning).

Changes in the total length in free embryos are presented at Figure 9.

Tropheus simultaneously exhausted yolk sac supplies and developed fin rays in all fins (25th day after spawning). The 25th day from spawning was considered as the beginning of juvenile period, when young fishes resemble adult specimens from this species (Figure 10). These typical vertical stripes can be seen on the adult specimens of *Tropheus moorii* Chaitika too, but they disappear on the fishes which are very high in social hierarchy (for example territorial males have no stripes at all).

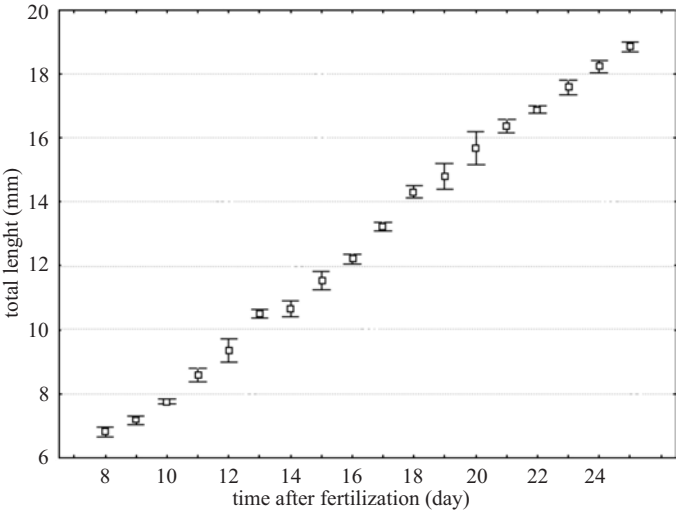

Fig. 9. Changes in total length in the *Tropheus moorii* embryos. Vertical bars indicate SD

Fig. 10. *Tropheus moorii* Chaitika begining the juvenile period

Discussion

African Cichlids exercise very wide range of breeding strategies, which are demonstrated not only by specific mating behavior of individual species, but by color manifestation (egg dummy in mouthbrooders) and even by strong size dimorphism (small females and big males in shell dwellers) (SCHÜTZ, TABORSKY 2000). The breeding strategy is related to the characteristic of their early development type: from egg size to size and structure of the embryo (BALON 1977).

Cichlids can protect their offspring in two main ways: the eggs can be laid on the substrate in some kind of nest (substrate brooders) or can be hidden in mouth of one or both parents (mouthbrooders) (KUWAMURA, MICHIGO 1988). *Tropheus* is a mouthbrooder. Mouthbrooders can be found in other fishes families, like *Osphronemidae* (RÜBER et al. 2004) or *Apogonidae* (VAGGELI 1999)

for example. Most of mouthbrooding species lay comparatively bigger eggs, number of eggs in one spawning is reduced, early ontogeny is longer compared to the substrate brooders, and mouthbrooders lack the larva stadium (BALON 1977, 1986).

There are hypotheses trying to explain the origin of this specific parental care demonstrated by mouthbrooders. One of them suggests that mouthbrooding is a reaction to strong spawning place and nesting place competition (KONINGS 1998). Another hypothesis suggests that the determining factor is the availability of food to young stadiums of fishes. According to this hypothesis, the enlargement of eggs' size (therefore suppling embryos with bigger yolk sac needed for endogenous feeding) makes the prolongation of early ontogeny period possible, thus the fishes make their first attempts to exogenous feeding later, when they are bigger compared to young non--mouthbrooders fishes (KUWAMURA, MIHIGO 1988). As a consequence, the range of available food for young mouthbrooders is very wide. This factor, together with highly developed parental care, is probably the reason of better survivability of mouthbrooders' offspring (BALON 1991). It is very probable that the diversity of types of early ontogeny in many species of fishes, is one of the types of environmental adaptation (ARAUJO-LIMA 1994).

Eggs of *Tropheus* have no glutinous membranes, which prevents glutination of eggs in mother's mouth in one conglomerate. Some mouthbrooders, belonging to other fishes families, lay their eggs with glutinous membrane and incubate the eggs glued in one egg mass (for example some *Apogonidae*), but they develop additional behavior mechanisms enabling to supply the oxygen to the eggs: the egg mass is being spit out and quickly catched back into the parent's mouth (VAGELLI 1999). Eggs with glutinous membrane are also laid by *Sarotherodon galigaeus* (*Cichlidae* – mouthbrooder). Those eggs are connected with each other by sticky filaments, but the filaments disappear in short time period because of water movement in parent's mouth (BALSHINE--EARN 1997).

