# Polish Journal of Natural Sciences

**26**

(4/2011)

Statistical editor Anna Wiśniewska

Executive editor Agnieszka Orłowska-Rachwał

The print edition is the primary version of the Journal

The Journal is also available in electronic form on the web site http://wydawnictwo.uwm.edu.pl (subpage *Czytelnia*)

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# EFFECTIVENESS OF SELECTED FUNGICIDES IN POTATO PROTECTION AGAINST *PHYTOPHTHORA INFESTANS* AND *ALTERNARIA* SPP.

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K e y   w o r d s: potato, late blight, early blight, fungicidal control, leaves, fungi.

A b s t r a c t

An exact micro-plot experiment was conducted to determine the effect of fungicides (treatments: Sandofan Manco 64 WP, Penncozeb 80 WP, Tanos 50 WG; Tanos 50 WG applied three times; Tanos 50 WG, Penncozeb 80 WP, Tanos 50 WG) on the severity of late blight and early blight in three potato cultivars and on the composition of fugal communities colonizing potato leaves. The tested fungicides significantly reduced the intensity of late blight and early blight. The efficacy of fungicidal control was affected by weather conditions and potato cultivars. The highest efficacy (30–36%) against late blight and early blight was noted in potato plants cv. Aster sprayed with Sandofan Manco 75 WG, Penncozeb 80 WP, Tanos 50 WG and Tanos 50 WG applied three times in the first year of the study. Sandofan Manco 75 WG, Penncozeb 80 WP, Tanos 50 WG, and Tanos 50 WG and Penncozeb WP 80 used alternately during the last two last growing seasons were most effective in late blight control in potato cv. Tara and Salto. In these cultivars, early blight was best controlled with Tanos 50 WG applied three times, and Tanos 50 WG and Penncozeb 80 WP used alternately. The lowest counts of *Alternaria* spp. – the causal agent of early blight – were isolated from potato leaves in the above treatments.

## OCENA WYBRANYCH FUNGICYDÓW W OCHRONIE ZIEMNIAKA PRZED *P. INFESTANS ALTERNARIA* SPP.

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S ł o w a   k l u c z o w e: ziemniak, zaraza ziemniaka, alternarioza, ochrona chemiczna, liście, grzyby.

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Abstrakt

W ścisłym doświadczeniu mikropoletkowym badano wpływ opryskiwania fungicydami (kombinacje ochronne: Sandofan Manco 64 WP, Penncozeb 80 WP, Tanos 50 WG, 3-krotnie Tanos 50 WG, Tanos 50 WG, Penncozeb 80 WP, Tanos 50 WG) trzech odmian ziemniaka na nasilenie zarazy i alternariozy oraz na skład zbiorowiska grzybów kolonizujących liście.

Fungicydy istotnie ograniczały nasilenie zarazy i alternariozy na roślinach ziemniaka. Wpływ na skuteczność prowadzonej ochrony miały warunki pogodowe i odmiany. Największą skuteczność (30–36%) zanotowano w ochronie przed zarazą i alternariozą roślin odmiany 'Aster' opryskiwanych fungicydami: Sandofanem Manco 75 WG, Penncozebem 80 WP, Tanosem 50 WG oraz 3-krotnie fungicydem Tanos 50 WG w pierwszym roku badań. W ochronie ziemniaka odmiany 'Tara' i 'Salto' przed zarazą najefektywniejsze były fungicydy: Sandofan Manco 75 WG, Penncozeb 80 WP, Tanos 50 WG oraz przemiennie stosowany Tanos 50 WG i Penncozeb WP 80 w ostatnich dwóch sezonach wegetacyjnych. Nasilenie alternariozy na badanych odmianach najbardziej ograniczał 3-krotnie stosowany Tanos 50 WG oraz na przemian użyty Tanos 50 WG i Penncozeb 80 WP. Z liści badanych odmian ziemniaka w tych kombinacjach otrzymano najmniejszą liczebność sprawcy alternariozy.

# Introduction

The severity of late blight and early blight, two serious diseases of potato crops worldwide, is affected by weather conditions (CIF in 1996... 1997, ABD-EL-KHAIR and WAFLA 2007, BERNAT 2008) and cultivation measures (WIGGINS and KINKEL 2005, BOUWS and FINCKH 2008). Late blight resistance is an important consideration in breeding programs aimed to develop new potato varieties with improved disease resistance (GAWIŃSKA-URBANOWICZ and KAPSA 2006, SADOWSKI 2006). Fungicide application during the growing season may considerably reduce the incidence of early blight and late blight. Fungicides can prevent or delay the occurrence of the first infections caused by dangerous pathogens, including *Phytophthora infestans* and fungi of the genus *Alternaria*, thus inhibiting their spread and further development (KAPSA 2004, KURZA-WIŃSKA and GAJDA 2004, SCHEPERS 2004, SHAILBALA and PUNDHIR 2008).

The objective of this study was to determine the effect of fungicides applied to potato plants on the severity of late blight and early blight during the growing season, and on the composition of fugal communities colonizing potato leaves.

# Materials and Methods

Three potato cultivars, very early Aster (medium resistance to late blight: leaves – 4º, tubers 5º), medium early Tara (medium susceptibility to late blight: leaves – 2º, tubers – 4.5º) and medium late Salto (medium resistance to late blight: leaves – 5.5º, tubers – 5º), were grown in an exact micro-plot experiment conducted in Tomaszkowo (NE Poland). The experiment was carried out in

a randomized block design, with cultivars as blocks and fungicide treatments as sub-blocks. Every treatment comprised six plants per plot, in four replications. Plots with unprotected plants served as the control treatment. In experimental treatments, potato plants were sprayed three times at 10-day intervals with the following fungicides:

– Sandofan Manco 64 WP at 3 kg ha$^{-1}$ (1 application), Penncozeb 80 WP at 2 kg ha$^{-1}$ (2 applications), Tanos 50 WG at 0.7 kg ha$^{-1}$ (3 applications),

– Tanos 50 WG at 0.7 kg ha$^{-1}$,

– Tanos 50 WG at 0.7 kg ha$^{-1}$ (1 application), Penncozeb 80 WP at 2 kg ha$^{-1}$ (2 applications), Tanos 50 WG at 0.7 kg ha$^{-1}$ (3 applications).

Certified seed potato tubers were planted. Winter wheat was grown as a forecrop. Agricultural practices recommended by the Institute of Soil Science and Plant Cultivation in Puławy were applied.

During the growing season, two weeks from the last fungicide treatment, the severity of late blight and early blight was evaluated twice using a nine-point scale (PIETKIEWICZ 1985, where 1 – no symptoms, 9 – most severe symptoms). The results were presented in the form of an infection index.

$$\text{Infection index } I_i = \frac{\Sigma\,(ab) \cdot 100\%}{NI}$$

where:
$\Sigma\,(ab)$ – the sum of the products resulting from multiplying the number of the plants analyzed and the Ninen scale degree,
$N$ – total number of the plants analyzed,
$I$ – the highest scale degree. The efficacy (%) of the applied control methods was calculated using Abbott's formula, as recommended by EPPO.

After flowering, 20 leaves were collected randomly from the middle layer in plots of each treatment. 1 cm$^2$ samples of leaf tissue cut out at the base were placed in 200 ml flasks containing 90 ml sterile water. The flasks were shaken for 10 minutes. 0.2 ml samples of the suspension were transferred to Petri dishes containing PDA medium with rose Bengal and streptomycin. Fungal colonies were inoculated onto agar slants for later identification of species. The results were verified statistically by an analysis of variance for a randomized block design (STATISTICA® 8.0 2007–2008 software). Means were compared by Duncan's test (significance level 0.05). Meteorological data for the experimental period are shown in Table 1.

Weather conditions (Meteorological Station in Tomaszkowo)

| Month | 2000 | 2001 | 2002 | Mean for 1961–1995 |
|---|---|---|---|---|
| Temperature [ºC] | | | | |
| May | 14.0 | 12.8 | 16.2 | 12.7 |
| June | 16.0 | 13.9 | 16.5 | 15.9 |
| July | 15.9 | 20.0 | 20.1 | 17.7 |
| August | 16.9 | 18.1 | 19.8 | 17.2 |
| Monthly mean | 15.7 | 16.2 | 18.2 | 15.9 |
| Rainfall [mm] | | | | |
| May | 53.5 | 33.2 | 81.5 | 49.1 |
| June | 34.8 | 77.9 | 48.6 | 82.9 |
| July | 98.7 | 148.6 | 27.5 | 71.3 |
| August | 110.8 | 53.0 | 61.0 | 67.1 |
| Monthly total | 297.8 | 312.7 | 218.6 | 270.4 |

## Results and Discussion

The severity of late blight and early blight on potato plants was determined by weather conditions. The highest rate of infection by *P. infestans,* ranging from 47.7% to 53.4% (Table 2) was noted in cv. Aster and Tara in the control treatment, in the first two years of the study – wet with moderate temperatures (Table 1). In the analyzed period, the lowest infection rate was reported for cv. Salto. In the dry and warm growing season of 2002, the development of late blight was inhibited and the infection index did not exceed 25%.

As demonstrated by LENC (2007), high moisture levels resulting from, among others, sprinkler irrigation, may stimulate the development of late blight, particularly in susceptible potato cultivars (MATKOWSKI et al. 2004). The severity of late blight and early blight on the aboveground parts of potato plants, and the percentage of affected tubers are determined by the growth stage of the potato when the first infections caused by spores take place (RUBIO-COVARRUBIAS and GRUNWALD 2000). According to SAWICKA (2005) and GAWIŃSKA-URBANOWICZ and KAPSA (2006), weather conditions during the growing season affect also the response of potato cultivars to *P. infestans*. The cited authors observed the weakest symptoms of late blight in medium-late and late potato cultivars, and the strongest – in very early and early cultivars. Also in the present study the intensity of late blight symptoms was significantly lower in the late cultivar Salto than in early cultivars.

Table 2

Efficacy of fungicidal control of late blight caused by *P. infestans* in the investigated period

| Treatments | Aster | | Tara | | Salto | | Mean for treatments |
|---|---|---|---|---|---|---|---|
| | $I_i$ [%] | Ef [%] | $I_i$ [%] | Ef [%] | $I_i$ [%] | Ef [%] | |
| 2000 | | | | | | | |
| Control | 49.2[a] | – | 47.7[a] | – | 36.7[bc] | – | 44.5[a] |
| S, P, T | 34.8[cd] | 34 | 38.6[b] | 17 | 32.5[de] | 9 | 35.3[b] |
| 3 x T | 33.5[cd] | 36 | 34.5[cd] | 34 | 30.1[e] | 22 | 32.7[c] |
| Mean for cultivar | 39.2[a] | | 40.3[a] | | 33.1[b] | | |
| 2001 | | | | | | | |
| Control | 53.4[a] | – | 50.7[b] | – | 33.6[g] | – | 45.9[a] |
| S, P, T | 37.2[ef] | 24 | 38.3[ef] | 26 | 28.7[h] | 11 | 34.7[c] |
| 3 x T | 42.7[c] | 13 | 41.5[cd] | 20 | 27.1[h] | 22 | 37.1[b] |
| T, P, T | 39.5[de] | 18 | 36.5[f] | 29 | 26.9[h] | 24 | 34.3[c] |
| Mean for cultivar | 43.2[a] | | 41.8[b] | | 29.1[c] | | |
| 2002 | | | | | | | |
| Control | 29.7[a] | – | 27.8[ab] | – | 23.5[cd] | – | 27.0[a] |
| S, P, T | 25.8[bc] | 14 | 24.4[cd] | 12 | 17.3[e] | 35 | 22.5[b] |
| 3 x T | 24.6[cd] | 15 | 22.5[d] | 18 | 19.2[e] | 26 | 22.1[b] |
| T, P, T | 25.0[cd] | 22 | 23.8[cd] | 17 | 17.1[e] | 33 | 22.0[b] |
| Mean for cultivar | 26.3[a] | | 24.6[b] | | 19.3[c] | | |

Explanations: *S, P, T* – Sandofan Manco 64 WP, Penncozeb 80 WP, Tanos 50 WG, 3 x *T* – Tanos 50 WG applied three times, *T, P, T* – Tanos 50 WG, Penncozeb 80 WP, Tanos 50 WG, $I_i$ % – infection index [%], Ef% – efficacy [%], values marked by the same letter are not significantly different in years

During the three-year experimental period, the severity of late blight was significantly reduced in fungicide-protected potato plants. Tanos 50 WG applied three times was most effective in late blight control in early potato cultivars during the first growing season (efficacy of 34% to 36%). Tanos 50 WG applied three times as well as Sandofan Manco 64 WP, Penncozeb 80 WP and Tanos 50 WG provided the best control of late blight in cv. Tara in the second growing season (efficacy of 26% to 29%). In 2002, the lowest disease incidence in the above treatments was noted in cv. Salto, and the efficacy of the applied fungicides reached the highest level of 33% to 35%. According to many authors (HOWARD et al. 1996), the development of *P. infestans* after infection is inhibited to the greatest degree by cymoxanil. ANDRIEU et al. (2001) reported that famoxate was effective as a preventive agent against late blight, however the level of control after infection was insufficient. According to BASU et al. (2003), mancozeb and mancozeb with metalaxyl offered the best control of late blight on potato leaves and tubers. As shown by STEIN and KIRK (2002), the combined application of dimethomorph and mancozeb, pyraclostrobin

or chlorotalonil, is equally effective in potato protection against *P. infestans*. In a long-term study, MANTECON (2009) confirmed the high efficacy of mancozeb-based products in controlling *P. infestans*. The results of research conducted in Poland (KURZAWIŃSKA and GAJDA 2004) also indicate that mancozeb-based fungicides (Tattoo 750 SC, Curzate M 72,5 WP, Unikat 75 WG, Brestanid 502 SC) are highly effective in reducing *P. infestans* infections on the aboveground parts and tubers of potatoes. The following latest fungicides provide the best control of late blight: Ridomil Gold MZ 68 WG, Tattoo C 750 SC, Melody Med 69 WG, Altima 500 SC, Infinito 687.5 SC, Curzate M 72.5 WP (BERNAT 2008) and Ridomil MZ 72 (SHAILBALA and PUNDHIR 2008).

In contrast to late blight, the highest intensity of early blight was observed in the warm and dry growing season of 2002, and the highest infection rate was noted in cv. Salto in the control treatment (40.8%, Table 3). SADOWSKI (2006) reported that the development of early blight is supported by long drought periods interspersed by showers and potato growing in light, sandy soils. The factors that substantially reduce the incidence of early blight include the cultivation of varieties with increased disease resistance.

Table 3

Efficacy of fungicidal control of early blight caused by *A. alternata* in the investigated period

| Treatments | Aster | | Tara | | Salto | | Mean for treatments |
|---|---|---|---|---|---|---|---|
| | $I_i$ [%] | Ef [%] | $I_i$ [%] | Ef [%] | $I_i$ [%] | Ef [%] | |
| 2000 | | | | | | | |
| Control | $23.4^{bcd}$ | | $27.5^a$ | | $25.8^{ab}$ | | $25.6^a$ |
| S, P, T | $18.3^{ef}$ | 30 | $22.8^{cd}$ | 21 | $23.2^{bcd}$ | 16 | $21.4^b$ |
| 3 x T | $17.5^f$ | 33 | $24.4^{bc}$ | 16 | $20.8^{de}$ | 27 | $20.9^b$ |
| Mean for cultivar | $19.7^c$ | | $24.9^a$ | | $23.3^b$ | | |
| 2001 | | | | | | | |
| Control | $22.8^c$ | | $246^{bc}$ | - | $29.5^a$ | - | $25.6^a$ |
| S, P, T | $16.5^d$ | 35 | $19.0^d$ | 26 | $25.8^b$ | 21 | $20.4^b$ |
| 3 x T | $18.9^d$ | 22 | $18.3^d$ | 29 | $24.3^{bc}$ | 16 | $20.5^b$ |
| T, P, T | $17.4^d$ | 31 | $17.7^d$ | 34 | $25.9^b$ | 15 | $20.3^b$ |
| Mean for cultivar | $18.9^b$ | | $19.9^b$ | | $26.4^a$ | | |
| 2002 | | | | | | | |
| Control | $31.1^c$ | | $33.8^b$ | | $40.8^a$ | | $35.2^a$ |
| S, P, T | $26.4^{def}$ | 16 | $24.8^{efg}$ | 23 | $31.5^c$ | 23 | $27.6^b$ |
| 3 x T | $25.3^{efg}$ | 16 | $22.5^h$ | 31 | $27.4^{de}$ | 34 | $25.1^c$ |
| T, P, T | $25.8^{def}$ | 17 | $23.1^{gh}$ | 33 | $28.0^d$ | 30 | $25.6^c$ |
| Mean for cultivar | $27.2^b$ | | $26.1^b$ | | $31.9^b$ | | |

Explanations as in Table 2

In unprotected potato cv. Aster and Tara, the infection rate did not exceed 25% only in the second year of the study. In 2001 and 2002, cv. Salto was most affected by fungi of the genus *Alternaria*, and the difference between this cultivar and the other analyzed cultivars was statistically significant. The applied fungicides significantly reduced disease severity, except in cv. Salto treated with Sandofan Manco 64 WP, Penncozeb 80 WP and Tanos 50 WG. In the first two years of the experiment, the efficacy of fungicides in controlling early blight was higher in early potato cultivars (35%) than in late cultivars. In the last growing season, Tanos 50 WG applied three times and Tanos 50 WG and Penncozeb 80 WP used alternately offered the best control of early blight in cv. Tara and Salto. The high efficacy of Tanos 50 WG against early blight has been previously reported by KUCIŃSKA (2005). In a study by KAPSA (2004), the contact fungicides Unikat 75 WG and Antracol 70 WP provided effective control of early blight, contributing to a 34% increase in tuber yield. As demonstrated by SINGH (2008), Ridomil MZ 72 applied three times considerably inhibited the development of late blight and early blight, whereas Melody 50 WG was found to be least effective.

The fungal communities isolated from potato leaves (10 435 isolates) were dominated by yeast-like fungi (50% of all isolates) and filamentous fungi (29 species, Table 4). All fungal communities comprised species of the genera *Cladosporium, Epicoccum* and *Penicillium* and the order *Mucorales*, which is consistent with the author's previous findings (CWALINA-AMBROZIAK et al. 2007). The following potential pathogens were frequently isolated from potato leaves (approx. 30%): *Alternaria alternata, Botrytis cinerea, Colletotrichum coccodes, Fusarium avenaceum, F. culmorum, F. concolor, F. oxysporum, F. solani* and *R. solani*. The potential pathogens colonized potato leaves in all growing seasons. A decrease in the population size of potential pathogens was noted in fungicide treatments. The causal agent of early blight was isolated most frequently in 2002, when it accounted for 25.5% of all fungal isolates, which is why the infection rate was highest in this growing season. The lowest counts of this potential pathogen were reported from the aboveground parts of potato plants protected with Tanos 50 WG applied three times and Tanos 50 WG used alternately with Penncozeb 80 WP. The effect of fungicidal control on the abundance of the remaining pathogens varied. The smallest population of *Fusarium* fungi was noted in 2002, in the community isolated from potato leaves treated with Tanos 50 WG applied three times and Tanos 50 WG used alternately with Penncozeb 80 WP. JEANDET et al. (2000) pointed to the inhibitory effect of aluminum chloride and potassium phosphate on the growth of *Fusarium* fungi due to the activation of systemic resistance, including the induction of phytoalexin synthesis.

