UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

(4/2010) 25

PUBLISHER UWM
OLSZTYN 2010

The Polish Journal of Natural Sciences is indexed and abstracted
in Biological Abstracts and Biosis Previews

The Journal is also available (from volume 22) in electronic form. The online edition
is hosted by MetaPress (www.metapress.com) in partnership with Versita
(www.versita.com)

PL ISSN 1643-9953

PUBLISHER UWM OLSZTYN

Address
ul. Jana Heweliusza 14
10-718 Olsztyn-Kortowo, Poland
tel.: (48) (089) 523-36-61
fax: (48) (089) 523-34-38
e-mail: wydawca@uwm.edu.pl

Ark. wyd. 10,62, ark. druk. 8,75, nakład 140 egz.
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 632

TABLE OF CONTENTS

Agriculture

Animal Breeding and Husbandry

Environmental Protection

Fishery

Food and Nutrition Sciences

SPIS TREŚCI

Rolnictwo

Chów i Hodowla Zwierząt

Ochrona Środowiska

Rybactwo

Nauka o Żywności i Żywieniu

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 313–322, Y. 2010

DOI 10.2478/v10020-010-0028-4

A COMPARISON OF SOLUBLE SUGAR ACCUMULATION IN ZYGOTIC AND SOMATIC PEA EMBRYOS

***Kamilla Górska-Koplińska*[1], *Anna Źróbek-Sokolnik*[2], *Ryszard J. Górecki*[1], *Lesław Lahuta*[1]**

[1] Department of Plant Physiology and Biotechnology
[2] Department of Botany and Nature Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: desiccation, somatic embryogenesis, somatic embryos, soluble sugars.

Abbreviations: DAF – day after flowering; DW – dry weight; FW – fresh weight; RFOs – raffinose family oligosaccharides

A b s t r a c t

This study compares the soluble sugar content of zygotic and somatic pea embryos. It was noted that mature somatic embryos differed from zygotic embryos with respect to carbohydrate composition. Mature zygotic pea embryos contained glucose, *myo*-inositol, sucrose, maltose, galactinol, galactosyl-cyclitols, raffinose, stachyose and verbascose. The presence of maltose, galactosyl-cyclitols, stachyose and verbascose was not determined in somatic embryos, and their total soluble sugar content was below that of zygotic embryos. High sucrose levels in somatic embryos most probably resulted from the presence of sucrose in the growth medium. Monocotyledonous and irregular somatic embryos were characterized by a different sugar profile than regularly shaped somatic embryos and seeds.

PORÓWNANIE GROMADZENIA WĘGLOWODANÓW ROZPUSZCZALNYCH W ZYGOTYCZNYCH I SOMATYCZNYCH ZARODKACH GROCHU

***Kamilla Górska-Koplińska*[1], *Anna Źróbek-Sokolnik*[2], *Ryszard J. Górecki*[1], *Lesław Lahuta*[1]**

[1] Katedra Fizjologii i Biotechnologii Roślin
[2] Katedra Botaniki i Ochrony Przyrody
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: desykacja, somatyczna embriogeneza, somatyczne zarodki, węglowodany rozpuszczalne.

Skróty: DAF – dzień po kwitnieniu, DW – sucha masa, FW – świeża masa; RFO – cukry rodziny rafinozy.

Address: Anna Źróbek-Sokolnik, University of Warmia and Mazury, pl. Łódzki 1, 10-719 Olsztyn, Poland, phone: +48 (89) 523 49 27, e-mail: a.zrobeksokolnik@uwm.edu.pl

Abstrakt

W pracy porównano zawartość węglowodanów rozpuszczalnych w zygotycznych i somatycznych zarodkach grochu. Odnotowano, iż skład węglowodanowy dojrzałych zarodków somatycznych jest zmodyfikowany w porównaniu z zarodkami zygotycznymi. W dojrzałych zarodkach zygotycznych grochu występowały glukoza, *myo*-inozytol, sacharoza, maltoza, galaktinol, galaktozylo-cyklitole, rafinoza, stachioza i werbaskoza. W zarodkach somatycznych nie wykryto maltozy, galaktozylo--cyklitoli, stachiozy i werbaskozy, a całkowita zawartość cukrów rozpuszczalnych była niższa niż w zarodkach zygotycznych. Wysoka zawartość sacharozy w zarodkach somatycznych była prawdopodobnie skutkiem jej obecności w podłożu wzrostowym. Jednoliścieniowe oraz nieregularne zarodki somatyczne wykazywały odmienny profil cukrowy niż prawidłowe zarodki somatyczne i nasiona.

Introduction

Storage materials are accumulated at the final stages of embryo maturation. The seeds and embryos of legumes accumulate different types of soluble sugars (GÓRECKI et al. 2000). Maturing zygotic seeds store sucrose, raffinose family oligosaccharides (RFOs), cyclitols and galactosyl-cyclitols (GÓRECKI and OBENDORF 1997), and their starch content is subject to fluctuation (GÓRECKI et al. 2000).

Starch is one of the main reserve sugars in the pea. It is accumulated during the development of zygotic embryos, but this process is not always observed during somatic embryogenesis. Starch accumulation in somatic embryo cells was investigated by LOISEAU et al. (1998). There is a general scarcity of studies exploring the accumulation of soluble sugars in somatic embryos (e.g. GÓRECKI et al. 2000), therefore, the objective of this experiment was to compare the accumulation of soluble sugars during zygotic and somatic embryogenesis of the pea, which significantly affects the process of embryo desiccation.

Materials and Methods

Plant material

The experimental material comprised the seeds of pea var. Oskar and HM-6 supplied by AGRITECH Ltd. of the Czech Republic. To obtain zygotic embryos, seeds were placed in pots filled with compost soil and sand (4:1, v/v). The seeds were regularly watered with tap water. The moisture content of the substrate was maintained at 60–70%. The Florovit fertilizer was applied three times: at the stage of five leaves, at the beginning of flowering and at fruiting. The first maturing pods were harvested 10 days after flowering (DAF), and then every

4 days until full maturity. The collected seeds' fresh weight, dry weight, vigor and viability were determined.

The material for culturing somatic embryos was excised from four-day-old, etiolated, axenically raised seedlings. To obtain axenically grown seedlings, seeds were surface sterilized in a 5% aqueous solution of Chloramine B for 15 minutes, followed by three washes with sterile distilled water. Disinfected seeds were placed in sterile tubes (25 ml capacity) containing moist cotton wool. After germination (in darkness at 25–26°C for four days), shoot apices were excised from seedlings using a dissecting microscope, and they were placed on the induction medium.

Explants were subjected to 14-day induction on the basal medium described by Griga (1998) that contained MS salts (MURASHIGE and SKOOG 1962), Gamborg B5 vitamins (GAMBORG et al. 1968), 3% sucrose and 2.5 μM picloram. After induction, all cultures were transferred to the differentiation medium – a basal medium without phytohormones. The cultures were kept in a growth chamber under the 16:8 photoperiod (light : darkness) and at temperatures of 23–24°C during the day and 19–20°C at night.

Determination of the soluble sugar content of zygotic and somatic embryos

Soluble sugars were extracted by the modified method proposed by GÓRECKI et al. (1997). The pea flour obtained from ground pea seeds or parts thereof (around 50 mg) was combined with 100 μg of internal standard (xylitol) and 800 μl of 70% ethanol solution, and it was placed in a water bath with a temperature of 60–65°C for 35 minutes. The samples were cooled to room temperature and centrifuged at 22 000 *g* for 30 minutes. The supernatant was passed through Dovex 50Wx8-100 and Dover 2x8 ion exchange columns. After centrifuging (22 000 *g*; 10 min; 20°C), 200 μl of the supernatant was transferred to chromatographic vials and dried in a vacuum centrifuge. Dried samples were stored in a desiccator over silica gel (Silica Gel Blue, Fluka). Soluble sugars were extracted from live somatic embryos using the same procedure.

Prior to chromatographic separation, the sugars were dissolved in a mixture of TMSi (N-trimethyl-silylimidazole) and pyridine at a temperature of 90°C for 60 minutes. TMS-derivatives were separated using a capillary column in the GC-2010 gas chromatograph (SHIMADZU). The sugars were separated in a temperature gradient of 150 to 325°C. The carrier gas was helium, applied at a flow rate of 1.25 cm^2/min. The sugars were identified by comparing their retention times (total and relative) against commercially available standards.

The number of sugars was determined based on simple regressions calculated for changes in the ratio of the sugar surface area to the surface area of the internal standard.

Results and Discussion

In legumes, the accumulation of soluble sugars during seed maturation is related to the development of desiccation resistance. This effect is attributed mainly to the accumulation of sucrose, raffinose, stachyose and verbascose. The stachyose+raffinose:sucrose ratio approximates 1 when seeds become resistant to dessication and reach full maturity (BAILLY et al. 2001). The presence of fructose, glucose, *myo*-inositol, sucrose, maltose, galactinol, galactosyl-cyclitol, raffinose, stachyose and verbascose was determined in maturing zygotic embryos. Changes in the carbohydrate composition of zygotic embryos were observed during maturation (Figure 1). The embryos of mature pea seeds contained mostly sucrose, galactinol, raffinose, verbascose and stachyose as well as trace amounts of fructose, glucose, maltose and *myo*-inositol (Table 1). Somatic pea embryos contained fructose, glucose, *myo*-inositol, sucrose, raffinose and galactinol. The presence of maltose, galactosyl-cyclitol, stachyose and verbascose was not found, and the total soluble sugar content of somatic embryos was several times lower in comparison with zygotic embryos (Table 2). The sugar profile of normal (dicotyledonous) somatic embryos was most similar to that of several-days-old zygotic embryos. Sucrose was the main soluble sugar accumulated by somatic embryos, and it had more than a 75% share of all soluble sugars in the somatic embryos of var. HM-6. In var. Oskar, sucrose levels reached 65.2% in dicotyledonous embryos and 52.4% in monocotyledonous embryos. Similar quantities of sucrose were observed during seed maturation, ranging from 27.1% to 89.7% in var. HM-6, and from 17.6% to 90.5% in var. Oskar (Table 1). The noted results are consistent with the findings of Blöchl et al. (2005) who studied the somatic embryos of alfalfa. In zygotic embryos, an increase in sucrose levels is accompanied by the onset of maturation which is characterized by the rapid growth of fresh and dry weight, a drop in water content, the emergence of starch granules and albuminous substances (e.g. SANCHEZ-ROMERO et al. 2002). The rise in sucrose levels in mature somatic embryos is generally attributed to intensive embryo metabolism (IRAQUI et al. 2005), sucrose conversion to oligosaccharides (LIN et al. 1998) and direct sucrose uptake from the culture medium by explant and embryo cell enzymes without prior hydrolysis (ŻUR et al. 2002, IRAQUI et al. 2005).

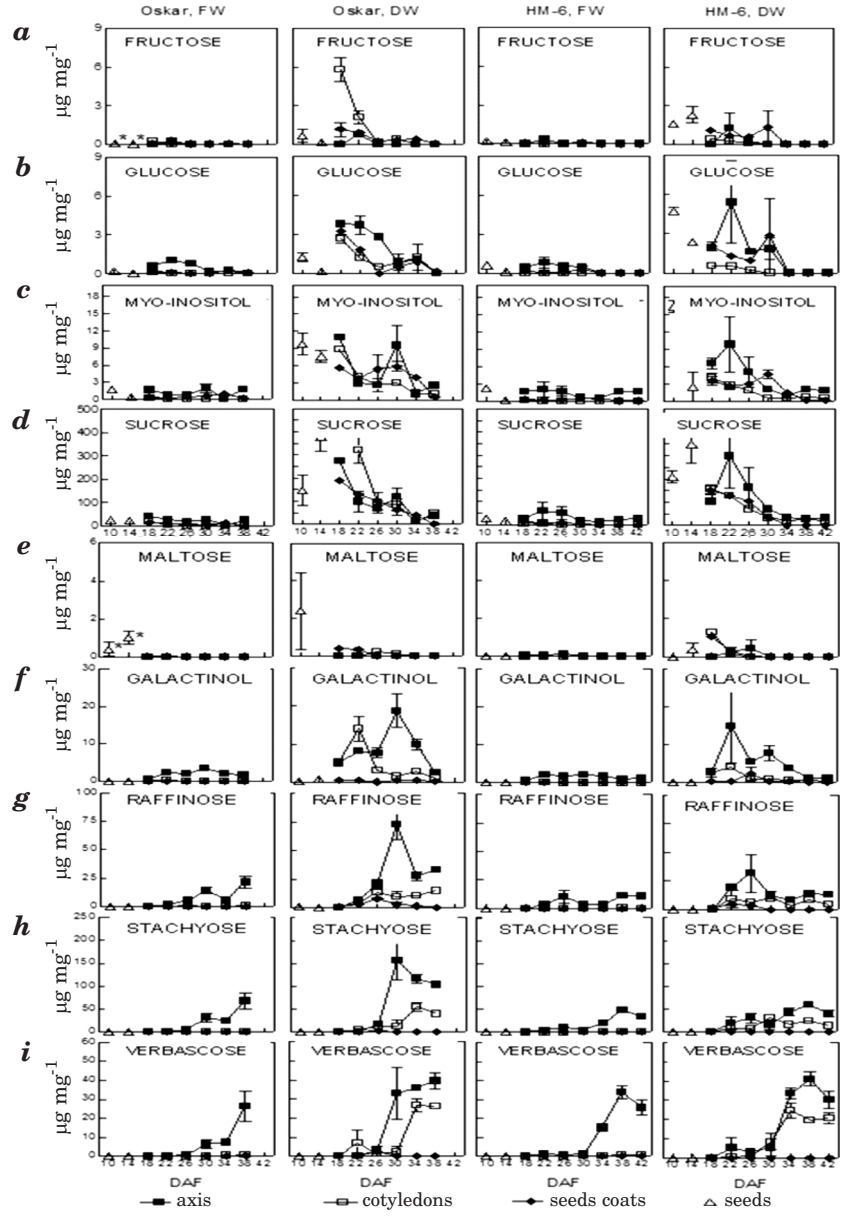

Fig. 1. The soluble sugars concentration [µg mg^{-1}] in pea seeds, embryo axes, cotyledons and seed coats (measured to one milligram of fresh and dry weight). DW – dry weight, FW – fresh weight. *a* – fructose, *b* – glucose, *c* – myo-inositol, *d* – sucrose, *e* – maltose, *f* – galactinol, *g* – raffinose, *h* – stachyose, *i* – verbascose.
* the seeds from 10 and 14 DAF were not part to embryo axes, cotyledons and seed coats.

Table 1

Soluble sugar accumulation in pea seeds

Soluble carbohydrate accumulation in seeds [μg/mg FW] (sugars contents converted to dry weight [μg/mg DW])				
Carbohydrate	Oskar		HM-6	
	10 DAF	mature seeds (38 DAF)	10 DAF	mature seeds (38 DAF)
Fructose	0.108 (0.632)	0.020 (0.053)	0.212 (1.587)	0
Glucose	0.205 (1.232)	0.084 (0.197)	0.631 (4.728)	0
Myo-inositol	1.657 (9.847)	2.001 (4.470)	2.201 (16.504)	1.748 (2.723)
Sucrose	25.253 (147.900)	26.949 (87.494)	27.751 (208.643)	28.771 (52.445)
Maltose	0.411 (2.403)	0	0	0
Galactionol	0.033 (0.191)	1.834 (3.780)	0.026 (0.201)	1.150 (1.812)
Gal-cyclitols	0.100 (0.587)	2.760 (6.156)	0.049 (0.375)	1.766 (3.018)
Raffinose	0.119 (0.697)	22.270 (48.502)	0.041 (0.320)	11.081 (17.532)
Stachyose	0	69.557 (143.688)	0	35.195 (54.195)
Verbasose	0	27.341 (65.790)	0	26.429 (50.749)
Raffinose/sucrose	0.005 (0.005)	0.083 (0.55)	0.001 (0.001)	0.38 (0.33)
Total soluble sugar acumulation	27.886 (163.488)	152.982 (360.130)	30.911 (232.358)	106.140 (182.474)

In the seeds of many plant species, the highest monosaccharide concentrations are observed at the early stages of embryo development, while trace amounts of monosaccharides are found in mature seeds (SANCHEZ-ROMERO et al. 2002). The results of the existing research suggest that mature pea seeds contain no reducing sugars (GÓRECKI and OBENDORF 1997), yet certain varieties demonstrate small quantities of fructose and glucose (GÓRECKI et al. 2000). The results of this study confirm the above observations. Monosaccharides were not found in the mature seeds of var. HM-6, while trace amounts of reducing sugars were determined in Oskar seeds (around 0.07%) – Table 1. Fluctuations in total glucose and fructose concentrations were also noted during the maturation of zygotic embryos (Figure 1). The seeds of other legume species, such as lupine, are marked by low glucose levels (GÓRECKI et al. 1997). In normal, dicotyledonous embryos of var. Oskar, reducing sugars had an estimated 8.70% share, whereas in var. HM-6, the total concentrations of fructose and glucose accounted for 9.46% of all determined sugars (Table 2). In irregular, monocotyledonous embryos of var. Oskar, monosaccharide concentrations were very high at 43% and 100% of total soluble sugars. The above can probably be attributed to the physiological immaturity of embryos.

Table 2

Soluble carbohydrate accumulation in somatic embryos (mean ± S.E)

Soluble carbohydrate accumulation in somatic embryos [µg/mg FW]						
Carbohydrates	Oskar			HM-6		
	2-cotyledonous	1-cotyledonous	irregular	2-cotyledonous	1-cotyledonous	irregular
Fructose	0.246 ± 0.050	0.562 ± 0.0	0.0	0.3 ± 0.015	0.247 ± 0.002	0.361 ± 0.009
Glucose	0.718 ± 0.050	0.279 ± 0.001	0.596 ± 0.0	0.412 ± 0.011	0.456 ± 0.009	0.545 ± 0.017
Myo-inositol	1.589 ± 0.030	0.0	0.0	0.950 ± 0.011	0.759 ± 0.005	1.030 ± 0.007
Sucrose	7.221 ± 0.017	1.018 ± 0.0	0.0	5.644 ± 0.062	5.039 ± 0.133	7.286 ± 0.031
Galactinol	0.230 ± 0.017	0.0	0.0	0.0	0.0	0.0
Raffinose	1.071 ± 0.017	0.0	0.0	0.219 ± 0.011	0.0	0.0
Raffinose/Sucrose	0.15	–	–	0.04	–	–
Total soluble sugar acumulation	11.075 ± 0.008	1.943 ± 0.001	0.596 ± 0.0	7.525 ± 0.059	6.501 ± 0.147	9.222 ± 0.048

Myo-inositol was found in both the normal and abnormal somatic embryos of var. HM-6, whereas in var. Oskar it was determined solely in dicotyledonous embryos (Table 2). In comparison with zygotic embryos (1.64% to 7.12% soluble sugars in var. HM-6, and 1.31% to 5.94% in var. Oskar), regularly shaped somatic embryos were characterized by elevated levels of *myo*-inositol (12.62% and 14.35% soluble sugars, respectively). Despite relatively high *myo*-inositol concentrations in somatic embryos, galactosyl derivatives of this compound were not observed in noticeable amounts (galactinol was found solely in the dicotyledonous embryos of var. Oskar). According to GÓRECKI et al. (2000), the zygotic embryos of pea seeds contain 1.65% to 3.08% inositol and other cyclitols. In a study of maturing soybean seeds, Obendorf et al. (1998) observed that *myo*-inositol concentrations decrease with an increase in galactinol levels. Higher *myo*-inositol concentrations were observed in *in vitro*-matured zygotic embryos. This could suggest that elevated *myo*-inositol levels in somatic embryos are a characteristic feature of *in vitro* cultures. ŻUR et al. (2002) reported that the active uptake of *myo*-inositol from the medium by explant cells increased during the initiation of organogenesis in rapeseed cultures. An increased uptake of *myo*-inositol could testify to its key role in plant metabolism, such as signal transduction and resistance to stress (LOEWUS and MURTHY 2000).

Raffinose was the only raffinose family oligosaccharide detected in the dicotyledonous somatic embryos of both studied varieties. Raffinose content in var. Oskar and HM-6 was 9.67% and 2.91%, respectively. Raffinose levels in seeds also varied between the varieties (Table 1). In mature zygotic embryos, the raffinose to sucrose ratio was determined at 0.38 and 0.83 in var. HM-6 and Oskar, respectively. The values of the above ratio were very low in somatic embryos, reaching around 0.15 in var. Oskar and 0.04 in var. HM-6. Sucrose and raffinose also had a varied share of the total soluble sugar content. In mature zygotic embryos, sucrose proportions were determined at 0.28 in var. HM-6 and 0.24 in var. Oskar. In HM-6 somatic embryos, the sucrose to soluble sugars ratio reached 0.75, 0.77 and 0.79 for dicotyledonous, monocotyledonous and irregular embryos, respectively. In Oskar somatic embryos, the above ratio was determined at 0.65, 0.52 and 0 for dicotyledonous, monocotyledonous and irregular embryos, respectively. The raffinose to soluble sugars ratio reached 0.09 and 0.13 in mature zygotic embryos, and 0.03 and 0.09 in dicotyledonous embryos of var. HM-6 and Oskar, respectively. The absence of RFOs in monocotyledonous and irregular embryos could result from low *myo*-inositol levels (LOEWUS and MURTHY 2000). The raffinose to sucrose ratio in the above embryos was also low, suggesting that the produced somatic embryos did not reach physiological maturity.

Conclusions

The results of this study indicate that somatic embryos contain high levels of soluble sugars, such as sucrose, *myo*-inositol, raffinose and monosaccharides. Contrary to zygotic embryos, the presence of stachyose and verbascose was not noted in somatic embryos.

High sucrose levels in somatic embryos probably resulted from the presence of sucrose in the culture medium.

Monocotyledonous somatic embryos and irregular embryos were characterized by a different sugar profile than normal somatic embryos and seeds.

It can be concluded that selected developmental defects observed in somatic embryos (number of cotyledons) could be attributed to growing conditions and the accumulation of monosaccharides and raffinose family oligosaccharides.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 5.10.2010

References

BAILLY C., AUDIGIER C., LADONNE F. WAGNER M.H., COSTE F., CORBINEAU F., COME D. 2001. *Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality.* J. Exp. Bot., 52: 701–708.

BLÖCHL G., MARCH G.G. DE, SOURDIOUX M., PETERBAUER T., RICHTER A. 2005. *Induction of raffinose oligosaccharide biosynthesis by abscisic acid in somatic embryos of alfalfa (Medicago sativa L.).* Plant Science, 168: 1075–1082.

GAMBORG O.L., MILLER R.A., OJIMA K. 1968. *Nutrient requirements of suspension cultures of soybean root cells.* Exp. Cell Res., 50: 11–15.

GÓRECKI R.J., LAHUTA L.B., JONES A.D., HEDLEY C.L. 2000. *Soluble sugars in maturing pea seeds of different lines in relation to desiccation tolerance.* [In:] *Seed Biology. Advences and applications,* Eds. M. Black, K.J. Bradford, J. Vasquez-Ramos, 67–74.

GÓRECKI R.J., OBENDORF R.L. 1997. *Galactosyl cyclitols and raffinose family oligosaccharides in relation to desiccation tolerance of pea and soybean seedlings.* [In:] *Basic and applied aspects of seed biology*. Eds. R.H. ELLIS, M. BLACK, A.J. MURDOCH, T.D. HONG. Kluwer Academic Publishers, 119–128.

GÓRECKI R.J., PIOTROWICZ-CIEŚLAK A., OBENDORF R.L. 1997. *Soluble sugars and flatulence-producing oligosaccharides in maturing yellow lupine (Lupinus luteus L.) seeds.* Seed Sci. Res., 7: 185–193.

GRIGA M. 1998. *Direct somatic embryogenesis from shoot apical meristems of pea, and thidiazuron-induced high conversion rate of somatic embryos.* Biol. Plant., 41: 481–495.

IRAQUI D., LE V.Q., LAMHAMEDI M.S., TREMBLAY F.M. 2005. *Sucrose utilization during somatic embryo development in black spruce: involvement of apoplastic invertase in the tissue and of extracellular invertase in the medium.* J. Plant Physiol., 162: 115–124.

LIN T.P., YEn W.L., CHIEN C.T. 1998. *Disappearance of desiccation tolerance of imbibed crop seeds is not associated with the decline of oligosaccharides.* J. Exp. Bot., 49: 1203–1212.

LOEWUS F.A., MURTHY P.P.N. 2000. *Myo-inositol metabolism in plants.* Plant Science, 150: 1–19.

LOISEAU J., MICHAUX-FERRIERE N., DENUFFY Y. LE. 1998. *Histology of somatic embryogenesis in pea.* Plant Physiol. Biochem., 36: 683–687.

MURASHIGE T., SKOOG F. 1962. *A revised medium for rapid growth and bioassays with tobacco tissue culture.* Physiol. Plant., 15: 437–497.

OBENDORF R.L., DICKERMAN A.M., PFLUM T.M., KACALANOS M.A., SMITH M.E. 1998. *Drying rate alters soluble carbohydrates, desiccation tolerance, and subsequent seedling growth of soybean (Glycine max L. Merrill) zygotic embryos during in vitro maturation.* Plant Science, 132: 1–12.

SANCHEZ-ROMERO C., PERAN-QUEADA R., BARCELO-MUNOZ A., PLIEGO-ALFRO F. 2002. *Variations in storage protein and carbohydrate levels during development of avocado zygotic embryos*. Plant Physiol. Biochem., 40: 1043–1049.

ŻUR I., SKOCZOWSKI A., PIEŃKOWSKI S., DUBERT F. 2002. *Kinetics of 14*C-labelled sucrose, myo-inositol and phosphatidylocholine uptake during induction and differentiation in Brassica napus callus culture. Acta Physiol. Plant., 24: 11–17.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 323–331, Y. 2010

DOI 10.2478/v10020-010-0029-3

THE EFFECT OF MULTI-COMPONENT FERTILIZERS ON SPRING WHEAT YIELD, THE CONTENT AND UPTAKE OF MACRONUTRIENTS*

Anna Nogalska, Jerzy Czapla, Małgorzata Skwierawska

Department of Agricultural Chemistry and Environmental Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: spring wheat, yield, macronutrients, multi-component fertilizers.

A b s t r a c t

The aim of this study was to determine the effect of mixed multi-component fertilizers on spring wheat yield, the content and uptake of macronutrients. A three-year field experiment (2005–2007) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 3 and Amofosmag 4. The tested crop was spring wheat cv. Eta.

The application of Amofosmag 4 significantly increased the yield of spring wheat grain and straw. The concentrations of the analyzed macronutrients in wheat were similar in all fertilization treatments, thus pointing to a comparable effect of the applied fertilizers. More pronounced differences in the chemical composition of wheat plants were observed between successive years of the study. The highest total uptake of nitrogen, phosphorus, calcium and magnesium by spring wheat was noted in plots fertilized with Amofosmag 4, which indicates that the nutrients contained in this product were more readily available to plants.

WPYW NAWOZÓW WIELOSKŁADNIKOWYCH NA PLON, ZAWARTOŚĆ I POBRANIE MAKROSKŁADNIKÓW PRZEZ PSZENICĘ JARĄ

Anna Nogalska, Jerzy Czapla, Małgorzata Skwierawska

Katedra Chemii Rolnej i Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: pszenica jara, plon, makroskładniki, nawozy wieloskładnikowe.

Address: Anna Nogalska, University of Warmia and Mazury, ul. Michała Oczapowskiego 8, 10-719 Olsztyn, Poland, phone: +48 (89) 523 32 50, e-mail: anna.nogalska@uwm.edu.pl
* This study was financed by Agrochem Ltd., Dobre Miasto.