In the mouthbrooders group relatively large diversification of the eggs size can be observed, but in the majority of species the eggs are bigger than in non-mouthbrooding group. In mouthbrooders group some species with relatively small eggs can be found – *Microdontochromis* sp. or *Perrisodus microlepis* for example, and some species with very big eggs (*Tropheus* or *Cyphotilapia frontosa*). These facts give evidence for existence of different steps of the breeding specialization in the mouthbrooders group itself (STIASSNY, GERSTNER 1992). *Tropheus* females in incubation period catch small food particles (for example *Artemia* nauplius) to feed embryos (YANAGISAWA, SATO 1990). Feeding the offspring can be of additional help to parental care prolongation, therefore offspring can be bigger and consequently able to have

greater chance to survive in the beginning of the self-dependent period of their life. *Tropheus*' eggs have no fat globules, which is probably related to buoyancy. The eggs of mouthbrooders (for example *Labeotropheus')* have negative buoyancy and after being laid they sink to the substrate. The mother pick eggs almost immediately (BALON 1977). *Tropheus* do the same. At the spawning time, the movement of adult fishes is very fast and intense, they cause strong water movements as well. If egg would have had positive buoyancy, it could have been unintentionally removed from spawning place and consequently from safe mother's mouth. Mouthbrooders spawn is a very fast process, eggs are picked by female almost immediately after they had been laid, so they can hardly be seen. Negative buoyancy is related not only to relatively big yolk sac, but also to higher yolk density (BALON 2001).

Intensive orange color of *Tropheus*' eggs comes probably from high content of carotenoids in yolk mass. Presence of carotenoids in yolk ensure oxygen supplies for developing embryo in periods of oxygen deficiency (BALON 1981, 1991). The presence the carotenoids in yolk is another factor which lets mouthbrooders' embryos hatch later and prolongates early ontogeny period (BALON 2002).

Hatched embryos have fully pigmented eyes, which is common in mouthbrooders group (BALON 1977, KUWAMURA, NAGOSHI, SATO 1989, HOLDEN, BRUTON 1994), some mouthbrooders can hatch without eye pigmentation – for example *Haplotaxodon microlepis* – mouthbrooder from Lake Tanganyika (KUWAMURA 1988).

Tropheus has simple development, without larva. According to BALON (2002), the embryonic period is characterized by exclusively endogenous feeding and the beginning of exogenous feeding marks the beginning of the larva period (complex development) or juvenile period (simple development). In juvenile period fishes lose most embryonic (or larval) organs, have ossificated skeleton, scales and occupy the same ecological niche, as the adult fishes of the same species. They also look similar to adults (PAVLOV 1999). The beginning of exogenous feeding in mouthbrooders' embryos take place when they have a lot of energy supplies aggregated in yolk sac. According to Balon, these stages should be called "juveniles" (BALON 2002), not "larva", because they exhibit many similarities to adult specimens (ossification) and the difference from the adults is the presence of embryonic organs, such as primary fold. *Tropheus*' embryos start to feed exogenously when their primary fold is still present and fishes have no ventral fins yet. These specimens should be rather called "free embryo" according to HOLDEN, BRUTON (1992, 1994). Many debates and controversy about precise definition of "larva" and "juvenile" demonstrate how difficult is to create clear criteria (KOVAČ, COPP 1999, HENSEL 1999, BALON 2002).

Mouthbrooders' free embryos demonstrated very intensive pectoral fins movements, while their caudal peduncle was rather small and almost immobile with comparison to non-mouthbrooders. In the mouthbrooders yolk sac, including *Tropheus* embryos, there is high concentration of carotenoids, which work as oxygen reserve in low-oxygen periods (BALON 1991). *Tropeheus* free embryos shown very strong photophobic reaction immediately after hatching, which probably prevent unintentional leaving the parent's mouth in natural conditions. Cement glands on the head of *Tropheus* embryos were not visible, this lack of head glands can be seen in almost all mouthbrooders embryos. Very interesting exception from this rule is Tanganyikan mouthbrooder *Haplotaxodon microlepis* (KUWAMURA 1988).

In many species of fishes which lays big eggs, embryos can begin exogenous feeding later than the species laying small eggs (KUWAMURA 1988, BALON 1977, MIHIGO 1986). This hypothesis is true for many cichlid species, for *Tropheus* as well.

In *Tropheus*, the fins became fully developed and the yolk sac supplies depleted on the same day, 25th since the mating. Since this species keeps its offspring in the mouth for 30–35 days (YANAGISAWA, SATO 1990), the young stay in they shelter for another one to two weeks. When they are let out for the first time, they are in an advanced stage of their development, mature enough to live like the adults of their species. Since their yolk sack supplies are depleted before the termination of parental care period, it is obvious that they must be intrabucally fed in this time (YANAGISAWA, SATO 1990). It is confirmed by the fact that in aquarium conditions incubating females pick the food particles as the non-incubating females do. The food had been found on a regular basis in incubating females mouths when acquiring eggs and embryos for observations. Since the young *Tropheus* already can ingest exogenous food while being still in mothers mouth, it is on purpose to feed crushed food to the incubating females in aquarium conditions.

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