Table 4

Fungi isolated from potato leaves (number of isolates)

| Species | 2000 | | | 2001 | | | | 2002 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | control | S, P, T | 3 x T | control | S, P, T | 3 x T | T, P, T | control | S, P, T | 3 x T | T, P, T |
| *Alternaria alternata* (Fr.) Keissler | 157 | 87 | 87 | 109 | 61 | 60 | 44 | 246 | 134 | 137 | 102 |
| *Arthrinium sphaeospermum* Fuckel | 4 | – | 1 | – | – | – | – | – | – | – | – |
| *Botrytis cinerea* Pers. | 117 | 67 | 9 | 178 | 88 | 138 | 114 | 47 | 32 | 40 | 30 |
| *Chaetomium globosum* Hughes | 14 | 21 | 9 | – | – | – | – | – | – | – | – |
| *Cladosporium cladosporioides* Fres.de Vries | 13 | 29 | 14 | 20 | 12 | 2 | 18 | 33 | 8 | 46 | 22 |
| *Cladosporium herbarum* Link ex Fries | 14 | 2 | 1 | 22 | 11 | 2 | 6 | 13 | 20 | 35 | 30 |
| *Cladosporiumr macrocarpum* Preuss | 8 | 4 | 1 | – | – | – | – | – | – | – | – |
| *Colletotrichum coccodes* (Wallr) Hughes | 35 | 26 | 26 | 24 | 24 | 18 | 21 | 58 | 48 | 43 | 53 |
| *Endothia* spp. | 4 | 8 | 1 | 2 | 5 | 3 | – | 22 | 18 | 41 | 14 |
| *Epicoccum* spp. | 26 | 30 | 28 | 37 | 23 | 33 | 21 | 48 | 46 | 41 | 30 |
| *Fusarium avenaceum* (Fr.) Sacc. | 6 | 1 | 4 | 17 | 8 | 13 | 5 | 5 | 1 | 1 | – |
| *Fusarium concolor* Reinking | 11 | 6 | 6 | 13 | 9 | 12 | 13 | 2 | 5 | 2 | 5 |
| *Fusarium culmorum* (W. G. Sm.) Sacc. | 14 | 19 | 11 | 19 | 21 | 11 | 12 | 14 | 11 | 8 | 9 |
| *Fusarium oxysporum* Schlecht. | 16 | 15 | 14 | 16 | 18 | 15 | 13 | 4 | 7 | 8 | 6 |
| *Fusarium solani* (Mart.) Sacc. | 10 | 4 | 1 | 4 | 2 | 2 | 2 | 5 | 1 | – | 1 |
| *Geotrichum candidum* Link. | 6 | 4 | 1 | – | – | – | – | – | – | – | – |
| *Humicola brevis* Gillman and Abbott | 14 | 4 | 4 | – | – | – | 2 | 39 | 45 | 50 | 22 |
| *Humicola fuscoatra* Traaen | 5 | 4 | 1 | – | – | – | – | 16 | 32 | 31 | 7 |
| *Humicola grisea* Traaen | – | – | – | 6 | – | 2 | – | – | – | – | – |
| *Mortierella alpina* Peyronel | 7 | 10 | 8 | 12 | 7 | 6 | – | 18 | 14 | 16 | 30 |
| *Mortierella isabelina* Quadem | 2 | 1 | 5 | – | – | – | – | 7 | 8 | 26 | 9 |
| *Mucor circinelloides* van Tieghem | 7 | 7 | 7 | 7 | 11 | 1 | – | 22 | 6 | 36 | 10 |
| *Mucor hiemalis* Wehmer | 14 | 3 | 6 | 8 | 18 | 4 | – | 18 | 6 | 51 | 5 |
| *Penicillium* spp. | 48 | 83 | 54 | 39 | 56 | 65 | 50 | 27 | 23 | 46 | 38 |
| *Rhizoctonia solani* Kuhn | 7 | 7 | 3 | 9 | 8 | 7 | – | 5 | 5 | 4 | 4 |
| *Rhizopus nigricans* Ehrenberg | 8 | 6 | 11 | – | – | – | – | – | – | – | – |
| *Sporotrichum olivaceum* Fries | 14 | 5 | 8 | – | – | – | – | – | – | – | – |
| *Trichoderma hamatum* (Bon.) Bain | 12 | 2 | 2 | 13 | 2 | 4 | – | 4 | 2 | 1 | – |
| *Trichoderma harzianum* Rafii | – | – | – | – | – | – | – | 2 | 1 | – | – |
| Yeast-like fungi | 509 | 368 | 452 | 717 | 601 | 683 | 651 | 308 | 231 | 263 | 264 |
| Total | 1102 | 723 | 857 | 1280 | 994 | 1106 | 977 | 966 | 706 | 931 | 693 |

Explanations as in Table 2

It may be concluded that the tested fungicides significantly reduced the severity of late blight and early blight in the studied potato cultivars. The efficacy of fungicidal control was determined by the cultivar and the date of analysis. Cv. Salto was least affected by *P. infestans*, and most affected by fungi of the genus *Alternaria*. Protective treatment involving the use of systemic, contact and translaminar fungicides is highly recommendable for potato protection against *P. infestans*, while translaminar and contact fungicides can be recommended for potato protection against *Alternaria* spp.

Translated by Aleksandra Poprawska

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# APPLICATION EXPERIENCE OF AGRICULTURAL LANDS PRODUCTIVITY IMPROVEMENT METHODS

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K e y   w o r d s: agricultural lands, bioactive substances, soil chemical characteristics, cropping capacity, soil-protective technologies.

### A b s t r a c t

In a present article the results of land fund investigation of Vasylivka Rural Counsil in Onufriivka region of Kirovohrada oblast in Ukraine and agricultural lands productivity monitoring of land use entity Limited Liability Company "Maryivske" are given. It was established that lands are low-yielding (2.5 Mg ha$^{-1}$ of crops and leguminous plants) and ploughed up to 71%. The implementation of measures of agricultural chemicals rational use showed that their productivity reached 100%. It is reasonable to calculate the rates of local fertilizer distribution, taking into account soil chemical characteristics, provided by plants protection products adding and bioactive substances.

### BADANIE ZASTOSOWANIA METOD ZWIĘKSZENIA PRODUKCJI ROLNEJ ZIEMI

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S ł o w a   k l u c z o w e: grunty rolne, substancje biologicznie czynne, właściwości chemiczne gruntu, plony, technologia ochrony gruntu.

### A b s t r a k t

W artykule przedstawiono wyniki badań zasobów ziemi obszaru Vasylivka w powiecie Onufriskim (województwo kirovohradskie) na Ukrainie i wydajność monitorowanych gruntów rolnych użytkownika Ltd. „Maryivske". Ustalono, że grunty orne stanowią 71% i są niskowydajne (2,5 Mg ha$^{-1}$ upraw zbożowych i roślin strączkowych) Wykazano, że po uwzględnieniu racjonalnego wykorzystania nawozów nastąpił 2-krotny wzrost plonów. Uzasadniona jest więc taka dystrybucja i rozdysponowanie nawozów, która uwzględni właściwości chemiczne gleby, odpowiednie środki ochrony roślin i substancje biologicznie czynne.

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# Introduction

In the modern period of state evolution the most important is the guard of land reserves. The problems of large-scale ploughing and low-yield of agricultural lands demand a great attention. It is of great importance to work-out the economic instruments that have to guarantee reliable and effective land maintenance. The soil actual price, effective system of operational monitoring and state ecological policy are required. To work out precise and clear methods of assessment of damages from soil erosion in monetary terms is very essential. In conditions of law-based state, the land-user has to provide obvious evidences of adequate assessment of damages caused by his activities or, on the contrary, he has to be sure that improving the soil condition one will offset the expenditures on soil-protective technologies. The state has to conduct scientifically-based policy orientated on soil guard and regeneration for land-users (SHYKULA 2006).

# Materials and Methods

The soil of Vasylivka village soviet Rural Council in Onufriivskyi region of Kirovohrada oblast is the main research object. From 2005 till 2009 the researches of the land stock of the country and monitoring of the soil productivity of the limited liability company "Maryivske" using a complex approach to fertilizers applying have been held. The standards of mineral fertilizers regarding the level of nutrients have been established, taking the planned harvest scope into account. In order to establish more exact standards, the indices of the actual content of feed elements, agrochemical cartograms, ecological and agrochemical certificate and formulae were used (DEMYDENKO 2006, ZHUCHENKO 2006):

– winter wheat and maize, grain:

– nitrogen = $(4.0 - 0.16 \cdot N) \cdot Y - Ncor;$     (1)

– phosphorus = $(3.2 - 0.213 \cdot P) \cdot Y;$     (2)

– potassium = $(3.1 - 0.155 \cdot K) \cdot Y.$     (3)

Sunflower: nitrogen = $(5.8 - 0.193 \cdot N) \cdot Y - Ncor;$

  phosphorus = $(6.6 - 0.44 \cdot P) \cdot Y;$

  potassium = $(5.6 - 0.233 \cdot K) \cdot Y,$

where:

  N – the nitrogen content, mg/100 gr,

  P – the phosphorus content in the soil,

  K – the potassium content in the soil,

  Y – the crops height,

  Ncor – the forerunner correction.

# Results and Discussion

The total area of Vasylivka Rural Council soviet is 6168.00 ha, including agricultural lands, among them: 4360.88 ha of arable lands (70.7%), 880.87 ha of pastures (14.28%), 59.7 ha of hayfields (0.97%), 62.0 ha of gardens (1.0%), 84.76 ha (1.37%) of built-up lands, 627.2 ha of forests and forest-plantations (10.16%), 26.7 ha of underwater territories (0.43%) and other lands – 65.89 ha (1.06%) – Figure 1.
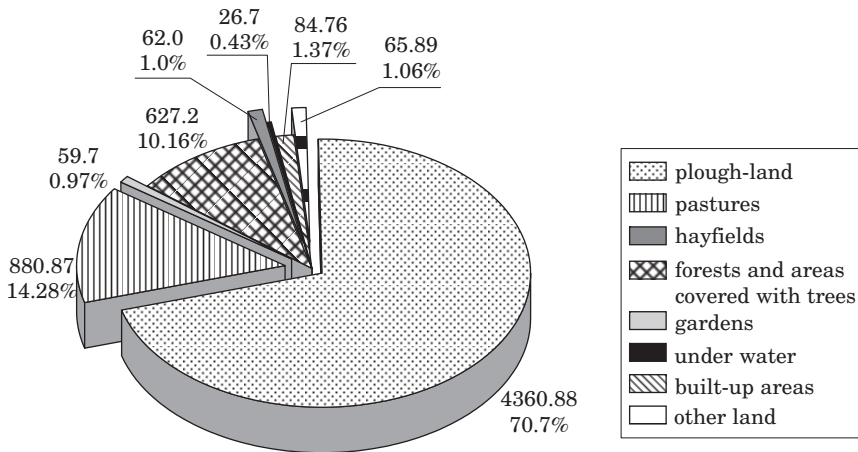


Fig. 1. Structure of agricultural lands on the having a special purpose setting

During denationalization and privatization of agricultural lands 2244.76 ha of lands (36%) have been attached to the State property and 3923.24 ha (64%) of lands – to the private property (Figure 2). Some lands have been transferred to the temporary use, including 2663.12 ha (43.2%) on lease terms, 429.93 ha (7%) on long-use terms, 2233.19 ha (36.2%) on short-use terms.
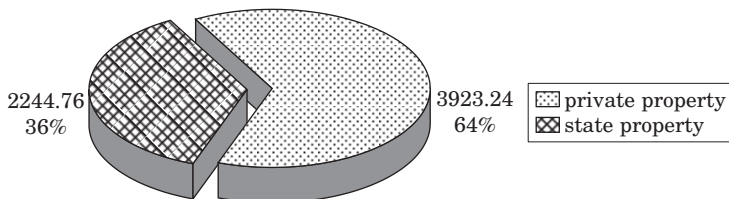


Fig. 2. Distributing of the landed fund is on the patterns of ownership

The land area within a settlement makes up 481 ha. Agricultural enterprises take up 6.07 ha (1%), personal peasant households take up 254.70 ha (53%), civil lands comprise 4.40 ha (1%), commercial lands take up 0.40 ha, lands of technical infrastructure – 0.21 ha, motor transport lands comprise 7.00 ha (2%), lands for general use take up 208.22 ha (43%). Agricultural (arable) lands are used on tenant rights and also by the citizens who own land shares (Figure 3).

Fig. 3. The functional use of earths is within the limits of vill

Fig. 4. Structure of the use of plough-land by Lands-users

The main land-users on the territory Vasylkivka Rural Council are the following juridical entities: Limited Liability Company "Maryivske", which possesses the plot of land (arable land) with the total area 836.85 ha (19%),

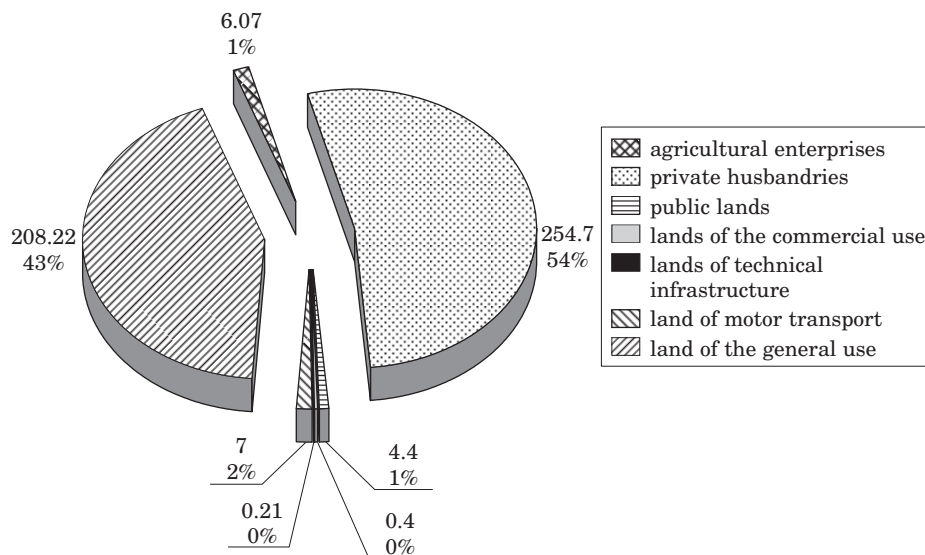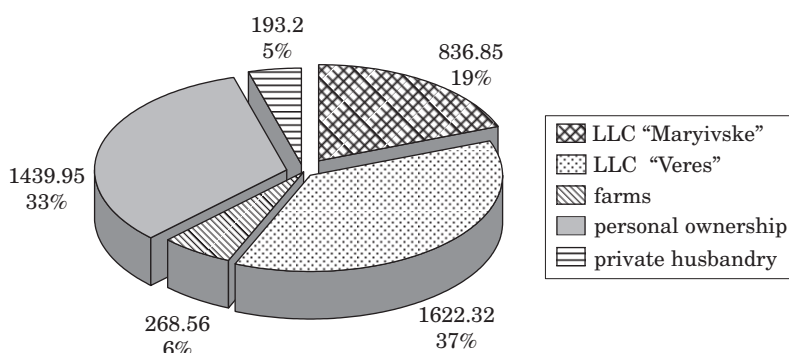Limited Liability Company "Veres" with the total area of 1622.32 ha (37%). The usage of individuals: farms – 268.56 ha (6%); private usage of land plots (Shares) which were transferred into ownership – 1439.95 ha (33%), and the lands for private husbandry – 193.2 (5%) ha (Figure 4).

The main reasons, which determine the modern conditions of erosional danger of the solid are: the high rate of the cultivated agricultural holdings, the total area of the cultivated lands on the territory oh the Vasylkivka Rural Council reaches more than 71% (Figure 5), the rapid formation of new types of the land-tenures, the absence of the state, regional and local solid-protection programs and the law level of financial implementation of politics directed to the protection of solid from erosion (KANASH 2005).



4360.88 ha
71%

1807.12 ha
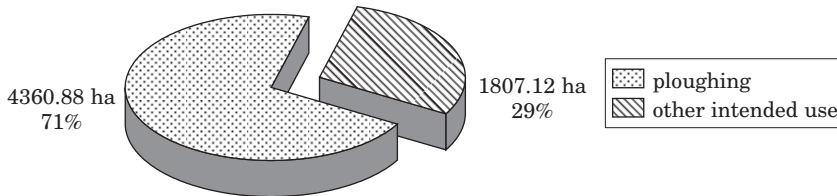29%

ploughing
other intended use

Fig. 5. Thrown of agricultural lands open

Most of the land-users do not abide the solid-protection technologies of the crop growing. The drawback in the providing of such technologies is the absence of the direct financial concernment of the land-users in the maintenance of the solid both with the absence of the system of the antierosion equipment for the work at inclination more than $3^o$. The land users are not responsible for the damage they inflict to the soil. Their work is estimated by the profitability rate of the manufactured products (KIRSANOV 2007, MEDVEDIEV 2007).

During the years 2005–2009 on the territory of Limited Liability Company "Maryivske" the system the efficient usage of the agricultural holdings was provided: the calculation of the norm of fertilizers in each case, their local adding with bioactive substance and for plants protection products.