Abstrakt

Celem pracy była ocena wpływu zastosowanych nawozów wieloskładnikowych mieszanych na plon, zawartość i pobranie makroskładników przez pszenicę jarą. Trzyletnie doświadczenie polowe (2005–2007) przeprowadzono w Zakładzie Dydaktyczno-Doświadczalnym w Tomaszowie należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Doświadczenie, założone metodą losowanych bloków, obejmowało trzy obiekty nawozowe w czterech powtórzeniach: obiekt kontrolny (nawozy jednoskładnikowe), Amofosmag 3 i Amofosmag 4. Rośliną testowaną była pszenica jara odmiany Eta.

Z przeprowadzonych badań wynika, że nawożenie Amofosmagiem 4 miało istotny wpływ na zwiększenie plonu ziarna i słomy pszenicy jarej. Zawartość badanych makroskładników w pszenicy w poszczególnych obiektach nawozowych była zbliżona, a zastosowane nawozy wykazywały działanie równorzędne. Większe zróżnicowanie w składzie chemicznym badanej rośliny wystąpiło między poszczególnymi latami badań. Największe łączne pobranie azotu, fosforu, wapnia i magnezu przez pszenicę jarą stwierdzono w obiektach z Amofosmagiem 4. Świadczy to o lepszej przyswajalności składników pokarmowych z tego nawozu.

Introduction

Due to their balanced and complete composition, mixed fertilizers can meet the specific nutrient requirements of each plant species and can be adjusted properly to soil fertility, which is an important consideration in developing fertilization programs. Multi-component fertilizers, which provide three primary macronutrients, N, P and K, and secondary nutrients, Mg, Ca, S and Na, in varying proportions, are applied to correct magnesium deficiency in the soil and to reduce sulfur emissions, thus preventing nutrient deficiency in plants (FILIPEK 2001, FILIPEK-MAZUR, GONDEK 2005). A clear advantage of multi-component fertilizers over simple fertilizers is that the former supply a combination of nutrients at a time (GLABISZ et al. 1992). Compound fertilizers provide crops with essential nutrients in adequate amounts and proportions, and they help prevent or reduce nutrient leaching (CZUBA 1998, ZAWARTKA, SWIERAWSKA 2004a). The aim of this study was to determine the effect of mixed multi-component fertilizers, Amofosmag 3 and Amofosmag 4, on spring wheat yield, the content and uptake of macronutrients.

Materials and Methods

A three-year field experiment (2005–2007) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment, which comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 3 and Amofosmag 4, was established on proper brown soil developed from sandy loam, of quality class III b and very good rye

complex. The physicochemical properties of soil in each year of the study are presented in Table 1. The tested crop was spring wheat (*Triticum aestivum* L. *mend)* cv. Eta. The preceding plants were winter triticale in the first year, and winter rapeseed in the following two years. Plot surface area was 10 m.

Table 1

Selected physicochemical properties of soil used in the experiment (mg kg^{-1})

Year	pH w 1 M KCl	Available forms		
		P	K	Mg
2005	6.15	56.7	112.0	31.0
2006	7.20	112.9	145.3	25.0
2007	5.60	116.0	224.0	87.0

Based on the average levels of available phosphorus in the soil, 300 kg ha^{-1} Amofosmag 3 (NPKMg 3:14:20:2 + 22% CaO + 9% SO_3; 9 kg N, 18 kg P, 50 kg K on pure ingredient basis) and Amofosmag 4 (NPKMg 4:15:15:2 + 24% CaO + 9% SO_3; 12 kg N, 20 P, 37 kg K on pure ingredient basis) were applied pre-sowing. The nitrogen rate of 100 kg per ha was supplemented with two doses of ammonium nitrate applied by top-dressing in all treatments, including control. In the control treatment, the following fertilizers were applied pre-sowing: 12 kg N in the form of urea, 45 kg P_2O_5 (20 kg P) in the form of triple superphosphate and 45 kg K_2O (37 kg K) : ha^{-1} in the form of potash salt.

Samples of spring wheat were collected at the stage of full maturity. The grain and straw harvested in each plot was dried and weighed individually. Wet mineralized samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium and potassium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). The results of chemical analyses were verified statistically by a two-factorial analysis of variance for a randomized block design. The experimental factors were as follows: a – fertilization, b – duration of the experiment. The least significant difference was assumed at $p = 0.05$.

Results and Discussion

The distribution of air temperatures in 2005 differed insignificantly from the long-term average (Table 2). Precipitation total in April was substantially lower than the long-term average, which could have contributed to uneven

emergence, whereas July was too wet. In 2006, mean monthly temperatures were similar to the long-term average. The highest temperature was noted in July. Precipitation levels differed considerably from the average values in July and August. Precipitation total in July in August was over 2.5-fold lower and nearly 2.5-fold higher, respectively, than the long period average, which made harvest difficult. In 2007, air temperatures during the growing season were slightly above the long-term average. July was wet, with a difference of 99.9 mm between mean monthly rainfall and the long period average. Weather conditions could have affected the yield of spring wheat.

Table 2
Weather conditions in 2005–2007 – data provided by the Meteorological Station in Tomaszkowo

Month	Mean daily temperature (°C)				Precipitation total (mm)			
	2005	2006	2007	1970–2000	2005	2006	2007	1970–2000
April	8.2	7.3	7.5	6.9	22.0	25.6	24.7	36.1
May	11.6	12.5	13.8	12.7	68.2	89.2	93.5	51.9
June	14.2	16.0	17.7	15.9	35.4	79.2	88.1	79.3
July	19.7	20.9	17.7	17.7	83.9	29.3	173.7	73.8
August	16.9	17.2	18.3	17.2	39.6	165.0	68.0	67.1
September	18.1	14.8	12.6	12.5	17.9	51.0	57.9	59.0

In the first year of the experiment (2005), the yield of spring wheat grain ranged from 4.38 to 4.86 t ha^{-1} , depending on the applied fertilizer (Table 3). The highest average yield was attained in the treatment fertilized with Amofosmag 4 – it was by 7.8% and 11% higher than in the control treatment (simple fertilizers) and in the Amofosmag 3 treatment, respectively. In an experiment with winter wheat conducted by SZTUDER (2007), multi-component fertilizers had a more desirable yield-forming effect than simple fertilizers. Different results were reported by STĘPIEŃ and MERCIK (2001). In the present study, wheat straw yield corresponded to grain yield. In 2006, the yield of spring wheat grain varied between 6.14 and 6.50 t ha^{-1}, and it was considerably higher (by 37% on average) than the value noted in 2005. This could have resulted from favorable temperatures. Amofosmag 4 contributed to an increase in the yield of wheat grain and straw, in comparison with the remaining treatments. The lowest wheat grain yield was attained in 2007 – it was lower by 13.3% and 37% than in 2005 and 2006, respectively. The above could be due to less favorable weather conditions. Also in 2007 Amofosmag 4 had the most beneficial influence on wheat grain yield, which was found to increase signifi-

cantly (by around 15%), compared with the control treatment and the treatment fertilized with Amofosmag 3. Wheat straw yield was affected by the applied fertilizers to a lower degree.

Table 3

Spring wheat yield after the application of Amofosmag 4 and Amofosmag 3 (t ha^{-1})

Treatment	Grain				Straw			
	2005	2006	2007	mean for *a*	2005	2006	2007	mean for *a*
NPK	4.51	6.22	3.80	4.85	5.33	8.55	5.90	6.59
Amofosmag 4	4.86	6.50	4.34	5.23	5.77	9.07	6.35	7.06
Amofosmag 3	4.38	6.14	3.76	4.76	5.44	8.48	5.85	6.59
Mean for *b*	4.58	6.29	3.97	–	5.51	8.70	6.03	–
$LSD_{p=0.05}$ for *a*	0.39				n.s.			
b	0.42				0.71			
a · *b*	n.s.				n.s.			

Legend: *a* – fertilization, *b* – duration of the experiment

Amofosmag 4 increased the yield of spring wheat grain and straw by approximately 8% and 10%, respectively, in comparison with the control treatment. An increase in the yield of spring barley grain following the application of mixed fertilizers was also reported by ZAWARTKA and SKWIERAWSKA (2004b), and by MAZUR et al. (2001).

The results of chemical analyses of spring wheat grain and straw, presented in Table 4, show that the concentrations of the analyzed macronutrients varied insignificantly between fertilization treatments, and in most cases they remained within normal limits (CZUBA 1996). Significant differences were observed in this respect between successive years of the study. In the first year, the grain of spring wheat contained significantly less nitrogen and significantly more potassium and magnesium, compared with the values noted in the two consecutive years. In the second year of the experiment, wheat grain contained larger amounts of phosphorus and calcium than in the first and third year, and larger quantities of nitrogen than in the first year. The above differences were statistically significant. The highest nitrogen content (26.7 g kg^{-1} d.m. on average) of wheat grain was observed in 2007, and it was significantly higher than in 2005 and 2006 (by 76% and 24%, respectively). Nevertheless, the findings of numerous authors (KRZYWY et al. 2000, FILIPEK 2001, KRZYWY et al. 2001, MAZUR et al. 2001) suggest that multi-component fertilizers have an insignificant effect on the macronutrient content of the tested plants.

Macronutrient uptake (kg per ha) was estimated based on the yield and macronutrient content of spring wheat grain and straw. The highest nitrogen ptake by wheat plants (177.19 kg N ha^{-1}) was noted in the second year of the

Table 4
Macronutrient content of spring wheat after the application of Amofosmag 4 and Amofosmag 3 (g kg^{-1} d.m.)

Macronutrient	Treatment	Grain				Straw			
		2005	2006	2007	mean for *a*	2005	2006	2007	mean for *a*
Nitrogen	NPK	15.8	21.6	25.6	21.0	6.2	4.0	4.6	4.9
	Amofosmag 4	15.4	21.4	26.8	21.2	6.8	4.2	4.5	5.2
	Amofosmag 3	14.1	21.7	27.6	21.1	6.5	4.2	4.9	5.2
Mean for *b*		15,1	21.6	26.7		6.5	4.1	4.7	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		1.07				0.79			
a · *b*		n.s.				n.s.			
Phosphorus	NPK	4.2	5.7	2.6	4.2	0.5	1.1	0.9	0.8
	Amofosmag 4	4.1	5.7	2.5	4.1	0.5	1.1	0.8	0.8
	Amofosmag 3	4.2	5.9	2.6	4.2	0.6	1.1	0.8	0.8
Mean for *b*		4.2	5.8	2.6		0.5	1.1	0.8	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.21				0.20			
a · *b*		n.s.				n.s.			
Potassium	NPK	5.3	3.3	4.0	4.2	13.1	6.8	10.5	10.1
	Amofosmag 4	5.5	3.1	3.7	4.1	12.4	5.9	11.1	9.8
	Amofosmag 3	5.4	3.3	3.8	4.2	13.0	6.3	10.8	10.0
Mean for *b*		5.4	3.2	3.8		12.8	6.3	10.8	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.24				1.07			
a · *b*		n.s.				n.s.			
Calcium	NPK	0.39	1.00	0.43	0.61	2.8	2.5	1.6	2.3
	Amofosmag 4	0.45	1.01	0.45	0.64	2.8	2.3	1.5	2.2
	Amofosmag 3	0.40	1.02	0.44	0.62	2.5	2.2	1.6	2.1
Mean for *b*		0.41	1.01	0.44		2.7	2.3	1.6	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.05				0.42			
a · *b*		n.s.				n.s.			
Magnesium	NPK	1.6	1.0	1.0	1.2	0.43	0.58	0.48	0.50
	Amofosmag 4	1.6	1.0	1.0	1.2	0.41	0.55	0.48	0.48
	Amofosmag 3	1.6	1.1	1.0	1.2	0.40	0.56	0.46	0.47
Mean dla *b*		1.6	1.0	1.0		0.41	0.56	0.47	
$LSD_{p=0.05}$ for *a*		n.s.				n.s.			
b		0.05				0.09			
a · *b*		n.s.				n.s.			

Explanations as in Table 3

experiment, following the application of Amofosmag 4. Nitrogen uptake was correlated with wheat yield (Table 5). Phosphorus uptake levels were comparable in all treatments, and they were found to increase in plots fertilized with Amofosmag 4. Phosphorus uptake varied between years, reaching the highest level in 2006 (44.85 to 47.02 kg P ha^{-1}) when the highest yield of wheat grain

Table 5

Nutrient uptake by spring wheat grain and straw (kg ha^{-1})

Treatment	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
2005					
NPK	104.6	21.4	94.4	16.8	9.4
Amofosmag 4	114.4	22.7	98.3	18.3	10.1
Amofosmag 3	96.9	21.5	94.4	15.3	9.2
2006					
NPK	168.5	44.8	78.7	27.6	11.2
Amofosmag 4	177.2	47.0	73.7	27.4	11.5
Amofosmag 3	174.8	45.5	73.7	24.8	11.5
2007					
NPK	124.4	16.0	87.0	11.1	6.6
Amofosmag 4	130.4	15.9	86.5	11.5	7.4
Amofosmag 3	132.4	14.4	77.5	11.0	6.4

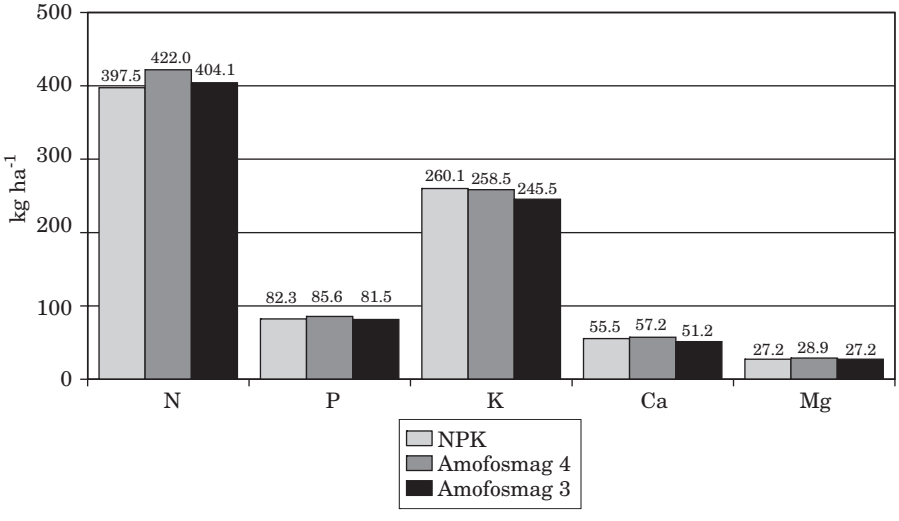

Fig. 1. Total macronutrient uptake by spring wheat over a three-year experimental period

and straw was obtained, and the lowest (around three-fold lower than in the preceding year) in 2007. A similar, albeit less pronounced, relation was observed with regard to the uptake of calcium and magnesium, which was highest after the application of Amofosmag 4. Potassium uptake by wheat plants was in the range of 73.66–98.27 kg K ha^{-1}. The highest potassium uptake was reported in the first year of the experiment in the Amofosmag 4 treatment, and it was related to the high concentrations of this element in wheat grain and straw. In the long term, simple fertilizers improved potassium

uptake by spring wheat (Figure 1). The highest total uptake of nitrogen, phosphorus, calcium and magnesium by spring wheat (mean values of three years) was noted in plots fertilized with Amofosmag 4, which indicates that the nutrients contained in this product were more readily available to plants. STĘPIEŃ and MERCIK (2001), ŚWIERCZEWSKA and SZTUDER (2006), and SZTUDER (2007) demonstrated that multi-component fertilizers, compared with simple fertilizers, contributed to higher nutrient uptake by various plants.

Conclusions

1. Amofosmag 4 had the most beneficial influence on spring wheat yield – the application of this fertilizer enabled to increase grain yield by 9% on average, in comparison with the remaining treatments.

2. The concentrations of the analyzed macronutrients in spring wheat grain and straw varied insignificantly between fertilization treatments. Simple and mixed multi-component fertilizers exerted a comparable effect on the mineral composition of the tested crop. Significant differences were observed in this respect between successive years of the study.

3. The highest total uptake of nitrogen, phosphorus, calcium and magnesium by spring wheat was noted in plots fertilized with Amofosmag 4, which indicates that the nutrients contained in this product were more readily available to plants.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 9.09.2010

References

CZUBA R. 1998. *Optymalny asortyment podstawowych nawozów wieloskładnikowych warunkowany krajową strukturą zasiewów i zasobnością gleb*. Folia Univ. Agric. Stetin., Agricultura, 190(72): 43-48.

CZUBA R. 1996. *Nawożenie mineralne roślin*. Zakłady Chemiczne „POLICE" SA.

GŁABISZ U., KIC B., GRZMIL B. 1992. *Manufacture of low chloride multicomponent fertilizers based on conversion in aqueous solution*. J. Agric. Food Chem., 40: 1393–1397.

KRZYWY E., JAKUBOWSKI W., KRZYWY J. 2001. *Wpływ nawozów wieloskładnikowych i jednoskładnikowych na wysokość i niektóre cechy jakościowe plonów roślin*. Cz. I. *Wpływ nawozów wieloskładnikowych i jednoskładnikowych na wysokość plonów roślin oraz zawartość w nich azotu, fosforu, potasu, wapnia, magnezu i siarki*. Folia Univ. Agric. Stetin., Agricultura, 223(89): 99–106.

KRZYWY E., JAKUBOWSKI W., PASIKOWSKI J. 2000. *Wpływ nawozów wieloskładnikowych i jednoskładnikowych na wysokość plonów i niektóre cechy jakościowe pszenżyta ozimego i jęczmienia jarego*. Folia Univ. Agric. Stetin., Agricultura, 204(81): 163–168.

FILIPEK T. 2001. *Zawartość składników pokarmowych a zastosowanie nawozów wieloskładnikowych z KIZPS „Siarkopol"*. Folia Univ. Agric. Stetin., Agricultura, 223(89): 41–46.

FILIPEK-MAZUR B., GONDEK K. 2005. *Plonowanie i zawartość siarki w gorczycy białej jako efekt stosowania wieloskładnikowych nawozów zawierających siarkę*. Acta Agrophysica, 6(2): 343–351.

MAZUR T., MAZUR Z., WOJTAS A., GRZEŚKOWIAK A. 2001. *Wpływ nawozów wieloskładnikowych na wielkość i jakość plonów roślin uprawianych w 4-polowym zmianowaniu.* Folia Univ. Agric. Stetin., Agricultura, 223(89): 113–120.

STĘPIEŃ W., MERCIK S. 2001. *Działanie na rośliny i glebę nawozów wieloskładnikowych i pojedynczych w zmianowaniu pięciopolowym.* Folia Univ. Agric. Stetin., Agricultura, 223(89): 165–168.

ŚWIERCZEWSKA M., SZTUDER H. 2006. *Wskaźniki efektywności i wykorzystania składników pokarmowych przy różnych sposobach aplikacji nawozów w uprawie kukurydzy*. Zesz. Probl. Post. Nauk Rol., 508: 181–186.

SZTUDER H. 2007. *Produkcyjna i ekologiczna ocena różnych sposobów aplikacji nawozów w uprawie pszenicy ozimej.* Inżynieria Rolnicza, 3(91): 167–172.

ZAWARTKA L., SKWIERAWSKA M. 2004a. *Wpływ nawozów wieloskładnikowych na wymywanie fosforu i innych makroelementów z gleby.* Prace Nauk. AE Wrocław, 1017: 69–77.

ZAWARTKA L., SKWIERAWSKA M. 2004b. *Wpływ nawozów wieloskładnikowych na plon i zawartość fosforu i innych makroelementów w jęczmieniu jarym.* Prace Nauk. AE Wrocław, Chemia, 1017: 149–157.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 332–340, Y. 2010

DOI 10.2478/v10020-010-0030-x

THE EFFECT OF FUNGICIDE SEED TREATMENT ON THE PRODUCTIVITY AND HEALTH OF HUSKED OAT GRAIN*

Agnieszka Pszczółkowska, Gabriel Fordoński, Jacek Olszewski, Adam Okorski, Krystyna Płodzień

Department of Diagnostics and Plant Pathophysiology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: oat, photosynthesis, transpiration, fungal infections, correlation, protein fractions.

A b s t r a c t

The experiment investigated the effect of Raxil 060 FS fungicide treatment on the yield, photosynthesis and transpiration rates, and the health status of husked oat grain cv. Flämingsstern. The results indicate that Raxil 060 FS had a beneficial influence on the total oat grain yield and selected yield components. Gas exchange parameters (photosynthesis and molar transpiration) were not affected by the experimental factor in the first year of the study, whereas in the second year tebuconazole was found to exert a positive effect on the analyzed parameters. Raxil 060 FS contributed to a decrease in the abundance of *Fusarium* spp. on oat grain, but it had varying effects on the remaining fungal species. Fungicide seed treatment had no significant influence on the content of the analyzed protein fractions.

WPŁYW ZAPRAWY FUNGICYDOWEJ NA PRODUKTYWNOŚĆ I ZDROWOTNOŚĆ ZIARNA OWSA OPLEWIONEGO

Agnieszka Pszczółkowska, Gabriel Fordoński, Jacek Olszewski, Adam Okorski, Krystyna Płodzień

Katedra Diagnostyki i Patofizjologii Roślin
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: owies, fotosynteza, transpiracja, porażenie grzybami, korelacja, frakcje białek.

Address: Agnieszka Pszczółkowska, University of Warmia and Mazury, pl. Łódzki 5, 10-727 Olsztyn, Poland, phone: +48 (89) 523 35 11, e-mail: agnieszka.pszczolkowska@uwm.edu.pl
* This study was supported by the Ministry of Education and Science, grant PBZ-KBN-09/P06/2003

A b s t r a k t

W doświadczeniu badano wpływ zaprawy fungicydowej Raxil 060 FS na plon ziarna, intensywność fotosyntezy i transpiracji oraz zdrowotność ziarna owsa oplewionego odmiany Flämingsstern. W badaniach wykazano korzystny wpływu zaprawy Raxil 060 FS na plon ziarna owsa i niektóre elementy struktury plonu. Wskaźniki wymiany gazowej (fotosynteza i transpiracja molowa) pod wpływem badanego czynnika nie uległy zróżnicowaniu w pierwszym roku badań. W drugim roku eksperymentu wykazano natomiast korzystny wpływ tebukonazolu na badane parametry wymiany gazowej. Zaprawa nasienna Raxil 060 FS wpłynęła na obniżenie liczebności *Fusarium* spp. na ziarnie owsa, nie stwierdzono zaś jednoznacznego wpływu zaprawy w przypadku pozostałych gatunków grzybów. Nie wykazano wyraźnego zróżnicowania w zawartości frakcji białek pod wpływem badanego czynnika.

Introduction

Owing to its chemical composition, oat grain has a high dietary value and health-promoting properties. Oat grain contains proteins with balanced amino acid levels, high concentrations of unsaturated fatty acids, water-soluble beta-glucans and antioxidants (BARTNIKOWSKA et al. 2003). Cereal grains intended for human consumption should be characterized by high quality and be free from pathogens that cause contamination and produce mycotoxins, thus posing a health risk for humans and animals (MIELNICZUK 2001).

According to ZAWIŚLAK and ADAMIAK (1998), oat is a cereal crop with relatively low fungicide requirements, while JAŃCZAK (1999) demonstrated that seed dressing is recommended as an effective protective measure against pathogens colonizing seeds and the soil environment. KORBAS and KUBIAK (2000) also reported that seed dressing is an important consideration in spring cereals due to the high rates of seedling infections responsible for yield decrease. According to ROŻEK and WNUK (1994), plant protection products have a minor phytotoxic effect on crops, and they significantly inhibit disease incidence and maximize seed yield.

The following research problems were formulated in the present study: is seed dressing treatment justified? What is the effect of seed dressing treatment on the productivity and health of oat grain? The objective of this study was to determine the effect of the seed dressing fungicide Raxil 060 FS on the yield, yield components, selected gas exchange parameters, health status and the content of protein fractions in husked oat grain cv. Flämingsstern.

Materials and Methods

A large-area experiment was carried out at the Production and Experimental Station in Bałcyny (NE Poland) in 2005 and 2006. The experimental

material consisted of husked oat grain cv. Flämingsstern. The investigated parameters were determined in four replications per each treatment with an area of 20 m^2. The seeds were divided into two groups:

1. seeds treated with the fungicide Raxil 060 FS (tebuconazole)
2. untreated seeds.

The scope of the study was as follows:

1. Determination of the key biometric parameters of oat plants (plant height, number of grains per panicle, TGW) and grain yield per hectare at 15% moisture content.

2. Determination of gas exchange parameters.

Gas exchange parameters were determined using a LI-COR 6400 portable gas analyzer. The studied indicators (photosynthesis and molar transpiration rates) were determined at a fixed CO_2 concentration of 400 ppm and light intensity of 1000 μmol m^{-2} s^{-1}. The photon source was a LED Light Source lamp emitting light with the main peak spectrum at 670 nm and the second peak at 465 nm. Measurements were carried out at the following growth stages: I – at the stem elongation stage, II – beginning of heading stage, III – at the flowering stage (at the highest, fully developed leaf). The noted values were registered, the measurements were carried out in five replications, and the presented results contain average values.

3. Determination of the health status of oat grain by traditional methods.

The harvested oat grain was subjected by a mycological analysis using the artificial culture method. A phytopathological evaluation by the artificial culture method was carried out on 100 randomly selected kernels which were rinsed with water and surface disinfected with 70% ethanol and 1% sodium oxochlorate. The kernels were placed on Petri dishes with solidified PDA. The cultures were incubated for 7–10 days at a temperature of 20–24°C. Fragments of the emerged mycelia were transferred onto PDA slants. The fungi colonizing oat kernels were identified to genus and species by traditional microscopic observation, based on the available monographs (ELLIS 1971, GILMAN 1957, KWAŚNA et al. 1991).

4. Determining the content of protein fractions in oat grain.

A 3 g seed sampled was ground in the IKA A10 (Labortechnik) analytical mill, and the resulting particles were passed through a sieve with 400 μm mesh size (particles smaller than 250 μm ether had a 90% share). The solvent was evaporated, 100 mg of the powdered seeds was placed in Eppendorf test tubes, and three protein fractions were extracted according to the method proposed by WIESER et al. (1998):

1. albumins + globulins – 1 cm^3 of the mixture (0.4 mol/L NaCl + 0.067 mol/L $HKNaPO_4$ with pH of 7.6) was extracted in two replications;

2. prolamin – 1 cm^3 of the mixture (60% ethanol) was extracted in three replications;

3. glutelins – 1 cm^3 of the mixture (50% propanol-1 + 2 mol/L urea + 0.05 mol/L Tris HCl with pH of 7.5) was extracted with 1% DTE under nitrogen, in two replications.

The first two protein fractions were extracted at room temperature using the Eppendorf thermomixer (10-minute extraction). Glutelins were extracted in the thermomixer at 60°C. After each extraction, proteins were centrifuged at 11000 x *g*. The collected fractions were freeze-dried, dissolved in 2 cm^3 of the corresponding phase (1–3), purified using the Spartan-3NY 0.45 μm filter and transferred to glass vials. Protein fractions were identified using the Hewlett Packard Agilent 1050 HPLC with the following parameters: RP-18 Vydac 218TPP54 column, 5 μm, 250 x 4,6 mm, Zorbax 3000SB-C18 precolumn, 4.6 x 12.5 mm, column temperature – 45°C, mobile phase flow rate – 1 ml/min, injection volume – 20 μl. The separation was performed using a two-component gradient. Share of component A: 0 min 75%, 5 min 65%, 10 min 50%, 17 min 25%, 18 min 15%, 19 min 75% (the first component, *A*, was water with the addition of 0.1% TFA, the second component, *B*, was ACN with the addition of 0.1% TFA). A HP detector was applied at a wavelength of 210 nm. The results were analyzed using the Hewlett Packard HPLC 3D Chem Station application. Protein fraction analyses were carried out at the Department of Plant Raw Materials Processing and Chemistry, Faculty of Food Sciences at the University of Warmia and Mazury in Olsztyn.