In order to get 2.6 Mg ha$^{-1}$ (Figure 6) of the winter wheat grain and 1.5 Mg ha$^{-1}$ sunflower, the norm of fertilizers under the actual provision of the fields with nitrogen – 9.4, phosphorous – 6.0 and potassium – 9.3 mg per 100 g of the soil (Figure 7) is: by nitrogen – 95 kg ha$^{-1}$, by phosphorus – 50 kg ha$^{-1}$, by potassium – 45 kg ha$^{-1}$ (winter wheat); by nitrogen – 75 kg ha$^{-1}$, by phosphorus – 50 kg ha$^{-1}$, by potassium – 50 kg ha$^{-1}$ (sunflower).
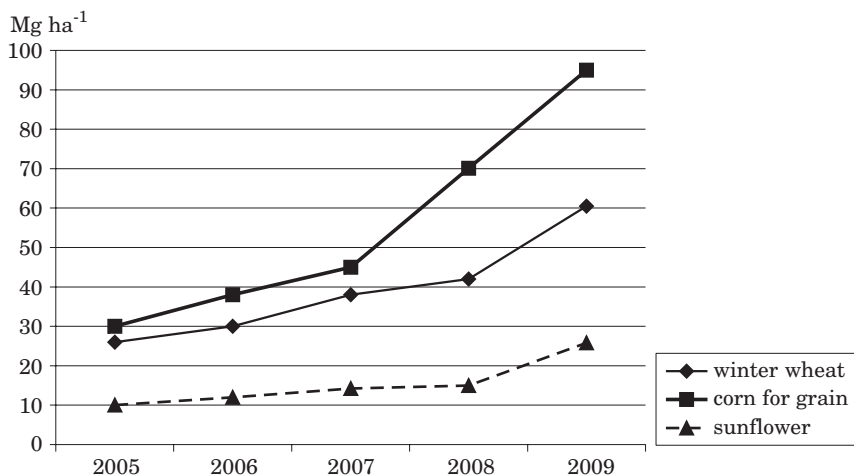
Fig. 6. Productivity of agricultural cultures [Mg ha$^{-1}$]

The formula (1–3) evaluation resulted in the more accurate norm of the fertilizers, 2005 year:

at nitrogen = $(4.0 - 0.16 \cdot 9.4) \cdot 26 - 12 = 52.89$ kg ha$^{-1}$, (Figure 8);

at phosphorus = $(3.2 - 0.213 \cdot 6.0) \cdot 26 = 49.97$ kg ha$^{-1}$;

at potassium = $(3.1 - 0.155 \cdot 9.3) \cdot 26 = 43.12$ kg ha$^{-1}$.
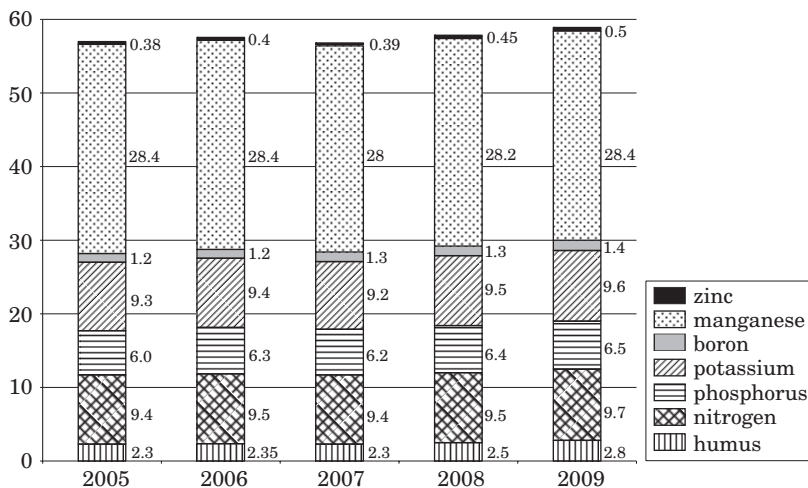


Fig. 7. Feeding elements content the soil layer per
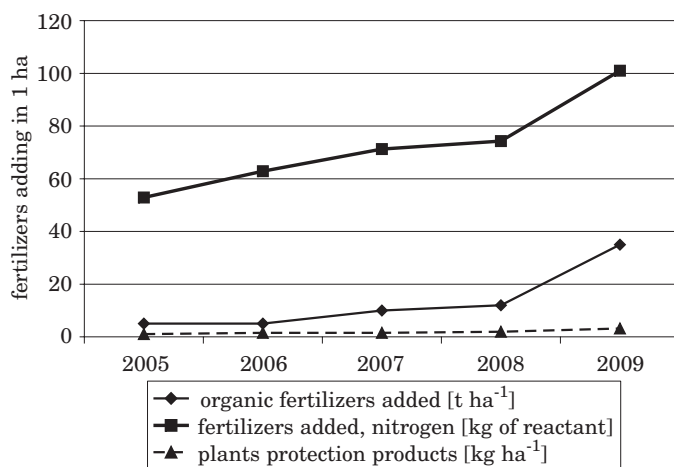
Fig. 8. Agricultural lands fertilizers adding

For sunflower (2.1–2.3), 2008 year:
– at nitrogen = $(5.8 - 0.193 \cdot 9.4) \cdot 15 - 3 = 56.79$ kg ha$^{-1}$;
– at phosphorus = $(6.6 - 0.44 \cdot 6.0) \cdot 15 = 59.4$ kg ha$^{-1}$;
– at potassium = $(5.6 - 0.233 \cdot 9.3) \cdot 15 = 51.4$ kg ha$^{-1}$.

The findings showed that such an approach of adding the fertilizers has a perspective of soil quality improvement and productivity increasing more than 100%.

The above mentioned norms are calculated for the spreaded adding, which by the local appliance can be decreased by 30–40%. During the period of the plants vegetation the mineral fertilizers were used according to the plants diagnostics. Such approach permits to apply fertilizers only when the plants need, to decrease their consumption to 10–15%, to secure the stability of the environment and to obtain output without surplus of nitrates (SOZINOV 2004, SYNYTSKYI 2007).

Fertilizers are the elements of the whole system of agrochemical programs (the regulation of the soil acidity, abatement with the weedages, diseases and plant pests, the selection of the best sorts, the confining of the optimal seed time).

Use of fertilization in crop rotation is an important part of high-yielding agriculture. However increasing volumes of its use (especially in the time of low output yield) results in environmental pollution (TARARIKO 2004, TOPOL-NYI 2007).

# Conclusions

1. Main reasons of soil agrochemical properties decreasing is a repeated cultivation by means of different tools with the help of powerful and heavy wheel-tire tractors and high level of ploughed of agricultural lands.

2. The analysis of present condition of Vasylivka Rural Council agricultural land shows their deep degradation which is manifested in large increasing of erosion lands areas, areas of medium and high eroded soil. It happens because lands are situated on the surface with inclination $1–15^\circ$ that is why because of soil erosion the areas of arable land and meadows are getting lost.

Experience of rational use of fertilizers by the Limited Liability Company "Maryivske" showed the perspective of land quality improvement and, as a result, decreasing of areas of ploughed land.

Translated by INNA PIROG

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# EFFECT OF PROTEIN LEVEL IN DIETS OF GROWER-FINISHER PIGS ON GROWTH PERFORMANCE, NITROGEN EXCRETION AND CARCASS VALUE

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K e y   w o r d s: dietary protein, fatteners, performance, carcass, N-retention.

## A b s t r a c t

Possibility of reduction of protein level in feed mixtures of similar content of amino acids digestible in the small intestine and its influence on fattener's performance and nitrogen retention were determined. Thirty pigs (35 kg) were divided into 3 groups. The control animals were fed with diets with crude protein level suggested by polish pig feeding standards (*Normy żywienia...* 1993) – 17% in grower and 15% in finisher diet. In the experimental groups (I and II) the protein level was reduced about 10 or 20% respectively. No significant effect on feed intake, weight gains and feed utilization was found. The animals from the both experimental group utilized less crude protein ($P=0.001$) than in Control group. The significant ($P=0.001$) reduction in nitrogen excreted in urine and total N-excretion in both group with reduced protein level in feed mixtures, were observed. Nitrogen retention in g/day was higher for group I compared to other groups and N-retention : N intake were significantly higher ($P<0.05$) in both experimental groups. The reduction of crude protein level in the experimental diets did not affect slaughter performance but in group II the tendency to increase ($P=0.064$) in back fat thickness and decrease meat content in carcass, were observed. The results suggest that lowering the protein level in diets for high-meat crossbred pigs (Polish Large White $\times$ Polish Landrace) $\times$ (Hampshire $\times$ Pietrain) from 170 and 150 g kg$^{-1}$ to 136 g and 115 g kg$^{-1}$ in grower and finisher diets, respectively, but supplemented with synthetic amino acids, did not impact negatively production results of fatteners, but decreased nitrogen emission to the environment and cost of feed.

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## WPŁYW POZIOMU BIAŁKA W MIESZANKACH DLA ŚWIŃ W OKRESIE GROWER-FINISZER NA WYNIKI PRODUKCYJNE, WYDALANIE AZOTU I JAKOŚĆ TUSZY

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A b s t r a k t

Celem badań było określenie wpływu obniżenia poziomu białka w mieszankach dla tuczników, zbilansowanych pod względem zawartości aminokwasów strawnych w jelicie cienkim, na wyniki produkcyjne, wydalanie azotu i jakość tuszy. Trzydzieści świń (35 kg) podzielono na 3 grupy. Zwierzęta kontrolne otrzymywały mieszanki z poziomami białka rekomendowanymi przez *Normy żywienia...* 1993 – 17% w mieszance grower i 15% w mieszance finiszer. W grupach doświadczalnych (I i II) poziom białka był obniżony o odpowiednio 10 lub 20% w stosunku do grupy kontrolnej. Nie stwierdzono znaczących różnic w pobraniu pasz, dziennych przyrostach masy ciała oraz wykorzystaniu paszy. Zwierzęta z obu grup doświadczalnych pobierały mniej białka ogólnego niż zwierzęta z grupy kontrolnej (*P*=0.001). Stwierdzono istotne (*P*=0.001) obniżenie ilości całkowitego wydalonego azotu i azotu wydalonego w moczu w obu grupach doświadczalnych w porównaniu z grupą kontrolną. Retencja azotu w g/dzień oraz stosunek retencji azotu do azotu pobranego były istotnie wyższe (*P*<0.05) w obu grupach doświadczalnych w stosunku do grupy kontrolnej. Redukcja poziomu białka w mieszankach doświadczalnych nie wpłynęła istotnie na wydajność rzeźną świń, jednakże w grupie II zaobserwowano tendencję (*P*=0.064) do wzrostu grubości słoniny i obniżenie udziału mięsa w tuszy.

Wyniki badań sugerują, że obniżenie poziomu białka w mieszankach dla wysoko mięsnych tuczników (wbp × pbz) × (Ha × Pi) ze 170 na 136 g kg$^{-1}$ w mieszance grower i ze 150 na 115 g kg$^{-1}$ w mieszance finiszer, z jednoczesnym uzupełnieniem niedoborów aminokwasów za pomocą aminokwasów krystalicznych, nie wpływa negatywnie na wyniki produkcyjne tuczników, ale obniża wydalanie azotu do środowiska i koszt żywienia.

## Introduction

Monitoring and reduction of nitrogen emission from animal farms is one of strategic objectives of the European Union (ERISMAN et al. 2003). It is estimated that nearly 50% of global production of the main source of nitrogen, ammonia, comes from pig farms (HAYES et al. 2004). The reduction of protein level in finisher pigs diet with appropriate feed ration balance and meeting real demand for amino acids digestible in the small intestine decreases nitrogen emission to the environment (KERR and EASTER 1995, KERR et al. 1995, NYACHOTI et al. 2006) without lowering pig's performance (KERR et al. 1995, HINSON et al. 2009).

To pork production high-meat crossbred pigs are generally used. New hybrid-lines of pig are very sensitive and stressful for feed quality and their specific demand for nutrients are not well recognized (ŠPRYSL et al. 2010). Also,

the production and deposition of fat and meat in the carcass are limited by the genotype of the animals (ZRŮSTOVAL et al. 2009) and different for specific pig lines. Differences occurring in this area should be taking into account in the feeding of adjusting the level of protein and amino acids most often deficient to the actual pig demand. It seems, that the reduction in total protein levels recommended by polish pig feeding standards (*Normy żywienia...* 1993) with simultaneous addition of crystalline amino acids will not lead to deterioration of performance results. Excessive lowering of the protein level in diets can cause a deficiency of some essential amino acids and lowering pig performance.

The aim of the experiment was to determine the possibility of reducing the level of protein in diets for high-meat fatteners (PLW × PL) × (Pi × Ha) and its influence on growth performance, nitrogen retention and carcass quality.

## Material and Methods

### Animals and diets

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Poznań (#34/2009).

Thirty crossbred pigs (Polish Large White × Polish Landrace) × (Hampshire × Pietrain) with an average initial body weight of 35 kg were divided into 3 groups of 10 animals each. The animals were sorted to the groups by body weight and sex – 5 barrows and 5 gilts to each group, and placed in individual pens.

The pigs were fed *ad libitum* a standard grower diet by 35 days (to about 65 kg of body weight) and a finisher diet by 41 days (to 105 kg of body weight). The control animals were fed the diets with crude protein level recommended by polish pig feeding standards (*Normy żywienia...* 1993) – 17% in grower and 15% in finisher diet. In the experimental groups (I and II) the protein level was reduced about 10 or 20% respectively (Table 1). All the diets had a similar metabolically energy level. Due to the demand for digestible lysine, methionine + cystine, threonine and tryptophan, diets were supplemented with crystalline amino acids according to *GfE system* (2006). Digestible amino acids concentration in feed mixtures was calculated base on content of digestible amino acids in component as recommended *NRC* (1998). Water was freely available from pig drinker bowl. During the experiment, individual feed intake and body weight were controlled and average daily feed intake (ADFI), average daily gains (ADG), and feed conversion ratio (FCR) were calculated.

In the last week of the grower-phase, 4 animals from each group were transferred to metabolic cages for N-balance trial. After 3-day adaptation period, total excreta were collected for 3 days, separately urine and faeces,

Table 1

Composition and nutritional value of the experimental diets fed to pigs

| Item Ingredients (g kg$^{-1}$) | Grower | | | Finisher | | |
|---|---|---|---|---|---|---|
| | control | I | II | control | I | II |
| Wheat | 450.0 | 500.0 | 550.0 | 430.0 | 475.2 | 530.0 |
| Rye | 90.2 | 89.4 | 89.8 | 98.1 | 96.8 | 92.8 |
| Barley | 250.0 | 250.0 | 250.0 | 200.0 | 200.0 | 200.0 |
| Wheat bran | – | – | – | 150.0 | 150.0 | 150.0 |
| Soybean meal | 180.0 | 128.0 | 75.0 | 100.0 | 55.0 | – |
| Monocalcium phosphate | 8.0 | 8.0 | 8.0 | 4.0 | 3.5 | 4.0 |
| Limestone | 14.0 | 14.0 | 14.0 | 13.0 | 12.5 | 13.5 |
| Sodium chloride | 3.5 | 3.5 | 3.5 | 2.2 | 2.2 | 2.2 |
| Premix grower[1]/finisher[2] | 3.3 | 3.3 | 3.3 | 2.2 | 2.2 | 2.2 |
| Lysine 76% | 1,0 | 2.6 | 4.0 | 0.5 | 1.7 | 3.2 |
| Methionine 98% | – | 0.5 | 0.7 | – | 0.3 | 0.7 |
| Threonine 98% | – | 0.5 | 1.2 | – | 0.4 | 1.0 |
| Tryptophan 98% | – | 0.2 | 0.5 | – | 0.2 | 0.4 |
| Nutritional value of diets (g kg$^{-1}$) | | | | | | |
| ME[3] (MJ kg$^{-1}$) | 12.9 | 12.9 | 13.0 | 12.5 | 12.5 | 12.5 |
| Crude protein | 172.2 | 154.1 | 135.7 | 149.7 | 134.2 | 115.0 |
| ME/crude protein (MJ/1%) | 0.75 | 0.84 | 0.96 | 0.83 | 0.93 | 1.09 |
| Digestible lysine[4] | 7.6 | 7.7 | 7.7 | 5.7 | 5.7 | 5.7 |
| Digestible methionine[4] | 5.2 | 5.3 | 5.2 | 4.5 | 4.5 | 4.5 |
| Digestible threonine[4] | 5.0 | 4.9 | 5.0 | 4.1 | 4.1 | 4.1 |
| Digestible tryptophan[4] | 1.8 | 1.8 | 1.9 | 1.5 | 1.5 | 1.5 |
| Digestible protein | 142.1 | 127.8 | 113.1 | 122.0 | 109.8 | 97.0 |

[1] Premix grower – Ca 260 g kg$^{-1}$, Vit A – 1 500 000 IU kg$^{-1}$, Vit D$_3$ – 300 000 IU kg$^{-1}$, Vit E – 10 500 mg kg$^{-1}$, Vit K$_3$ – 220 mg kg$^{-1}$, Vit B$_1$ – 220 mg kg$^{-1}$, Vit B$_2$ – 600 mg kg$^{-1}$, Vit B$_6$ – 450 mg kg$^{-1}$, Vit B$_{12}$ – 3 700 µg kg$^{-1}$, Folic acid – 300 mg kg$^{-1}$, Pantothenic acid – 1 500 mg kg$^{-1}$, Nicotinic acid – 3 000 mg kg$^{-1}$, Biotine – 15 000 µg kg$^{-1}$, Choline chloride – 40 000 mg kg$^{-1}$, Mn – 6 000 mg kg$^{-1}$, Zn – 15 000 mg kg$^{-1}$, Fe – 15 000 mg kg$^{-1}$, Cu – 4 000 mg kg$^{-1}$, Co – 60 mg kg$^{-1}$, I – 120 mg kg$^{-1}$, Se – 30 mg kg$^{-1}$, Antioxidant (BHA and BHT);

[2] Premix finisher – Ca 280 g kg$^{-1}$, Vit A – 1 000 000 IU kg$^{-1}$, Vit D$_3$ – 200 000 IU kg$^{-1}$, Vit E – 7 000 mg kg$^{-1}$, Vit K$_3$ – 150 mg kg$^{-1}$, Vit B$_1$ – 150 mg kg$^{-1}$, Vit $_2$ – 400 mg kg$^{-1}$, Vit B$_6$ – 300 mg kg$^{-1}$, Vit B$_{12}$ – 2 500 µg kg$^{-1}$, Folic acid – 200 mg kg$^{-1}$, Pantothenic acid – 1 000 mg kg$^{-1}$, Nicotinic acid – 2 000 mg kg$^{-1}$, Biotine – 10 000 µg kg$^{-1}$, Choline chloride – 20 000 mg kg$^{-1}$, Mn – 4 000 mg kg$^{-1}$, Zn – 8 000 mg kg$^{-1}$, Fe – 10 000 mg kg$^{-1}$, Cu – 4 000 mg kg$^{-1}$, Co – 40 mg kg$^{-1}$, I – 80 mg kg$^{-1}$, Se – 20 mg kg$^{-1}$, Antioxidant (BHA and BHT);

[3] ME was calculated using program Win Pasze;

[4] Digestible AA in diets were calculated as recommended by NRC (1998).

weighted and sampled for nitrogen determination. After the end of the experiment, the animals were carried to a slaughterhouse where carcass values were evaluated.

## Analyses

Crude protein and amino acid analyses of fresh excreta samples and diets were carried out according to the *AOAC* (2005). Backfat and meat content from *Musculus longissimus dorsi* were estimated according to a standard procedure used in the Polish Pig Testing Stations (Różycki 1996) using Ultra Fom 300.

One-way analysis of variance was performed (ANOVA) and an alpha level of 0.05 was used to assess significance between means by Duncan's test. The statistical analysis was performed using SAS 9.1 Software (USA).

## Results

Daily feed intake, weight gains and feed utilization did not differ significantly ($P>0.05$) after reduction of digestible protein level in the examined diets in grower and finisher periods (Table 2). The animals from experimental groups utilized significantly ($P=0.001$) less crude protein per 1 kilogram of weight gain, about 9.4 and 19.1% in the grower phase; 5.2 and 17.0% in the finisher phase; and 7.1 and 18.0% in the whole experiment, respectively in the group I and II, in comparison to the Control one.

In N-balance (Table 2) trials a significant decrease ($P=0.001$) in nitrogen consumption was observed together with the reduction of protein content in feed mixtures. In both experimental groups significant ($P=0.001$) reduction (about 30%) in nitrogen excreted in urine was noted. No significant differences in daily amount of nitrogen excreted in faeces were found, however, N amount in faeces was reduced by 7% and 17% for group I and II, respectively, in comparison to the Control group. Additionally a significant decreasing ($P=0.001$) in the total N-excretion by about 26% and 29%, in the group I and II, respectively, was observed. Absorbed amount of N was significantly higher ($P=0.001$) in group I in comparison to other groups (48% vs. Control and 36% vs. group II). Significantly higher N retention/N intake ($P=0.001$) in both experimental groups were observed compared to the Control one.

The lowering of digestible protein level in the experimental feed mixtures did not adversely affect slaughter performance in comparison to the Control group (Table 3). The animals from the experimental groups had lower slaughter weight and slightly lower hot and cold carcass weight. In the group II

a tendency to increase ($P$=0.064) in back fat thickness was observed, comparing to the group I, together with decrease in carcass meat content (by 3 percentage points), comparing to the other groups.

Table 2

Performance (ADFI, ADG, FCR), crude protein efficiency and N-balance trial results

| Item | Units | Group | | | | |
|---|---|---|---|---|---|---|
| | | control | I | II | SEM | $P$ |
| Grower ($n$=10 animals/group) | | | | | | |
| Start weight | [kg] | 32.4 | 31.9 | 32.9 | 1.1 | 0.455 |
| Finish weight | [kg] | 63.6 | 63.3 | 64.2 | 2.5 | 0.745 |
| ADFI | [kg] | 2.51 | 2.43 | 2.46 | 0.1 | 0.425 |
| ADG | [g] | 890 | 899 | 896 | 12 | 0.956 |
| FCR | [kg kg⁻¹] | 2.84 | 2.71 | 2.75 | 0.04 | 0.438 |
| Crude protein/kg of gain | [g kg⁻¹] | 512$^a$ | 464$^b$ | 414$^c$ | 8 | 0.001 |
| Finisher ($n$=10 animals/group) | | | | | | |
| Start weight | [kg] | 63.6 | 63.3 | 64.2 | 2.5 | 0.745 |
| Finish weight | [kg] | 102.1 | 100.9 | 102.2 | 1.03 | 0.644 |
| ADFI | [kg] | 2.96 | 2.90 | 3.02 | 0.10 | 0.108 |
| ADG | [g] | 940 | 915 | 927 | 13 | 0.704 |
| FCR | [kg kg⁻¹] | 3.16 | 3.19 | 3.27 | 0.03 | 0.371 |
| Crude protein/kg of gain | [g kg⁻¹] | 501$^a$ | 475$^b$ | 416$^c$ | 2.5 | 0.001 |
| Overall | | | | | | |
| ADFI | [kg] | 2.74 | 2.66 | 2.74 | 0.02 | 0.113 |
| ADG | [g] | 915 | 907 | 911 | 9.95 | 0.940 |
| FCR | [kg kg⁻¹] | 3.00 | 2.95 | 3.00 | 0.07 | 0.320 |
| Crude protein/kg of gain | [g kg⁻¹] | 506$^a$ | 470$^b$ | 415$^c$ | 4.52 | 0.001 |
| N-balance ($n$= 4 animals/group) | | | | | | |
| N-intake | [g/day] | 57.7$^a$ | 53.9$^b$ | 46.6$^c$ | 0.00 | 0.001 |
| N – in urine | [g/day] | 34.2$^a$ | 23.7$^b$ | 23.3$^b$ | 0.90 | 0.001 |
| N – in faeces | [g/day] | 8.36 | 7.85 | 6.95 | 0.27 | 0.152 |
| N – excrection | [g/day] | 42.59$^a$ | 31.57$^b$ | 30.20$^b$ | 0.86 | 0.001 |
| N – retention | [g/day] | 15.15$^b$ | 22.30$^a$ | 16.41$^b$ | 0.75 | 0.001 |
| N – retention/N intake | [%] | 26.23$^a$ | 41.42$^b$ | 35.20$^b$ | 1.57 | 0.011 |

$^{a, b, c}$ – values in the same rows with different letters differ significantly at $P$<0.05

Table 3

Carcass characteristic of pigs

| Item | Units | Group | | | | |
|---|---|---|---|---|---|---|
| | | control | I | II | SEM | $P$ |
| Slaughter weight | [kg] | 102.1 | 100.9 | 102.2 | 1.03 | 0.644 |
| Hot carcass weight | [kg] | 79.1 | 76.70 | 77.40 | 0.80 | 0.462 |
| Cold carcass weight | [kg] | 77.91 | 75.92 | 76.32 | 0.79 | 0.510 |
| Carcass yield | [%] | 76.62 | 75.98 | 75.78 | 0.33 | 0.554 |
| Backfat | [mm] | 19.86 | 19.29 | 22.08 | 0.48 | 0.064 |
| Meat | [%] | 57.51 | 57.52 | 54.58 | 0.24 | 0.794 |

# Discussion

In the current research fatteners of all the groups similarly gained weight and utilized feed, as can be expected in appropriate meeting animal demand for amino acids digestible in the small intestine, especially lysine, an amino acid responsible for the synthesis and accumulation of protein in a carcass and occurring the most frequently in deficiency (OTTO et al. 2003, SHRIVER et al. 2003, RUUSUNEN et al. 2007). It is associated with the improvement in nitrogen retention in relation to the amount of nitrogen consumed in feed mixtures. Mixtures with lower protein content but supplemented with crystalline amino acids (lysine, methionine, threonine, tryptophan) are usually characterized by higher protein biological value than control mixtures (FIGUEROA et al. 2003), which may compensate the lower nitrogen consumption, what also indicates a greater daily nitrogen retention found in our experiment in group I. The animals receiving the diets with a lower crude protein content better utilized nitrogen (N retention : N intake) what implicated less nitrogen excreted to the environment, mainly in the urine (LE BELLEGO et al. 2001, NOBLET et al. 2001, FIGUEROA et al. 2002, LE BELLEGO and NOBLET 2002, ZERVAS and ZIJLSTRA 2002, HAYES et al. 2004). This has a great importance in reduction of nitrogen excreted to the environment. Polish pig feeding standards (*Normy żywienia...* 1993) are not as restricted in pig requirements as other modern systems – GfE or NRC, what impact negatively environmental and, especially, economical conditions. In the experimental diets soybean meal level was lowered compared to the control diet what reduced the cost of pigs feeding.

In the present experiment, in the grower and finisher diet used in group II, per 1% of the protein accounted about 30% more ME than in the Control group, what effected higher fat synthesis. This was not reflected in daily body weight gains, but increased the dorsal fat thickness and lowered meat content in carcasses (about 3% points). The results obtained by KERR et al. (1995) and FIGUREOA et al. (2002) corresponding to our own findings, i.e. observed that dietary protein level influences fat metabolism, mainly causing the growth of back fat. Additionally some authors (KERR et al. 1995, RUUSUNEN et al. 2007) reported a decreasing in carcass meat content, when pigs were fed the diets with lowered protein content. The increase in carcass fat deposition resulted from direct influence of dietary nitrogen level on carcass quality parameters, regardless of dietary supplementation with crystalline amino acids. What is more, low-protein diets also have higher energy value (KERR et al. 2005), which results in the deposition of the excess energy in the form of fat.

Because no negative effect on production results and carcass value all the mixtures are recommended in pig production.

# Conclusions

The obtained results showed that reduction of crude protein level from 170 g kg$^{-1}$ to 154 g kg$^{-1}$ and 136 g kg$^{-1}$ and from 150 g kg$^{-1}$ to 134 g kg$^{-1}$ and 115 g kg$^{-1}$ in grower and finisher diets, respectively, but supplemented with synthetic amino acids (according to *GfE system*. 2006) did not impact negatively growth performance results of high-meat fatteners (Polish Large White × Polish Landrace) × (Hampshire × Pietrain) but significantly decreased nitrogen emission to the environment and reduced the cost of feed because of lower level of soybean meal in mixtures. All of the presented feed mixture with reduced protein level may find practical use in swine nutrition.

Translated by MAGDALENA BŁAŻ

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# THE EFFECT OF DIETARY SUPPLEMENTATION WITH A BLEND OF HERBAL EXTRACTS AND ALUMINOSILICATES ON NUTRIENT DIGESTIBILITY AND THE GROWTH PERFORMANCE OF WEANED PIGLETS

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K e y   w o r d s: pigs, nutrition, herbal extract, aluminosilicates, apparent digestibility, growth performance.

## A b s t r a c t

Two complete diets for weaned piglets were analyzed. Control diet I was composed of soybean meal, ground barley, ground wheat, premix and acids. Experimental diet II was supplemented with 0.1% Vilocym, a blend of herbal extracts *(Azadirachta indica, Curcuma longa, Allium sativum, Andrographis paniculata, Solanum nigrum)* and sodium-calcium aluminosilicates. Nutrient digestibility was determined by a simple balance method, on 16 young barrows [(Polish Large White x Polish Landrace) x Duroc] with average initial body weight of 25 kg, assigned to two dietary treatments. A five-day experimental period proper was preceded by a seven-day adjustment period. A production trial was carried out on a pig farm, and it involved 240 weaners with average initial body weight of 20.5 kg, divided into two groups. Each group comprised four pens, with 30 animals per pen. The experiment lasted 30 days. Dietary supplementation with herbal extracts and aluminosilicates improved the digestibility of crude fat, total protein and organic matter. Weaned piglets fed Vilocym were characterized by higher daily gains than control group animals (725 g vs. 665 g, $P \leq 0.05$). Feed consumption per kg body weight gain was significantly lower in group II (2.14 g/kg vs. 2.33 g/kg, $P \leq 0.05$).

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## WPŁYW DODATKU MIESZANINY EKSTRAKTU Z ZIÓŁ I GLINOKRZEMIANÓW NA STRAWNOŚĆ SKŁADNIKÓW POKARMOWYCH I WYNIKI ODCHOWU WARCHLAKÓW

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S ł o w a   k l u c z o w e: świnie, żywienie, ekstrakt ziołowy, glinokrzemiany, strawność pozorna, odchów.

A b s t r a k t

Badano dwie mieszanki pełnoporcjowe przeznaczone dla warchlaków. W skład mieszanki kontrolnej (I) wchodziła śruta poekstrakcyjna sojowa, śruta jęczmienna, śruta pszenna, premiks oraz preparat zakwaszający. Mieszanka doświadczalna (II) zawierała ponadto 0,1% preparatu Vilocym, który był mieszaniną ekstraktów z ziół *(Azadirachta indica, Curcuma longa, Allium sativum, Andrographis paniculata, Solanum nigrum)* i glinokrzemianów wapniowo-sodowych. Badania strawności składników pokarmowych przeprowadzono metodą bilansową bezpośrednią na 16 wieprzkach [(wbp x pbz) x duroc] o średniej początkowej masie ciała 25 kg podzielonych na dwie grupy. Okres wstępny trwał 7 dni, a właściwy 5 dni. Badania produkcyjne przeprowadzono na fermie tuczu trzody chlewnej na 240 warchlakach o początkowej masie ciała 20,5 kg, podzielonych na dwie grupy. Jedną grupę stanowiły zwierzęta umieszczone w 4 kojcach po 30 sztuk w każdym. Okres badań trwał 30 dni. Wprowadzenie do mieszanki dla warchlaków dodatku ekstraktu z ziół i glinokrzemianów wpłynęło na poprawę strawności tłuszczu surowego, białka ogólnego i substancji organicznej. Stwierdzono także wyższe przyrosty dobowe masy ciała u warchlaków otrzymujących mieszankę z dodatkiem preparatu Vilocym w porównaniu z grupą kontrolną (725 g vs. 665 g, *P* ≤ 0,05). Wykorzystanie paszy na kg przyrostu również było u warchlaków grupy II istotnie lepsze (2,14 g/kg vs. 2,33 g/kg, *P* ≤ 0,05).

## Introduction

Due to the ban on the use of antibiotics as feed additives in pig production, producers have to search for alternatives to antibiotic growth promoters in pig diets. Alternative additives are expected to naturally enhance the immune system of animals, and to stabilize the gut microflora. Herbs and essential oils have been shown to modulate the gut microflora (HASHEMI and DAVOODI 2011, YANG et al. 2009). Glycosides, tannins, essential oils and saponins contained in herbal plants can naturally increase appetite, stimulate peristalsis and the secretion of gastric acid (NAMKUNG et al. 2004, WINDISCH et al. 2008). Numerous plants contain natural antimicrobial agents that strongly suppress the growth of pathogenic bacterial strains (HASHEMI and DAVOODI 2011). In a study by MANZANILLA et al. (2004), piglets weaned at 21 days of age were fed diets supplemented with plant extracts, alone or in combination with formic acid. The supplements were effective in modifying the gastrointestinal system, stomach contents and stomach emptying rate. Yucca saponins added

to animal feed have been shown to reduce ammonia production and excretion (FRANCIS et al. 2002). Research results also indicate that dietary supplementation with herbal extracts could affect nutrient digestibility in monogastric animals (HERNANDEZ et al. 2004, NAMKUNG et al. 2004, GERRITSEN et al. 2010). As demonstrated by YAN et al. (2011), herbal extracts influence the serum levels of LDL, HDL, total cholesterol and triacylglycerols in finishing pigs.

The objective of this study was to determine nutrient digestibility and the growth performance of weaned piglets fed a diet supplemented with a blend of herbal extracts and sodium-calcium aluminosilicates.

## Materials and Methods

Two complete diets for weaned piglets were analyzed. The diets, formulated in accordance with the pig nutrient requirements (*Normy żywienia...* 1993), were composed of soybean meal, ground barley, ground wheat, premix and acids. Experimental diet II was supplemented with Vilocym (1 kg t$^{-1}$, 0.1%), a blend of herbal extracts *(Azadirachta indica, Curcuma longa, Allium sativum, Andrographis paniculata, Solanum nigrum)* and natural sodium-calcium aluminosilicates (Table 1).

Table 1

Composition [%] and calculated feeding value of experimental diets

| Specification | Diets | |
|---|---|---|
| | I | II |
| Ingredients | | |
| Ground wheat | 45.70 | 45.60 |
| Ground barley | 30.00 | 30.00 |
| Soybean meal | 20.00 | 20.00 |
| Premix* | 4.00 | 4.00 |
| Acids | 0.30 | 0.30 |
| Vilocym | – | 0.10 |
| Calculated nutrient profile | – | – |
| Metabolizable energy [MJ kg$^{-1}$] | – | – |
| Crude protein [%] | 13.03 | 13.01 |
| Lysine [%] | 19.30 | 19.30 |
| Methionine [%] | 1.28 | 1.28 |
| Threonine [%] | 0.73 | 0.73 |
| Tryptophan [%] | 0.80 | 0.80 |
| Calcium [%] | 0.23 | 0.23 |
| Available phosphorous [%] | 0.84 | 0.85 |
| Salt [%] | 0.46 | 0.46 |
| | 0.20 | 0.20 |

* lysine – 10.5%, methionine – 3%, threonine – 4%, Ca – 17.2%, P – 5,5%, available P – 3.26%, Na – 4.4%, Vit. A – 400 000 IU, Vit. D$_3$ – 50 000 IU, Vit. E – 3 004 mg, Vit. K$_3$ – 100 mg, Vit. B$_1$ – 60 mg, Vit. B$_2$ – 160 mg, Vit. B$_6$ – 120 mg, Vit. B$_{12}$ – 1 mg, niacin – 800 mg, pantothenic acid – 400 mg, folic acid – 80 mg, biotin – 4 mg, choline – 10 000 mg, Mn – 1 800 mg, Zn – 4 000 mg, Fe – 3 500 mg, Cu – 4 000 mg, I – 40 mg, Co – 20 mg, Se – 20 mg, enzyme (+)

Nutrient digestibility was determined by a simple balance method, on 16 young barrows [(Polish Large White x Polish Landrace) x Duroc] with average initial body weight of 25 kg, assigned to two dietary treatments by the analogue method. The animals were kept in individual metabolism cages. A five-day experimental period proper was preceded by a seven-day adjustment period. The weaners were fed twice daily, at 7.00 a.m. and 3.00 p.m., and they had free access to drinking water.

A production trial was carried out on a pig farm, and it involved 240 hybrid weaners [(Polish Large White x Polish Landrace) x (Duroc x Pietrain)] with average initial body weight of 20.5 kg, divided into two experimental groups. Each group comprised four pens, with 30 animals per pen. The experiment lasted 30 days. The animals were fed dry feed *ad libitum*. Feed intake and the health status of weaners were monitored throughout the experiment. The animals were weighed at the beginning and at the end of the experiment. The following growth performance parameters were determined: total body weight gain (TBG), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

The nutrient content of feed and feces was determined by the Weende analysis (*AOAC*, 2000). The gross energy content of feed and feces was determined using an adiabatic bomb colorimeter. The metabolizable energy content of diets was calculated using the equations proposed by Hoffman and Schiemann (*Normy żywienia...* 1993), based on proximate chemical composition and coefficients of nutrient digestibility.

The results were processed statistically by one-way ANOVA and Duncan's test. Arithmetic means (x), standard errors of the mean (SEM) and significance level (*P*) were calculated with the use of STATISTICA 7 software.

## Results and Discussion

The proximate chemical composition of pig diets is presented in Table 2. The total protein content of diets I and II was 18.08% and 18.09%, respectively, and it corresponded to the pig nutrient requirements (*Normy żywienia...* 1993). The metabolizable energy content of diets, calculated based on chemical composition and digestibility coefficients, was 12.10 MJ kg$^{-1}$ in control group I and 12.42 MJ kg$^{-1}$ in experimental group II. Both values were lower than recommended in the pig nutrient requirements (*Normy żywienia...* 1993) (12.7 MJ kg$^{-1}$).