5. Statistical analysis

The results were processed statistically in STATISTICA software, version 6 (StatSoft, Inc. 2003), using an analysis of variance. Differences between means were determined at a significance level of $p = 0.05$ for grain yield and biometric parameters, and at $p = 0\ 01$ for photosynthesis and molar transpiration rates. The mean values of the investigated parameters were classified into uniform groups with the use of Fisher's test.

Results and Discussion

A significantly higher oat grain yield was noted in the treatment where seeds were dressed with the fungicide Raxil 060 FS (Table 1). The harvested grain was marked by high TGW and a higher number of grains per panicle (Table 1). The use of tebuconazole had no significant effect on plant height.

ŚWIDERSKA-OSTAPIAK and STANKOWSKI (2006) found that the seed dressing fungicides Sarfun 500 SC (carbendazim) and Dithane 75 WG (mancozeb) had an insignificant influence on oat yield, compared with the control treatment. The difference in yield levels between both treatments was small, at 1 dt ha^{-1}, which suggests that oat did not respond to seed dressing. The cited authors

Table 1

Selected biometric parameters of husked oat (means of 2005–2006)

Cultivar	Treatment	Plant height [cm]	Number of seeds per panicle	Thousand seeds weight [g]	Seeds yield [t ha^{-1}]
Flämingsstern	seeds dressed	79.6^{a}	56.5^{b}	36.4^{b}	5.6^{b}
	seeds undressed	80.5^{a}	41.1^{a}	31.5^{a}	5.1^{a}

Homogeneous groups *a, ab, b*, according Fisher's LSD test

also demonstrated that yield components were not affected by fungicide seed treatment. In a study by SZUMIŁO and RACHOŃ (2006), the applied protection levels had no significant effect on oat grain yield, although intensive chemical protection contributed to a 9.2% increase in productivity. The above authors also reported that intensive plant protection measures caused a significant increase in panicle density regardless of cultivar, whereas different levels of chemical protection had a minor effect (within the limits of statistical error) on the number and weight of grains per panicle and TGW.

Apart from the significant impact of seed dressing with tebuconazole on the total oat yield and yield components, the results of this study point also to positive correlations between yield and the number of grains per panicle (correlation coefficient $R = 0.58$) – Figure 1a, between yield and plant height ($R = 0.38$) – Figure 1b, and between plant height and the number of grains per panicle ($R = 0.39$) – Figure 1c.

The applied fungicide did not affect the studied gas exchange parameters in the first year of the study (Table 2). In the second year, Raxil 060 FS caused a significant increase in photosynthesis and transpiration rates at the stem elongation stage and at the beginning of heading (Table 3). PSZCZÓŁKOWSKA (2008) and OLSZEWSKI (2004) who investigated the response to fungicides in winter wheat cv. Kris at various growth stages and in faba bean grown under field and greenhouse conditions, respectively, observed no significant effect of plant protection chemicals on gas exchange parameters in the studied crops.

No significant correlations between the rate of photosynthesis and oat yield were observed in the present study (Figure 1d). As demonstrated by PALA (2002), high photosynthetic efficiency is not always accompanied by high yield potential. Such relationships have been shown for potatoes, soybeans and wheat (PALA 2002). In this experiment, there was a negative correlation between molar transpiration and oat yield (Figure 1e). A higher yield was obtained from plants characterized by a lower rate of transpiration, and a lower yield – from plants with high transpiration efficiency.

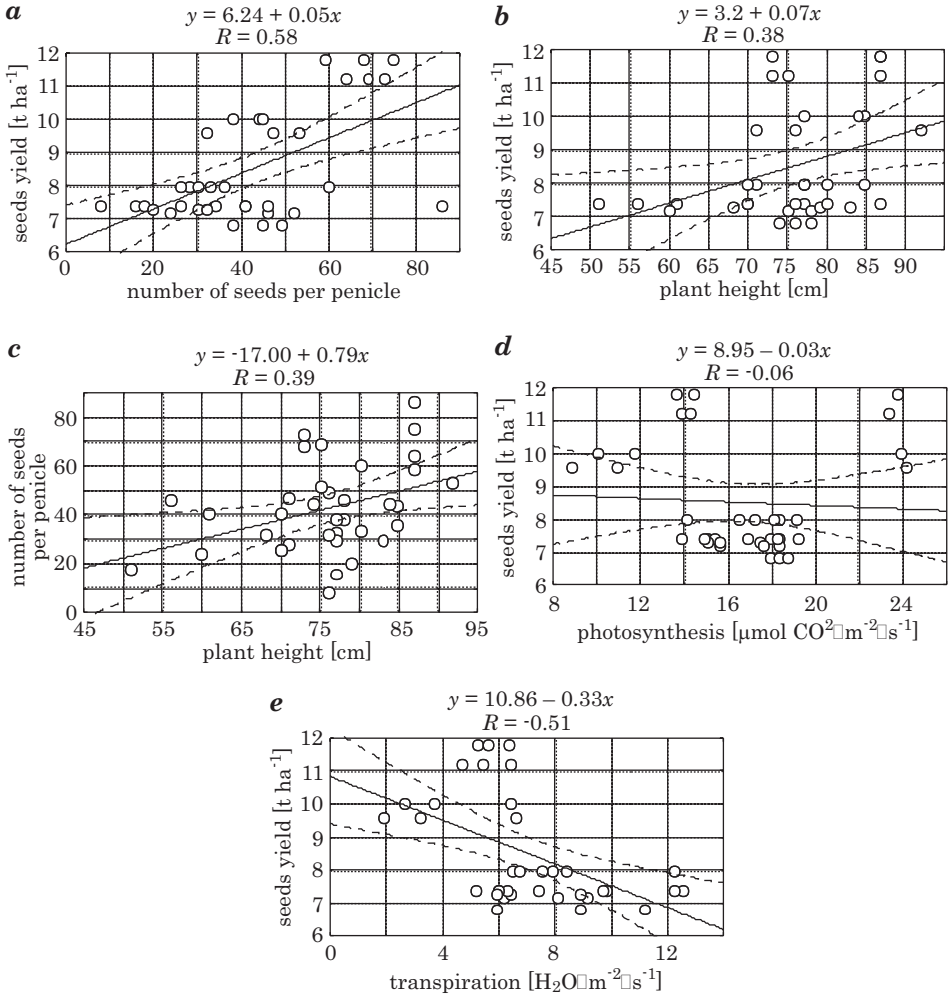

Fig. 1. Linear regresion betwen: *a* – seeds yield and number of seeds per penicle; *b* – seeds yield and plant height; *c* – number of seeds per penicle and plant height; *d* – seeds yield and photosynthesis; *e* – seeds yield and transpiration

Table 2

Gas exchange parameters of husked oat in 2005

Cultivar	Treatment	Photosynthesis (μmol $CO_2 m^{-2} s^{-1}$)			Transpiration (mmol $H_2O m^{-2} s^{-1}$)		
		I	II	III	I	II	III
Flämingssterm	Seeds dressed	17.95^A	15.62^A	16.57^A	6.02^A	10.00^A	6.85^A
	Seeds undressed	16.95^A	17.25^A	17.55^A	6.57^A	11.12^A	8.22^A

I – measurement of gas exchange parameters at the stem elongation stage; II – measurement of gas exchange parameters beginning of heading stage; III – measurement of gas exchange parameters at the flowering stage

Table 3

Gas exchange parameters of husked oat in 2006

Cultivar	Treatment	Photosynthesis (μmol $CO_2m^{-2}s^{-1}$)			Transpiration (mmol $H_2Om^{-2}s^{-1}$)		
		I	II	III	I	II	III
Flämingssterm	Seeds dressed	13.95^A	14.05^A	23.65^A	5.52^A	5.26^A	6.46^A
	Seeds undressed	11.30^B	10.38^B	24.00^A	3.45^B	2.87^B	6.41^A

I – measurement of gas exchange parameters at the stem elongation stage; II – measurement of gas exchange parameters beginning of heading stage; III – measurement of gas exchange parameters at the flowering stage

Oat grain samples from both treatments were characterized by similar fungicide infection levels (Table 4 and Table 5). The predominant species was *Alternaria alternata*, whereas toxin-producing fungi of the genus *Fusarium* were represented by single isolates of *Fusarium poae* and *Fusarium avenaceum*. It should be stressed that oat grain harvested in the treatment where seeds were dressed with fungicide showed a lower degree of colonization by *Fusarium* spp. (Table 4 and Table 5). MIELNICZUK (2001) and KIECANA et al. (2005) also reported the presence of *Fusarium avenaceum* and *Fusarium poae* on oat grain. KIECANA et al. (2005) found that *Fusarium poae* greatly contributed to fusariosis on oat panicles and grain infection. According to JAŃCZAK (1999), KORBAS and KUBIAK (2000), seed dressing fungicides effectively protect seedlings against pathogens colonizing seeds and the soil environment. In a study by BURGIEŁ and PISULEWSKA (2003), the predominant fungal species on oat kernels were *Alternaria alternata*, *Epicoccum purpurascens*, *Penicillium* spp. and *Fusarium culmorum*.

Table 4

Number of fungal isolates in husked oat seeds cv. Flämingssterm in 2005

Fungal specied	Seeds dressed	Seeds undressed
Species of the genus *Fusarium*		
Fusarium avenaceum (Fr.) Sacc.	1	1
Fusarium poae (Peck) Wollenw.	–	2
Total	1	3
Other fungal species		
Alternaria alternata Keissler Nees	42	44
Cladosporium cladosporioides (Fres) de Vries	–	3
Drechslera sorokiniana (Sacc.) Subram. and Jain	1	–
Epicoccum purpurascens Ehrenberg	16	11
Penicillium ssp.	1	1
Total	60	59
Total isolated fungi	61	62

Table 5

Number of fungal isolates in husked oat seeds cv. Flämingssterm in 2006

Fungal specied	Seeds dressed	Seeds undressed
Species of the genus *Fusarium*		
Fusarium avenaceum (Fr.) Sacc.	–	3
Fusarium poae (Peck) Wollenw.	1	1
Fusarium spp.	–	1
Total	1	3
Other fungal species		
Alternaria alternata Keissler Nees	42	45
Cladosporium cladosporioides (Fres) de Vries	2	4
Bipolaris sorokiniana (Sacc.) Shoemaker	2	5
Epicoccum purpurascens Ehrenberg	16	10
Penicillium ssp.	4	1
Total	66	65
Total isolated fungi	67	70

As demonstrated by the results of this experiment, fungicide treatment had no significant effect on the content of the investigated protein fractions (albumins+globulins, prolamin and glutelins) in control and experimental oat grain samples (Table 6).

Table 6

Content of protein fractions in of husked oat seeds cv. Flämingssterm (calculated as mAU·s)

Treatment	Albumins +globulins	Prolamins	Glutelins
Seeds undressed	34 075	17 910	12 844
Seeds dressed	33 268	17 722	13 596

Conclusions

1. Raxil 060 FS had a beneficial influence on the total oat grain yield and selected yield components.

2. Gas exchange parameters were not affected by the experimental factor in the first year of the study, whereas in the second year tebuconazole was found to exert a positive effect on the analyzed parameters.

3. Raxil 060 FS contributed to a decrease in the abundance of *Fusarium* spp. on oat grain, but it had varying effects on the remaining fungal species.

Translated by Aleksandra Poprawska

Accepted for print 12.10.2010

References

BARTNIKOWSKA E. 2003. *Przetwory z ziarna owsa jako źródło ważnych substancji prozdrowotnych w żywieniu człowieka*. Biul. IHAR, 229: 235–245.

BURGIEŁ Z., PISULEWSKA E. 2003. *Grzyby zasiedlające ziarno owsa nagonasiennego*. Biul. IHAR, 229: 205–210.

ELLIS M.B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute Kew, Surrey, England.

GILMAN J.C. 1957. *A manual of soil fungi*. The Iowa State University, Ames USA.

JAŃCZAK C. 1999. *Zaprawianie ziarna – konieczne w ochronie zbóż jarych*. Plon, 11: 6.

KIECANA I., MIELNICZUK E., PERKOWSKI J., GOLIŃSKI P. 2005. *Infection of panicles with Fusarium poae* (*Peck*) *Wollenw. and mycotoxin content in oat grain*. Acta Agrobot., 59(2): 91–102.

KORBAS M., KUBIAK K. 2000. *Ochronić ziarno siewne*. Top Agrar Polska, 1: 44–45.

KWAŚNA H., CHEŁKOWSKI J., ZAJKOWSKI P. 1991. *Grzyby*. Tom XXII. (*Flora Polska*). PAN Instytut Botaniki, Warszawa-Kraków.

MIELNICZUK E. 2001. *The occurrence of Fusarium on panicles oat* (*Avena sativa L.*). J. Plant Protection Research, 41(2): 173–180.

OLSZEWSKI J. 2004. *Wpływ wybranych stresów abiotycznych i biotycznych na intensywność fotosyntezy i transpiracji, plonowanie oraz zdrowotność bobiku i grochu siewnego*. Rozprawy i Monografie, 85. Wydawnictwo UWM, Olsztyn, 109.

PALA J. 2002. *Genetyczne, fizjologiczno-biochemiczne i ekologiczne uwarunkowania plonowania roślin*. [W:] *Fizjologia plonowania roślin*. Red. R.J. Górecki, S. Grzesiuk. Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego, Olsztyn, 27–73.

PSZCZÓŁKOWSKA A. 2008. *Diagnostyka patogenów grzybowych metodą PCR i tradycyjną oraz produktywność pszenicy ozimej* (*Triticum aestivum* L.) w warunkach zróżnicowanej ochrony fungicydowej. Rozprawy i monografie, 140, UWM Olsztyn.

ROŻEK S., WNUK A. 1994. *Wpływ wybranych pestycydów na zawartość niektórych składników w nasionach i w kiełkach dwóch odmian bobiku*. Acta Agraria et Silvestria, 32: 75–83.

SZUMIŁO G., RACHOŃ L. 2006. *Porównanie plonowania i jakości owsa nagoziarnistego i oplewionego w warunkach zróżnicowanej ochrony chemicznej*. Biul. IHAR, 239: 85–92.

ŚWIDERSKA-OSTAPIAK M., STANKOWSKI S. 2006. *Wpływ ilości wysiewu i zaprawiania ziarna na plon i komponenty plonu owsa nagoziarnistego i oplewionego*. Biul. IHAR, 239: 93–102.

WIESER H., ANTES S., SEILMEIER W. 1998. *Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography*. Cerael Chem., 75(5): 644–650.

ZAWIŚLAK K., ADAMIAK E. 1998. *Płodozmian i pestycydy jako czynniki integrowanej uprawy owsa*. Acta Acad. Agricult. Tech. Olst. Agricultura, 66: 131–142.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 341–351, Y. 2010

DOI 10.2478/v10020-010-0031-9

FATTENING RESULTS OF FINISHING PIGS FED SECOND-STAGE DIETS WITH A HIGH CONTENT OF OAT BRAN AND SOYBEAN OIL

***Aniela Falkowska*[1], *Dorota Bugnacka*[2], *Wojciech Kozera*[2], *Janusz Falkowski*[2]**

[1] Department of Animal Nutrition and Feed Science
[2] Department of Pig Breeding
University of Warmia and Mazury in Olsztyn

K e y w o r d s: feeding, pigs 70–110 kg BW, oat bran, slaughter value, blood, liver.

A b s t r a c t

The experiment was performed on 48 crossbred (♀Polish Landrace x ♂Duroc) finishing pigs divided into three feeding groups: group I (control) – fed a cereal-soybean diet (diet 1), group II – fed a diet containing 15% oat bran and 3% soybean oil (diet 2), group III – fed a diet containing 30% oat bran and 5% soybean oil (diet 3). The pigs were kept in straw-litter pens (two animals, one gilt and one barrow, in each), and they were fed *ad libitum* from 70 to 110 kg live weight. A second-stage diet containing 30% oat bran and 5% soybean oil (group III) significantly decreased average daily weight gains and feed conversion efficiency, compared with control group I and group II. Different feeding had no significant effect on the lean meat and fat content of carcasses and the proximate chemical composition of meat. Diet supplementation with oat bran and soybean oil contributed to a significant increase in alpha-linolenic acid concentrations in the lipids extracted from *m. longissimus dorsi* (*m.l.d.*). The higher content of crude fiber and crude fat in diets resulted in a highly significant increase in HDL concentrations and a significant increase in triacylglycerol levels in the blood serum of pigs, yet it had no influence on total cholesterol levels in meat (*m.l.d.*) and liver samples.

WYNIKI TUCZU ŚWIŃ ŻYWIONYCH W DRUGIM OKRESIE TUCZU DIETAMI Z WYSOKĄ ZAWARTOŚCIĄ OTRĄB OWSIANYCH I OLEJU SOJOWEGO

***Aniela Falkowska*[1], *Dorota Bugnacka*[2], *Wojciech Kozera*[2], *Janusz Falkowski*[2]**

[1] Katedra Żywienia Zwierząt i Paszoznawstwa
[2] Katedra Hodowli Trzody Chlewnej
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: żywienie, świnie 70–110 kg masy ciała, otręby owsiane, wartość rzeźna, krew, wątroba.

Address: Aniela Falkowska, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (89) 523 49 36, e-mail: aniela.falkowska@uwm.edu.pl

Abstrakt

Do doświadczenia użyto 48 tuczników mieszańców (♀ polska biała zwisłoucha x ♂ duroc) podzielonych na 3 grupy żywieniowe: I (kontrolną) – żywioną mieszanką (1) zbożowo-sojową, II – żywioną mieszanką (2) z udziałem 15% otrąb owsianych i 3% oleju sojowego, III – żywioną mieszanką (3) z udziałem 30% otrąb owsianych i 5% oleju sojowego. Świnie utrzymywane były w kojcach ściołowych (po 2 szt., 1 loszka i 1 wieprzek), żywiono je do woli, a tucz obejmował przedział masy ciała 70–110 kg. Żywienie tuczników w drugim okresie tuczu mieszanką z udziałem 30% otrąb owsianych i 5% oleju sojowego (gr. III) wpłynęło na istotne obniżenie ich średnich dziennych przyrostów masy ciała oraz istotne pogorszenie wykorzystania paszy, w porównaniu z grupą kontrolną (I) i II. Zróżnicowane żywienie badanych tuczników nie wywarło jednak istotnego wpływu na mięsność i otłuszczenie pochodzących od nich tusz oraz na podstawowy skład chemiczny mięsa. Wprowadzenie otrąb owsianych i oleju sojowego do diet spowodowało istotne zwiększenie udziału kwasu linolenowego w tłuszczu mięśnia najdłuższego grzbietu (*m.l.d.*). Podwyższenie zawartości włókna surowego i tłuszczu surowego w mieszankach wpłynęło także na wysoko istotny wzrost koncentracji HDL i istotny wzrost poziomu triacylogliceroli w surowicy krwi badanych świń, jednak nie miało wpływu na poziom cholesterolu całkowitego w mięsie (*m. longissimus dorsi*) oraz wątrobie.

Introduction

Recent years have witnessed an increasing interest in food products of animal origin that satisfy human nutritional requirements and provide health benefits. Numerous research studies have been conducted with the aim to lower cholesterol levels and increase the concentrations of unsaturated fatty acids in meat. The enrichment of muscle tissue lipids with polyunsaturated fatty acids, particularly n-3 PUFAs, improves the nutritional quality of pork. The inclusion of vegetable oils in diets for growing-finishing pigs contributes to an increase in PUFA concentrations in tissues (BAROWICZ, PIESZKA 2001, DAZA et al. 2005, APPLE et al. 2009), and the observed changes are determined by the amount of vegetable oil added to feed (BAROWICZ et al. 2000, REALINI et al. 2010). Research results suggest that feeding unsaturated fatty acids to pigs may reduce the total cholesterol content of muscles, depot fat (BAROWICZ 2000, MIGDAŁ et al. 2005) and blood serum (BAROWICZ, PIETRAS 1998, RAMJIGANESH et al. 2002).

According to many authors, the quantity and quality of dietary fiber may affect the fatty acid composition of meat lipids and cholesterol metabolism (FERNANDEZ 1995, BAROWICZ et al. 1998). Short-chain fatty acids, produced during the microbial fermentation of fiber in the lower gastrointestinal tract, are almost entirely absorbed into the hepatic portal vein and transported to the liver. Due to their effect on the reduction of enzymes involved in cholesterol synthesis, short-chain fatty acids may affect lipid metabolism (CAMERON-SMITH et al. 1994, LEVRAT et al. 1994). KRITCHERSKY (1997) demonstrated that crude fiber, in particular its soluble fractions, is capable of binding bile acids,

thus limiting their absorption in the digestive system. DEMIGNE et al. (1995) studied isolated hepatic cells in rats and found that propionic acid inhibited cholesterol synthesis in the liver.

The objective of this study was to determine the effects of different inclusion levels of crude fiber and crude fat in complete diets for finishing pigs on production results, the fatty acid content of *m. longissimus dorsi*, and selected blood and liver lipids.

Materials and Methods

The experiment was conducted at the research laboratory of the Department of Pig Breeding, University of Warmia and Mazury in Olsztyn. The experimental materials comprised three second-stage complete diets for finishing pigs. Control diet 1 was composed of soybean meal, ground barley, ground wheat, mineral and vitamin supplements. Experimental diets 2 and 3 were supplemented with 15% and 30% oat bran, respectively, and their energy level was raised to reach the targeted level by the addition of 3% and 5% soybean oil, respectively. The diets were supplemented with synthetic amino acids (lysine, methionine), in accordance with the Pig Nutrient Requirements (1993). Diet composition is presented in Table 1.

Table 1

Composition of experimental diets [%]

Specification	Diets		
	1	2	3
Ground wheat	40.00	20.00	20.00
Ground barley	41.94	40.95	22.91
Soybean meal	13.50	16.50	17.50
Oat bran	–	15.00	30.00
Dicalcium phosphate	1.50	1.50	1.50
Limestone	1.00	1.00	1.00
Grower premix	1.50	1.50	1.50
NaCl	0.30	0.30	0.30
Soybean oil	–	3.00	5.00
L-lysine	0.24	0.21	0.23
DL-methionine	0.02	0.04	0.06

The experiment was performed on 48 crossbred (♀ Polish Landrace x ♂Duroc) finishing pigs with average initial body weight of 71.2 kg, selected from our own herd, divided into three feeding groups by the analogue method.

The pigs were kept in straw-litter pens (two animals, one gilt and one barrow, in each). Feed was provided *ad libitum*, in pelleted form, from automatic feeders. The animals had free access to water from automatic drinkers. Feed intake was monitored and registered daily. The pigs were weighed at two-week intervals. Blood samples were collected from the jugular vein before slaughter.

The animals were slaughtered at body weight of approximately 110 kg. Right half-carcass dissection was carried out as recommended by the Pig Testing Station. Samples of *m. longissimus dorsi* (*m.l.d.*) and liver were also collected. The proximate chemical composition of meat was determined, including the content of dry matter, total protein and ether extract, in accordance with the AOAC (1990). The fatty acid composition of lipids extracted from *m.l.d.* was determined using a PV-4600 gas chromatograph with a flame ionization detector (FID) and a capillary column (30 m x 0.32 mm i.o. x 0.25 fm film thickness), under the following conditions: detector temperature – 250°C, column temperature – 170°C, injection mode – 50:1 split ratio, carrier gas – helium. Total cholesterol levels were estimated in lipid extracts from *m.l.d.* and liver samples, as described by ARNETH and AL.-AHMAD (1995). The serum concentrations of total cholesterol, HDL and triacylgltcerols were determined by enzymatic methods, using Cormay diagnostic test kits. The chemical composition of feed was analyzed in accordance with AOAC guidelines (1990), using TECATOR analyzers. NDF and ADF fractions were determined by the method proposed by GOERING and SOEST VAN (1970), in the Fibertec System (Foss Tecator). The gross energy content of diets was determined in an adiabatic bomb calorimeter.

The results were verified statistically by a one-way analysis of variance and Duncan's test, using Statistica 6.0 software.

Results and Discussion

The addition of oat bran (chemical composition: dry matter – 93.87%, crude ash – 3.19%, crude protein – 8.45%, crude fat – 3.57%, crude fiber – 20.25%, N-free extracts – 58.41%) to experimental diets increased their crude fiber content. The level of this component amounted to 3.15%, 5.37% and 9.45%, respectively. The inclusion of soybean oil in experimental diets 2 and 3 increased their crude fat content (Table 2).

Production results are presented in Table 3. The initial body weight of pigs was similar in all groups, at 71.2 kg on average. The growth rate of pigs was high, and their daily gains reached 919 g, 973 g and 868 g in groups I, II and III, respectively. Group II animals, fed a diet containing 15% oat bran and 5.37% crude fiber, were characterized by the highest average daily gains.

Table 2

Chemical composition of experimental diets [%]

Specification	Diets		
	1	2	3
Dry matter	87.29	89.30	89.83
Crude ash	5.23	5.63	6.44
Organic matter	82.06	83.67	83.39
Crude protein	16.19	16.92	16.07
Crude fat	2.39	4.35	5.98
Crude fibre	3.15	5.37	9.45
N-free extractives	60.33	57.03	51.89
NDF	13.93	20.09	26.10
ADF	4.94	7.18	12.28
Hemicellulose	8.91	12.91	13.82
Gross energy MJ kg^{-1}	15.863	16.749	17.288

Table 3

Fattening performance of experimental pigs

Specification	Statistical measures	Group		
		I	II	III
Initial body weight [kg]	$\bar{x}$	71.10	71.30	71.31
	s	8.57	7.41	7.24
Final body weight [kg]	$\bar{x}$	109.6	109.8	106.5
	s	5.62	3.64	3.80
Daily gain [g]	$\bar{x}$	919^{A}	973^{A}	868^{B}
	s	61.4	62.8	68.5
Daily feed intake [kg]	$\bar{x}$	3.77	3.85	4.34
	s	0.25	0.34	0.66
Feed/gain ratio [kg kg^{-1}]	$\bar{x}$	4.12^{A}	3.97^{A}	5.00^{B}
	s	0.35	0.41	0.64

A,B – $P \leq 0.01$

The addition of 30% oat bran (group III) to diet 3 increased its crude fiber content to 9.45%, which significantly decreased ($P \leq 0.01$) average daily gains and feed conversion efficiency, compared with groups I and II (Table 3).

No significant changes were noted in the parameters of carcass quality (Table 4). Finishing pigs fed high-fiber diets (groups II and III) had insignificantly lower back fat thickness and an insignificantly higher carcass lean content. KREUZER et al. (2002) reported lower average daily gains (625 g vs. 566 g) in growing pigs fed diets whose crude fiber content was increased (from 5.5% to 8.3%) by adding sugar beet pulp, rye bran and citrus pulp. However,

the cited authors observed no differences in the body weight gains of pigs fed high-fiber diets whose crude fat content was increased to 7.9% by adding an oil blend. KENNELLY and AHERNE (1980) increased the crude fiber content of diets for pigs from 4.1% to 10.25% by adding 22% oat hulls and reported significantly lower body weight gains only at the first stage of fattening (22–63 kg). Lower daily gains and higher feed consumption per kg weight gain resulting from a higher crude fiber content of diets for fatteners were also observed by O'DOHERTY et al. (2002) and SHRIVER et al. (2003).