Table 3 shows the coefficients of nutrient digestibility determined in the study. A blend of herbal extracts and aluminosilicates, added to the complete experimental diet for weaners, had a statistically significant effect on nutrient

Table 2

Chemical composition and nutritive value of experimental diets

| Specification | Diets | |
|---|---|---|
| | I | II |
| Dry matter [%] | 88.34 | 88.68 |
| Crude ash [%] | 4.33 | 4.55 |
| Organic matter [%] | 84.01 | 84.13 |
| Crude protein [%] | 18.09 | 18.08 |
| Crude fat [%] | 1.55 | 1.66 |
| Crude fiber [%] | 3.45 | 3.26 |
| N-free extractives [%] | 60.92 | 61.13 |
| Metabolizable energy [MJ kg$^{-1}$] | 60.92 | 61.13 |

Table 3

Apparent fecal digestibility coefficients [%]

| Specification | Diets | | SEM | P |
|---|---|---|---|---|
| | I | II | | |
| Organic matter | 83.47[b] | 84.82[a] | 0.366 | 0.019 |
| Crude protein | 77.14[b] | 78.81[a] | 0.349 | 0.039 |
| Crude fat | 55.29[B] | 64.90[A] | 2.816 | 0.006 |
| Crude fiber | 32.45 | 35.45 | 0.912 | 0.063 |
| N-free extractives | 89.68 | 90.12 | 0.280 | 0.516 |
| Gross energy | 80.92 | 81.92 | 0.275 | 0.097 |

a b – $P \leq 0.05$
AB – $P \leq 0.01$

digestibility. A significant increase in total protein digestibility was noted. KONG et al. (2009), who studied the effects of *Acanthopanax senticasus* extracts (1 g/kg feed) on the apparent ileal digestibility of amino acids in weaned piglets, reported that the supplement enhanced the digestibility of lysine, methionine, isoleucine, histidine, threonine, arginine, cysteine and valine. The ileal digestibility of some amino acids (isoleucine, leucine, threonine, valine) was higher in piglets fed the AS extract-supplemented diet than in those receiving an antibiotic.

In the present study, a significant increase in crude fat digestibility was also noted in weaned piglets fed a Vilocym-supplemented diet. The coefficient of crude fat digestibility increased from 55.29% in the control group to 64.90% in experiment group animals fed a diet supplemented with herbal extracts (Table 3). Organic matter digestibility also improved in the experimental group. MANZANILLA et al. (2004) examined the apparent ileal and fecal digestibility of organic matter in pigs weaned at 21 days of age, fed a mixture of plant extracts (oregano, cinnamon, Mexican pepper), alone or in combination with

formic acid. An increase in organic matter digestibility was reported only in the group receiving plant extracts. In an experiment conducted by GERRITSEN et al. (2010), piglets weaned at 26 days of age were fed (from 9 kg to 18 kg BW) diets supplemented with essential oils (thyme, oregano, cloves) and formic acid or a blend of organic acids. The combination of essential oils and formic acid insignificantly enhanced the digestibility of total protein and crude fat, and significantly increased crude fiber digestibility. YAN et al. (2011) observed no effect of Saururus chinensis extract supplementation on the digestibility coefficients of dry matter, total protein and energy in pigs (ca. 54 kg BW).

Table 4 shows the results of a production trial that involved growing pigs. The average initial body weight of animals was 20.5 kg in both groups. No disease symptoms were observed in weaners over a 30-day experiment, and their survival rate reached 100%. After 30 days, the average body weight of weaners was 39.87 kg in the control group and 42.27 kg in the experimental group. The inclusion of 0.1% herbal extracts and aluminosilicates in pig diets had a significant effect on average daily gains, which reached 725 g in group II and 645 g in group I. The growth rate of weaned piglets was relatively high, and comparable with that noted in previous studies (BUGNACKA and FALKOWSKI 2001). PASCHMA and KACZOR (2008) added a herbal mixture (chamomile, stinging nettle, caraway, fennel) at 1% and 1.5% to a diet for sows, from 100 days of pregnancy to 21 days of lactation, and found that piglets aged 21 days were characterized by high body weights in the experimental group, compared with the control group. KONG et al. (2007) and FANG et al. (2009) studied the effects of Chinese herbal ultra-fine powder and Acanthopanax senticosus extracts as dietary additives for early-weaned piglets, and reported that the above supplements improved daily gains and the gain/feed ratio, and reduced the incidence of diarrhea in piglets. LIPIŃSKI and TYWOŃCZUK (2008) also observed an improvement in the production parameters of piglets fed diets supplemented with Macleaya mordata extract.

Table 4

The effect on the growth performance of pigs

| Specification | Diets | | SEM | $P$ |
|---|---|---|---|---|
| | I | II | | |
| No. of pigs | 120 | 120 | | |
| Days of experiment | 30 | 30 | | |
| Initial weight [kg] | 20.50 | 20.50 | 0.180 | 1.000 |
| Final weight [kg] | 39.87[b] | 42.27[a] | 0.630 | 0.031 |
| TBG [kg] | 19.37[b] | 21.77[a] | 0.603 | 0.017 |
| ADG [g/day] | 645[b] | 725[a] | 20.083 | 0.017 |
| ADFI [kg/day] | 1.50 | 1.55 | 0.017 | 0.258 |
| FCR [kg/kg] | 2.33[b] | 2.14[a] | 0.049 | 0.021 |

*a b* – $P \leq 0.05$

Significantly better feed conversion efficiency was noted in experimental group animals fed Vilocym (2.14 kg/kg on average), in comparison with the control group (2.33 kg/kg).

## Conclusions

Dietary supplementation with herbal extracts and aluminosilicates (Vilocym) improved the digestibility of crude fat, total protein and organic matter. Weaners fed Vilocym were characterized by higher daily gains (by 12%) and better feed conversion, compared with control group animals.

Translated by ALEKSANDRA POPRAWSKA

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# NITRIFICATION ONTO A ROTATING ELECTRO-BIOLOGICAL CONTACTOR

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K e y  w o r d s: electrolysis, nitrification, rotating electro-biological contactor.

## A b s t r a c t

The goal of the conducted experiment was to determine the electric current impact on the efficiency of nitrogen compounds oxidation at COD to total Kjeldahl's nitrogen ratio of 20, 10, 5. The experiments were run in bench scale, in a rotating electro-biological contactor (REBC) under both, conventional conditions (i.e. without electric current) and with electric current passage at the following densities: $0.2$ A m$^{-2}$, $0.8$ A m$^{-2}$, and $1.5$ A m$^{-2}$. The cathode comprised stainless steel discs with immobile biofilm, and the anode a stainless steel electrode, submerged in waste water contained in the flow-tank of the contactor. The process of nitrogen oxidation was the most effective with electric current passage at the density of $1.5$ A m$^{-2}$ regardless of applied COD/NTK ratio.

# NITRYFIKACJA NA ELEKTROBIOLOGICZNYM ZŁOŻU TARCZOWYM

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S ł o w a  k l u c z o w e: elektroliza, nitryfikacja, elektrobiologiczne złoże tarczowe (REBC).

## A b s t r a k t

Celem doświadczenia było określenie wpływu przepływu prądu elektrycznego na sprawność utleniania związków azotu gdy stosunek ChZT do azotu ogólnego Kjeldahla wynosił 20, 10 i 5. Badania prowadzono w skali laboratoryjnej na elektrobiologicznym złożu tarczowym (REBC) gdy nie przepływał prąd elektryczny, oraz gdy przepływał prąd elektryczny o gęstości: 0,2 A m$^{-2}$, 0,8 A m$^{-2}$,

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1,5 A m$^{-2}$. Katodą były tarcze ze stali nierdzewnej z unieruchomioną błoną biologiczną, anodą – elektroda ze stali nierdzewnej, zanurzona w ściekach komory przepływowej złoża. Najefektywniej proces utleniania azotu przebiegał gdy przepływał prąd elektryczny o gęstości 1,5 A m$^{-2}$, niezależnie od stosunku ChZT:NTK.

## Introduction

Biological nitrification and denitrification processes are employed to remove nitrogen from wastewater. Since the requirement for nutrient removal is becoming increasingly stringent, a high efficiency of nitrogen removal is necessary to achieve a low total nitrogen concentration in the effluent. Often, when nitrogen removal fails, it is due to poor nitrification.

Removal of ammonium by biological nitrification using activated sludge system is process that is widely used in the treatment of domestic and industrial wastewater. Nitrification is an obligate aerobic process and is carried out sequentially by ammonia and nitrite oxidizing groups of bacteria, such as *Nitrosomonas* and Nitrobacter nitrifying microorganisms. The nitrifying bacteria are sensitive to changes in temperature, pH, substrate and product concentrations affecting the rate process. Unfortunately, the kinetic of nitrification is slower and more susceptible to environmental conditions than organic matter oxidation by heterotrophs. Generally, simultaneous growth of nitrifiers and heterotrophs in a single reactor leads to low nitrification specific rates due to overwhelming action of heterotrophs, when treating municipal and industrial wastewaters with a high C:N ratio (BERISTAIN-CARDOSO et al. 2009).

The low growth rate of nitrifying bacteria and the relatively poor capacity of activated sludge units to retain nitrifying biomass require large settlers. The most common problem is the apparition of wash out (CAMPOS et al. 2007). For these reasons the activated sludge units can not treat high nitrogen loadings rates. One of the cheapest ways to improve the sludge retention time in immobilization.

As compared with the activated sludge systems, the reactors with immobilized biomass are characterized by a higher concentration of biomass in the reactor, a higher surface area for the biological development of microorganisms, higher hydraulic loads, better stability of treatment, higher rates of pollutants removal, and lower susceptibility to toxic substances (LI et al. 2003, WILDERER 1995). What is more, low investment costs enable carrying out an economical and advanced process of pollutions disposal (BRINKE-SEIFERTH 1999). Microorganisms gain the maximum rate of growth, which in turn facilitates their physiological adaptation and makes the biofilm resistant to rapid changes of the hydraulic load (WOOLARD 1997). In addition, immobiliz-

ation prevents the washing out of slowly-growing nitrifying bacteria that are retained on the carrier irrespective of the hydraulic retention time (LINDEMANN and WIESMANN 2000, TIJHUIS et al. 1995).

The rotating biological contactor (RBC) is an attached growth bioreactor that offers an alternative technology to the conventional activated sludge process. Rotating biological contactors constitute a very unique and superior alternative for biodegradable matter and nitrogen removal on account of their feasibility, simplicity of design and operation, short start-up, low land area requirement, low energy consumption, low operating and maintenance cost and treatment efficiency. More often RBCs have been successfully used to nitrify municipal wastewater and oxide carbon compounds (CORTEZ et al. 2008).

An increasing load of data is being published that point to the possibility of enhancing the nitrification – denitrification processes as a result of electrolytic excitation. The essence of that method is running the processes in an electric field. Thus, the microbial activity is to be facilitated by hydrogen and oxygen produced onto a cathode and anode, respectively (BESCHKOV et al. 2004).

KRZEMIENIEWSKI and RODZIEWICZ (2005) as well as RODZIEWICZ et al. (2011) carried out a study aimed at combining electrochemical processes induced during the passage of electric current in sewage medium with biological processes proceeding on a rotating biological contactor. Technological design of the experiment assumed that the process of nitrogen elimination would occur during electric current passage, since gaseous hydrogen is produced on the cathode's surface during water hydrolysis which is then used as a source of electron donor by autotrophic bacteria in the denitrification process. In the system discussed, processes of nitrification and denitrification proceeded simultaneously owing to the formation of aerobic and anoxic areas. The anoxic medium was generated in deeper areas of the aerobic biofilm due to limited penetration of oxygen.

Even at low DO concentration in the solution, the nitrification rate is very high due to the use of oxygen generated on the anode. Denitrification rate is relatively high at high DO concentrations due to the application of gaseous hydrogen on the inner side of the cathode-biofilm. A higher rate of total nitrogen removal is achieved at lower DO concentrations and higher densities of an electric current. On this ground, one can conclude that the bio-electrochemical process can be applied in nitrification/denitrification due to the creation of stable aerobic and anoxic regions in a single reactor and that it is beneficial compared to the processes carried out separately, because of the reduction of reactor volume and time (WATANABE et al. 2002). Anoxic conditions in the cathode-biofilm are created as a result of DO use along with the use of hydrogen by the aerobic microorganisms not participating in denitrification.

## Materials and Methods

### Characteristics of waste waters

Experiments were carried out in Weinberger model wastewaters (*Woda i ścieki*... PN-87/C-04616/10). The inflowing and treated wastewaters were determined for the following indicators of contamination: concentration of total nitrogen – with the Kjeldahl's method; concentration of ammonia nitrogen – with the colorimetric method; concentration of nitrate nitrogen III and nitrite nitrogen V – with the colorimetric method, chemical oxygen demand – COD; reaction, and temperature of wastewaters and environment – according to the Polish Standard Methods.

Parameters of oxidation of nitrogen compounds by biofilm were determined using computational formulas presented in the work of KRZEMIENIEWSKI and RODZIEWICZ (2005).

## Research station

Investigations were carried out in a rotating biological contactor (RBC) working in a bench scale (Figure 1). The contactor consisted of four sections. Each section was made of packets of disks mounted coaxially on a horizontal shaft 0.42 m in length. Each packet contained eight disks 0.22 m in diameter each. The disks were made of stainless steel. Each section was fixed in a half-round container with a volume of 2 dm$^3$. The disks rotated with the speed of 60 rpm by means of an electric motor.
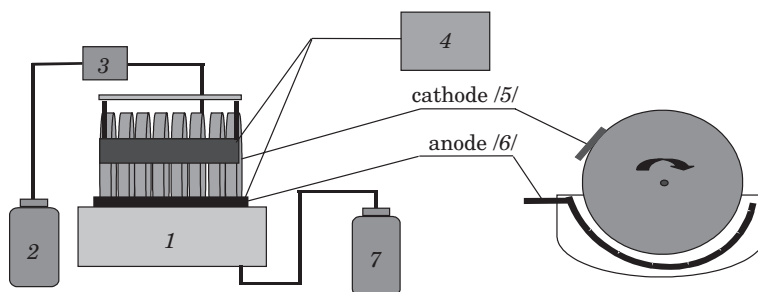


Fig. 1 The scheme of an experimental post: *1* – rotating biological contactor, *2* – tank with untreated waste water, *3* – peristaltic pump, *4* – electric current source, *5* – cathode, *6* – anode, *7* – tank with treated waste water

## Design of kinetic analyses

Kinetic analyses were carried out at COD to total Kjeldahl's nitrogen ratio of 20, 10, 5 and were used as a basis for determining the kinetics of nitrogen compounds oxidation depending on density of the flowing electric current. Samples to be analyzed were collected from a tank of biological contactor in 2.0 h intervals for 10 h, and the last sample was collected after 24 h.

Likewise technological experiments, the kinetic assays were conducted under the flow of electric current with the following densities: $0.0$ A m$^{-2}$, $0.2$ A m$^{-2}$, $0.8$ A m$^{-2}$, and $1.5$ A m$^{-2}$.

Results of the kinetic analyses were used to determine reaction order, rate of ammonia nitrogen oxidation. Experimental results obtained were a backbone for formulating a model that was used for the approximation of measuring points of changes in concentrations of oxidized nitrogen.

The rate of ammonia nitrogen oxidation was described by the following equations:

$$C_{Nox}^{t} = (k_{Nox} \cdot t) + C_{Nox}^{i} \tag{1}$$

where:
$C_{Nox}^{t}$ – concentration of oxidized nitrogen after time $t$ [mg N L$^{-1}$],
$k_{Nox}$ – constant of the rate of ammonia nitrogen oxidation [mg L$^{-1}$ h$^{-1}$],
$C_{Nox}^{i}$ – initial concentration of oxidized nitrogen [mg N L$^{-1}$].

$$C_{Nox}^{t} = C_{Nox}^{i} \cdot (1 - e^{-k_{Nox} \cdot t}) \tag{2}$$

$C_{Nox}^{t}$ – concentration of oxidized nitrogen in the contractor after time $t$ [mg N L$^{-1}$],
$C_{Nox}^{i}$ – initial concentration of oxidized nitrogen [mg N L$^{-1}$],
$k_{Nox}$ – constant of the rate of ammonia nitrogen oxidation [h$^{-1}$].

Reaction rate constants were determined based on experimental data with the method of non-linear regression using STATISTICA software.

## Results and Discussion

The rate of nitrification was computed based on changes in the concentration of oxidized ammonia nitrogen in the biological contractor at 24-h time of sewage retention.