Table 4

Selected slaughter parameters of finishing pigs

Specification	Statistical measures	Group		
		I	II	III
Dressing percentage [%]	$\bar{x}$	78.92	79.02	78.32
	s	1.36	1.47	2.59
Carcass length [cm]	$\bar{x}$	80.50	80.90	79.7
	s	1.75	1.28	2.44
Carcass lean content [%]	$\bar{x}$	55.03	57.49	56.60
	s	3.01	2.47	3.01
Loin eye area [cm^2]	$\bar{x}$	58.20	58.19	59.74
	s	7.63	6.47	9.84
Back fat thickness mean of 5 measurements [mm]	$\bar{x}$	24.40	23.71	22.48
	s	2.91	2.76	4.12

There were no significant changes in the chemical composition of meat from the studied pigs (Table 5). The higher concentrations of crude fiber and crude fat in experimental diets caused an insignificant decrease in the crude fat content of *m.l.d.*, from 1.35% in the control group to 1.05% and 1.09% in groups II and III, respectively. The increase in the content of crude fiber and crude fat in diets affected the fatty acid composition of *m.l.d.* (Table 6). The concentrations of alpha-linolenic acid (18:3) increased significantly, from 0.42% in the control group to 0.60% and 0.81% in groups II and III, respectively. An increase was also noted in the linoleic acid (18:2) content of meat samples collected from experimental group pigs, but the differences were statistically non-significant. Total PUFA concentrations were also higher in experimental groups (9.20% in group II and 9.41% in group III) than in the control group (7.44%).

There were no significant differences in total cholesterol levels in *m.l.d.* between groups, but the cholesterol content of meat was somewhat lower in pigs fed diets with an increased content of crude fiber and crude fat (Table 7). The increase in the crude fiber and crude fat content of experimental diets

Table 5

Chemical and physico-chemical properties of meat

Specification	Statistical measures	Group		
		I	II	III
Dry matter [%]	$\bar{x}$	25.52	24.79	24.88
	s	1.39	0.63	0.57
Crude protein [%]	$\bar{x}$	22.47	22.31	22.29
	s	0.73	0.64	0.83
Crude fat [%]	$\bar{x}$	1.35	1.05	1.09
	s	0.52	0.40	0.30
Crude ash [%]	$\bar{x}$	1.11	1.14	1.15
	s	0.09	0.06	0.05
pH_{45}	$\bar{x}$	5.97	5.94	6.03
	s	0.25	0.23	0.23
pH_{24}	$\bar{x}$	5.21	5.25	5.30
	s	0.11	0.13	0.20

Table 6

Fatty acid composition of *m. longissimus dorsi* lipids [in % of total acids]

Fatty acids	Group			SEM
	I	II	III	
C 14:0	1.39	1.35	1.42	0.016
C 16:0	24.92	24.33	24.42	1.692
C 16:1	4.32	4.14	3.81	0.199
C 17:0	0.19	0.25	0.22	0.007
C 17:1	0.28	0.30	0.28	0.012
C 18:0	12.01	11.91	11.50	0.861
C 18:1	48.20	47.33	47.56	8.004
C 18:2	5.85	7.06	7.33	2.748
C 18:3	0.42^{B}	0.60^{b}	0.81^{Aa}	0.022
C 20:0	0.25	0.29	0.26	0.017
C 20:1	0.95	0.88	1.00	0.021
C 20:4	1.17	1.47	1.26	0.305
SFAs	38.78	38.14	37.80	–
PUFAs	7.44	9.20	9.41	–
MUFAs	53.76	52.65	52.85	–

a,b – $P \leq 0.05$
A,B – $P \leq 0.01$
SEM – standard error of the mean

caused a highly significant ($P \leq 0.01$) increase in the serum concentrations of HDL cholesterol (groups II and III), although the above feed ingredients had no effect on total serum cholesterol. The diet containing 9.45% crude fiber and 5.98% crude fat (group III) contributed to a significant increase ($P \leq 0.05$) in serum triacylglycerol levels. No significant changes were noted in cholesterol concentrations in fresh liver samples. Total cholesterol levels in the livers of finishing pigs reached 3.78 mg g^{-1}, 3.55 mg g^{-1} and 3.82 mg g^{-1}, respectively.

Table 7

Level of biochemical indices in blood serum, *m. longissimus dorsi* and in the liver

Specification	Statistical measures	Group		
		I	II	III
Blood serum:				
– total cholesterol [mg dl^{-1}]	$\bar{x}$	148.8	150.7	150.8
	s	15.1	20.5	15.4
– HDL [mg dl^{-1}]	$\bar{x}$	41.7^{B}	48.0^{A}	50.2^{A}
	s	4.7	4.8	4.8
– triacylglycerols [mg dl^{-1}]	$\bar{x}$	41.2^{a}	45.0^{a}	55.2^{b}
	s	10.9	11.6	18.2
M. longissimus dorsi:				
– total cholesterol [mg/g fresh tissue]	$\bar{x}$	0.59	0.48	0.50
	s	0.14	0.14	0.10
Liver:				
– total cholesterol [mg g^{-1} fresh tissue]	$\bar{x}$	3.78	3.55	3.82
	s	0.56	0.55	0.65

a,b – $P \leq 0.05$
A,B – $P \leq 0.01$

Research results indicate that that the type of fat added to pig diets affects the fatty acid composition of intramuscular fat. In a study by APPLE et al. (2009), a 5% addition of soybean oil to pig diets resulted in an increase in the alpha-linolenic acid (18:3n-3) content of intramuscular fat, from 0.65% (control group) to 1.94%. The most desirable n-6/n-3 fatty acid ratio was reported for fattening pigs fed a diet supplemented with linseed oil (REALINI et al. 2010). KOZERA et al. (2006) observed an increase in PUFA concentrations and a decrease in MUFA levels in fat samples extracted from the meat (*m.l.d.)* of pigs fed diets with an increased content of crude fiber (whose source was ground wheat straw) and crude fat (whose source was soybean oil). As reported by KREUZER et al. (2002), an increase in the crude fiber content of diets following the addition of sugar beet pulp significantly decreased the serum concentrations of total, LDL and HDL cholesterol in pigs. However, the crude

fiber content of the ration had no significant effect on total cholesterol concentrations in muscle tissue lipids. Serum cholesterol levels were not affected by the composition of crude fiber (cellulose, hemicellulose, pectins), either. Yet, an increase in the crude fiber and crude fat content of pig diets led to an increase in the serum concentrations of total cholesterol, HDL and triacylglycerols (KREUZER et al. 2002).

The dietary supply of fatty acids may affect lipid metabolism in the liver and blood cholesterol concentrations. FIEDOROWICZ et al. (2000) noted no significant differences in cholesterol levels in the liver and blood serum of pigs fed diets supplemented with 4% lard or linseed oil. MARTINEZ-FLORES et al. (2004) studied the effect of high-fiber diets containing oat hulls, and observed a significant decrease in total cholesterol and triacylglycerol levels in the livers of hamsters. The liver plays an important role in the control of cholesterol esterification and breakdown, and it is also involved in lipoprotein synthesis which affects serum cholesterol concentrations. Crude fiber contained in pig diets affects the populations of intestinal bacteria and the production of short-chain volatile fatty acids (FURGAŁ 2005), which may influence lipid metabolism in the liver (KREUZER 2002). As demonstrated by DEMIGNE et al. (1995), propionic acid lowered blood cholesterol concentrations by inhibiting cholesterol synthesis in the liver. Crude fiber, which is a source of volatile fatty acids, probably affects pancreatic insulin and glucagon secretion, thus influencing lipid metabolism in the liver and tissues (SILEIKIENE et al. 2005).

Conclusions

The second-stage diet containing 30% oat bran and 5% soybean oil (group III) significantly ($P \leq 0.01$) decreased average daily weight gains and feed conversion efficiency, compared with control group I and group II. Different feeding had no significant effect on the lean meat and fat content of carcasses and the proximate chemical composition of meat (*m.l.d.*). Diet supplementation with oat bran and soybean oil contributed to a significant increase in alpha-linolenic acid concentrations in the lipids extracted from *m. longissimus dorsi*. The higher content of crude fiber and crude fat in experimental diets resulted in a significant ($P \leq 0.01$) increase in HDL concentrations and increase ($P \leq 0.05$) in triacylglycerol levels in the blood serum of pigs, yet it had no influence on total cholesterol levels in meat (*m.l.d.*) and liver samples.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 9.11.2010

References

ARNETH W., AL.-AHMAD H. 1995. *Cholesterol. Bestimmung in Muskel und Fettgeewbe sowie in Innereine mittels HPLC*. Fleischwirtsch, 75: 185–187.

AOAC. 1990. *Association of Official Analytical Chemists. Official Methods of Analysis.* 15th Edition, Arlington.

APPLE J.K., MAXWELL C.V., GALLOWAY D.L., HAMILTON C.R., YANCEY J.W. S. 2009. *Interactive effects of dietary fat source and slaughter weight in growing-finishing swine:* III. *Carcass and fatty acid compositions.* J. Anim. Sci., 87: 1441–1454.

BAROWICZ T., PIETRAS M. 1998. *Wpływ źródła nienasyconych kwasów tłuszczowych w dawce pokarmowej oraz płci zwierząt na wybrane wskaźniki lipidowe krwi i w mięśniu najdłuższym u tuczników*. Rocz. Nauk. Zoot., 25: 83–97.

BAROWICZ T., BRZÓSKA F., PIETRAS M. 1998. *Influence of source of dietary fiber in some indicators of lipid metabolism in fattening pigs.* Proc. Int. Conf. On Pig Production, Pekin 6–8.07.1998. China Int. Acad. Publ., 404–406.

BAROWICZ T. 2000. *Wpływ źródła NNKT w dawce pokarmowej dla tuczników na skład kwasów tłuszczowych i zawartość cholesterolu w mięśniu najdłuższym.* Rocz. Nauk. Zoot., Supl., 6: 33–38.

BAROWICZ T., PIESZKA M. 2001. *Using linseed oil in fattening pig rations to modify chemical composition and dietetic value of pork*. Suppl., Polish J. Food Nutr. Sci., 10/51(3): 42–45.

CAMERON-SMITH D., COLLIER G. R., O'DEA K. 1994. *Effect of propionate on in vivo carbohydrate metabolism in streptozotocin-induced diabetic rats.* Metabolism, 43: 728–734.

DAZA A., REY A.J., RUIZ J., LOPEZ-BOTE C.J. 2005. *Effect feeding in free-range conditions or in confinement with different dietary MUFA/PUFA rations and l-tocopheryl acetate on antioxidants effects accumulation and oxidative stability in Iberian pigs.* Meat Sci., 69: 151–163.

DEMIGNE C., MORAND C., LEVRAT M.A., BESSON C., MOUNDRAS C., REMESY C. 1995. *Effect of propionate on fatty acid and cholesterol synthesis and acetate metabolism in isolated rat hepatocytes.* British J. of Nutr., 74: 209–219.

FERNANDEZ M.L. 1995. *Distinct mechanisms of plasma LDL lowering effect of pectin, guar gum and psyllium.* J. Lipid RES., 36: 2394–2404.

FIEDOROWICZ S., SAWOSZ E., BIELECKI W., LECHOWSKI R. 2000. *Wpływ dodatku różnych rodzajów tłuszczu oraz octanu tokoferolu na skład kwasów tłuszczowych i zawartość cholesterolu w wątrobie świń.* Zesz. Nauk. PTZ, 48: 339–348.

FURGAŁ-DIERŻUK I. 2005. *The effect of different dietary fibres on microbial population and short chain fatty acid concentration in the caecum on pigs.* J. Anim. Feed Sci., 14 (Supl. 1): 345–348.

GOERING H.K., SOEST P.J. VAN 1970. *Forage fiber analyses – apparatus, reagents, procedures and some applications*. Agriculture Handbook 379, ARS-USDA, Washington DC.

KENNELLY J. J., AHERNE F.X. 1980. *The effect of fiber addition to diets formulated to contain different levels of energy and protein on growth and carcass quality of swine.* Can. J. Anim. Sci., 60: 385–393.

KOZERA W., FALKOWSKI J., BUGNACKA D., FALKOWSKA A. 2006. *Fattening results of crossbred (Polish Landrace x Pietrain) pigs fed diets with a high wheat straw content.* Pol. J. Natur. Sc., 20: 193–203.

KREUZER M., HANNEKEN H., WITTMANN M., GERDEMANN M.M., MACHMULER A. 2002. *Effects of different fibre source and fat addition on cholesterol and cholesterol-related lipids in blood serum, bile and body tissues of growing pigs.* J. Anim. Physiol. a. Anim. Nutr., 89: 253–259.

KRITCHERSKY D. 1997. *Cereal fiber and lipidemia.* Cereal Food World, 42, 2: 81–85.

LEVRAT M.A., FAVIER M.L., MOUNDRAS C., REMESY C., DEMIGNE C., MORAND C. 1994. *Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats.* J. of Nutr., 124: 531–538.

MARTINEZ-FLORES H.E., CHANG Y.K., MARTINEZ-BUSTOS F., SGARBIERI V. 2004. *Effect of high fiber products on blood lipids and lipoproteins in hamster.* Nutr. Res., 24: 85–93.

MIGDAŁ W., BAROWICZ T., PIESZKA M., ŻIVKOWIĆ B. 2005. *Effect of mono- and polyunsaturated fatty acids on muscle cholesterol level in fattening pigs.* Bioch. Anim. Husb., 21(1–2): 49–54.

Normy żywienia świń. Wartość pokarmowa pasz. 1993. Omnitech Press, Warszawa.

O'DOHERTY J.V., MCGLYNN S.G., MURPHY D. 2002. *The influence of fibre level and fat supplementation in expander-processed diets on grower-finisher pig performance.* J. Sci. Food Agric., 82: 1036–1043.

RAMJIGANESH T., ROY S., FREAKE H.C., MCINTYRE J.C., FERNANDEZ M.L. 2002. *Corn fiber oil lowers plasma cholesterol by altering hepatic cholesterol metabolism and up-regulating LDL receptors in guinea pigs.* J. Nutr., 132: 335–340.

REALINI C.E., DURAN-MONTGE P., LIZARDO R., GISPERT M., OLIVER M.A., ESTEVE-GARCIA E. 2010. *Effect of source of dietary fat on pig performance, carcass characteristics and carcass fat content, distribution and fatty acid composition.* Meat Science, 85: 606–612.

SHRIVER J.A., CARTER S.D., SUTTON A.L., RICHERT B.T., SENNE B.W., PETTEY L.A. 2003. *Effect of adding fiber sources to reduced-crude protein, amino acid- supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs.* J. Anim. Sci., 81: 492–502.

SILEIKIENE V., MOSENTHIN R., TAFAJ N., KRUSZEWSKA D., WESTROM B., MATTSSON J., PIERZYMOWSKI S.G. 2005. *Effect of short chain fatty acids infused intraileally on interdigestive exocrine pancreatic secretions in growing pigs.* J. Anim. Physiol. a. Anim. Nutr., 89: 253–259.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 352–359, Y. 2010

DOI 10.2478/v10020-010-0032-8

QUALITY ASSESSMENT OF THE COMMON FOX (*VULPES VULPES*) PELTS OBTAINED IN TWO REGIONS OF POLAND ON THE BASIS OF SELECTED INDICES

Paweł Janiszewski, Andrzej Gugołek, Marta Kowalewska, Justyna Cilulko

Chair of Fur-bearing Animals Breeding and Game Management
University of Warmia and Mazury in Olsztyn

K e y w o r d s: *Vulpes vulpes*, pelt quality, hair length.

A b s t r a c t

The aim of this study was to characterise and compare the pelts of common foxes (*Vulpes vulpes*) obtained through reduction shootings in two Polish hunting districts: Olsztyn and Warsaw. The study material was a hundred pelts obtained by the hunters from the two districts. It has been concluded that the average weight and length of the pelts reflect the sexual dimorphism of the common fox. It has been further concluded that the area of origin does not affect the examined parameters. The extent of the silvering effect in the pelt did not depend on the sex or the area. It has been shown that male foxes in general and the foxes from the Olsztyn district (as drawn by Polish Hunting Association) were marked by both longer guard hair as well as longer down hair. The nature of the defects detected in the hides as well as the frequency of their occurrences has been similar and suggests improper initial treatment.

OCENA JAKOŚCI SKÓR LISÓW POSPOLITYCH (*VULPES VULPES*) POZYSKANYCH W DWÓCH REJONACH POLSKI NA PODSTAWIE WYBRANYCH WSKAŹNIKÓW

Paweł Janiszewski, Andrzej Gugołek, Marta Kowalewska, Justyna Cilulko

Katedra Hodowli Zwierząt Futerkowych i Łowiectwa
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: *Vulpes vulpes*, jakość skór, długość włosa.

Address: Paweł Janiszewski, University of Warmia and Mazury in Olsztyn, ul. Michała Oczapowskiego 5, 10-917 Olsztyn, Poland, phone: +48 (89) 523 44 42, janisz@uwm.edu.pl

A b s t r a k t

Celem pracy była charakterystyka i porównanie skór lisów pospolitych pozyskanych w wyniku odstrzału redukcyjnego przez myśliwych z olsztyńskiego i warszawskiego okręgu Polskiego Związku Łowieckiego. Materiał do badań stanowiło 100 skór lisów pospolitych. Stwierdzono, że średnia masa i długość skór potwierdza występowanie dymorfizmu płciowego u lisów pospolitych, natomiast teren pozyskania nie wpłynął na badane parametry. Stopień wysrebrzenia skóry nie jest uzależniony od płci lisa i rejonu bytowania. Wykazano, że dłuższymi włosami pokrywowymi i puchowymi charakteryzowały się samce oraz osobniki pochodzące z olsztyńskiego okręgu Polskiego Związku Łowieckiego. Częstotliwość i charakter stwierdzonych wad skór była podobna i wskazuje także na niewłaściwą obróbkę wstępną skóry.

Introduction

There exist numerous subspecies of the *Vulpes vulpes* and they inhabit nearly entire Northern Hemisphere. Depending on the geographical location they may differ in colour and body size and quality of the hair coat. In Poland there occurs the *Vulpes vulpes crucigera.* The predominant fox in Poland is the common fox with various intensity of colouration. Far less common is the fox of the cross colour variant (GOSZCZYŃSKI 1995). The *Vulpes vulpes* is also common in breeding farms with melanistic silver fox being the most popular colour variant. Apart from that numerous other variants have been farm-bred (e.g. gold, platinum, ring neck, pastel) (NES et al. 1989).

The fox hunting season in Poland is between 1st June and 31st March. The majority of shooting however takes place between November and the end of February. Various methods of hunting are allowed. The technique, weapons and ammunition used determine the quality of hides (GOSZCZYŃSKI 1995).

According to The Polish Hunting Association records (www.pzlow.pl) in the season 2008/2009 most foxes were hunted in the Province of Wielkopolska (over 20 000). In the provinces of Mazowsze as well as Warmia and Mazury 12 993 and 10 383 were shot respectively.

For the fur industry the common fox pelt is a valuable material of universal use. The examples include making coats, hats and women's coat collars. The pelts are either dyed or used in their natural form (DUDA 1992).

The aim of the paper was to characterise and compare the pelts of the *Vulpes vulpes* obtained through the density reduction shooting by the hunters of the Olsztyn and Warsaw districts of The Polish Hunting Association.

Materials and Methods

The study material was the pelts of the *Vulpes vulpes* obtained in the season 2007/2008 by the hunters of the Olsztyn and Warsaw districts (as classified by The Polish Hunting Association.) Data of a hundred pelts have

been collected, out of which fifty originated from the Olsztyn district and another fifty from the Warsaw district. In either group there have been twenty five pelts of male foxes and twenty five pelts of female foxes. The pelts were chosen randomly in both areas, and after the hunting season ended they were tanned at a tanner's in central Poland. The hides were only evaluated after they had been tanned, and therefore the differences which might result from the various method of treatment such as tightening, forming and drying were minimized (PIÓRKOWSKA 1998).

During the research the physical parameters of the hides such as the weight and the length were defined. The length of both the tail and the white spotted tail tip were measured and the extent of the silvering effect was defined. The weight was measured with electronic scales to an accuracy of one gram. The length of the tail and the tail tip were measured with a tape measure to an accuracy of one centimetre. The silvering effect was given in percentage terms.

Also measured were both the underfur and guard hair length. The measurements were taken in nine points on the left side of the skin – three in each of the following areas: the front, the middle and the back. To define these points the findings of other fur animal researchers' were modified and then used (BARABASZ et al. 2000, PIÓRKOWSKA 2008). The points are illustrated in Figure 1. The measurements were taken to the accuracy of one millimetre.

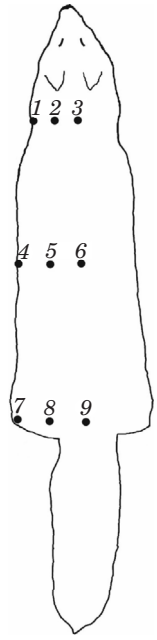

Fig. 1. Hair length measuring points. Explanations are in the text

The numerical data obtained was subjected to a statistical analysis based on a single-factor orthogonal analysis of variance (STATISTICA 8.0 PL).

Results and Discussion

All of the pelts examined from both sources have been classified as the typical red fox in its dark colour variant. The physical parameters of the pelts evaluated are presented in Table 1.

Table 1

Physical parameters of pelts ($\bar{x} \pm$ SD), $n = 50$

Measurement	Sex		District	
	♂	♀	Warsaw	Olsztyn
Pelt weight (g)	$320.84^{A} \pm 44.12$	$257.51^{B} \pm 42.47$	294.80 ± 52.10	282.28 ± 54.76
Pelt length (cm)	$85.24^{A} \pm 5.72$	$81.10^{B} \pm 5.20$	83.24 ± 5.48	83.02 ± 6.20
Length of white tail tip (cm)	7.35 ± 2.47	6.95 ± 2.37	6.96 ± 2.23	7.37 ± 2.60
Extent of silvering effect (%)	38.58 ± 7.99	39.23 ± 8.58	39.21 ± 5.71	38.62 ± 10.24

A, B - $p \leq 0.01$

The results obtained from the pelts weight measurements have led to the conclusion that the pelts of male foxes are statistically significantly heavier than the pelts of vixen. This may result from the sexual dimorphism present in the species *Vulpes vulpes* (GOSZCZYŃSKI 1995). The above thesis is supported by the results of the skin length measurements. The pelts of male foxes were significantly longer in comparison with those of vixen. A similar difference in body weight, with male specimens heavier than female, was found by NOWICKI et al. (*2000*). It should, however, be noted that the pelts obtained from the farm-bred common foxes are noticeably longer (BRZOZOWSKI 2002). For instance the research by PIÓRKOWSKA (2008) shows the average weight of a tanned common fox hide (*Vulpes vulpes*) of the pastel colour variant was 378 grams and the length was 101 centimetres. The statistically significant occurrence of sexual dimorphism in farm-bred foxes was proven by LOREK et al. (2001). Moreover, PIÓRKOWSKA (1998) established that the tanned hides obtained from male specimens of another species – the arctic fox – were 11 per cent heavier than those obtained from the females.

No statistically significant difference has been observed between the pelts with regard to the area of acquisition. This may indicate that there exist no differences in body size between the fox population in Warmia and Mazury and Mazowsze (Warsaw) regions.

A characteristic of the *Vulpes vulpes* is the white tail tip. The feature is a subject of selection in the farm-bred fox populations (*Wzorzec oceny*... 1998). In wild fox populations the white tail tip is less pronounced and tends to be more changeable (GOSZCZYŃSKI 1995, NES et al. 1989). In the hides examined no difference in the length of the white tip has been observed either between sexes or the regions studied. The average length is seven centimetres (Table 1).

Also characteristic of the common fox (*Vulpes vulpes*) regardless of its colour variant is the silvering effect, or guard hair depigmentation. Depending on the extent of trunk depigmentation common foxes can be classified as being 0, 25, 50, 75 and 100 per cent silver (DUDA 1992, *Wzorzec oceny*... 1998). The extent of the silvering effect in the skins examined is similar in male and female specimens and fluctuates between 38.58 and 39.23 per cent. There is also no significant difference between the regions and the extent of silvering effect amounted to 39.21 per cent and 38.62 per cent in the Warsaw and Olsztyn districts respectively.

Table 2 shows the guard hair length of the examined skins. It has been observed that the male *Vulpes vulpes* specimens are marked by longer coat than the female specimens at the measurement points located at the front (point 1, 2 and 3) as well in the middle of the pelt (points 4, 5 and 6). These differences are a result of sexual dimorphism and have been proven highly significant. The male foxes are characterized by longer guard hairs on the neck that form the so called mane. The same phenomenon occurs in farm-bred foxes but it is of lesser intensity. This is considered undesirable as fox farm-breeding aims for the evenness in the guard hair length throughout the animal's body (*Wzorzec oceny*... 1998).

Table 2

Guard hair length ($\bar{x}$ ± SD)

Measurement point	Sex		District	
	♂	♀	Warsaw	Olsztyn
1.	6.32^{A} ± 1.27	5.33^{B} ± 1.10	5.59 ± 1.22	6.03 ± 1.31
2.	6.32^{A} ± 1.19	5.38^{B} ± 1.05	5.60^{b} ± 1.14	6.08^{a} ± 1.24
3.	6.24^{a} ± 1.24	5.35^{B} ± 1.14	5.52^{b} ± 1.24	6.04^{a} ± 1.24
4.	5.56^{A} ± 1.14	4.92^{B} ± 1.04	5.10 ± 1.06	5.42 ± 1.17
5.	5.51^{A} ± 1.15	4.93^{B} ± 1.00	4.99^{b} ± 1.04	5.44^{a} ± 1.14
6.	5.59^{A} ± 1.15	4.97^{B} ± 1.07	5.06 ± 1.10	5.49 ± 1.16
7.	7.89 ± 1.21	4.49 ± 0.95	4.44^{b} ± 1.02	4.93^{a} ± 1.12
8.	4.89 ± 1.10	4.51 ± 0.49	4.43^{b} ± 0.92	4.91^{a} ± 1.14
9.	4.82 ± 1.22	4.43 ± 0.95	4.43^{B} ± 1.02	4.91^{A} ± 1.14
$\bar{x}$	5.51^{A} ± 1.18	4.94^{B} ± 1.02	4.99^{B} ± 1.07	5.44^{A} ± 1.17

A, B ≤ 0.01; *a, b* ≤ 0.05

The guard hair length measurements demonstrate that the foxes from the Olsztyn hunting district are characterized by longer hair coat in comparison with those of the Warsaw district, the observation being scientifically significant. This might indicate the foxes' adaptation to a harsher climate (BOBEK et al. 1984).

The length of underfur hairs is shown in Table 3. It has been marked that in most measurement points the down hairs on skins of male foxes were noticeably longer than those on the skins of female foxes. This has been proven to be of high statistical significance. The foxes obtained in the Olsztyn district as classified by The Polish Hunting Association are characterized by longer underfur hair than those obtained in central Poland. Also PIÓRKOWSKA (2008) proved that the underfur hairs in the common fox (*Vulpes vulpes*) of the pastel colour variant are statistically longer in male specimens than in female ones.

Table 3

Underfur hair length ($\bar{x}$ ± SD)

Measurement point	Sex		District	
	♂	♀	Warsaw	Olsztyn
1.	1.46 ± 0.54	1.31 ± 0.41	1.32 ± 0.51	1.45 ± 0.45
2.	1.51^{a} ± 0.46	1.33^{b} ± 0.35	1.36 ± 0.43	1.47 ± 0.41
3.	1.56^{A} ± 0.46	1.31^{B} ± 0.40	1.34^{b} ± 0.45	1.52^{a} ± 0.43
4.	1.83^{a} ± 0.52	1.59^{b} ± 0.41	1.60^{b} ± 0.50	1.82^{a} ± 0.44
5.	1.89^{A} ± 0.45	1.63^{B} ± 0.43	1.68^{b} ± 0.46	1.84^{a} ± 0.44
6.	1.87^{A} ± 0.48	1.63^{B} ± 0.43	1.65^{b} ± 0.49	1.84^{a} ± 0.44
7.	2.18^{A} ± 0.51	1.89^{B} ± 0.45	1.90^{B} ± 0.46	2.16^{A} ± 0.51
8.	2.22^{A} ± 0.55	1.94^{B} ± 0.45	1.96^{b} ± 0.51	2.19^{a} ± 0.51
9.	2.24^{A} ± 0.53	1.95^{B} ± 0.45	1.95^{B} ± 0.48	2.24^{A} ± 0.51
$\bar{x}$	1.81^{A} ± 0.52	1.62^{B} ± 0.42	1.64^{B} ± 0.47	1.79^{A} ±0.46

A, B ≤ 0.01; *a, b* ≤ 0.05

According to DUDA (1992) the length of guard hairs in the common fox can fluctuate between 6 and 11 centimetres. In the case of the down hairs it is between 3 to 5.50 centimetres. PRZYSIECKI et al. (2004) show that the guard hair height in the farm-bred common foxes of gold variant is within 6.85 and 7.05 centimetres in male and within 6.38 and 6.70 centimetres in female specimens. As far as the down hairs are concerned the respective values are 3.25–3.37 centimetres and 3.11–3.21 centimetres.

Table 4 presents the defects in the pelts examined. In the case of 21 pelts (which amount to 11.2 per cent of the total number) felted hair coating in the back regions has been observed. The degree to which the coat is felted is not

very noticeable, and the defect can be repaired through combing. Another defect that has been noticed is the bald areas on the fur. This is due to the local decay that can form because of improper drying or fleshing processes. Similar instances of defects are noted by DUDA (1992) as the ones most frequently found in the skins of the *Vulpes vulpes*. Furthermore, the defects are more frequently observed in the pelts obtained by hunting than those obtained from the breeding farms.

Table 4

Pelt defects

Type of defect	District				Total	
	Warsaw		Olsztyn			
	number	%	number	%	number	%
Felted hair areas	11	9.65	10	13.70	21	11.23
Bald areas	9	7.89	4	5.48	13	6.95
Blood stained areas	37	32.46	25	34.25	62	33.15
Improper fleshing result	14	12.28	17	23.29	31	16.58
Shot holes	19	16.67	5	6.85	24	12.83
Rips	13	11.40	6	8.22	19	10.16
Lack of tail	6	5.26	4	5.48	10	5.35
Lack of limb	5	4.39	2	2.74	7	3.74
Total	114	100	73	100	187	100

The pelt defect of highest occurrence is the blood stained areas, which are noticeable even after tanning. The blood stains are most often located in the rear parts of the pelts on the abdomen or limbs. This particular defect indicates that the hunters have not been using blood absorbents such as sawdust.

Improper fleshing that leads to hair bulb damage has been identified in as many as 31 pelts (which amounts to 16.58 per cent of their total number). What has also been observed is high occurrence of pelt rips and holes due to the methods of hunt used. In ten of the pelts the tails have been missing; in seven there have been limbs missing. The defects have been caused by the negligent or unskilful processing and preserving of the raw pelts. On the basis of the data obtained the conclusion has been drawn that the defects are of similar occurrence in both regions analysed.

Based on the research conducted with regard to the fox pelts obtained in the Olsztyn and Warsaw hunting areas (as defined by the Polish Hunting Association) it has been concluded that the average weight and length of the pelts confirms the occurrence of sexual dimorphism in the common fox (*Vulpes vulpes*). The area of origin did not affect the examined parameters. The extent

of the silvering effect on the pelt does not depend on the sex or the habitat of the fox. It has also been observed that the male foxes and the specimens originating from the Olsztyn hunting area (as classified by the Polish Hunting Association) were characterised by a longer hair coat. The high frequency of occurrence and the nature of the observed pelt defects indicate improper initial treatment of pelts.

Translated by MAŁGORZATA KURPIEWSKA

Accepted for print 10.11.2010

References

BARABASZ B., BAGDONAS I.I., PIÓRKOWSKA M., KORCZYŃSKA M. 2000. *Badania parametrów okrywy włosowej norek po implantacji melatoniny*. Zesz. Nauk. Prz. Hod., 53: 195–204.

BOBEK B., MOROW K., PERZANOWSKI K. 1984. *Ekologiczne podstawy łowiectwa*. PWRiL, Warszawa.

BRZOZOWSKI M. 2002. *Zmiany pokroju mięsożernych zwierząt futerkowych jako efekt pracy hodowlanej*. Zesz. Nauk. Prz. Hod., 66: 81–86.

DUDA I. 1992. *Skóry surowe futrzarskie*. Wydawnictwo Akademii Ekonomicznej. Kraków.

GOSZCZYŃSKI J. 1995. *Lis*. Oficyna Wydawnicza OIKOS, Warszawa.

Kopański R. 1976. *Obróbka skór łownych zwierząt futerkowych*. PWRiL, Warszawa

Lorek M.O., Gugołek A., Hartman A. 2001. *Studies on the relationship between body weight, trunk lenght and pelt size in common foxes (Vulpes vulpes)*. Czech J. Anim. Sci., 46:481-484.

Łowiectwo. 1994. Ed. P. Havet. Oficyna Wydawnicza Delta W-Z. Warszawa, 335 pp.

NES N.N., EINARSSON E.J., LOHI O., JORGENSEN G. 1989. *Beautiful Fur Animals and their colour genetics*. Scientifur, Hillerod.

NOWICKI W., BRUDNICKI W., JABŁOŃSKI R., WILAND C. 2000. *Charakterystyka populacji lica (Vulpes vulpes L.) w okręgu bydgoskim*. Materiały z konferencji: *Zwierzyna drobna jako element bioróżnorodności środowiska przyrodniczego*, Włocławek, 200–208.

PIÓRKOWSKA M. 1996. *Relation between the body weight of arctic foxes and physical parameters of their pelts*. Apel. Sci. Rep., Prog. Animal Sci., 167–174.

PIÓRKOWSKA M. 1998. *Charakterystyka parametrów fizycznych skór surowych i wyprawionych. Aktualne badania w hodowli zwierząt futerkowych*. Sympozjum naukowe. Kazimierz Dolny, 139–140.

PIÓRKOWSKA M. 2008. *Ocena jakości okrywy włosowej lisów pastelowych utrzymywanych w Zakładzie Doświadczalnym IZ-PIB Chorzelów*. Rocz. Nauk. PTZ, 3(4): 271–277.

Polski Związek Łowiecki, http://www.pzlow.pl, access: 1.07.2010.

PRZYSIECKI P., NOWICKI S., PAWLAK F., NAWROCKI Z., STANISŁAWSKI D. 2004. *Wpływ niektórych zabiegów zootechnicznych na użytkowość produkcyjną lisów pospolitych płomienistych*. Zesz. Nauk. Prz. Hod., 72(6): 111–117.

Wzorzec Oceny Lisów Pospolitych. 1998. Centralna Stacja Hodowli Zwierząt, Warszawa.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 360–368, Y. 2010

DOI 10.2478/v10020-010-0033-7

CARCASS QUALITY AND PRODUCTION RESULTS OF GEESE FATTENED ON OAT FOLLOWING A RESTRICTED FEEDING REGIME

Daria Murawska, Danuta Michalik, Roman Bochno, Włodzimierz Makowski

Department of Commodity Science and Animal Improvement
University of Warmia and Mazury in Olsztyn

K e y w o r d s: geese, restricted feeding, oat fattening, carcass quality, feed consumption, feed cost.

A b s t r a c t

The aim of this study was to determine the carcass quality and production results of geese fattened on oat following a restricted feeding regime. The experimental materials comprised White Kołuda geese (56 ♂ and 56 ♀) reared to 15 weeks of age. During the first 12 weeks, the amount of feed given to birds was limited by 20% in comparison with the control ration, as follows: group II – from 7 to 12 weeks, group III – from 2 to 6 weeks, and group IV – from 2 to 12 weeks of age. Over the finishing period of three weeks (week 13 to 15), geese of all groups were fed oat grain *ad libitum*. At 15 weeks of age, the birds were slaughtered and carcass quality was evaluated.

Group II geese, compared with control group birds, were characterized by significantly higher body weight (6566 g vs. 6043 g), but their carcasses had a lower lean meat content (44.55% vs. 47.19%) and a higher content of skin including subcutaneous fat (36.00% vs. 32.33%). In comparison with the control group, group II geese consumed significantly less oat per kg body weight gain (9.36 kg vs. 11.56 kg) and less feed per kg body weight (3.86 kg vs. 4.31 kg), which resulted in a lower feed cost per kg body weight in group II. The lean meat content of the carcass was similar in the control group and in groups III and IV (47.19%, 45.26% and 45.13% respectively). However, group III and IV geese were characterized by significantly higher carcass fatness than control group birds (35.76%, 35.23% and 32.33% respectively), and they consumed larger amounts of feed per kg body weight. Feed cost was comparable in the above groups.

Address: Daria Murawska, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-718 Olsztyn, Poland, phone: +48 (89) 523 41 28, e-mail: daria.murawska@uwm.edu.pl

JAKOŚĆ TUSZKI I WYNIKI ODCHOWU GĘSI DOTUCZANYCH ZIARNEM OWSA PO OKRESIE ŻYWIENIA RESTRYKCYJNEGO

Daria Murawska, Danuta Michalik, Roman Bochno, Włodzimierz Makowski

Katedra Towaroznawstwa Ogólnego i Doświadczalnictwa
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: gęsi, restrykcyjne żywienie, dotucz owsem, wartość rzeźna, zużycie paszy, koszty paszy.

A b s t r a k t

Celem podjętych badań była analiza jakości tuszki oraz wyników odchowu gęsi dotuczanych ziarnem owsa, żywionych wcześniej ograniczonymi ilościowo dawkami mieszanek przemysłowych. Badania wykonano na gęsiach Białych Kołudzkich (56 ♂ i 56 ♀) odchowywanych do wieku 15 tygodni. W okresie żywienia paszowymi mieszankami przemysłowymi (do wieku 12 tyg.) zastosowano w trzech różnych okresach wiekowych dawki żywieniowe ograniczone ilościowo o 20% w stosunku do ilości paszy pobranej przez ptaki z grupy kontrolnej (grupa I): od 7. do 12. tygodnia (grupa II), od 2. do 6 tygodnia (grupa III) oraz od 2. do 12. tygodnia (grupa IV). Następnie od 13. do 15. tygodnia odchowu gęsi ze wszystkich grup żywiono *ad libitum* ziarnem owsa. W wieku 15. tygodni ptaki poddano ubojowi i ocenie wartości rzeźnej.

Stwierdzono, że gęsi z grupy II w porównaniu z ptakami grupy kontrolnej charakteryzowała istotnie większa masa ciała (odpowiednio 6566 i 6043 g), lecz ich tuszki zawierały mniej mięsa (odpowiednio 44.55 i 47.19%), a więcej skóry z tłuszczem podskórnym (36.00 i 32.33%). Ptaki z tej grupy, w stosunku do kontrolnej, zużyły istotnie mniej owsa na 1 kg przyrostu masy ciała (9.36 i 11.56 kg) i paszy łącznie w przeliczeniu na 1 kg masy ciała (3.86 i 4.31 kg), co skutkuje mniejszymi kosztami paszy poniesionymi na uzyskanie 1 kg masy ciała. Ptaki z grup III i IV charakteryzowało podobne umięśnienie do ptaków z grupy kontrolnej (45.26; 45.13 i 47.19%), lecz istotnie większe otłuszczenie tuszki (35.76; 35.23 i 32.33%) i większe zużycie paszy przy równoczesnych podobnych kosztach paszy w przeliczeniu na 1 kg masy ciała.

Introduction

The oat-fattened goose is one of the specialties of Polish agriculture. Due to its unique amino acid composition of protein and a high biological value, oat is an important component of diets for geese. The properties of oat were investigated in detail 80 years ago. During the finishing period of three weeks, geese are fattened on oat grain (KSIĄŻKIEWICZ 2006). The type and amount of feed determine both carcass quality and production profitability, which is why efforts are made to reduce the overall costs of goose fattening (BIELIŃSKA et al. 1984, 2002). According to BIELIŃSKA and CZECHLOWSKA (1999), an optimal solution enabling to both decrease production costs and maintain high carcass quality is to feed geese concentrate with a reduced total protein content for the first 12 weeks. The results of other studies (BIELIŃSKA et al. 1980, BOCHNO and BRZOZOWSKI 1992, JANISZEWSKA et al. 2000 and 2002, BOCHNO et al. 2007)

conducted on young slaughter geese show that temporary feed restriction, compared with *ad libitum* feeding, allows to decrease carcass fat content and to improve feed conversion efficiency. In an experiment performed by BIELIŃSKA et al. (1980), White Italian geese were given a daily ration limited by 15% from 4 to 8 weeks of age, which led to a slower growth rate and a prolonged rearing period, but had a beneficial effect on breast muscle yield and meat quality. However, more severe feed restriction (30%) caused emaciation. BOCHNO and BRZOZOWSKI (1992) limited the amount of feed offered to young slaughter geese by 20% (in comparison with *ad libitum* feeding) and reported a lower fat content of the carcass, a more desirable meat to fat ratio and lower feed consumption. The results of later studies were similar (BOCHNO et al. 2007).

The positive effects of feed restriction programs noted in young geese and a scarcity of information regarding the combination of restricted feeding and satiation feeding prompted the authors to conduct the present study, aimed to determine the carcass quality and production results of geese fattened on oat following a restricted feeding regime.

Materials and Methods

The experimental materials comprised a total of 112 White Kołuda geese (56 ♂ and 56 ♀) randomly divided into four feeding groups, each of two pens of males and two pens of females. The birds were reared to 15 weeks of age. During the first 12 weeks, geese were fed commercial diets, starter (to 5 weeks) and grower/finisher (from 6 to 12 weeks), containing 20.16% and 19.14% total protein and 12.34 MJ and 12.10 MJ metabolizable energy respectively. From 13 to 15 weeks of age, birds of all groups were fed *ad libitum* oat grain with a total protein content of 8.88% and a metabolizable energy content of 10.12 MJ. Over the first week of rearing, all birds were fed to appetite, and from week 2 to 12 the amount of feed offered was as follows:

– group I – continuation of *ad libitum* feeding (control);

– group II – continuation of *ad libitum* feeding until week 6, followed by feeding a ration restricted by 20% (in comparison with the control diet) until week 12;

– group III – feeding a ration restricted by 20% until week 6, followed by *ad libitum* feeding in the amount not higher than in the control group;

– group IV – feeding a ration restricted by 20% (in comparison with the control diet) from week 2 to 12.

At the completion of the rearing period, ten males and ten females were selected randomly for slaughter and post-slaughter analysis. Chilled carcasses were dissected. The collected data were used to calculate:

– feed cost (based on purchase prices of March 2008) per kg body weight,

– carcass dressing percentage as total body weight and giblets weight expressed as a percentage of live weight.

The statistical analysis (STATISTICA 8.0) included:

– the characteristics of the analyzed traits ($\bar{x}$, v),

– feed consumption per bird, feed, total protein and metabolizable energy intake per kg body weight, carcass weight and lean weight,

– the determination of the significance of differences between feeding groups with respect to the mean values of feed consumption and carcass quality; a two-factorial analysis of variance with two elements in subgroups (feed consumption and cost) or ten elements in subgroups (carcass quality parameters).

Results and Discussion

Body weight

During oat fattening, geese subjected previously to feed restriction (groups II–IV) were characterized by a faster growth rate than control group birds (group I). The body weight of geese of experimental groups II, III and IV increased over that period by 1355, 1098 and 1369 g respectively, compared with 647 g in the control group. Due to the above gains, the live body weight of group II and III birds before slaughter was 6566 g and 6335 g respectively, compared with 6043 g in the control group ($p \leq 0.05$, Table 1). The live weight of group IV geese (6109 g) was similar to the live weight of control group birds, but the former were lighter ($p \leq 0.05$) than group II and III geese. Rapid weight gain following a period of feed restriction has been previously observed in chickens, turkeys (PLAVNIK and HURVITZ 1991), ducks (WILKIEWICZ-WAWRO 1994, SZEREMETA et al. 2000) and geese (BOCHNO and BRZOZOWSKI 1992, JANISZEWSKA et al. 2000, BOCHNO et al. 2007).

Females, compared with males, had lower body weight, which is consistent with the results of earlier studies of that species (BIELIŃSKA et al. 1980, 2002, MAZANOWSKI and BERNACKI 1998 a,b, JANISZEWSKA et al. 2000).

Feed consumption and cost

As expected, feed consumption per bird during the first 12 weeks was highest in the control group (21.73 kg) and lowest in group IV (18.05 kg). Feed intake was lower in group II than in group III (18.57 kg vs. 20.66 kg).

Table 1

Body weight of geese, feed consumption and cost

Specification	Statistical measures	Group				Sex	
		I	II	III	IV	♂	♀
Body weight (g) at the age of:							
12 weeks	$\bar{x}$	5396^{A}	5209^{A}	5237^{A}	4740^{B}	5302*	5030
	v	12.71	9.69	8.92	9.63	11.94	10.14
15 weeks	$\bar{x}$	6043^{a}	6566^{b}	6335^{b}	6109^{a}	6506*	6036
	v	14.63	11.41	11.55	10.52	11.93	11.46
Feed consumption [kg/bird]	$\bar{x}$	21.73^{A}	18.57^{Ba}	20.66^{A}	18.05^{Bb}	20.15*	19.34
Feed and oat consumption [kg/bird]	$\bar{x}$	32.53	31.35	33.01	32.11	32.56	31.85
Oat consumption [kg] per kg body weight gainbetween week 13 and 15	$\bar{x}$	11.56^{a}	9.36^{b}	10.39^{ab}	9.32^{b}	9.80	10.37
Total feed and oat consumption [kg] per kg body weight gain	$\bar{x}$	4.40^{A}	3.96^{B}	4.81^{C}	4.76^{C}	4.43	4.55*
Feed cost [PLN] per kg body weight	$\bar{x}$	5.23^{A}	4.48^{B}	5.35^{A}	5.20^{A}	5.01	5.12*

Means followed by different superscript letters (feeding groups) or by* (sex) are significantly different: capital letters or ** – at $\alpha = 0.01$, small letters or * – at $\alpha = 0.05$.

Restricted feeding, in comparison with *ad libitum* feeding, affected not only the final body weight of geese aged 15 weeks, but also oat consumption and conversion levels. It should be stressed that birds of experimental groups were more efficient users of oat grain. Oat consumption per kg body weight gain was significantly lower in groups II and IV (9.36 and 9.32 kg respectively) than in the control group (11.56 kg, Table 1). Group III geese consumed less oat (10.39 kg, Table 1) than control group geese, but more than birds of the remaining groups. The above indicates that feed restriction from week 2 or 6 to 12 has a beneficial influence on oat intake. Significant differences were also noted with respect to total feed consumption per kg body weight gain over the entire experiment. The lowest feed conversion ratio was reported in group II (3.96 kg kg^{-1}), and highest – in groups III and IV (4.8 kg kg^{-1}), compared with 4.40 kg kg^{-1} in the control group.

Feed cost per kg body weight was significantly lower in group II (PLN 4.48) than in the control group (PLN 5.23) and in the remaining experimental groups (above PLN 5.20, Table 1). The total cost of feed consumed in groups III and IV reached 102.3% and 99.4%, respectively, of that noted in the control group. Those results suggest that feeding geese aged 7 to 12 weeks a ration reduced by 20%, in comparison with the control diet, may contribute to a significant decrease in the overall production costs of oat-fattened geese.

Feed consumption per kg body weight, depending on feeding levels at different rearing stages (Table 2), was similar to feed consumption per kg body

weight gain (Table 1). Over the entire experiment, group II geese consumed by approximately 12% less feed per kg body weight than birds of the remaining groups. Feed consumption per kg carcass weight was significantly lower in group II (6.42 kg) than in the control group (7.29 kg) and in groups III and IV (above 7.54 kg). A similar trend was observed with regard to feed consumption per kg lean meat weight.

Table 2

Feed [kg], total protein [g] and metabolizable energy [MJ] intake per kg body weight, carcass weight and lean weight

Specification	Group				Sex	
	I	II	III	IV	♂	♀
Feed intake [kg] per kg:						
body weight	4.31^{A}	3.86^{B}	4.71^{C}	4.66^{C}	4.34	4.45*
carcass weight	7.29^{b}	6.42^{a}	7.54^{b}	7.56^{b}	7.17	7.23
lean meat weight	15.86^{ABa}	14.82^{Aa}	17.13^{Bb}	17.09^{Bb}	16.19	16.32
Total protein intake [g] per kg:						
body weight	779.9^{AB}	664.4^{B}	802.4^{Aa}	770.4^{Ab}	746.6	759.4
carcass weight	1314.8^{B}	1103.7^{Aa}	1284.1^{Bb}	1249.5^{b}	1234.4	231.2
lean meat weight	2859.5^{A}	2548.9^{Bb}	2916.2^{A}	2823.5^{a}	2785.0	2779.0
Metabolizable energy intake [MJ] per kg:						
body weight	51.1^{A}	45.4^{B}	54.5^{A}	53.6^{A}	51.0	51.3
carcass weight	86.1^{a}	75.4^{b}	87.3^{a}	86.9^{a}	84.2	83.3
lean weight	187.3^{AB}	171.1^{A}	198.2^{B}	196.5^{B}	190.1	188.0

Means followed by different superscript letters (feeding groups) or by* (sex) are significantly different: capital letters or ** – at $\alpha = 0.01$, small letters or * – at $\alpha = 0.05$.

Total protein and metabolizable energy intake per kg body weight, carcass weight and lean meat weight was also lower in group II than in the control group and in groups III and IV (Table 2). Total protein and metabolizable energy intake per kg body weight was by respectively 116 g and 5.7 MJ lower in group II than in the control group. Compared with the control group, group II geese needed also less protein (by 311 g) and metabolizable energy (by 16 MJ) to produce 1 kg lean meat.

Carcass quality

At the end of the oat fattening period, the average carcass weight of geese subjected to early feed restriction was slightly higher (≥ 3834 g) than the carcass weight of control group birds (3613 g, Table 3). The weights of abdominal fat, gizzard and liver were also higher in experimental groups.

Heart weight was significantly ($p \leq 0.05$) higher in group II than in group I. Carcass dressing percentage was similar in all groups (from 68.63% to 69.04%).

Table 3

Carcass quality parameters (arithmetic means and coefficients of variation)

Specification	Statistical measures	Group				Sex	
		I	II	III	IV	♂	♀
Carcass weight [g]:							
hot	$\bar{x}$	3613	3899	3961	3834	3938	3710
	v	13.27	9.81	8.95	12.43	12.44	9.97
cold	$\bar{x}$	3547	3812	3885	3762	3851	3646
	v	13.43	9.10	9.04	12.56	12.37	10.12
Carcass dressing percentage (%)	$\bar{x}$	68.50	68.63	68.92	69.04	68.56	68.94
	v	2.98	3.15	2.14	2.61	3.09	2.30
Total giblets weight including: [g]	$\bar{x}$	335.6	383.7	368.7	367.9	387.3	340.5
	v	13.96	12.62	11.17	15.21	13.34	11.14
liver	$\bar{x}$	90.6	107.9	112.2	109.0	111.9	97.7
	v	27.15	17.40	27.75	24.29	28.35	20.12
heart	$\bar{x}$	36.6^{a}	43.6^{b}	41.1	38.2	41.6	37.9
	v	21.01	16.65	17.76	13.38	17.45	18.18**
gizzard	$\bar{x}$	208.4	232.2	215.4	220.8	233.9	204.9
	v	14.97	16.19	10.56	16.27	14.36	11.99
Abdominal fat weight [g]:	$\bar{x}$	291.0	332.9	333.2	308.8	309.2	319.5
	v	30.32	30.57	23.33	27.11	31.15	24.86
Relative weight of carcass							
lean meat	$\bar{x}$	1667	1694	1755	1691	1749	1660
	v	10.53	8.40	8.51	10.77	9.08	9.50
skin and subcutaneous fat	$\bar{x}$	1159^{a}	1377^{b}	1392^{b}	1330	1329	1285
	v	23.61	16.56	14.18	18.19	21.55	17.17**
bones	$\bar{x}$	561	570	562	554	595	533
	v	10.06	14.18	11.05	16.73	12.52	10.39
Percentage content in carcass							
lean meat	$\bar{x}$	47.19^{a}	44.55^{b}	45.26	45.13	45.67	45.62
	v	5.55	5.92	5.85	6.60	6.69	5.90
skin and subcutaneous fat	$\bar{x}$	32.33^{a}	36.00^{b}	35.76^{b}	35.23^{b}	34.20	35.08
	v	11.45	11.48	8.25	10.27	11.96	10.25
bones	$\bar{x}$	15.93^{a}	14.96	14.45^{b}	14.71^{b}	15.50*	14.67
	v	9.72	10.82	5.97	9.11	9.53	9.17

Means followed by different superscript letters (feeding groups) or by* (sex) are significantly different: capital letters or ** – at $\alpha = 0.01$, small letters or * – at $\alpha = 0.05$.

Feeding geese a restricted ration prior to oat fattening resulted in significant differences in carcass tissue composition, compared with *ad libitum* feeding. The carcasses of group II geese, in comparison with group I birds, had a similar content of lean meat (1694 g vs. 1667g) and bones (570 g vs. 561 g), but a higher content of skin and subcutaneous fat (1377 g vs. 1159 g, $p \leq 0.05$). The percentage share of skin and subcutaneous fat was higher (by 3.67%), and that of lean meat lower (by 2.64%, Table 3) in group II. The carcasses of group III geese, compared with group I birds, had a similar percentage content of lean

meat (45.26% vs. 47.19%), but a higher percentage share of skin and subcutaneous fat (35.76 vs. 32.33%, Table 3). The carcasses of group IV geese, in comparison with the control group, had a similar content of lean meat, but a higher (by 2.9%) percentage share of skin and subcutaneous fat and a lower (by 1.22%) bone content ($p \leq 0.05$, Table 3).

Females, in comparison with males, had somewhat lower carcass weight as well as a lower content of the analyzed tissue components and insignificantly higher abdominal fat weight, which is consistent with the findings of other authors (BIELIŃSKA et al. 2002, MAZANOWSKI and BERNACKI 1998 b).

Conclusions

1. Group II geese (fed a restricted ration from week 7 to 12, prior to oat fattening), compared with control group birds, were characterized by significantly higher body weight (6566 g vs. 6043 g), but their carcasses had a lower lean meat content (44.55% vs. 47.19%) and a higher content of skin including subcutaneous fat (36.00% vs. 32.33%). In comparison with the control group, group II geese consumed significantly less oat per kg body weight gain (9.36 kg vs. 11.56 kg) and less feed per kg body weight (3.86 kg vs. 4.31 kg), which resulted in a lower feed cost per kg body weight. Total protein and metabolizable energy intake per kg body weight, carcass weight and lean weight was also lower in group II than in the control group.

2. The body weight and carcass tissue composition of group III geese (fed a restricted ration from week 2 to 6, prior to oat fattening) and group II birds were similar, but the former were characterized by significantly higher levels of total oat and feed consumption per kg body weight gain and higher intake of total protein and metabolizable energy. Feed cost per kg body weight was highest in group III.

3. The body weight and carcass lean meat content of group IV geese (fed a restricted ration from week 2 to 12, prior to oat fattening) and group I birds were similar (6109 g and 6043 g respectively and 45.13% and 47.19% respectively), but the former had a higher carcass fat content (35.23% vs. 32.33%). Total feed consumption was higher in group IV (4.76 kg vs. 4.40 kg), while feed cost per kg body weight was comparable in both groups.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 22.09.2010

References

Bielińska K., Bielińska H., Bieliński K., Trojan M., Pakulska E., Jamroz D., Krasiński D. 1980. *Effect of periodical limitation of feed consumption on geese broiler slaughter maturity*. Rocz. Nauk. Zoot., 7(2): 269–280.

Bielińska H., Czechlowska T. 1999. *Technologia odchowu i żywienia gęsi tuczonych*. Wyd. własne IZZZD, Kołuda Wielka.

Bielińska H., Koreleski J., Pakulska E., Rosiński A., Badowski J. 2002. *Effect of enzyme supplementation to ground barley–based diet fed from 5 to 14 weeks on rearing and fattening performance of White Koluda geese*. Rocz. Nauk. Zoot., supl., 16: 185–191.

Bielińska K., Skarżyński L., Bieliński K. 1984. *Effect of concentrates intake on fattening performance of 4-month-old pasture-raised geese*. Rocz. Nauk. Zoot., 11(2): 185–196.