In all systems, changes in the concentration of oxidized ammonia nitrogen proceeded following the first order reaction.
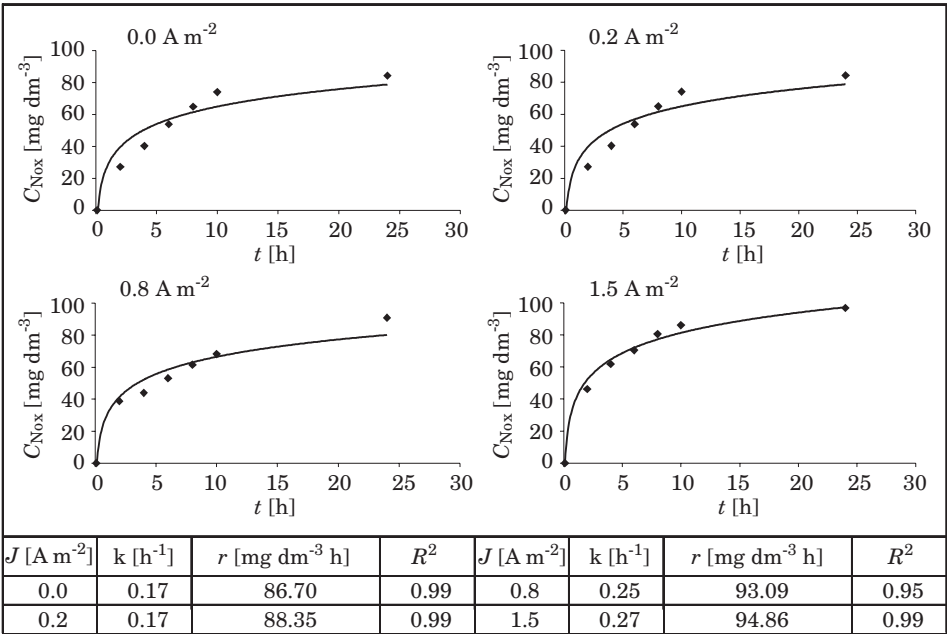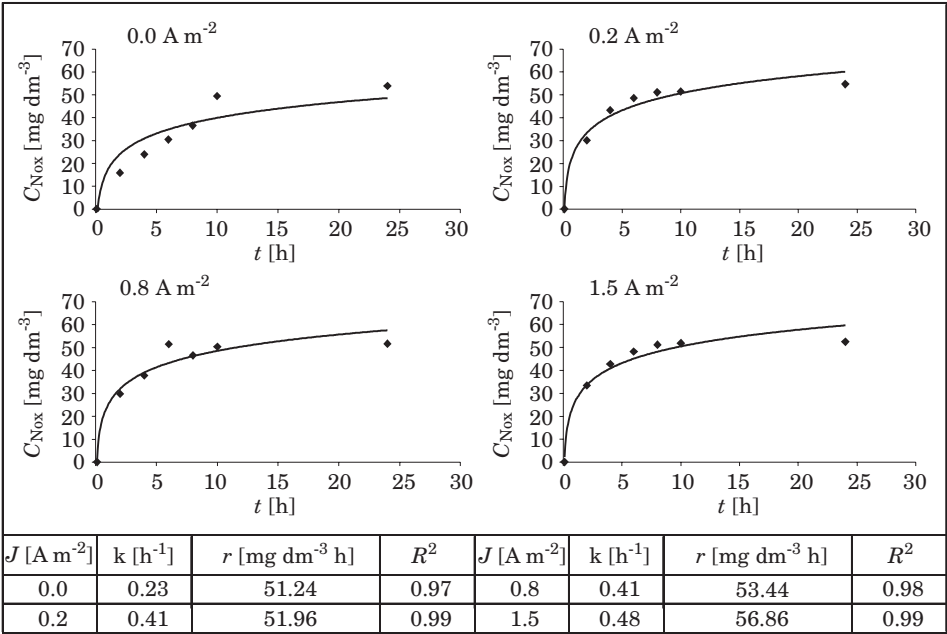
Fig. 2. Changes in the concentration of oxidized ammonia nitrogen ($C_{Nox}$) in time at COD:NTK = 5.0
(the table provides values of rate constant k, initial rate $r$ and conformity coefficient $R^2$)

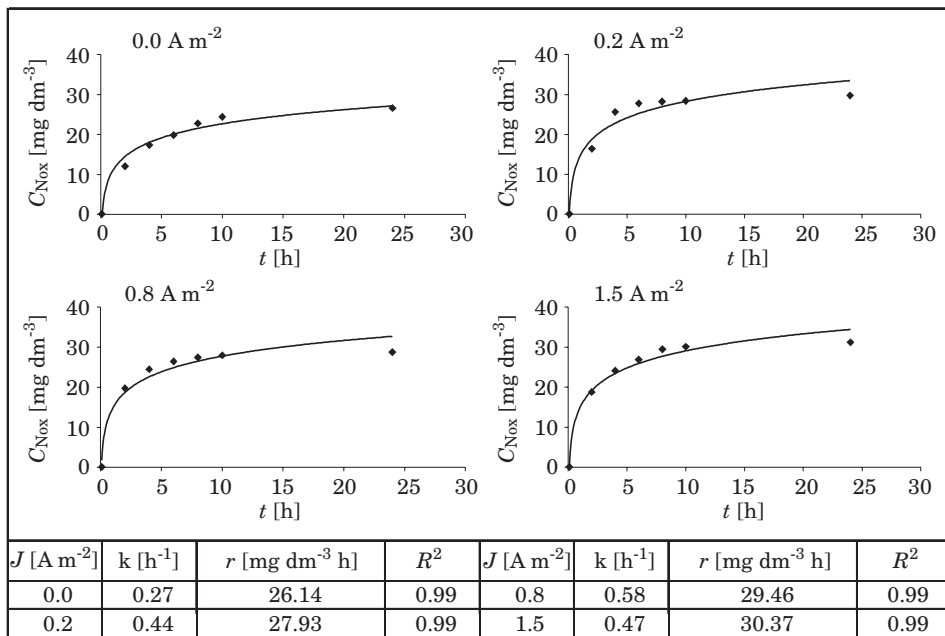| $J$ [A m$^{-2}$] | k [h$^{-1}$] | $r$ [mg dm$^{-3}$ h] | $R^2$ | $J$ [A m$^{-2}$] | k [h$^{-1}$] | $r$ [mg dm$^{-3}$ h] | $R^2$ |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.17 | 86.70 | 0.99 | 0.8 | 0.25 | 93.09 | 0.95 |
| 0.2 | 0.17 | 88.35 | 0.99 | 1.5 | 0.27 | 94.86 | 0.99 |



Fig. 3. Changes in the concentration of oxidized ammonia nitrogen ($C_{Nox}$) in time at COD:NTK = 10.0
(the table provides values of rate constant k, initial rate $r$ and conformity coefficient $R^2$)

| $J$ [A m$^{-2}$] | k [h$^{-1}$] | $r$ [mg dm$^{-3}$ h] | $R^2$ | $J$ [A m$^{-2}$] | k [h$^{-1}$] | $r$ [mg dm$^{-3}$ h] | $R^2$ |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.23 | 51.24 | 0.97 | 0.8 | 0.41 | 53.44 | 0.98 |
| 0.2 | 0.41 | 51.96 | 0.99 | 1.5 | 0.48 | 56.86 | 0.99 |

At COD:NTK = 5, the highest value of the constant of ammonia nitrogen oxidation rate k equal to 0.27 $h^{-1}$ was obtained at the flow of electric current with density of 1.5 A $m^{-2}$ (Figure 2). In turn, the lowest oxidation rate constant (0.17) was reported in the conventional system and at the flow of electric current with density of 0.2 A $m^{-2}$. Under conditions of the flow of electric current with density of 0.8 A $m^{-2}$ the value of constant k accounted for 0.25.

At COD:NTK = 10, the highest value of the constant of ammonia nitrogen oxidation rate k equal to 0.48 $h^{-1}$ was obtained at the flow of electric current with density of 1.5 A $m^{-2}$ (Figure 3). In turn, the lowest oxidation rate constant (0.23) was reported in the conventional system. Under conditions of the flow of electric current with densities of 0.2 A $m^{-2}$ and 1.5 A $m^{-2}$ the values of constant k accounted for 0.41.

At COD:NTK = 20, the highest value of the constant of ammonia nitrogen oxidation rate k equal to 0.58 $h^{-1}$ was obtained at the flow of electric current with density of 0.8 A $m^{-2}$ (Figure 4). In turn, the lowest oxidation rate constant (0.27) was reported in the conventional system. Under conditions of the flow of electric current with densities of 0.2 A $m^{-2}$ and 1.5 A $m^{-2}$ the values of constant k accounted for 0.44 and 0.43, respectively.



| $J$ [A $m^{-2}$] | k [$h^{-1}$] | $r$ [mg $dm^{-3}$ h] | $R^2$ | $J$ [A $m^{-2}$] | k [$h^{-1}$] | $r$ [mg $dm^{-3}$ h] | $R^2$ |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.27 | 26.14 | 0.99 | 0.8 | 0.58 | 29.46 | 0.99 |
| 0.2 | 0.44 | 27.93 | 0.99 | 1.5 | 0.47 | 30.37 | 0.99 |

Fig. 4. Changes in the concentration of oxidized ammonia nitrogen ($C_{Nox}$) in time at COD:NTK = 20.0 (the table provides values of rate constant k, initial rate $r$ and conformity coefficient $R^2$)
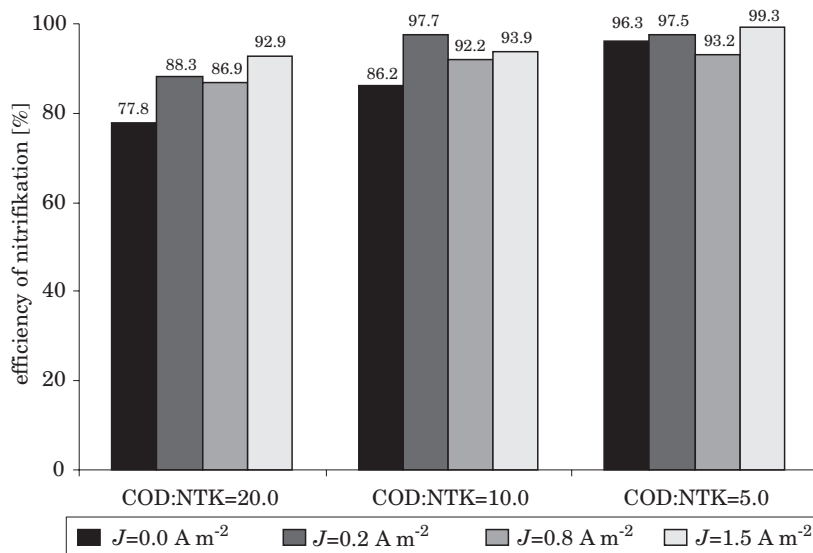
Fig. 5 Efficiency of nitrification onto a rotating electro-biological contactor

In the reported experiment efficiency of nitrification depended on the applied COD:NTK ratio and the flow of electric current. The highest efficiency of nitrification (99.3%) was obtained under conditions of the flow of electric current with density of $1.5\ A\ m^{-2}$ and at COD:NTK = 5. The lowest efficiency of nitrification, i.e. 77.8%, was observed under conventional conditions and at COD:NTK = 20.

One of more important parameters that determine the efficiency of ammonia nitrogen oxidation is biomass load with organic contaminants. A number of authors point to a dependency of nitrification efficiency on the concentration of biomass immobilized in the reactor. Once investigating nitrifying biomass colonizing pieces of chalk in a fluidized bed, GREEN et al. (2001) reported a higher rate of ammonia nitrogen oxidation at a higher biomass concentration. The higher concentration of biomass restricts diffusion. In turn, HEM et al. (1994) analyzed the effect of organic matter on nitrification efficiency and demonstrated that at a load over 5 g $BOD_7$ $m^{-2}\ d^{-1}$ the rate of nitrification decreases to values close to zero. At the load ranging from 2 to 3 g $BOD_7\ m^{-2}\ d^{-1}$ the rate of ammonia nitrogen oxidation fluctuates between 0.3 to 0.8 g $N\text{-}NO_x m^{-2}\ d^{-1}$. The results of PATUREAU et al. (1997) showed that the inhibition of nitrifying microorganism activity could be caused by higher organic compound levels in environment. Alike results were reported by PASTORELLI et al. (1997a, 1997b) as well as RUSTEN et al. (1994). According to the authors, the decreasing rate

of ammonia nitrogen oxidation upon the increasing load of organic contaminants is linked with excessive growth of aerobic heterotrophic microorganisms, which results in diminished percentage of nitrifiers in the biomass. The effect of the C:N ratio in sewage on spatial distribution of nitrifiers and heterotrophs in a biofilm was the subject of research of numerous authors (GIESEKE et al. 2001, GIESEKE et al. 2002, OHASI et al. 1995, OKABE et al. 1996, SATOH et al. 2000, VAN LOOSDRECHT et al. 1995). They demonstrated that increasing the C:N ratio in the inflowing sewage leads to the competition for oxygen and ammonia nitrogen between nitrifying and heterotrophic bacteria, which in turn evokes a decrease in the percentage of nitrifiers in the outer aerobic layer of the nitrifying membrane. According to ITOKOWA et al. (2001) nitrate accumulation, lack of ammonium in the effluent and high rate of nitrification were observed when C:N ratio in waste was 2.4 and 3.5.

ØDEGAARD (2000) and ØDEGAARD et al. (1999), who studied changes in a reactor with biomass immobilized on plastic carriers being in motion, demonstrated that at the load higher than 4 g $BOD_7$ $m^{-2}$ $d^{-1}$ the nitrification process required high oxygen concentration, i.e. over 6 mg $O_2$ $L^{-1}$.

The reported experiment was carried out at the COD:NTK ratio of 20 which corresponds to organic contaminants load of 7.0 g COD $m^{-2}$ $d^{-1}$. Similar loading with organic contaminants was applied by KARNCHANAWONG and POLPRASER (1990) in a circulating reactor with immobilized biomass. The COD load used by those authors ranged from 3.56 to 10.16 g $m^{-2}$ $d^{-1}$. The highest efficiency was obtained at the load of 5 g COD $m^{-2}$ $d^{-1}$.

In our study, the flow of electric current turned out to affect both the rate and efficiency of nitrogen compounds removal. Under conventional conditions, the efficiency of nitrification appeared to be the lowest. The application of electric current and increasing its density led to an increase in the efficiency of nitrogen compounds oxidation, which might have been due to the utilization of oxygen generated on the anode by the nitrifiers (VAN LOOSDRECHT et al. 1995).

## Conclusions

In the conducted experiment, the passage of electric current was observed to affect the effectiveness of nitrification and the rate of nitrogen compounds oxidation depending on the value of the COD:NTK ratio applied.

At the high loading of biomass with organic compounds (COD:NTK = 20), the application of an electric current was resulting in an increase in nitrification effectiveness. Under conventional conditions the effectiveness of nitrogen compounds oxidation accounted for 77.8%, whereas under the flow of an electric current with a density of 1.5 A $m^{-2}$ – for nearly 93%.

At COD:NTK = 5 the effectiveness of nitrogen compounds oxidation was similar in all treatment systems.

Translated by JOANNA MOLGA

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# EFFECTIVENESS OF FLOCCULATION OF MODEL SILICA SUSPENSION BY ORGANIC POLYELECTROLYTES

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### A b s t r a c t

Coagulation/flocculation of a suspension of silica $SiO_2$ was carried out with the use of macro-mlecular organic polymers which supported the action of two inorganic coagulants – PACl and $Al_2(SO_4)_3$. The results indicate the exceptional effectiveness of cationic and anionic flocculants in coagulation with $Al_2(SO_4)_3$ and an anionic polymer P 2540 in combination with PACl. They also show the correlation between the degree of purification of the liquid phase with 20 mg Al $dm^{-3}$ from $Al_2(SO_4)_3$, supported by flocculants and the fractal dimension D of the aggregates obtained with $Al_2(SO_4)_3$ and organic polymers. An increase in coagulation of the liquid phase corresponds to an increase in the fractal dimension D = 1.52–1.97.

## SKUTECZNOŚĆ FLOKULACJI MODELOWEJ ZAWIESINY KRZEMIONKI PROWADZONEJ ZA POMOCĄ POLIELEKTROLITÓW ORGANICZNYCH

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### A b s t r a c t

Proces koagulacji/flokulacji zawiesiny krzemionki $SiO_2$ prowadzono z zastosowaniem wysokocząsteczkowych polimerów organicznych wspomagających działanie dwóch koagulantów nieorganicznych – PACl i $Al_2(SO_4)_3$. Uzyskane wyniki badań wskazują na szczególną skuteczność

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flokulantów kationowych i anionowych w koagulacji prowadzonej z $Al_2(SO_4)_3$ i anionowym polimerem P 2540 we współpracy z PACl. Dowiedziono, że istnieje korelacja między stopniem oczyszczenia fazy ciekłej za pomocą 20 mg Al $dm^{-3}$ z $Al_2(SO_4)_3$ wspomaganego flokulantami a rozmiarem fraktalnym D agregatów otrzymanych za pomocą $Al_2(SO_4)_3$ i polimerów organicznych. Wzrostowi stopnia skoagulowania fazy ciekłej odpowiada wzrost rozmiaru fraktalnego D = 1.50–1.99.

## Introduction

Organic polymers are frequently used in water and wastewater treatment. Water-soluble polymers whose chains contain polar or ionic groups are called polyelectrolytes. Depending on the group's character, they are classed as cationic, anionic or non-ionic. Organic polymers used in water and wastewater treatment can effectively destabilise colloids, but they are usually used in order to support inorganic coagulants.

Literature reports sometimes use the term coagu-flocculation to refer to the process which consists of three consecutive stages: reagent mixing, coagulation and flocculation. Coagulation involves formation of micro-flocs with a size of less than 5 μm, which in the next stage increase in size, forming macro-flocs (< 1 mm) (HANHUI et al. 2004). The addition of organic polyelectrolytes supports the flocculation phase and makes the flocs cluster into larger agglomerates.

Characteristic features of organic polymers include charge density, which is defined as the percentage ratio of ionic groups to all the groups in the chain (BOLTO 1995). When characterising flocculants in terms of their molecular weight and charge density, it is assumed that with a molecular weight of $10^7$ the charge density ranges from 50 to 100%; the value for polymers with the molecular weight of $10^8$–$10^6$ is about 25%, and when the molecular weight is about $10^4$–$10^5$ it is about 10%. Both the molecular weight and the charge density play an important role in creating the right conformation of a polymer chain in a solution which, in turn, affects the mechanism of flocculation and sedimentation (BESRA 2004). Emerging new technologies of polymer molecular architecture indicate that highly branched, spatial and interacting polymer conformations are more effective than linear ones and that their action results in more compact and less hydrated flocs (PEARSE 2001). The bridging properties of organic polymers increase the degree of aggregation of colloidal particles, which creates flocs able to sediment. The effectiveness of polymer adsorption depends both on the polymer properties (degree of hydrolysis, spatial structure, ionic strength) and on the characteristics of the systems which are being purified (e.g. the type and size of particles and the available surface and the electrokinetic potential of the dispersed particles and pH of the solution) (GUNGOR and KARAOGLAN 2001).

Research into the phenomenon of aggregation in combination with elements of fractal analysis has led to the development of different models of aggregation (simulation of diffusion-limited aggregation particles DLA, monomer-cluster aggregation, cluster-cluster aggregation controlled diffusion). Being a widely recognised tool, fractal analysis allows users to quantitatively assess the degree of folding or fraying irregular surface of the objects under analysis. When fractal analysis is applied to a description of aggregation, the structure and properties of the post-coagulation sludge can be described in a new, qualitative way (LEE 2002). Characterisation of flocs by this method allows one to determine the compactness of the aggregate structure and the degree of hydration of the sludge (BOTTERO 1989).

The aim of this study was to determine the effect organic polymers on the flocculation effectiveness of a model system of silica suspension with inorganic coagulants of the PACl and $Al_2(SO_4)_3$ type.

## Experimental methods

The results of coagulation-flocculation of colloidal solution of silica ($SiO_2$) at the concentration of 300 mg $dm^{-3}$ are presented. The system turbidity was removed with two aluminium coagulants: PACl and $Al_2(SO_4)_3$ at two levels of dosing. The supporting effect of three macromolecular flocculants has been examined: cationic Z 63, anionic P 2540 and non-ionic PFC 108.

The coagulants contained different amounts of the main ingredients:
– PAC type coagulants contained 47–52 mg Al $dm^{-3}$ and about 97 g $Ca^{2+}$ $dm^{-3}$,
– $Al_2(SO_4)_3$ contained about 9.1% Al.