Bochno R., Brzozowski W. 1992. *The influence of quantitative limitation of diet in various growing periods on feed utilization and carcass yield of white Italian geese*. Acta Acad. Agricult. Techn. Olst., Zoot., 37: 131–141.

Bochno R., Makowski W., Murawska D. 2007. *Effect of quantitatively restricted feeding on feed consumption and slaughter quality of young geese*. Pol. J. Natur. Sc., 22(2): 204–213.

Janiszewska M., Bochno R., Lewczuk A., Rymkiewicz J. 2000. *Changes in the body weight and slaughter value of White Kołudzka geese fed ad libitum or subjected to feed restriction*. Natur. Sc., 4: 147–159.

Janiszewska M., Bochno R., Lewczuk A., Rymkiewicz J. 2002. *Body weight and slaughter value of young geese fed ad libitum or given restricted feed*. Biul. Nauk. UWM, 17: 245–252.

Książkiewicz J. 2006. *Gęś Pomorska dawniej i obecnie*. Pol. Drob., 6: 42–45.

Mazanowski A., Bernacki Z. 1998a. *Results of oat fattening of 17- and 24-week-old crossbred geese from genetic reserve flocks compared with White Koludzka Geese*. Rocz. Nauk. Zoot., 25(4): 175–190.

Mazanowski A., Bernacki Z. 1998b. *Evaluation of meat traits of intensively reared crossbred geese from reserve flocks compared with White Koluda Geese*. Rocz. Nauk. Zoot., 25(4): 159–174.

Plavnik J., Hurvitz S. 1991. *Response of broiler chickens and turkey poults to food restriction of varied severity during early life*. Brit. Poult. Sci., 32: 343–352.

Szeremeta J., Bochno R., Brzozowski W. 2000. *Influence of restricted feeding on growth performance and slaughter value of young ducks*. Pol. J. Natur. Sc., 10(1): 153–162.

Wilkiewicz-Wawro E. 1994. *Effect of rearing and slaughter value of interspecific hybrid ducks fed ad libitum or a limited ration*. Acta Acad. Agricult. Techn. Olst., Zoot., 4 0, B: 3–37.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 369–375, Y. 2010

DOI 10.2478/v10020-010-0034-6

INFLUENCE OF DIFFERENT AREAL POLLUTION SOURCES ON SOME COMPOUNDS CONTENT IN WATER OF DEJGUNY LAKE

Danuta Domska, Marek Raczkowski, Krzysztof Stankiewicz

Chair of Foundations of Safety
University of Warmia and Mazury in Olsztyn

K e y w o r d s: Dejguny Lake, water, mineral compounds content.

A b s t r a c t

The purpose of the work was determination the area pollution influence on mineral compounds content in water of north and west part of Dejguny Lake. There was studied total-P, dissolved PO_4^{-3}(V), NH_4^+, NO_3^-, NO_2^-, total-Mg, total-Fe, SO_4^{-2} and Cl^- concentration in water. The most compounds content was in I class of water cleanness. The higher content of nitrate compounds was near the recreation area of Kronowo village and, ammonium and nitrate nitrogen, total phosphorus, ortho dissolved reactive phosphates and magnesium(II), near agricultural area depend on their content in water near the forested area. Moreover, in lake near agricultural area water side was higher content of sulphates(VI) in II class of water cleanness.

ODDZIAŁYWANIE OBSZAROWYCH ŹRÓDEŁ ZANIECZYSZCZEŃ NA ZAWARTOŚĆ NIEKTÓRYCH ZWIĄZKÓW W WODZIE JEZIORA DEJGUNY

Danuta Domska, Marek Raczkowski, Krzysztof Stankiewicz

Katedra Podstaw Bezpieczeństwa
Uniwersytet Warmińsko Mazurski w Olsztynie

S ł o w a k l u c z o w e: jezioro Dejguny, woda, zawartość substancji mineralnych.

Address: Danuta Domska, University of Warmia and Mazury, ul. Stefana Okrzei 1a, 10-266 Olsztyn, Poland, phone: +48 (89) 526 63 80, e-mail: danuta.domska@uwm.edu.pl

Abstrakt

Celem pracy było określenie wpływu obszarowych zanieczyszczeń na zawartość mineralnych związków w północnej i zachodniej części misy jeziora Dejguny. W badaniach oznaczono zawartość w wodzie P ogólnego, PO_4^{-3}(V) rozpuszczonych, NH_4^+, NO_3^-, NO_2^-, Mg ogólnego, Fe ogólnego, SO_4^{-2} i Cl^-. Stwierdzono, że nagromadzenie większości badanych związków było niskie i mieściło się w normach I klasy czystości wód. W porównaniu z ich zawartością w wodzie w okolicy zalesionej większe nagromadzenie związków azotu występowało przy terenie przeznaczonym na rekreację w pobliżu wsi Kronowo, a azotu amonowego i azotanowego oraz fosforu ogólnego, ortofosforanów rozpuszczonych i magnezu(II) przy gruntach użytkowanych rolniczo. W wodzie jeziora przy brzegu użytkowanym rolniczo znaleziono ponadto więcej siarczanów(VI) w ilościach odpowiadających II klasie czystości wód.

Introduction

The water properties, mainly the kind and quantity of the dissolved chemical substances are dicede about usefulness and possibilites to utilize for economy, living or recreation purposes (SZCZYGIELSKI 1996, KOC, SKONIECZYK 2007). In the case of the surface waters it depend not only on water kind, location of it or weather conditions but also on the area adaptation and the economy activity of this area. There is showing on the influence of the neighbouring area, particularly with agricultural activity or adapted for recreation, on some mineral compounds content (VOLLENWEIDER 1989, SZOSZKIEWICZ, SZOSZKIEWICZ 1997, RACZKOWSKI, WARECHOWSKA 2002, DOMSKA et al. 2005, PULIKOWSKI et al. 2005, DOMSKA et al. 2006, KOC, SIDORUK 2006). There is particularly dangerous too higher phosphorus and nitrate nitrogen content, not only on account of water quality deterioration but also on the risk to the degradation of the surface waters (SZOSZKIEWICZ, SZOSZKIEWICZ 1997).

The purpose of the work was the estimation of the different area pollution influence on phosphorus (total-P and PO_4^{-3} dissolved), mineral nitrogen (NO_3^-, NO_2^-, NH_4^+), soulphates(VI), chloride and some metal (Fe and Mg) content in water of some part of Dejguny Lake.

Experimental Procedures

The material of our investigation was Dejguny Lake (Figure 1) situeted about 8 km from Giżycko. It connected of the narrow canal with Mamry Lake, which is the second lake in Poland on account of the size. The maximum lenght of Dejguny Lake is 7.3 km, the breadth – 2.3 km, the surface – 8.36 ha, the average depth – 12 m and the maximum depth – 33 m (TOEPPEN 1995). The shore line is diversity, particularly flat in the south lake part and high, sometimes steep in the north lake part.

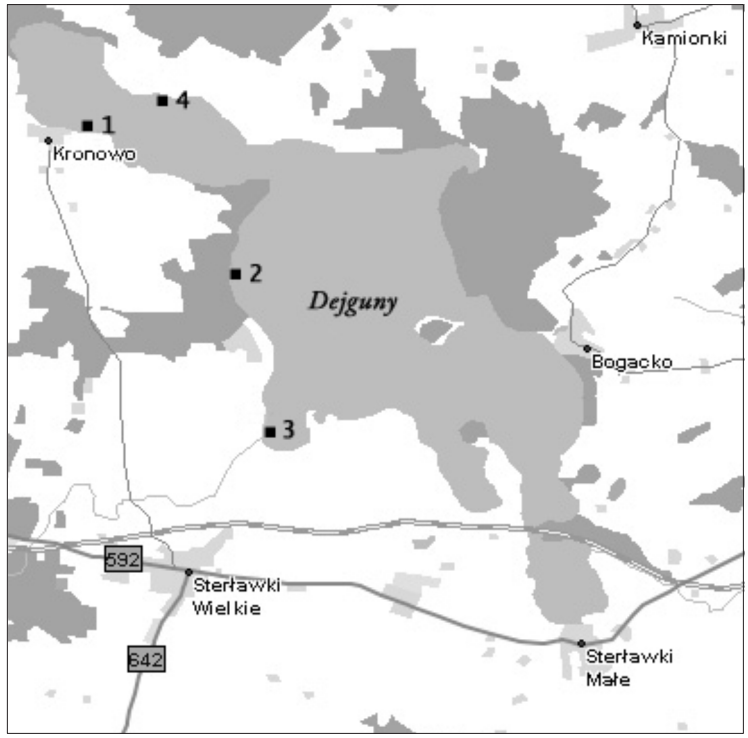

Fig. 1. Location of research sites: 1 – recreation area, 2 – forest, 3 – meadow, 4 – arable area

The studies were conducted in 2008 year. For determination of the area influence on water quality, there was sampled water in north and western part of the reservoir Dejguny Lake from 0.2 m depth and in place with lake depth not more than 1 m according to standard of *Jakość wody...* PN-EN ISO 5667-4. The water was sampled on April, every day, from 1-st to 5-th. The research sites location (Figure 1) was into direct contact with lake shore near the recreation area section of Kronowo village (site 1), forest (site 2) and agricultural areas – meadow and arable area (sites 3 and 4).

The study compounds content was determined immediately after water sampling. In the analytical methods have been used the universally accepted ways (DOJLIDO et al.): colometric (total-P, PO_4^{-3}, NO_2^-, Fe, Mg), titration (NH_4^+, Cl–), potentiometric (NO_3^-) and balance (SO_4^{-2}) analysis. The average results of 5 water samples were showed in the tables.

The obtained results was statistically calculated by Tukey test of the variant analyse at the considerable differences of the confidence interval $p = 0.05$.

Discussion of Results

There was showed that in time of the conducted studies were the significant different of some coumpunds content in water of Dejguny Lake (Table 1). Phosphorus coumpunds content, like to surface waters another water reservoirs (SZOSZKIEWICZ, SZOSZKIEWICZ 1997, DOMSKA et al. 2005) was low from 0.064 to 0.129 mg dm^{-3} of total phosphorus and from 0.03 to 0.26 mg dm^{-3} of phosphates. Total phosphorus and also reactive ortho-phosphates dissolved higher content was in lake water near agricultural areas (sites 3 and 4), particularly near meadow (site 3), but it was not higher than I class of water cleanness standard (Rozporządzenie MOŚ... 2004). DOMSKA et al. (2006) were showed higher phosphate content in water Narie Lake in II class of water cleanness near arable areas. BARTOSZEWICZ (2005) is also showing on the big influence of the intensive fertilized areas which are cultivated arable fields, on phosphate content in the surface waters. Moreover, PETTERSON and AMIARD (1998) and ZACHMAN et al. (2004) were showed the big influence of the lacustrine bottom deposits on the biogenes concentration in water, particularly in time of the summer stagnation with the higher bacteria activity. At the same time of the summer stagnation and of the anaerobic conditions, compounds of phosphorus connected with iron, after reduction Fe(III) to Fe(II) are set free from lacustrine bottom deposits to water bottomless and gradually migrated to surface waters.

Table 1

Phosphorus and nitrogen concentration in water of Dejguny Lake [mg dm^{-3}]

Research site	Total – P	Phosphates PO_4^{-3}	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$
1	0.064	0.003	0.020	0.124	0.124
2	0.083	0.003	0.030	0.060	0.060
3	0.129	0.029	0.260	0.224	0.224
4	0.100	0.015	0.200	0.250	0.200
LSD $p = 0.05$	0.019	0.008	0.009	0.010	0.005

The determined mineral nitrogen and phosphorus compounds content in Dejguny Lake water was low and it was in standard I class of water cleanness (Table 1). There was ammonium nitrogen content from 0.02 to 0.26 mg dm^{-3}, nitrate nitrogen(V) – from 0.06 to 0.25 mg dm^{-3} and nitrate nitrogen(III) – from 0.060 to 0.224 mg dm^{-3}. Depend on another studies (DOMSKA et al. 2005, 2006, WOJTAS, DĄBEK 2006) there was a relatively lot of nitrate nitrogen(V) content in Dejguny Lake water. The most ammonium and nitrate(III) nitrogen

content was in water near the lake shore with the perennial arable land, i.e. meadow (site 3) and nitrate nitrogen(V) content near arable field (site 4). The mineral nitrogen coumpounds content was also higher near the recreation area of Kronowo village (site 1) depend on the forest neighbourhood (site 2). Another authors were also showed the immediate influence of the agricultural areas, especially the ploughland or the intensive tourism on some mineral nitrogen coumpounds content. So, DOMSKA et al. (2005) were obtained a lot more nitrates content in water of Chełmżyński Lake with the shore near the recreation area. However, there one should to remember that nitrogen content in water much changed in year time (PULIKOWSKI et al. 2005). Among other things, WOJTAS and DĄBEK (2006) were remarked on the deciding influence of the vegetation time on nitrogen coumpounds content in water Olecko Wielkie Lake where nitrate nitrogen(III) and ammonium nitrate content decreased in spring and summer time and nitrate nitrogen(V) content was higher in summer time. Successively, DOMSKA et al. (2005) were showed that ammonium nitrogen content in water of the western part of Chełmżyński Lake reservoir was very low in the vegetation time and this nitrogen form was a little more in autumn time in water of lake shore near arable areas.

In the conducted studies was detrmined iron, magnesium(II) and chloride content in water of Dejguny Lake (Table 2). Iron content (0.20–0.25 mg dm^{-3}) and chloride content (13.0–15.5 mg dm^{-3}) was not different depend on the

Table 2
Iron, magnesium, sulphates and chlorides total concentration in water of Dejguny Lake [mg dm^{-3}]

Research site	Fe	Mg	SO_4^{-2}	Cl^-
1	0.20	10.1	147.9	15.5
2	0.20	10.2	146.9	13.0
3	0.25	13.9	155.3	13.2
4	0.25	12.8	160.3	13.5
LSD p = 0.05	0.05	1.8	3.4	2.5

location of water site and chloride content in water was lower than in RACZKOWSKI and WARECHOWSKA (2002) studies. These authors were showed the essential influence of the pollution points sources (storm basins) on chloride content in Jeziorak Mały Lake. Magnesium content in water Dejguny Lake was higher (about 3–4 mg dm^{-3}) near the acreage arables (sites 3 and 4). KRUK (1996) was showed that outflow of the mineral compounds, mainly metals ions to water, first of all, is from the boggy areas and it was higher than from the agricultural basins. The own results are showing that there was clear effect of the kind of the agricultural area adaption on sulphates(VI) content

in water of Dejguny Lake. In this case, sulphate content was 155.3 and 160.3 mg dm^{-3} and it was in II class of water cleanness and, it was considerably higher than in water near forest or recreation area. In contrast to these results, according to (1996) KRUK, there is a considerable risk of the intensive tourism and the area adaptation for the recreation.

Conclusions

1. The most compounds concentrations (total-P, ortho-phosphate (PO_4^{-3}) dissolved, Mg and Fe) in surface water of studed part of Dejguny Lake were low in standard I class of water cleanness.

2. In the lake water near the recreation area was higher mineral nitrogen compounds content but it was not higher than standard I class of water cleanness and, there was cosiderable more sulphates(VI) in the standard II class of water cleanness.

Translated by DANUTA DOMSKA

Accepted for print 2.07.2010

References

BARTOSZEWICZ A. 2005. *Migracja fosforanów w glebach i wodach zlewni rolniczej na terenie równiny kościańskiej*. Zesz. Probl. Post. Nauk Rol., 505: 41–49.

DOJLIDO J., DOŻAŃSKA W., HERMANOWICZ W., KOZIOROWSKI B., ZERBE J. 1999. *Fizyczno-chemiczne badanie wody i ścieków*. Arkady, Warszawa.

DOMSKA D., WOJTKOWIAK K., WARECHOWSKA M., RACZKOWSKI M. 2005. *Wpływ zróżnicowania obszarowego na zawartość niektórych związków w wodzie Jeziora Chełmżyńskiego*. Zesz. Probl. Post. Nauk Rol., 505: 95–99.

DOMSKA D., WOJTKOWIAK K., WARECHOWSKA M., RACZKOWSKI M. 2006. *Wpływ użytkowania rolniczego na zawartość związków azotu i fosforu wodzie Jeziora Narie*. Zesz. Probl. Post. Nauk Rol., 513: 81–86.

Jakość wody. Pobieranie próbek. 4. *Wytyczne dotyczące pobierania próbek z jezior naturalnych i sztucznych zbiorników zaporowych*. PN-ISO 5667-4: 2003.

KOC J., SIDORUK M. 2006. *Wpływ użytkowania zlewni na ładunek fosforu dopływający do jezior z wodami powierzchniowymi*. Zesz. Probl. Post. Nauk Rol., 505: 159–167.

KOC J., SKONIECZYK P. 2007. *Role of retention reservoir in protecting a lake from biogenic substances running off from rural areas*. Polish J. Envir., 16(2A): 292–296.

KRUK M. 1996. *Ocena redukcji składników mineralnych w odpływach a zabagnionych obszarów powierzchniowo bezodpływowych w krajobrazie rolniczym*. Acta Acad. Agricultura Tech. Olst., 22: 160–169.

KUBIAK J. 2000. *Ocena naturalnej tolerancji jezior Solińskiego Parku Narodowego na oddziaływanie antropogeniczne*. [w]: *Naturalne i antropogeniczne przemiany jezior*. Red. K. Lossow, H. Gawrońska. UWM w Olsztynie, 133–145.

PETTERSON K., AMIARD J. 1998. *Mechanisms for internal loading of phosphorus in lakes*. Hydrobiologia, 373–374(1–3): 21–25.

PULIKOWSKI K., PALUCH J., PARUCH A., KOSTRZEWA S. 2005. *Okres pojawiania się maksymalnych stężeń azotanów w wodach powierzchniowych*. Zesz. Probl. Post. Nauk Rol., 505: 339–345.

RACZKOWSKI M., WARECHOWSKA M. 2002. *Oddziaływanie różnych obszarowych i punktowych źródeł zanieczyszczeń na zawartość chlorków w wodzie jeziora Jeziorak Mały w Iławie*. Zesz. Probl. Post. Nauk Rol., 484: 529–535.

Rozporządzenie 2004. Rozporządzenie MOŚ z dnia 11 lutego 2004 r. w sprawie klasyfikacji dla prezentowania stanu wód powierzchniowych i podziemnych, sposobu prowadzenia monitoringu oraz sposobu interpretacji wyników i prezentacji stanu tych wód Dz.U. nr 32, poz. 284.

SZCZYGIELSKI D. 1996. *Gospodarka wodą na jeziorach i zbiornikach wodnych w aspekcie zanieczyszczeń rolniczych*. Wiad. Melior. Łąkarskie, 2: 51–53.

SZOSZKIEWICZ K., SZOSZKIEWICZ J. 1997. *Symptomy eutrifizacji jeziora Wielkie Partęczyny na Pojezierzu Brodzickim*. Rocz. AR w Poznaniu, CCXCII: 116–118.

WOJTAS A., DĄBEK M. 2006. *Dynamika zawartości związków azotowych w wodzie jeziora Olecko Wielkie*. Zesz. Probl. Post. Nauk Rol., 513: 547–553.

VOLLENWEIDER R.A. 1989. *Global problem sof eutrophication and its control*. Symp. Biol. Hung., 38: 19–41.

ZACHMANN D.W., VEEN A. VAN DER, MÜLLER S. 2004. *Distribution of phosphorus and heavy metals in the sediments of Lake Arendsee (Altmark, Germany)*. Stud. Quaternary, 21: 179–185.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 376–386, Y. 2010

DOI 10.2478/v10020-010-0035-5

DISTRIBUTION AND ABUNDANCE OF *PULSATILLA PATENS* POPULATIONS IN NATURE RESERVES IN NORTH-EASTERN POLAND

Barbara Juśkiewicz-Swaczyna

Department of Applied Ecology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: Easter pasque flower, threatened species, enviromental inventory, disappearance of sites.

A b s t r a c t

In the past most *Pulsatilla patens* sites were found in north-eastern Poland particulary in North--Podlasie Lowland, where the populations were also numerous. The purpose of my investigation has been to trace the current distribution of *Pulsatilla patens* in 16 nature reserves in north-eastern Poland. The data concerning the presence of this species have been verified following a few steps. First, the source references, such as the protection plans containing information about the selected nature reserves, were analyzed. Next, in each nature reserve the following components were analyzed: distribution of the stands, abundance of the populations, type of a community in which *P. patens* grows and type of the soil. Later, these data were verified by referring them to the current data of 2010, supplied by the Regional Directorate of National Forests (RDLP) in Białystok. Analysis of the documentation from the years 1955–2010 shows the gradual disappearance of *Pulsatilla patens* sites. Currently the sites of *Pulsatilla patens* have been preserved only in the 5 nature reserves (Krasne, Kukle, Góra Pieszczana, Kuriańskie Bagno, Szelągówka). At all the examined sites, the populations are very small, consisting of a few individuals. This suggests that the actions undertaken to protect it have failed.

WYSTĘPOWANIE I ZASOBY POPULACJI *PULSATILLA PATENS* W REZERWATACH PRZYRODY PÓŁNOCNO-WSCHODNIEJ POLSKI

Barbara Juśkiewicz-Swaczyna

Katerdra Ekologii Stosowanej
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: sasanka otwarta, gatunek zagrożony, inwentaryzacja przyrodnicza, zanik stanowisk.

Address: Barbara Juśkiewicz-Swaczyna, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (89) 523 37 42, e-mail: barbajus@uwm.edu.pl

A b s t r a k t

Większość stanowisk *Pulsatilla patens* w przeszłości występowała w północno-wschodniej Polsce, szczególnie na terenie Niziny Północno-Podlaskiej, gdzie populacje były reprezentowane przez liczne osobniki. Celem badań było opracowanie aktualnego rozmieszczenia stanowisk sasanki otwartej na terenie 16 rezerwatów przyrody oraz ocena zasobów populacji. Weryfikację danych o występowaniu i liczebności badanego gatunku przeprowadzono w kilku etapach. Najpierw dokonano analizy planów ochrony rezerwatów przyrody. Na terenie każdego obiektu analizowano: rozmieszczenie stanowisk, liczebność populacji, typ zbiorowiska w jakim sasanka występuje oraz typ gleby. Następnie dane te zweryfikowano, bazując na materiałach z 2010 r. otrzymanych z Regionalnej Dyrekcji Lasów Państwowych w Białymstoku oraz przeprowadzono badania terenowe w wybranych obiektach. W celu uzyskania pełnego obrazu zmian w występowaniu i zasobach populacji sasanki wykorzystano w pracy publikacje naukowe dotyczące rezerwatów. Analiza danych z lat 1955–2010 wskazuje na stopniowy zanik stanowisk sasanki. Aktualnie gatunek ten występuje tylko w pięciu rezerwatach przyrody (Krasne, Kukle, Góra Pieszczana, Kuriańskie Bagno, Szelągówka). Wszystkie populacje są bardzo nieliczne, obejmują po kilka osobników. Wskazuje to na nieskuteczność działań podjętych w celu ochrony tego gatunku.

Introduction

Pulsatilla patens (L.) Mill. (Eastern pasque flower) is a threatened plant species in Europe, listed in the Bern Convention and in Annex II of the European Habitats Directive. In Poland, this rare species has been legally protected since 1958. In Wielkopolska and Western Pomerania, *P. patens* is considered to be a threatened taxon (ŻUKOWSKI, JACKOWIAK 1995). It is included in the Polish Red Book of Plants, listed as a low risk (LR) taxon (WÓJTOWICZ 2001). In a recently published Red List of Vascular Plants in Poland (ZARZYCKI, SZELĄG 2006), *P. patens* is considered to be critically endangered.

Considering the fact that the number of sites has diminished significantly in many parts of the country (MICHALAK 1976, CIOSEK 1999, NOWAK et al. 2000, CHMURA 2003, *Zagrożone gatunki*. 2003), a national plan for the conservation of this species has been elaborated. It points to sources of the threat and specifies aims of the actions undertaken, such as active conservation of edge and scarce populations, improvement of the conditions for non-renewable populations, broadening our knowledge on the distribution and status of the population of *P. patens* and education of the general population on the need to maintain biological diversity (ZYCH 2007).

Within its geographical range, *P. patens* appears in different types of plant communities, including calcareous grasslands in Germany (RÖDER, KIEHL 2006), pine-dominated forests in Finland, (UOTILA 1996, KALLIOVIRTA et al. 2006), in steppe communities in Russia (RYSINA 1981), in alvar forests and shrublands, boreal heath forests, also in dry boreal forests in Estonia (PILT, KUKK 2002).

In Poland, the largest number of sites is found in the north-east. These are relatively abundant sites, comprising tens to hundreds of plants (WÓJTOWICZ 2000). Eastern pasque flower grows mainly in pine forests with elements of thermophilic vegetation. The presence of this species has been determined in *Peucedano-Pinetum* communities in Kurpiowska Forest (FALIŃSKI 1965, CZERWIŃSKI 1970), Augustowska Forest (SOKOŁOWSKI 1966, 1969, CZERWIŃSKI 1970, MATUSZKIEWICZ 1965, SOKOŁOWSKI 1968), Białowieska Forest (SOKOŁOWSKI 1966, 1991) and Knyszyńska Forest (CZERWIŃSKI 1995) as well as on Dobrzyń Moraine Plateau (KĘPCZYŃSKI 1965). In north-eastern Poland, *P. patens* has been considered as a species locally characteristic of *Peucedano-Pinetum* association and *Dicrano-Pinion* allion (CZERWIŃSKI 1978, 1995). Easter pasque flower also also occurs in *Serratulo-Pinetum* (CZERWIŃSKI 1995) and *Querco-Pinetum* associations (ENDLER 1979).

The above references, which document the presence of *P. patens*, are mainly of historical value and need to be verified. The purpose of my investigation has been to establish the current distribution of *Pulsatilla patens* in nature reserves in north-eastern Poland, to evaluate the abundance of these populations. The first step for such verification is the present analysis of the current occurrence of *P. patens* in nature reserves in north-eastern Poland, which deals with such issues as abundance of the populations and description of the characteristics of the ecological conditions. The author has chosen nature reserves for the research because she believes that areas under legally imposed conservation provide good environmental conditions for the existence and development of *P. patens* populations.

Material and Methods

Study area

Nature reserves where *P. patens* stands have been identified are in north-eastern Poland (Figure 1). According to the physical and geographical division of Poland into regions, these nature reserves lie in North Podlasie Lowland and Masurian Lake District. The area comprises part of the catchment basins of the rivers flowing to the Baltic Sea, i.e. the Vistula, Pregola and Neman Rivers. Another important component of the local hydrographic relations is the vast abudance of lakes and wetlands (KONDRACKI 2001). North-eastern Poland is in the Masurian and Podlasie climatic region, which stretches over the eastern part of the Masurian Lakes and Podlasie (WOŚ 1996). The weather is characterized by strong affinity to the continental climate with its typical length of seasons, such as a long and freezing winter (110 days), long summer

(90 days), but shorter spring and autumn than in the other parts of Poland. The snow cover remains here for a long time (85–96 days) and can be up to 10 cm thick. The average annual temperature is low (7°C) and the growing season is short, lasting about 200 days (GÓRNIAK 2000).

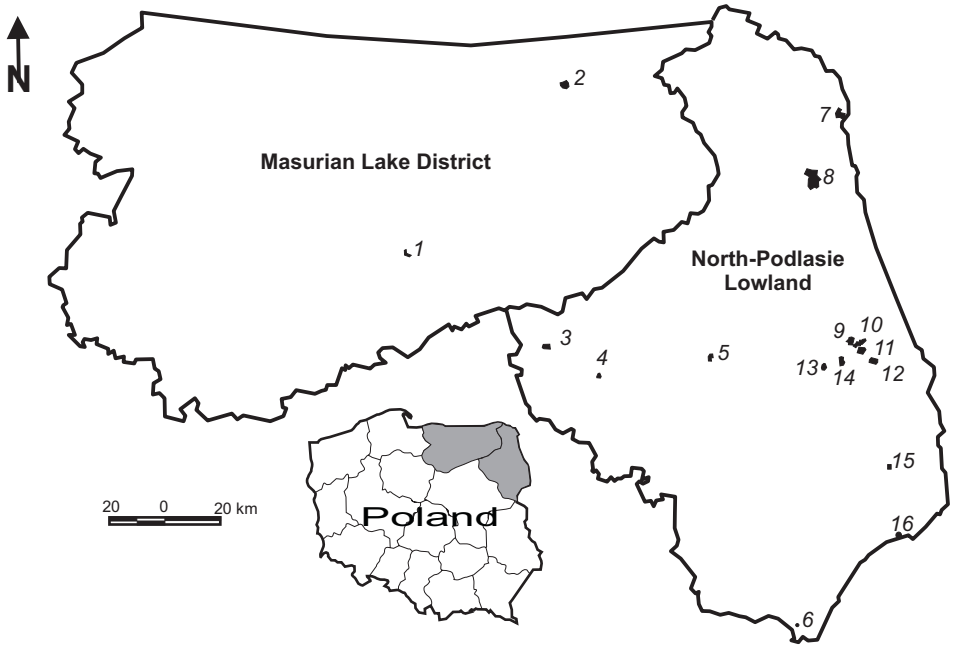

Fig. 1. Location of the sites (name of reserves – see Table 1)

Study species

Pulsatilla patens is a hemicryptophyte with an upright, branching rhizome, which makes older plants to form clumps. Two types of buds are produced annually: one replaces the terminal bud, which is transformed to a flowering apex and one becomes dormant, the resulting flower bud remains enclosed by the bud scales until the spring (mid-April until mid May) of the third season. The second type of bud produced annually is smaller and after producing protective bud scales enters what may be a prolonged dormancy. The reserve of viable dormant buds is augmented annually and enables the plant to regenerate new branches if terminal apices are damaged. The largest, most highly branched specimens which can form over 20 flowers and 50 leaves in one growing season are frequently found in locations that are grazed or burned (WILDEMAN, STEEVES 1982). The older individuals of *Pulsatilla patens* are very

sensitive to root damage (WÓJTOWICZ 2000). The seeds are dispersed by wind in June and July over short distances. In warm and moist weather germination occurs in late summer, but if the weather is cold and dry, it is delayed until the next spring or seeds may remain in the transient seed bank (PILT, KUKK 2002). Intensity of formation of leaf rosettes, flowering and fruit bearing depends on such weather conditions as winter temperatures, snow cover, autumn precipitations, temperature and sunlight in spring (WÓJTOWICZ 2000). *Pulsatilla patens* is a species that has a circumpolar distribution, with its western border runnig through Lusatia and Brandeburg (KRAWIEC 1932).

Methods

Based on an inventory of nature reserves in northern Poland (*Rezerwaty przyrody*... 2005), 16 objects have been selected, in which stands of *P. patens* are documented (Figure 1). The data concerning the presence and abundance of the examined species have been verified following a few steps. First, the source references, such as the protection plans containing information about the selected nature reserves, were analyzed. The protection plans of the nature reserves were made available by the Regional Directorate for Environmental Protection (RDOŚ) in Białystok. Next, in each nature reserve the following components were analyzed: distribution of the stands, abundance of the populations, type of a community in which *P. patens* grows and type of the soil. Later, these data were verified by referring them to the current data of 2010, supplied by the Regional Directorate of National Forests (RDLP) in Białystok. In order to obtain a more complete picture of the changes in the presence and abundance of Easter pasque flower, the author included the information from scietific publications concerning the nature reserves. A field investigations was also made in two nature reserves, such as Kulka and Piłackie Wzgórza. The observations are shown in Table 1, in which names for plant communities adhere to the terminology by MATUSZKIEWICZ (2007).

Results

In north-eastern Poland, the presence of Easter pasque flower has been determined in 16 nature reserves (Figure 1, Table 1). These wildlife refuges were established in 1970–1995 mainly to conserve woodland ecosystems, except Kulka Nature Reserve, which was set up in 1955 in order to conserve steppe flora. Almost all the nature reserves lie on North Podlasie Lowland, except Piłackie Wzgórza and Kulka, which are in the Masurian Lake District.

Table 1
Specification of data on *Pulsatilla patens* sites in nature reserves in north-eastern Poland

Nature reserve	Year of creation	Community	Data according to the reference – year, presence	Current state in 2010 year
1. Kulka	1955	Calcareous grassland	1955 (-)	(-)
2. Piłackie Wzgórza	1989	*Querco-Pinetum*	1990 (+)	(-)
3. Łokieć	1989	*Peucedano-Pinetum*	1997 (-)	(-)
4. Rycerski Kierz	1989	*Querco-Pinetum*	1996 (-)	(-)
5. Szelągówka	1995	*Peucedano-Pinetum*	2003 (-)	(+)
6. Góra Uszeście	1985	*Querco-Pinetum*	1994 (+)	(-)
7. Kukle	1983	*Peucedano-Pinetum*	2006 (+)	(+)
8. Kuriańskie Bagno	1985	*Peucedano-Pinetum*	1994 (+)	(+)
9. Międzyrzecze	1990	*Serratulo-Pinetum*	1991 (+)	(-)
10. Woronicza	1989	*Serratulo-Pinetum*	2000 (+)	(-)
11. Stare Biele	1987	*Serratulo-Pinetum*	2004 (+)	(-)
12. Góra Pieszczana	1987	*Serratulo-Pinetum*	2003 (+)	(+)
13. Krasne	1990	*Peucedano-Pinetum*	2007 (+)	(+)
14. Krzemienne Góry	1987	*Serratulo-Pinetum*	2007 (+)	(-)
15. Gnilec	1995	*Peucedano-Pinetum*	2002 (-)	(-)
16. Sitki	1979	*Peucedano-Pinetum*	1991 (+)	(-)

While making an enviromental inventory, which was essential for setting up a nature reserve, presence of *Pulsatilla patens* was determined in all these objects. However, the populations of this species were represented by a small number of individuals. Nonetheless, in the phytosociological tables prepared for these areas the contribution of this species was assigned (+).

In 7 nature reserves (Table 1), Easter pasque flower occurred in communities of the sub-continental pine forest *Peucedano-Pinetum*. The tree stands in such forests consist of pine trees, with single specimens of birch, oak and spruce trees. The undergrowth is created by *Convalaria majalis*, *Peucedanum oreoselinum*, *Polygonatum odoratum*, *Veronica officinalis*, *Anthericum ramosum*, *Solidago virgaurea*, *Carex ericetorum*, *Arctostaphylos uva-ursi*, *Antenaria dioica*, *Koeleria polonica* etc. These communities grow on rusty and brown-rusty soils. The subboreal multi-species forest *Serratulo-Pinetum* is a habitat of Easter pasque flower in four nature reserves (Table 1). The tree stand in this forest consists of spruce with a considerable share of pine and oak trees. The herbaceous plants contain, for example, *Vaccinium vitis-idaea*, *Melampyrum pratense*, *Chimaphila umbellata*, *Pirola chlorantha*, *Arctostaphylos uva-ursi*, *Goodyera repens*, *Polygonatum odoratum*, *Campanula persicifolia*, *Calamintha vulgaris*. These communities grow on rusty and brown-

-rusty soils. In three nature reserves (Rycerski Kierz, Góra Uszeście and Piłackie Wzgórza), *Pulsatilla patents* grew in a continental mixed forest *Querco-Pinetum*, growing on mesotrophic loamy and sandy soils. The oldest known stand of Easter pasque flower (documented since 1898) in Kulka Nature Reserve grows on xerothermic grasslands of the class *Festuco-Brometea*. Unfortunately, since 1955 the species has not been observed at this site. Analysis of the documentation regarding these nature reserves suggests that in 1991–1997 no stands of Eastern pasque flower was determined in 2 nature reserves (Rycerski Kierz, Łokieć). Another inventory study, carried out in 1999–2007, demonstrated that three more stands had disappeared (in the nature reserves called Gilec, Szelągówka, Międzyrzecze). The data collected in May 2010 show that the stands of *Pulsatilla patens* have been preserved only in the following nature reserves: Krasne, Kukle, Góra Pieszczana, Kuriańskie Bagno and Szelągówka (in this site the species has reappeared after a few years). At all the examined sites, the populations were very small, consisting of a few individuals. They have survived mainly at the edgesof forests, in open and well sunlit places.

Ever since Easter pasque flower became a legally protected species, the number of sites where it grew in the analyzed nature reserves has drastically diminished (about 60%).

Discussion

In the light of the Polish law, nature reserves are areas preserved in a natural or nearly unaltered natural state, which comprise ecosystems, wildelife havens and natural habitats, including stands of plants, fungi and forms of inanimate nature of outstanding natural, scientific, cultural and landscape-related values (*Ustawa o ochronie przyrody,* 2004). In Poland, the first nature reserve was established in 1827 to protect the common yew trees (*Taxus baccata*) in Wierzchlas. Before 1939, about 200 nature reserves had been created in Poland (*Rezerwaty przyrody*... 2005). At present, according to the data from the *Centralny Rejestr Form Ochrony Przyrody* (2006) there are 1,546 reserves, among which forest reserves are the most numerous. For nature reserves to function properly, a conservation plan has to be prepared every twenty years, in which the following are determined: conditions for any economic activity in a nature reserve as well as identification and ways of eliminating potential threats. In general, nature reserves are a good and effective form of nature conservation which enable us to preserve valuable natural ecosystems and rare species of plants and animals. Unfortunately, this cannot be said about sites of *Pulsatilla patens*. The present study indicates that

nature reserves as a form of nature conservation are not effective in protecting this rare species, as documented by the disappearance of *P. patens* in 11 out of the 16 examined nature reserves, where the species has been previously observed. The fact that the steppe flora in Kulka Nature Reserve in the Masurian Lake Disrict is threatened has been implied by POLAKOWSKI (1956) and ENDLER, ZIELIŃSKA (2003). Among the reasons for gradual disappearance of rare and protected plant species, including Easter pasque flower, these authors listed excessive growth of shrubs and increased forested area, which drastically change the access to light.

A negative influence of excessive shading of the bottom layer of a forest by an expansive species of the spruce *Picea abies* on xerothermic heliophytes has been described to occur in Sitki Nature Reserve in Białowieska Forest. When that reserve was being planned to be set up, it was suggested the forest undergrowth and some young spruce trees should be removed over a certain area and treated as the so-called 'unchangeable area' (SOKOŁOWSKI 1976). This would have been a treatment producing positive influence on natural stands of such species as *Neottianthe cucullata*, *Pulsatilla patens*, *Dianthus arenarius*, *Gypsophila fastigiata*, *Arctostaphylos uva-ursi*, *Potentilla arenaria*, all of which are rare in that region. Unfortunately, no such treatment has been carried out, and consequently the abundance of these plants has been decreasing, leading to gradual disappearance of their stands (SOKOŁOWSKI 1991). A similar situation occurs in other forest nature reserves, where any actions aimed at halting the natural succession of plants and maintaining good conditions for such plants as *P. patens* have been discontinued. In some nature reserves, all man's intervention has been stopped due to the protection zones of bird nests which exist in those reserves. It should be underlined that populations of *Pulsatilla patens* in the nature reserves described in this paper are very small – they were typically single specimens or groups of a few individuals. Considering the fact that at present Easter pasque flower appears in only 5 of the analyzed reserves, in very small numbers, it can be concluded that it is now a threatened taxon and the current conservation conditions are insufficient. This suggests that the actions undertaken to protect it have failed. It is possible that the causes for this gradual disappearance of *Pulsatilla patens* are excessive shrub cover and dense tree cover over large areas, which menas that sunlight conditions are inferior and as a result Easter pasque flower, like other heliophilous xerothermic plants, reced. No treatments have been introduced to shape proper ecological conditions for the occurrence of *P. patens*. Should this situation continue, it is likely that in the nearest future other valuable plant species will disappear as well.

A decrease in the populations of *P. patens* has also been reported in Harchinger Heide Nature Reserve in Germany. In 1991–2003, the abudance

of the local populations fell from 27,000 to about 9,700 specimens (RÖDER, KIEHL 2006). Although this is a relatively large number compared to populations in other parts of Europe, e.g. in Estonia, where populations of *Pulsatilla patens* consist of 100–1,000 specimens and only in some sites they are as large as about 10,000 individuals (PILT, KUKK 2002). At present, we know the data generated by the system of monitoring involved in Natura 2000, for example a total of 21,000 specimens of *P. patens* have been counted in Estonia and between 1994–2000 some of the populations rose in abudance owing to an improvement in the light conditions, whereas some other populations continued to diminish, mainly due to increased moss layer density. Long-term observations in Finland imply a similar tendency – out of the 24 analyzed populations of *P. patens*, 8 tended to increase, 12 were stabilized and 4 seemed to be decreasing. A growing tendecy was obserevd at the sites with semi-open grounds and fields as well as sufficiently scarce litter (KALLIOVITRA et al. 2003). Good conditions for the growth of Easter pasque flower are also found in a Nature 2000 wildlife refuge called the Grasslands in the Military Training Grounds in Orzysz, in the Masurian Lake District (NE Poland). One of the most numerous Polish populations of this plant species, consiting of 300 specimens, grows there (JUŚKIEWICZ-SWACZYNA 2010). A preliminary inventory carried out in 2007–2010 in north-eastern Poland by the Regional Management of National Forests in Białystok identified about 10 populations of *Pulsatilla patens*, containing between tens to hundreds of individual plants. The sites are localized in areas where regular forest economy is conducted. It should be therefore assumed that man's intervention is necessary for the existence of Easter pasque flower as it countreacts natural succession of plants, for example by extensive land use, removal of young trees and shrubs, grazing by animals. In order to establish successful conservation of *P. patens* in the Polish nature reserves described here, conservation plans should be elaborated that would take into consideration habitat requirements of this plant species; afterwards, the recommendations formulated in such plans should be strictly followed and the nature reserves should be monitored constantly. In nature reserves where *P. patent* has already disappeared, one might consider reintroduction of populations of this plant.

Acknowledgements

I am very grateful to the Regional Directorate for Nature Conservation (RDOŚ) and the Regional Directorate of State Forests (RDPL) in Białystok for making available the data for this study and their assistance in verifying presence of Easter pasque flower.

Translated by JOLANTA IDŹKOWSKA

Accepted for print 6.10.2010

References

Centralny Rejestr Form Ochrony Przyrody. 2006. http://rop.mos.gov.pl/, accessed: 16.05.2010.

CHMURA D. 2003. *Zagrożenia lokalnych populacji sasanki otwartej Pulsatilla patens na przykładzie stanowiska na Sodowej Górze w Jaworznie*. Chrońmy Przy. Ojcz., 59(5): 14–27, Kraków.

CIOSEK M. T. 1999. *Rodzaj Pulsatilla (Ranunculaceae) na Podlasiu i Mazowszu*. Fragm. Flor. Geobot. Ser. Polonica, 6: 15–19, Kraków.

CZERWIŃSKI A. 1970. *Bory sosnowe północno-wschodniej Polski*. Prace Kom. Biol. Pozn. TPN, 33(5): 1–99.

CZERWIŃSKI A. 1978. *Zbiorowiska leśne północno-wschodniej Polski*. Zesz. Nauk. Polit. Biał., 27: 1–326.

CZERWIŃSKI A. 1995. *Geobotanika w ochronie środowiska lasów Podlasia i Mazur*. Wyd. Polit. Biał., Białystok.

Czerwona księga Karpat Polskich. Rośliny naczyniowe. 2008. Red. Z. Mirek, H. Piękoś-Mirkowa. Inst. Bot. PAN, Kraków.

ENDLER Z. 1979. *Analiza geobotaniczna borów Wzniesień Piłackich*. Frag. Flor. Geobot. 25(4): 509–562, Kraków.

ENDLER Z., ZIELIŃSKA J. 2003. *Degradacja florystyczna rezerwatu stepowego Kulka na Pojezierzu Olsztyńskim*. Chrońmy Przy. Ojcz., 59(5): 14–27, Kraków.

FALIŃSKI J.B. 1965. *O roślinności Zielonej Puszczy Kurpiowskiej na tle stosunków geobotanicznych tzw. Działu Północnego*. Acta Soc. Bot. Pol., 34: 719–752.

GÓRNIAK A. 2000. *Klimat województwa podlaskiego*. Inst. Met. Gosp. Wod., Białystok.

JUŚKIEWICZ-SWACZYNA B. 2010. *Population structure of Pulsatilla patens* in relation to the habitat quality. Tuexenia, 30: 457–466.

KALLIOVITRA M., KUKK Ü., RYTTÄRI T. 2003. *Pulsatilla patens* (L.) Mill. *Monitoring of threatened vascular plants in Estonia and Finland. Methods and experiences*. Finnish Environment, 659: 37–47.

KĘPCZYŃSKI K. 1965. *Szata roślinna Wysoczyzny Dobrzyńskiej*. Wyd. UMK, Toruń.

KONDRACKI J. 2001. *Geografia regionalna Polski*. PWN, Warszawa.

KRAWIEC T. 1932. *O rozmieszczeniu sasanki (Pulsatilla Tourn.) w Wielkopolsce*. Acta. Soc. Bot. Pol., 9(3–4): 531–537.

MATUSZKIEWICZ W. 1965. *Die Kiefernwalder der Augustower Heide*. Mat. Zakł. Fitos. Stos. UW, 6: 113–119.

MATUSZKIEWICZ W. 2007. *Przewodnik do oznaczania zbiorowisk roślinnych Polski*. PWN, Warszawa.

MICHALAK S. 1976. *Wymieranie rodzimych składników flory na Równinie Opolskiej*. Phytocenosis, 5: 301–307.

NOWAK T., TOKARSKA-GUZIK B., CHMURA D. 2000. *Materiały do atlasu rozmieszczenia oraz stanu zasobów roślin chronionych i zagrożonych rejonu Górnośląskiego*. Cz. 7. *Pulsatilla patens (L.) Mill. (Ranunculaceae)*. Acta Biol. Siles., 35: 191–199.

POLAKOWSKI B. 1956. *Roślinność rezerwatu florystycznego Kulka*. (mscr.), Olsztyn.

PILT I., KUKK Ü. 2002. *Pulsatilla patens and Pulsatilla pratensis (Ranunculaceae) in Estonia: distribution and ecology*. Proc. Estonian Acad. Sci. Biol. Ecol., 51(4): 242–256.

Rezerwaty przyrody w Polsce północnej. 2005. Red. G. Rąkowski. Inst. Ochr. Środ. Warszawa.

RÖDER D., KIEHL K. 2006. *Population structure and population dynamic of Pulsatilla patens (L.) Mill. in relation to vegetation characteristics*. Flora, 201: 499–507.

RYSINA G.P. 1981. *On the biology of Pulsatilla patens* (L.) Mill. in the environs of Moscow. – Bull. Moscow Soc. Nat. N. S. 86: 129–134. Moscow.

SOKOŁOWSKI A.W. 1966a. *Fitosocjologiczna charakterystyka borów iglastych Puszczy Augustowskiej*. Prace IBL, 306: 107–125.

SOKOŁOWSKI A.W. 1966b. *Fitosocjologiczna charakterystyka borów iglastych ze związku Dicrano-Pinion Puszczy Białowieskiej*. Prace IBL, 305: 71–105..

SOKOŁOWSKI A.W. 1968. *Zespoły leśne nadleśnictwa Suwałki w Puszczy Augustowskiej*. Prace IBL, 349: 171–212.

SOKOŁOWSKI A.W. 1969. *Zespoły leśne nadleśnictwa Balinka w Puszczy Augustowskiej*. Monogr. Bot., 28: 1–79.

SOKOŁOWSKI A.W. 1991. *Roślinność rezerwatu Sitki w Puszczy Białowieskiej*. Ochr. Przyr., 48: 85–103.

SOKOŁOWSKI A.W. 2006. *Przyroda województwa podlaskiego i jej ochrona*. Łomż. Tow. Nauk. im. Wagów, Łomża.

Ustawa z dnia 16 kwietnia 2004 r. o ochronie przyrody. Dz.U. nr 92, poz. 880: 6056–6099.

UOTILA P. 1996. *Decline of Anemone patens (Ranunculaceae) in Finland*. – Acta. Univ. Ups. Symb. Bot. Ups. 31: 205–210. Uppsala.

WILDEMAN A.G., STEEVES T.A. 1982. *The morphology and growth cycle of Anemone patens*. Can. J. Bot., 60(7): 1126–1137.

WOŚ A. 1996. *Zarys klimatu Polski*. Wyd. UAM, Poznań.ą

WÓJTOWICZ W. 2000. *Biologia, wymagania siedliskowe i możliwości uprawy zachowawczej Pulsatilla patens (L.) Mill*. Biul. Ogr. Bot., 9: 45–54.

WÓJTOWICZ W. 2001. *Pulsatilla patens* (L.) Mill. In: *Polska Czerwona Księga Roślin*. Red. K. Zarzycki, R. Kaźmierczakowa. Inst. Bot. im. W. Szafera i Inst. Ochr. Przyr. PAN, Kraków, 142–144 pp.

Zagrożone gatunki flory naczyniowej Dolnego Śląska. 2003. Red. Z. KĄCKI. Inst. Biol. Uniw. Wrocł., Wrocław.

ZARZYCKI K., SZELĄG Z. 2006. *Red list of vascular plants in Poland*. [In:] *Red list of plants and fungi in Poland*. Red. Z. Mirek, K. Zarzycki, W. Wojewoda, Z. Szeląg. Inst. Bot. im. W. Szafera, Kraków, 9–20 pp.

ZYCH M. 2007. *Krajowy Plan Ochrony Gatunku. Sasanka otwarta Pulsatilla patens (L.) Mill*. Warszawa.

ŻUKOWSKI W., JACKOWIAK B. 1995. *Ginące i zagrożone rośliny naczyniowe Pomorza Zachodniego i Wielkopolski*. Pr. Zakł. Takson. Rośl. UAM, 3: 1–141, Poznań.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 387–400, Y. 2010

DOI 10.2478/v10020-010-0036-4

ZOOPLANKTON IN THE NIDA RIVER (THE UPPER WKRA RIVER) SUBJECTED TO REVITALIZATION TREATMENTS

***Anna Goździejewska*[1], *Andrzej Skrzypczak*[2], *Grażyna Furgała-Selezniow*[2], *Jacek Koszałka*[1], *Andrzej Mamcarz*[2]**

[1] Chair of Applied Ecology
[2] Department of Lake and River Fisheries
University of Warmia and Mazury in Olsztyn

K e y w o r d s: Wkra River, zooplankton, Rotatoria, Protozoa, revitalization.

A b s t r a c t

This paper focuses on the qualitative and quantitative characterization of zooplankton in a section of the Nida (the Upper Wkra) River subjected to revitalization treatments. At four established sites, biological samples were taken for analyses. In total, 44 taxa of zooplankton were determiend in the collected material. The upper section of the Wkra River, along which two sites were set up, was in general more varied in species composition, and the structure of biocenosis was shaped by rotifers and protozoa (mainly ameba species) in nearly equal halves. At the other two sampling sites, localized below, along a further section of the watercourse, the structure of zooplankton was poorer. It was colonized by larger numbers of individuals belonging to a few species of rotifers, among which *Keratella cochlearis* var. *tecta*, *Trichocerca similis* and *T. pusilla* are the species indicating a raised level of the trophy of the river water.

ZOOPLANKTON RZEKI NIDY (GÓRNA WKRA) PODDANEJ ZABIEGOM REWITALIZACJI

***Anna Goździejewska*[1], *Andrzej Skrzypczak*[2], *Grażyna Furgała-Selezniow*[2], *Jacek Koszałka*[1], *Andrzej Mamcarz*[2]**

[1] Katedra Ekologii Stosowanej
[2] Katedra Rybactwa Jeziorowego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: Wkra, zooplankton, Rotatoria, Protozoa, rewitalizacja.

Address: Anna Goździejewska, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (89) 523 41 89, e-mail: gozdzik@uwm.edu.pl

Abstrakt

W pracy skupiono się na charakterystyce jakościowej i ilościowej zooplanktonu odcinka rzeki Nidy (górna Wkra) poddanego zabiegom rewitalizacji. Na wyznaczonych czterech stanowiskach pobrano próby biologiczne do analizy. W zebranym materiale oznaczono łącznie 44 taksony zooplanktonu. Górny odcinek rzeki Wkry, na którym zlokalizowano dwa stanowiska, charakteryzował się ogólnie większym zróżnicowaniem gatunkowym, a struktura biocenozy kształtowana była niemalże po połowie przez wrotki i pierwotniaki (przeważnie gatunki ameb). W kolejnych punktach, w dalszym odcinku cieku, struktura zooplanktonu uległa zubożeniu. Dominowało kilka gatunków wrotków, spośród których *Keratella cochlearis* var. *tecta*, *Trichocerca similis* i *T. pusilla* wskazywały na stan podwyższonej trofii wód.

Introduction

For decades, watercourses have been among the water bodies most radically reshaped by man. The intensive civilization growth in the 20th century, development of technologies and methods which interfer with the nature, including use of flowing waters, and man's activity in river valleys, have caused great changes in natural ecosystems, which often lead to irriversible degradation. Due to such transformations, the biological function of some rivers has become limited, the biological diversity of plants and animals inhabiting watercourses has declined and the aesthetic role of rivers in landscape has deteriorated.

The evaluation of threats and degradation of rivers is based on guidelines and forms of actions suggested by the Water Framework Directive (WFD) of 2000, which should improve the ecological quality of surface waters. The following biological components are recommended to be taken as a basis for such evaluation: phytoplankton, phytobenthos, macrophytes, benthos invertebrates and fish. The picture of degradation, obtained according to these bioindicators and additional hydromorphological parameters, enables us to design and implement suitable renaturation actions in river valleys, with an aim of approximating the once lost natural character of such habitats (ŻELAZO and POPEK 2002, LÜDERITZ et al. 2004).

Success in restoring the wholesome nature of a water system depends mosty on good recognition and understanding of biological, chemical and physical processes which occur within the system as well as their mutual dependences/interactions.

It is therefore crucial to analyse very carefully the structure and ecology of organisms which dwell in a given watercourse, including the link omitted in bioindication, i.e. zooplankton. The role of plankton animals in an ecosystem is to take part in transformation and circulation of organic matter (ALLAN 1998), to regulate the biomass of phytoplankton (GRIFFIN et al. 2001) and

to serve as food for fish, especially for their earlier, larval stages and for fish fry (e.g. MILLS et al. 1986, MARMULLA and ROSCH 1990, SUTELA and HUUSKO 2000). Moreover, some specific zooplankton species help us to determine water purity of stagnant and flowing water bodies (PATUREJ and GOŹDZIEJEWSKA 2005), although they are not formally included in the control procedures established by the EU.

Zooplankton in flowing watercourses is much poorer than in stagnant waters, both in the variety of species and number of individuals. This is due to the the flow of water, which is a more demanding factor on many groups of organisms. The flow of water restricts the availability of food and makes it impossible for many groups of plankton to reproduce. The taxonomic structure and density of zooplankton in rivers are also shaped by the eutrophic effect of a river catchment basin (HILTON et al. 2006), presence of tributary rivers, river bends or old-river beds (RECKENDORFER et al. 1999), dam water reservoirs and lakes through which a watercourse flows (NIESLER and BIELAŃSKA-GRAJNER 2004), pressure produced by fish (JACK and THORP 2002) and some invertebrates, e.g. bivalves (LEVINTON and WALDMAN 2006).