Chemical coagulation was effected with the standard jar-test procedure:
– fast stirring (400 rpm) – 1min,
– slow stirring (30 rpm) – 15 min,
– sedimentation – 15 min.

Turbidity was determined by the absorption method with a DR 2000 HACH spectrophotometer. Testing organic flocculants was based mainly on the recorded changes in the studied system turbidity. The effectiveness of flocculation was determined from the formula (OZKAN 2003):

$$\text{flocculation effectiveness} = (T_0 - T_f)\, T_0^{-1}$$

where:
$T_0$ – initial turbidity of the dispersed silica suspension,
$T$  – sample turbidity following the coagulation/flocculation process.

## Results and Discussion

The properties of organic polymers were determined with respect to changes in the turbidity of a model system of silica after completing the coagulation/flocculation process. The process was carried out with the use of inorganic coagulants (PACl and $Al_2(SO_4)_3$), both with and without flocculants.

Figure 1 and Figure 2 show the results of examination of the process of coagulation-flocculation of silica suspension with the use of 10 and 20 mg Al $dm^{-3}$ with PACl and three selected organic flocculants with a different ionic character: cationic Z 63, anionic P 2540 and non-ionic PFC 108.
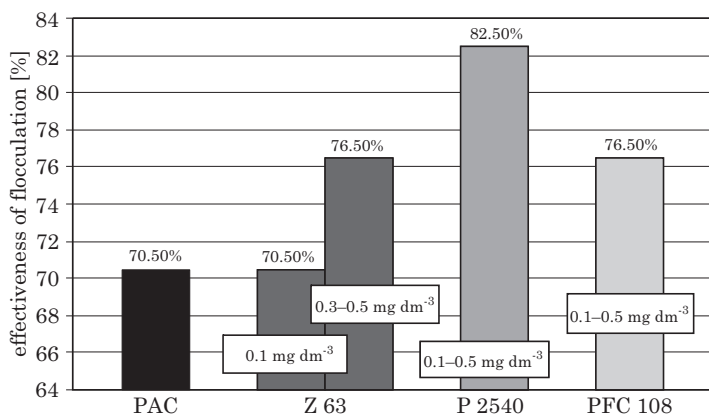
Fig. 1. The effect of the organic polymer dose on the effectiveness of flocculation performed with 10 mgAl $dm^{-3}$ from PACl
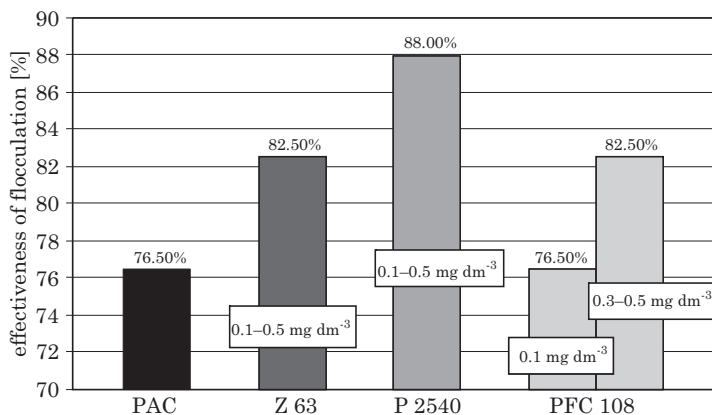
Fig. 2. The effect of the organic polymer dose on the effectiveness of flocculation performed with 20 mg Al $dm^{-3}$ from PACl

The peak effectiveness of flocculation was observed following the use of the lowest dose of 0.1 mg dm$^{-3}$ of the anionic flocculant P 2540 (at both levels of dispensing PACl). Such an amount of the flocculent caused the turbidity to decrease by 82.5% and 88%, respectively. The addition of 10 mg PACl with 0.3 mg of the cationic flocculant Z-63 and 0.1 mg of non-ionic polymer PFC 108 offsets the effect of the double dose of the inorganic coagulant. Anionic flocculants in the model system of silica suspension, used in combination with PACl (10 mg Al dm$^{-3}$), proved to be more effective in reducing turbidity than the cationic ones. The addition of anionic flocculant can considerably reduce the consumption of the inorganic coagulant. It is probable that the destabilisation of a greater number of positive particles of $(SiO_2)$n in the presence of anionic flocculants may result in the formation of larger flocs through direct "bridging". This may, in turn, significantly speed up the phase separation.

In general, according to LATTRIGE (1982), the process of destabilisation of electropositive sol of $(SiO_2)_n$ coagulated with PACl runs by bridging $(SiO_2)_n$ micelles with $AlO_4^{-5}$ anions, incorporated in the surface, with "$Al_{13}$" polycations. It is probable that supporting the process with a cationic flocculant is a result of increasing the number of "bridges" in the system. At the same time, molecules of the anionic flocculant can bridge positive $(SiO_2)_n$. micelles directly, without the need to incorporate negative ions $AlO_4^{-5}$.

Figure 3 and Figure 4 illustrate the effect of the type and dose of the flocculant on the residual turbidity in the coagulation-flocculation of silica suspension with 10 and 20 mg Al dm$^{-3}$ from another inorganic aluminium coagulant under examination – $Al_2(SO_4)_3$.

Surprisingly good results were achieved when $Al_2(SO_4)_3$ was used in combination with the cationic flocculant Z 63, which completely removed turbidity at a dose as low as 0.3 mg Al dm$^{-3}$. The data in Figures 1–4 show that
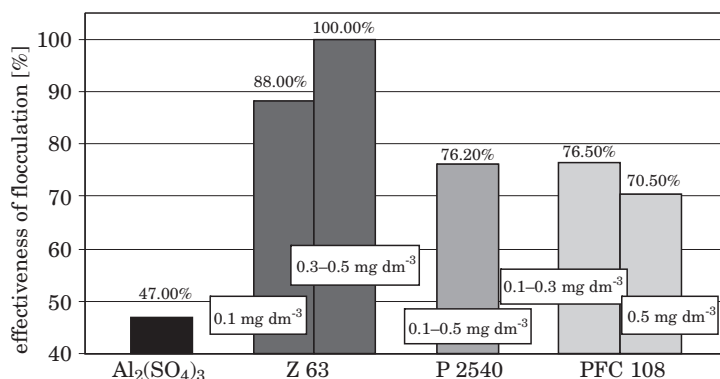


Fig. 3. The effect of the organic polymer dose on the effectiveness of flocculation performed with 10 mgAl from $Al_2(SO_4)_3$
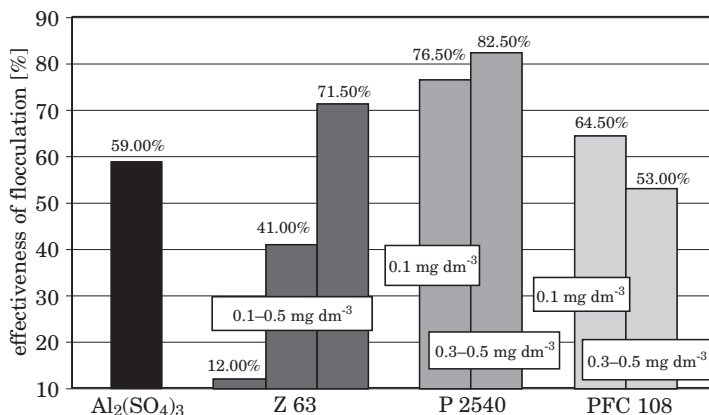
Fig. 4. The effect of the organic polymer dose on the effectiveness of flocculation performed with 20 mgAl dm$^{-3}$ from Al$_2$(SO$_4$)$_3$

the effectiveness of the flocculant used in the experiment largely depended on the type and effectiveness of the inorganic coagulant used. Identical doses of 10 mg Al dm$^{-3}$ from PACl and from Al$_2$(SO$_4$)$_3$, without any support, gave different effects of coagulation: 70.5% and 47%, respectively. The results confirm the higher coagulative ability of PACl as compared to Al$_2$(SO$_4$)$_3$, as suggested by literature reports (BOTTERO et al. 1989). However, it is in the presence of Al$_2$(SO$_4$)$_3$ that the process of destabilisation with the cationic flocculant Z 63 proved to be the most effective and resulted in total removal of turbidity in the liquid phase above the sediment after coagulation. This again confirms that an organic flocculant to support an inorganic one should be selected on a case-by-case basis, i.e. for a specific type of wastewater and for a specific type of inorganic coagulant.

Weak effect of the cationic flocculant was observed at 20 mg Al dm$^{-3}$ from Al$_2$(SO$_4$)$_3$ (Figure 4). Only the dose of 0.5 mg dm$^{-3}$ Z 63 reduced the turbidity by about 70.5%. Cationic flocculants in the other tests even had a negative effect on the effectiveness of coagulation-flocculation. Such results are difficult to interpret, but some explanation may be sought in the effect of the flocculant, which stabilises the colloidal system of (SiO$_2$)$_n$, or in some "consumption" of coagulative effect of Al from Al$_2$(SO$_4$)$_3$ by the flocculants used in the experiment. The consumption mentioned above could result in the formation of aggregates of the sol particles {Al(OH)$_3$}$_n$ and flocculant molecules. Possible unstable aggregates like {cationic flocculant}-AlO$_4$$^{-5}$-{SiO$_2$} can both release {SiO$_2$}$_n$ to the solution (increased turbidity) and restrict access of polycations (Al$_{13}$) to the surface of {SiO$_2$}$_n$. Due to its size and specific properties (large surface charge), one flocculant particle can disturb the effect of many inorganic coagulant particles (LEu and GOSH 1988). The standard jar test procedure can

effectively estimate the effect of organic polymers on the phase separation, expressed as the residue of turbidity liquid above the sludge.

From the point of view of coagulation effectiveness, adequate adjustment of the polyelectrolyte dose is a significant factor. Insufficient contact between the flocculant particles when a dose is too low results in insufficient de-stabilisation of colloidal particles and poor agglomeration. On the other hand, excessive volumes of polymer cause complete coverage of particles with flocculant, which enables inter-particle bridging and re-stabilisation of the colloidal particles. Optimum flocculation occurs when approximately half of the adsorption sites on the surface of the polyelectrolyte-saturated particles remain free.

The obtained results could be compared to the fraktal analysis of waste-water sludge (SMOCZYŃSKI and WIERZBICKA 2001). The application of a macro-scopic photography method allows for determination of the size, shape and properties of the obtained aggregates. Fractal analysis is one of the methods used in structural characterisation of flocs. The fractal dimension D is closely related to the aggregate density. An increase in the object density results in an increase in the fractal dimension D, which makes the aggregates more stable.

The highest values of the fractal dimension D were calculated for the aggregates obtained with $Al_2(SO_4)_3$ and the anionic flocculant P 2540 D = 1.988 and the cationic one Z 63 D = 1.978 (SMOCZYŃSKI and WIERZBICKA 2001). Such high values of D may indicate that aggregates with more compact structure were obtained. BOTTERO and BERSILLON (1989) claim that as the pH of the solution and PACl basicity (R = [OH] : [Al]) increase, the specific available surface $Al_{13}$ decreases. They found that the specific flocs surface, formed as a result of diluting and hydrolysis of polymeric coagulants such as PACl, is significantly larger than that obtained with a monomeric coagulant $Al_2(SO_4)_3$. Moreover, these researchers reported that the degree of contaminant sorption increases with the degree of unfolding of the studied flocs surfaces.

The relatively low values of D = 1.36 (PACl) and D = 1.56 ($Al_2(SO_4)_3$) (SMOCZYŃSKI and WIERZBICKA 2001) for the non-ionic flocculant N 300 may in turn indicate the existence of a loose structure of aggregates and their considerable hydration. The results indicate that there is a clear correlation between the degree of the liquid phase treatment with 20 mg Al $dm^{-3}$ from $Al_2(SO_4)_3$, supported by flocculants, and the fractal dimension D of aggregates obtained with $Al_2(SO_4)_3$ and organic polymers. An increase in the degree of coagulation of the liquid phase (64.5–82.5%) corresponds to an increase in the fractal dimension D = 1.5–1.988 (SMOCZYŃSKI and WIERZBICKA 2001). The values of $D_K$, $D_A$ and $D_N$ show that the flocculants applied significantly affected the structure and properties of the aggregates. Aggregates bind stronger in the flocculation phase than in coagulation (CHO et al. 2002), which most probably makes the structure of flocs formed by flocculation with an organic polyelec-

trolyte more stable. Examination of the fractal structures of sludge formed by coagulation and the effect of various factors on the value of their fractal dimension is very important from the practical point of view. Low values of fractal dimensions indicate a low density of aggregates and, in consequence, an increase in the size of voids in the space occupied by it.

## Conclusions

1. A positive effect of polyelectrolytes in coagulation using inorganic coagulants was observed in most trials (86%).

2. The highest effectiveness of flocculation (100%) was observed in the case of cationic flocculant Z63 in combination with 10 mg Al dm$^{-3}$ Al$_2$(SO$_4$)$_3$. In the other cases, anionic flocculants, acting in combination with PACl (10 and 20 mg Al dm$^{-3}$) and Al$_2$(SO$_4$)$_3$ (20 mg Al dm$^{-3}$), proved more effective in the model silica suspension.

3. The application of 10 mg PACl with 0.3 mg of the cationic flocculant Z-63 and 0.1 mg non-ionic polymer PFC 108 offsets the effect of the double dose of this coagulant.

4. There is a correlation between the degree of purification of the liquid phase with 20 mg Al dm$^{-3}$ from Al$_2$(SO$_4$)$_3$, used in combination with flocculants, and the fractal dimension D of aggregates obtained with Al$_2$(SO$_4$)$_3$ and organic polymers. An increase in the degree of coagulation of the liquid phase corresponds to an increase in the fractal dimension D = 1.52–1.97.

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# REARING OF BLACK MOLLY *POECILIA HYBRIDS* (VALENCIENNES, 1846) IN WATER OF DIFFERENT SALINITY

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K e y   w o r d s: *Poecilia* spp., aquarium fish, rearing, salinity, aquaristics.

### A b s t r a c t

This study analysed the rearing of Black Molly *Poecilia* hybrids. It is a crossbreed of sailfin molly *Poecilia latipinna* and short-finned molly *Poecilia sphenops*. Black mollies are very popular aquarium fish which are frequently put into marine aquaria to speed up the maturation of such aquaria. Without being acclimatised to high salinity, the fish are put into water with a salinity of SG 1.025 or 33 ‰. The results indicate that fish which are shorter than 20 mm die in water whose salinity is higher than SG 1.020. However, fish longer than 25 mm can adapt to higher salinity. The results indicate that fish which have been reared in water of higher salinity grow faster.

## PODCHÓW MOLINEZJI CZARNEJ "BLACK MOLLY" *POECILIA HYBRIDS* (VALENCIENNES, 1846) W WODZIE O RÓŻNYM ZASOLENIU

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S ł o w a   k l u c z o w e: molinezje, ryby akwariowe, podchów, zasolenie, akwarystyka.

### A b s t r a k t

Przeprowadzono badania nad podchowem molinezji czarnej "Black Molly" *Poecilia* hybrids. Jest ona krzyżówką molinezji szerokopłetwej *Poecilia latipinna* oraz molinezji ostroustej *Poecilia sphenops*. Molinezja "Black molly" jest bardzo popularną rybą akwariową, którą często umieszcza

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się w akwariach morskich, aby przyspieszyć proces dojrzewania tych zbiorników. Molinezje czarne bez wcześniejszej aklimatyzacji do dużego zasolenia, od razu umieszczane są w akwariach o zasoleniu SG 1,025, czyli 33‰. Uzyskane wyniki wskazują, iż ryby o długości poniżej 20 mm sną w wodzie o zasoleniu wyższym niż SG 1,020. Osobniki o długości powyżej 25 mm są natomiast w stanie przystosować się do wyższego zasolenia. Uzyskane wyniki wskazują, że ryby podchowywane w wodzie o wyższym zasoleniu szybciej przyrastają.

# Introduction

The results of recent studies in aquaculture have highlighted the need for a detailed elaboration of the technology associated with reproduction biotechniques (GLOGOWSKI et al. 1999, KUCHARCZYK et al. 2005, 2008a, KREJSZEFF et al. 2008, 2009, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2009, 2010, CEJKO et al. 2010, TARGOŃSKA et al. 2010), as well as the biotechnique of rearing larvae and juvenile stages (KUCHARCZYK et al. 1997, 1998, KUJAWA et al. 1997, 2010, KUPREN et al. 2008, ŻARSKI et al. 2009). The differences have not only been observed between species, but also between populations or shoals of fish of the same species (KUCHARCZYK et al. 2008a, KREJSZEFF et al. 2009, 2010a). Even small differences in the composition of water used to activate gametes, including its salinity, can affect the results of eggs fertilization (KUCHARCZYK et al. 2010b). All these details are later important in calculation of the production efficiency (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010) and may determine its economic success.

Aquaristics is a very popular branch of aquaculture. Tropical ornamental fish are bred in many places around the world, and trading in them brings huge profits (TLUSTY 2002). However, despite its popularity and considerable economic benefits, brought about by breeding and trading aquarium fish, publications about their biology and breeding have been scarce (KUCHARCZYK et al. 2008b, 2010a, KUPREN et al. 2008, TARGOŃSKA and KUCHARCZYK 2011). Fish of *Poecilia* spp. inhabit the fresh and slightly saline and even sea waters of Central America (COSTA and SCHLUPP 2010). *Poecilia sphenops* can be found even in the Atlantic, a long way off the coast. They reach a length of 10 cm and prefer medium hard to hard, alkaline water with the temperature ranging from 24 to 28°C. The fish are in a better shape when kept in water with a specific gravity of 1.002–1.005 than in fresh water. Black mollies have been used for a long time in newly established marine aquaria to start the nitrogen cycle.

*Poecilia* spp. seem to be particularly sensitive to nitrates and nitrites. The addition of salt to water reduces the toxicity of the compounds. Salt added to water is also an effective anti-parasitic and antifungal agent. Water with the salinity of 5 to 8‰ 7 is hard to live in for freshwater parasites, and changes

caused by fungi disappear quickly as compared to fish kept in fresh water. Fish of *Poecilia* spp. which are kept in fresh water are susceptible to many diseases, such as white-spot disease caused by *Ichthyophthirius multifiliis*, a fin necrosis caused by *Pseudomonas fluorescens*. The fish often suffer from molly disease and become unable to swim properly and they swing sideways. This is caused by deficit of electrolytes which are necessary for proper metabolism. The disease does not occur in mollies kept in slightly salty or salty water.

Carrying out pioneer research frequently requires constructing special systems which allow researchers to perform repeat studies and to achieve reliable results. Such systems have been developed to keep spawners (KUJAWA et al. 1999), to incubate eggs (KUCHARCZYK et al. 1996) or to rear larvae at different densities (Kujawa et al. 2000, Krejszeff et al. 2010b). However, no systems have been developed for simultaneously rearing larvae in water of different salinity.

The aim of the study was to determine the survival and growth rate of fish in fresh, slightly salty and sea water. However, to perform the task, special experimental runs have been developed and constructed.

## Materials and Methods

The fish used in the experiment were obtained from an aquarium fish breeding farm. Upon arrival, they were 16–17 mm long and weighed 0.057–0.081 g. All the fish (600) were put into a tank (192 dm$^3$) with fresh water where they were kept for 7 days. The water temperature was 25ºC, pH 7. No nitrites or ammonia were detected in the water while the fish were kept in it (Table 1). During the experiment, the fish were fed Artemis nauplii twice a day, 10 g of cysts per feeding. The fish were not fed on the day before being transferred to separate tanks. After a period of acclimatisation, the fish were put into 12 aquaria, 50 fish in each, the aquaria being parts of 6 independent rearing circulations with water of different salinity (Figure 1). Meersalz (produced by Aquamedic) synthetic sea salt was used, which contains the essential nutrients and has buffering properties. Salinity was measured with a refractometer of the same manufacturer. The salinity of the systems were:
   – system 1 – salinity SG 1.000; 0.0‰;
   – system 2 – salinity SG 1.005; 6.7‰;
   – system 3 – salinity SG 1010; 13.3‰;
   – system 4 – salinity SG 1.015; 20.0‰;
   – system 5 – salinity SG 1.020; 26.7‰;
   – system 6 – salinity SG 1.025; 33.3‰.

The water parameters during the experiment

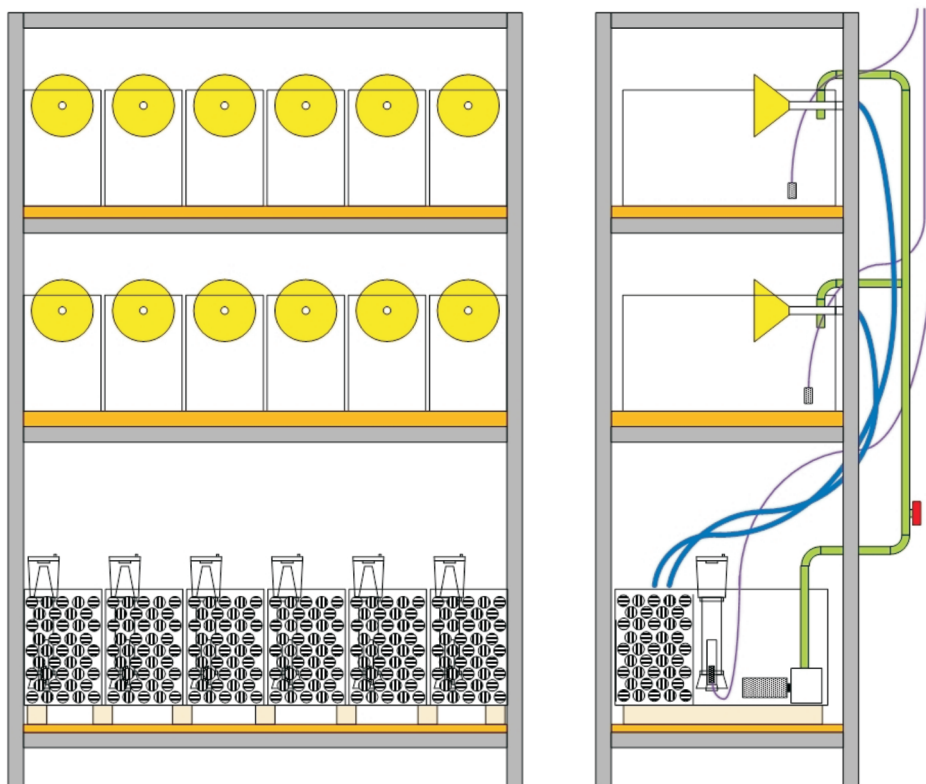| Parameter | Aquaria | |
|---|---|---|
| | experiment start | experiment end |
| Temperature [°C] | 25°C | 25°C |
| pH | 6.9–7.2 | 6.9–7.2 |
| NH$_3$, NO$_2$ [mg dm$^{-3}$] | 0 | < 1.0 |
| NO$_3$ [mg dm$^{-3}$] | 0 | < 10 |



Fig. 1. A diagram of the system of 6 aquaria with water of different salinity, used to conduct this study. The front view – on the left and the side view – on the right

Each system consisted of three interconnected aquaria with the working capacity of 25 dm$^3$ each. The fish were kept in two upper tanks, whereas in the lower tanks there were biological deposits as bio-bale with the total volume of 8 dm$^3$, a cube protein skimmer (Aquamedic miniflotor) and an Atman AT-306 pump. The pump pumped the water to the medium

and upper tanks, from where it was moved by gravitation to the lower tank through an outlet system with a meshed funnel at the end. The lower aquaria were placed in a 297 dm³ water bath with two 500 W heaters. The water temperature in the aquaria during the experiment was maintained at 25°C ± 0.2°C. The aquaria were lit with fluorescent light for 12 hours. Mollies were fed twice a day with JBL Novo Bea feed which consisted of raw protein (45.2%), raw fat (5%), raw fibre (1.5%) and raw ash (9.7%). The feed is intended for guppies and other small fish species of the family *Poeciliidae*, which also includes mollies. The feed has the form of small flakes, which initially float and then slowly sink. The feed was supplied through a bi-chamber automatic feeder, manufactured by Eheim. A single feed dose was 0.58 g per tank. The rearing was conducted for 70 days. The feed dose was increased every 10 days and was adapted to the weight of the fish.

The parameters measured during the experiment included water temperature, the number of dead fish, nitrates and ammonia content. The fish were measured every 4 days. A sample each time consisted of 10 fish from each tank. After being caught, the fish were put into a 5 dm³ tank, where they were anaesthetised with propiscin at 1 mL/dm³. After anaesthetising them, their total length *l.t.* was measured with an electronic calliper. Subsequently, the fish were transferred to a water tank placed on precise scales, where they were weighed.

The regression analysis fish length, weight in relationship to the days was conducted.

# Results

The results of the experiment have shown that using salt water in rearing juvenile stages of molly positively affected the growth rate and the final length (Figure 2, Table 2). The fish kept in fresh water grew much more slowly and achieved the smallest final length.

Using salt water in rearing juvenile stages of molly positively affected the growth rate and the final weight (Figure 3, Table 3). On the initial days of the experiment, the fish kept in water at the salinity level of SG 1.015 and 1.020 grew slower than those kept in fresh and slightly salty water. This was caused by acclimatisation of the fish to the salty environment and by achieving the osmotic equilibrium in fish. After 12 days, the fish kept in water of higher salinity started to grow faster, ultimately weighing more by 16 to 38% than those kept in fresh water.
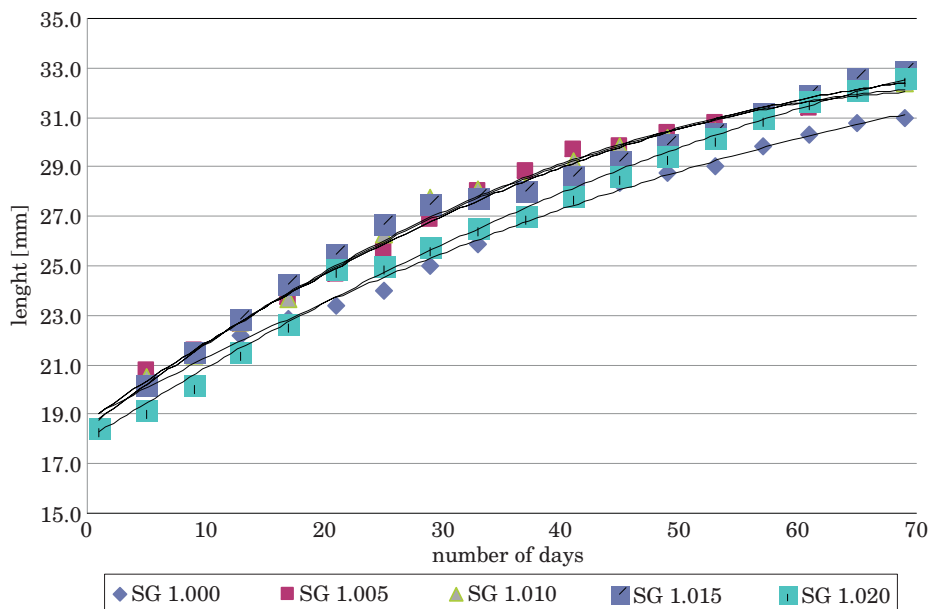
Fig. 2. The relationship between days of rearing and the total length *l.t.* of mollies kept in water of different salinity

Table 2

Equations of regression and coefficients of determination for the relationship between the days of rearing and the total length *l.t.* of mollies at different density levels

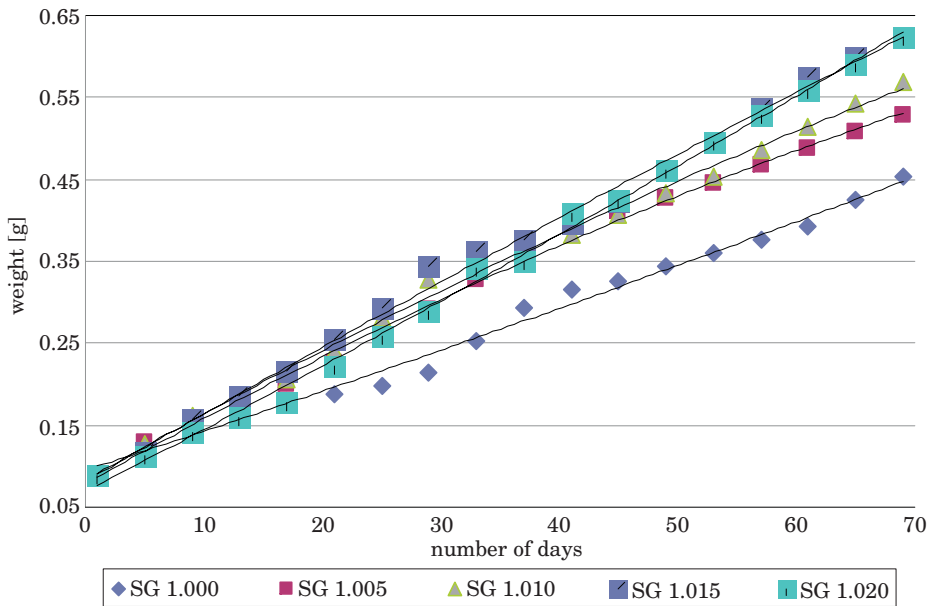| Salinity SG | Equation | Value of $R^2$ |
|---|---|---|
| 1.000 | $y = -0.001x^2 + 0.257x + 18.80$ | $R^2 = 0.992$ |
| 1.005 | $y = -0.002x^2 + 0.357x + 18.47$ | $R^2 = 0.995$ |
| 1.010 | $y = -0.002x^2 + 0.364x + 18.42$ | $R^2 = 0.994$ |
| 1.015 | $y = -0.002x^2 + 0.335x + 18.72$ | $R^2 = 0.987$ |
| 1.020 | $y = -0.001x^2 + 0.302x + 17.98$ | $R^2 = 0.994$ |

Fig. 3. The relationship between the days of rearing and the weight of mollies reared in water of different salinity

Table 3

Equations of regression and coefficients of determination for the relationship between the days of rearing and weight of mollies at different density levels

| Salinity SG | Equation | Value of $R^2$ |
|---|---|---|
| 1.000 | $y = 6\text{E-}06x^2 + 0.004x + 0.095$ | $R^2 = 0.988$ |
| 1.005 | $y = -2\text{E-}05x^2 + 0.008x + 0.078$ | $R^2 = 0.996$ |
| 1.010 | $y = -2\text{E-}05x^2 + 0.008x + 0.082$ | $R^2 = 0.995$ |
| 1.015 | $y = -7\text{E-}06x^2 + 0.008x + 0.081$ | $R^2 = 0.995$ |
| 1.020 | $y = 1\text{E-}05x^2 + 0.007x + 0.069$ | $R^2 = 0.997$ |

The results show that transferring the fish kept in fresh water to water with salinity exceeding 26.7‰ causes mass fish deaths (Figure 4, Table 4). Fish should be gradually acclimatised to higher salinity.
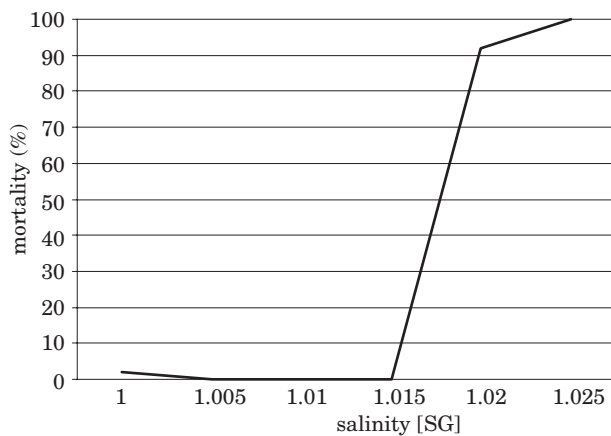
Fig. 4. Aggregated mortality of mollies in water at different salinity

Table 4

Mortality of mollies in water with different salinity

| Salinity SG | Mortality [%] | | | |
|---|---|---|---|---|
| | after 24 hours | after 48 hours | after 96 hours | total at the end of the rearing period |
| 1.000 | 0 | 1 | 1 | 2 |
| 1.005 | 0 | 0 | 0 | 0 |
| 1.010 | 0 | 0 | 0 | 0 |
| 1.015 | 0 | 0 | 0 | 0 |
| 1.020 | 82 | 6 | 4 | 96 |
| 1.025 | 100 | 0 | 0 | 100 |

The results show that the calculated partial growths (measured every 4 days) are uniform; however, they are 35 to 40% higher in salty water than in fresh water (Figure 5).
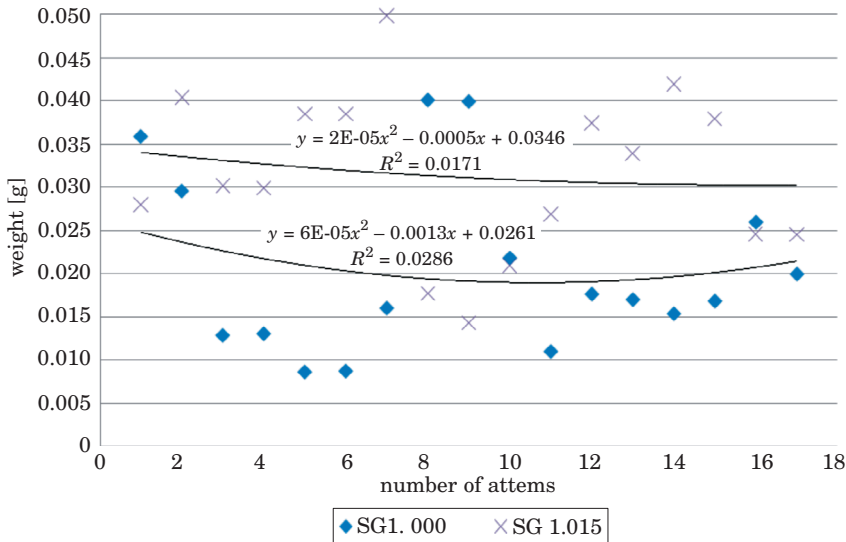
Fig. 5. Differences in increments of the fish weight in fresh water and in water of 20.0‰ (SG 1,015) salinity

## Discussion

There has been a rapid development of aquaculture in recent years, both in breeding of fish for consumption, such as the carp, but also among species which are less important, whose production is aimed at fry-stocking (KUJAWA et al. 2010). Tropical and ornamental species have also become increasingly popular (TLUSTY 2002). Moreover, recently there has been an increase in research into the conditions of rearing ornamental fish and the breeding of new species has been taken up. The most readily studied aspects include using different feeds, both live and artificial (LIM et al. 2003, SALES and JANSSENS 2003, TARGOŃSKA 2007). Ensuring proper environmental conditions for fish is one of the major problems in rearing fish larvae in controlled conditions (OPUSZYŃSKI 1983). Salinity in molly breeding is certainly one of these conditions.

Salinity affects many aspects associated both with reproduction and with rearing in many fish species. This is of particular importance in such fish as white sea bream (*Diplodus sargus* L.), gilthead sea bream *Sparus aurata* L. or flatfish, which live in salty or slightly salty waters (GAVLIK and SPECKED 2004, WANG et al. 2007, APPELBAUM and JESUAROCKIRAJ 2009, DIMITROGLOU et al. 2010). A properly selected level of salinity positively affects growth, but it also reduces disproportions among the reared fish, brings about synchronous

metamorphosis in Pleuronectidae and significantly affects survival rate (SMITH et al. 1999, GAVLIK and SPECKED 2004, WANG et al. 2007, KEARNEY et al. 2008, APPELBAUM and JESUAROCKIRAJ 2009, DIMITROGLOU et al. 2010, IMSLAND et al. 2010). Similar relationships have been observed in molly rearing in this study. The growth rate of mollies kept in fresh water was the lowest, but it was the highest when the fish were kept in water whose salinity was between 20 and 26.7‰. However, the aggregated mortality at the latter value exceeded 95%, whereas no deaths were recorded when the salinity level was 20‰. All the fish died at the beginning of the experiment in water with the highest salinity level tested in the experiment (33.3‰). The issue of water salinity is also used in breeding of other aquarium fish, such as the sea horse (MURUGAN et al. 2009). There may be several reasons for the positive – as seen from the aquarist;s perspective – effect of salt dissolved in water on the results of rearing fish, including the molly. One of these effects may be the intensive activity of gills and kidneys, as was observed by LIN et al. (2004) in the spotted green pufferfish *Tetraodon nigrovirdis*. When kept in water with the proper salinity level, fish used less energy for ion exchange and could grow faster. FURTHER-MORE and RILEY et al. (2002) reported that keeping Mozambique tilapia, *Oreochromis mossambicus*, in salt water significantly activates the growth hormone. Higher levels of secretion and higher activity of the growth hormone result in the higher growth rate of the fish.

The results of this experiment have indicated the need to rear mollies in water whose salinity is between 6.7 and 20.0‰. In these conditions, the highest growth rate is achieved with no deaths. Another possible solution to be applied in aquarium fish breeding, which requires salty water, is one proposed by APPELBAUM and JESUAROCKIRAJ (2009), in which salt was given to fish in their feed instead of in water. With properly selected doses of salt, an increase in growth and survival rate was achieved in juvenile stages of gilthead sea bream reared in slightly salty water.

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