This paper analyzes the qualitative and quantitative structure of zooplankton in the upper section of the Wkra River, locally named the Nida River (north-eastern Poland, Province of Warmia and Mazury, the administrative district of Nidzica). The section of the river is situated between the towns of Nidzica and Szymany. It has been subjected to revitalization treatments, including the planting of trees in the shore belt of the river bed, placing boulders and stones on the river bottom in order to diversity its structure and stocking with fish to rebuild the ichthyofauna of the river (SKRZYPCZAK et al. 2010).

The purpose of this revitalization effort was to restore the natural character of the ecosystem of the Wkra River, to rebuild and diversify plant and animal habitats and, consequently, to increase the biological diversity in the river channel. The underlying concept was that the river was a valuable natural component for the local forms of nature conservation and as an ecological channel in national and international nature networks. Additionally, the restoration of the natural values of the Wkra has a social and eco-sociological meaning. It should make the local community aware of the undeniable benefits of preserving clean nature, including the aspect of tourist assets of the whole region.

The aim of this paper has been to determine the qualitative and quantitative structure of the zooplaknton in the Nida River. The biocenosis of the Nida River was undergoing some changes stimulated by the restructuring of habitats and stocking with fish.

Materials and Methods

Zooplankton sampling and analysis methods

Samples of the zooplankton were taken on 2 and 15 of September 2009. Four sites were set up along the section of the Nida covered by the revitalization treatments (Figure 1). Stations varied in hydrology, habitat and due to the catchment impact (Table 1).

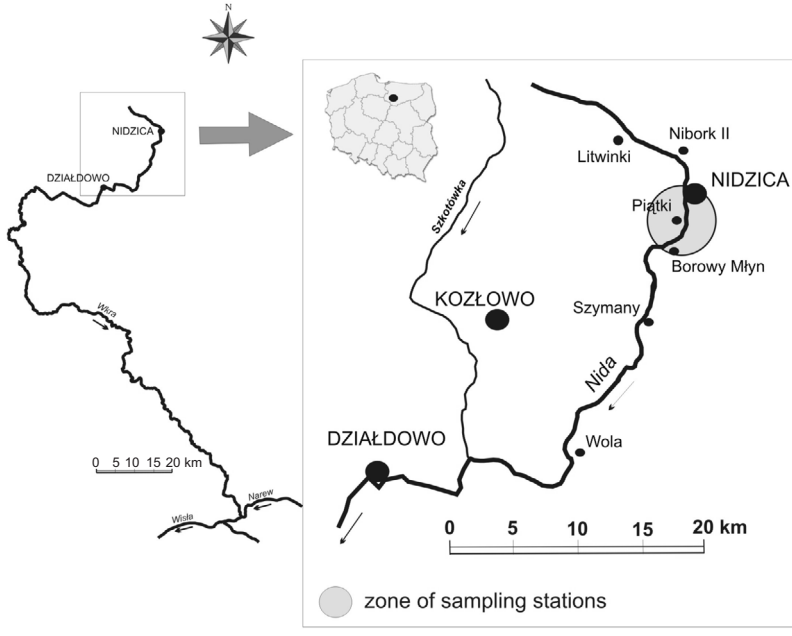

Fig. 1. Research area localization

Site 1 was situated in the southern outskirts of the town of Nidzica, in a developed area. Trees were planted in the shore belt between sites 1 and 2 (approximately 2 km) as part of the revitalization programme. Site 2 was set out near the location of sedimentation tanks of the wastewater treatment plant in Piątki. The surrounding area consists of fallow land and shrubs. Along two, 100-meter-long sections of the Nida River, between sites 3 and 4, the bottom of the river was made more diverse by placing boulders and pebbles. The immediate surroundings of the river, along these sections, comprise pastures, meadows and arable fields. No revitalization works were conducted at site 4, located the lowest along the river, in front of the weir in Borowy Młyn. The area on each side of the river channel comprises grasslands and arable fields.

Table 1
Characteristics of the zooplankton sampling sites. Modified from SKRZYPCZAK et al. (2010)

Sampling site / Parameter	1	2	3	4
Geographical position	N 53°21.29' E 20°25.27'	N 53°20.59' E 20°24.93'	N 53°20.44' E 20°24.41'	N 53°20.21' E 20°23.62'
River-bed width [m]	3	3.5	4	4.5
Mean depth [m]	0.3	0.7	0.7	0.8
Water flow [m s^{-1}]	0.4	0.3	0.3	0.2
Bottom substrate	sand with gravel and stones	muddy sand	sand	muddy sand
Submerged plants	*Elodea canadensis* filamentous algae	*Sagittaria sagittifolia*	*Elodea canadensis* filamentous algae	*Elodea canadensis* filamentous algae
Features of river chanel	regulated	regulated with seminatural elements	regulated with seminatural elements	regulated
Trees along banks [% of bank]	10	50	50	50
Adjacent area	meadows and pastures	bushes and wasteland	pastures and cultivated area	pastures and cultivated area

At each site, 20 liters of water were sampled using a calibrated measuring vessel. The sampled biological material was made denser on a 30 μm mesh size plankton net and then fixed in 4% formalin.

The analysis of the zooplankton material involved determination of the qualitative and quantitative composition and assessment of the biomass, Identification of the zooplankton was carried out down to the lowest identifiable taxonomic unit and the growth stage (FLÖSSNER 1972, STERBLE and KRAUTER 1978, RADWAN et al. 2004, RYBAK and BŁĘDZKI 2005). The stages of nauplius and copepodid larvae of the copepods were not assigned to approprate taxa. The number of individuals among the zooplankton (indiv. dm^{-3}) was estimated according to the Hansen's rule (STARMACH 1955). In order to determine the individual biomass of particular zooplankton individuals, standard weights for rotifers were applied (RADWAN et al. 2004). Regarding crustaceans and protozoa, particular organisms were measured under a microscope with a measuring lens at the maximum precision to 0.01 mm, using transmitted light. For the purpose of estimating biomass, it was assumed that the density of a zooplankton organism = 1. i.e. 1 mm^3 = 1 mg (HERNROTH 1985). Based on the results of the measurements, cubic volume of individuals was calculated, by comparing their shape to the basic geometrical solids.

Methods for evaluating the qualitative structure of zooplankton

The diversity of the qualitative structure of the zooplankton was estimated in respect of the species richness – Margalef index (MARGALEF 1957), general species diversity – Shannon-Wiener index (SHANNON 1948), eveness value of species – Pielou index (1966) – Table 2, the similarity and diversity in species between communities – Jackard index (MARCZEWSKI and STEINHAUS 1959) and Bray-Curtis index (CLARKE 1993) – Table 3.

Statistical significance of differences in values of Shannon-Wiener index between particular sites was verified with t-test at $p < 0.05$.

Table 2
Measures of the diversity in the qualitative structure of the zooplankton at sampling sites

Station / Biodiversity	1	2	3	4
Taxa Richness Index	4.98	5.82	3.42	5.44
Shannon's Index based on abundance	2.81	2.40	2.14	2.33
Pielou's Evenness	0.886	0.777	1.16	1.35

Table 3
Measures for evaluation of the similarity/difference in zooplankton communities between particular sites

Station / Measure/indicator	1–2	1–3	1–4	2–3	2–4	3–4
Faunal similarity	0.353	0.333	0.237	0.36	0.285	0.296
Faunal dissimilarity	0.575	0.625	0.735	0.422	0.426	0.622

Results

Diversity of the qualitative structure of zooplankton

The zooplankton in the Nida River was determined to comprise 31 taxa of rotifers, 11 taxa of protozoa, 2 taxa of cladocerans and a growth stage of nauplius of the copepods (Table 4). The species diversity of the biocenosis at each site depended on the number of species and differences in the abundance of populations belonging to particular taxa. The smallest number of taxa appeared at site 3 (12), higher and comparable at sites 1, 2 and 4 (24, 22 and 23).

Table 4

Species composition and abundance (indiv. dm^{-3}) of zooplankton in particular sections of the Nida River

Taxon	Station			
	1	2	3	4
Brachionus angularis Gosse				<1
Brachionus diversicornis Daday	2			
Brachionus urceolaris O.F. Müller	2			
Cephalodella auriculata O.F. Müller				13**
Cephalodella psammophila Koch-Althaus	2			2
Cephalodella sp.	2	<1		
Colurella colurus Ehrenberg	5	11		
Colurella sulcata Stenroos		1		<1
Colurella uncinata bicuspidata Ehrenberg	3		2*	2
Euchlanis dilatata Ehrenberg				1
Filinia longiseta Ehrenberg	2	<1	1	
Keratella cochlearis Gosse	9*	5**	6**	15**
Keratella cochlearis var. *hispida* Gosse		<1		
Keratella cochlearis var. *tecta* Gosse		<1		5*
Lecane closterocerca Schmarda	2	1	1	
Lecane hamata Stokes		<1		
Lecane imbricata Carlin				1
Lecane lunaris Ehrenberg		1		
Lecane stenroosi Meissner		1		
Lepadella rhomboides Gosse				1
Mytilina mucronata O.F. Müller		<1		<1
Polyarthra longiremis Carlin	2	1		
Polyarthra major Burckhardt			1	<1
Polyarthra remata Skorikov	5	3*	4**	6**
Polyarthra vulgaris Carlin	5	1		
Pompholyx sulcata Hudson	2			
Synchaeta sp.			1	
Trichocerca cylindrica Imhof		<1		
Trichocerca pusilla Lauterborn	2	1	1	<1
Trichocerca similis Wierzejski	5	1	3**	<1
Trichotria pocillum O.F. Müller				1
Rotatoria – total	44	17	18	51
Actinosphaerium eichhorni Ehrenberg	2			<1
Arcella discoides Ehrenberg	12**	8**	3**	2
Arcella gibbosa Penard		5**		
Arcella megastoma Penard		1	1	2
Centropyxis aculeata Ehrenberg	3			
Codonella cratera Leidy	11**	8**	4**	
Difflugia acuminata Ehrenberg	2			
Difflugia lobostoma Leidy	18**			
Difflugia pyriformis Ehrenberg	6**			
Difflugia sp.				1
Trachelophyllum sigmoides Kahl	3			
Protozoa – total	56	20	7	5
Alona sp.				<1
Chydorus sphaericus O.F. Müller				<1
nauplius Copepoda	2			
Crustacea – total	2			1
Zooplankton – total	101	37	25	57

** – strong domination (> 10%)

* – domination (5–10%)

The closest similarity in the zooplankton fauna between sites was determined for sites 1 and 4 (Table 3, Figure 2). The zooplankton at sites 2 and 4 was characterized by a high diversity defined by comparable values determined by Shannon index (2.40 and 2.33 respectively) and species richness index (5.82 and 5.44) – Table 2. No statistically significant differences were found in values of the species diversity index by Shannon-Wiener between sites 2, 3 and 4 (t-test, $p > 0.05$). The species diversity of the zooplankton was the highest at site 1 (2.81), and the eveness index at 0.886 accompanied by the smallest general abundance of zooplankton (101 indiv. dm^{-3}) suggests dominance of a few taxa which achieve high density: *Difflugia lobostoma* (18%), *Arcella discoides* (12%) and *Codonella cartera* (10.5%). The species diversity at site 1, measured with Shannon-Wiener index, was significantly different from the other sites (t-test, $p < 0.05$, site 2 and $p < 0.001$ sites 3 and 4).

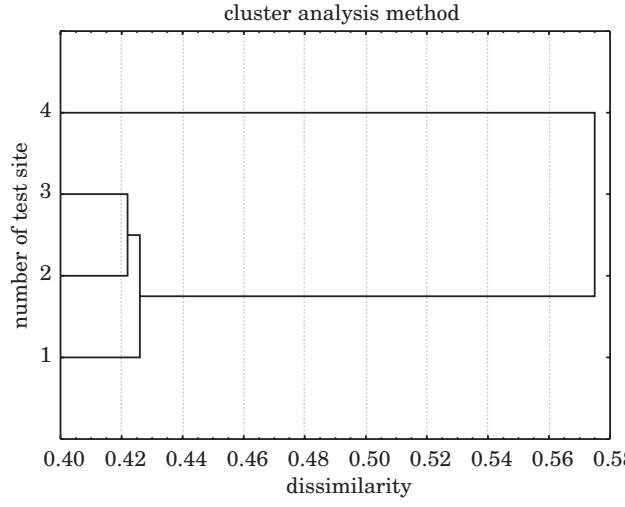

Fig. 2. A dendrogram of fauna dissimilarity between zooplankton communities in the analyzed sections of the Nida River

The most numerous and most diverse group of a higher order was Rotatoria (Table 4). Most of the taxa classified as rotifers were found at sites 2 and 4 (18 and 17, respectively), while the smallest number of such taxa was found at site 3 (9). Species which were present at all the analyzed sites along the river, being at the same time dominant ones in the structure of zooplankton, were *Keratella cochlearis* (max. 27.2% at site 4) and *Polyarthra remata* (max. 16% at site 3). Species of the genus *Trichocerca* were present constantly, although in smaller numbers.

T. similis appeared as a dominant (12%) at site 3. A large ecological group, especially at sites 2 and 4, consisted of small rotifers, usually dwelling amoung plants and/or characteristic for psammonic communities of the genera *Cephalodella*, *Colurella* and *Lecane* and *Lepadella*. *Cephalodella auriculata* reached up to 22.1% share in the total abundance of Rotatoria at site 4.

In general, the zooplankton at sites 3 and 4 was dominated in its abundance by rotifers, whose total share was 72 and 90.4%, respectively. At the other two sites, protozoa clearly dominated, constituting 55.2% (site 1) and 54.4% (site 4) of the total quantity of zooplankton.

The qualitative structure of the assemblage of protozoa consisted of ten identified species. Co-dominance of two species of amoeba was found, i.e. *Arcella* and *Difflugia*, as well as a typical plakton species of *Codonella cratera*.

Single specimens representing Crustacea were found only at site 4 (*Alona* sp. and *Chydorus sphaericus*) and at site 1 (growth stages nauplius of copepods)

Diversity of the quantitative structure of zooplankton

Values expressing the number of zooplakton at each analyzed site along the Nida River corresponded proportionally to the general volume of the biomass of plankton animals, which at all the sites was shaped by the weight of rotifers (Figure 3).

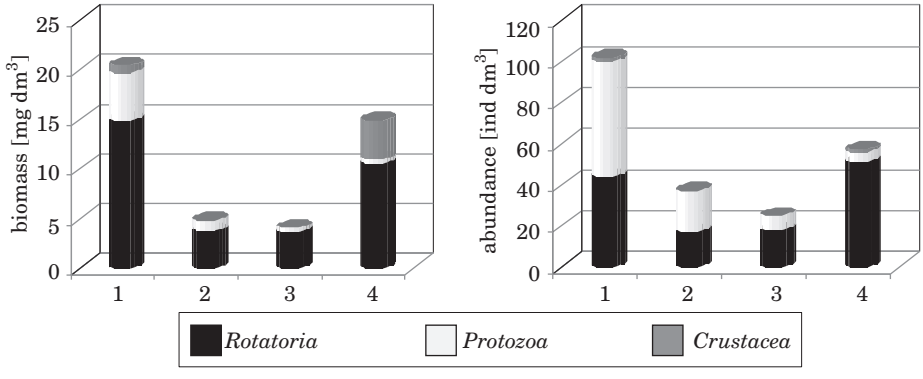

Fig. 3. Abundance and biomass of main groups of zooplankton at the analyzed sites along the Nida River

The highest zooplankton density and biomass were found at site 1 (101 indiv. dm^{-3} and 20.42 μg dm^{-3}) and the smallest ones – at site 3 (25 indiv. dm^{-3} and 4.09 μg dm^{-3}).

The prevailing number of Protozoa at the two sites located more upstream created just 32% (site 1) or 22.3% (site 2) of the total biomass, while the cladocerans present at the lowest sampling site along the river made up 37.4% of the value of this parameter (Figure 3).

Discussion

The general species diversity of the zooplankton along the analyzed section of the Nida River was not very high, which was due to the small number of identified taxa and their low density. This is, however, typical of lowland rivers similar in size to the Nida (second/third order rivers) in Poland. Plankton animals density fits in the range between dozen and maximum of two hundred in a litre. The highest values were recorded in spring and summer and along the sections where the water flow was slower, fed with tributaries from nearby lakes or dam reservoirs. It was confirmed by studies on the zooplankton of the Łyna River (BORKOWSKA 2001, DĄBROWSKA 2003) and its tributaries: the Symsarna (KUCHTA 2009, GOŹDZIEJEWSKA 2009) and Marózka Rivers, the Pasłęka River (GOŹDZIEJWSKA 2009) and the Wadąg River with its tributary, the Dymer (ENDLER et al. 2006). In general, the zooplankton in the Nida River was slightly less numerous than in the above watercourses, which could have been caused by a later date of water sampling (September), although the drastically decreasing tendency observed between sites 1 and 3 may have been due to the depressed quality of water caused by influx of pollutants from the river catchment basin and from the town of Nidzica. The diversity of the zooplankton biocensosis declines (10-fold less density and 30% fewer taxa) after the Dajna River passes through a catchment basin of agricultural and rural character (many villages), at a section 20 km in length, has been observed by GOŹDZIEJEWSKA (data not published).

The qualitative composition and proportions in the quantitative share of higher rank groups in the waters of the Nida River reveal an image typical of a river zooplankton biocenosis, associated with the dominance of Rotatoria, as observed in watercourses at different geographical latitudes and verified by researchers worldwide (SAUNDERS III and LEWIS Jr. 1988, ZARFDJIAN et al. 2000, MWEBAZA-NDAWULA et al. 2005, DJURKOVIC et al. 2008).

Adaptability of rotifers to flowing water and low concentration of foodstaff, such as phytoplankton, in water stems from a short time of generation in this group. At the same time, these two environmental factors limit the abundance of other assemblages, e.g. plankton crustaceans (cladocerans and copepods), which in stagnant water are a strong competition for shared food resources and often directly eliminate species of Rotatoria and Protozoa (LAMPERT and

SOMMER 2001). The qualitative and quantitative structure of the community of rotifers at all the sampling sites was mainly shaped by common eurytopic species: *Keratella cochelaris*, *Polyarthra remata*, *Trichocerca similis* and *T. pusilla*. While the two former species tolerate a very broad spectrum of environmental conditions, from lake ecosystems of different trophic relations, watercourses of variable flow (GOŹDZIEJEWSKA 2009), brackish water bodies (PATUREJ and GOŹDZIEJEWSKA 2005) to shallow ponds (GOŹDZIEJEWSKA and TUCHOLSKI, in press), the latter two species are quoted as indicators of high trophy RADWAN et al. (2004). The increasing role of these species in the structure of the zooplankton of the Wkra River occurred in its lower sections – sites 3 and 4. There, other indicators of eutrophication of river water appeared, i.e. *Brachionus angularis* and *K. cochlearis* var. *tecta*.

The upper section of the Wkra River was characterized by a generalny larger species diversity and the structure of biocenosis was shaped nearly equally by rotifers and protozoa. Noteworthy is the character of site 1, evidently different from the other sites. Higher species diversity determined at this site was attributable to a much faster regenerating population of protozoa, stimulated by a much more dynamic water flow in this part of the river, characterized by a narrower channel and shallower bottom. A similar growth in the number of Protozoa of the genus *Difflugia* sp. and *Arcella discoides* in water flowing into a river trough behind turbines of a power generating plant, coinciding with strong water turbulence, has been noticed by ENDLER et al. (2006). In turn, a biological factor which led to the dominance of plankton protozoa was most probably a very low (compared to the other sites) number of bivalves (SKRZYPCZAK et al. 2010), which while filtrating water eliminate both phytoplankton and small water animals (VAUGHN and HAKENKAMP 2001, LEVINTON and WALDMAN 2006). At the two following sites (downstream) on the Nida River, the genus *Difflugia* sp. was not noticed at all in the zooplankton, while the number of Bivalvia rose a hundred-fold (SKRZYPCZAK et al. 2010). Moreover, the poorest in taxa and the least numerous structure of rotifers and protozoa at site 3, versus all the other sites, could not have been caused, for example, by the pressure produced by fish because no presence of the analyzed Rotatoria species was determined in fish's digestive tracts (FURGAŁA-SELEZNIOW – oral report).

The single specimens representing cladocerans of the genera *Alona* sp. and *Chydorus sphaericus* captured at the site located most downstream (site 4) had most probably arrived from nearby fish culture ponds. The habitat conditions present in this section of the river favoured preservation of populations of small cladocerans and rotifers (e.g. *Cephalodella auriculata* was numerous), owing to an undisturbed, laminar flow of water and densly overgrown river bottom (mainly *Elodea canadensis*) (SKRZYPCZAK et al. 2010). Thus, the low

number of species belonging to Cladocera, as noticed in this study, was not caused by the hydrological and habitat-related factor but by the pressure produced by plankton-grazing fish living in the river (the stickleback, the ninespine stickleback) (FURGAŁA-SELEZNIOW – oral report). Therefore, plankton crustacea did not have any considerable effect on the general biomass of zooplankton along this section of the river. Moreover, such a low number of Cladocera could not have had any greater role in shaping the structure and abundance of rotifers and protozoa.

Recapitulating the above, it can be concluded that the structure and dynamics of changes in the biocenosis of the Nida River was more strongly conditioned by the hydrological and habitat-related conditions as well as inter-species interactions. Biotic factors, including pressure exerted by fish and predators, produced multidirectional effects favoured greater diversity and more rapid regeneration of the structure of zooplankton in the lower sections of the river.

Acknowledgements

Financial support for the research provided by: the Provincial Fund for Conservation of Nature and Water Management in Olsztyn, based on the subsidy agrement no 00029/09/62011/OP-PO/D, the district administrative office for the *Powiat* in Nidzica, the Polish Angling Association, the District in Ciechanów, the commune of Nidzica, the University of Warmia and Mazury in Olsztyn.

Translated by JOLANTA IDŹKOWSKA

Accepted for print 6.10.2010

References

ALLAN J.D. 1998. *Ekologia wód płynących*. Wyd. Nauk. PWN, Warszawa.

BORKOWSKA J. 2001. *Zooplankton rzeki Łyny na odcinku od mostu Obrońców Tobruku do mostu św. Jana*. UWM w Olsztynie. Zakład Ekologii i Hydrobiologii (praca magisterska).

CLARKE K.R. 1993. *Non-parametric multivariate analyses of changes in community structure*. Aust. J. Ecol., 18, 117–143.

DĄBROWSKA M.E. 2003. *Zooplankton rzeki Łyny na terenie Bartoszyc*. UWM w Olsztynie. Zakład Ekologii i Hydrobiologii (praca magisterska).

DJURKOVIC A., MIJOVIC S., CAJDO S. 2008. *Results of Zooplankton Analysis of the Sava River*. Balwois 2008 – Ohrid, Republic of Macedonia.

ENDLER Z., GOŹDZIEJEWSKA A., JAWORSKA B., GRZYBOWSKI M. 2006. *Wpływ małej elektrowni wodnej rzeki Dymer na organizmy planktonowe*. Acta Sci. Pol., Formatio Circumiectus, 5(2): 121–134.

FLÖSSNER D. VON. 1972. *Krebstiere, Crustacea. Kiemen- und Blattfüsser, Branchiopoda, Fischläuse*, Branchiura. VEB Gustav Fischer Verlag, Jena 1972.

GRIFFIN S., HERZFELD M., HAMILTON D.P. 2001. *Modelling the impact of zooplankton grazing on phytoplankton biomass during a dinoflagellate bloom in the Swan River Estuary, Western Australia*. Ecological Engineering, 16: 373–394.

GOŹDZIEJEWSKA A. 2009. *Wpływ małych elektrowni wodnych (MEW) na kształtowanie się struktury zooplanktonu na przykładzie wybranych rzek powiatu olsztyńskiego*. Mat. Zjazd. PTH. Wyd. UP w Lublinie, 119.

GOŹDZIEJEWSKA A., TUCHOLSKI S. *Zooplankton* of fish culture ponds periodically fed with treated wastewater. Polish J. Environ. Stud. (in press).

HERNROTH L. 1985. *Recommendations on methods for marine biological studies in the Baltic Sea. Mesozooplankton biomass assessment*. The Baltic Biologists Publication No. 10. Working Group 14.

HILTON J., O'HARE M., BOWES M.J., JONES J.I. 2006. *How green is my river? A new paradigm of eutrophication in rivers*. Science of the Total Environment, 365: 66–83.

JACK J.D., THORP J.H. 2002. *Impacts of fish predation on an Ohio River zooplankton community*. Journal of Plankton Research, 24(2): 119–127.

KUCHTA Ł. 2009. *Czy mała elektrownia wodna ma wpływ na rozmieszczenie zooplanktonu na rzece Symsarnie*? UWM w Olsztynie. Katedra Ekologii Stosowanej (praca magisterska).

LAMPERT W., SOMMER U. 2001. *Ekologia wód śródlądowych*. Wyd. Nauk. PWN, Warszawa. 195–271.

LEVINTON J.S., WALDMAN J.R. 2006. *The Huston River Estuary*. Cambridge University Press, 471: 217–227.

LÜDERITZ V., JÜPNER R., MÜLLER S., FELD Ch.K. 2004. *Renaturalization of streams and rivers- the special importance of integrated ecological methods in measurement of success*. An example from Saxony-Anhalt (Germany). Limnologica, 34: 249–263.

MARCZEWSKI E., STEINHAUS H. 1959. *O odległości systematycznej biotopów*. Zast. Mat., 4: 195–203.

MARGALEF R. 1957. *Information theory in ecology*. Gen. Syst., 3: 36–71.

MARMULLA G., ROSCH R. 1990. *Maximum daily ration of juvenile fish fed on living natural zooplankton*. J. Fish. Biol., 36: 789–801.

MILLS E.L., CONFER J.L., KRETCHMER D.W. 1986. *Zooplankton selection by young yellow perch: the influence of light, prey density, and predator size*. Transactions of American Fisheries Society, 115: 716–725.

MWEBAZA-NDAWULA L, SEKIRANDA S.B.K., KIGGUNDU V. 2005. *Variability of zooplankton community along a section the Upper Victoria Nile, Uganda*. Afr. J. Ecol., 43: 251–257.

NIESLER A., BIELAŃSKA-GRAJNER I. 2004. *Effect of the "Łąka" Dam Reservoir of planktonic Rotifers (Rotifera) communities in the Pszczynka River*. Teka Kom. Ochr. Środ. Przyr., I: 162–167.

PATUREJ E., GOŹDZIEJEWSKA A. 2005. *Zooplankton – based assessment of the trophic state of 3 three coastal lakes – Łebsko, Gardno, and Jamno*. Buul. Sea Fisch. Inst., 3(166): 7–26.

PIELOU E.C. 1975. *Ecological Diversity*. John Wiley & Sons Inc., New York.

RADWAN S., BIELAŃSKA-GRAJNER I., EJSMONT-KAROBIN J. 2004. *Fauna słodkowodna Polski. Wrotki (Rotifera)*. Zeszyt 32 A, B. Pol. Tow. Hydrobiol., UŁ, Oficyna Wydaw. Tercja, Łódź.

RECKENDORFER W., KECKEIS H., WINKLER G., SCHIEMER F. 1999. *Zooplankton abundance in the River Danube, Austria: the significance of inshore retention*. Freshwater Biology, 41: 583–591.

RYBAK J.I., BŁĘDZKI L.A. 2005. *Widłonogi. Copepoda: Cyclopoida. Klucz do oznaczania*. Insp. Ochr. Środ. Biblioteka Monit. Środowiska, Warszawa.

SAUNDERS III J.F., LEWIS JR. W.R. 1988. *Zooplankton abundance in the Caura River*, Venezuela. Biotropica, 20(3): 206–214.

SHANNON C.E. 1948. *A mathematical theory of communication*. Bell System Technical Journal, 27: 379–423.

SKRZYPCZAK A., FURGAŁA-SELEZNIOW G., KOSZAŁKA J., GOŹDZIEJEWSKA A., MAMCARZ A. 2010. *Rewitalizacja rzeki Nidy (górnej Wkry) w powiecie nidzickim. Raport ekspercki z projektu naukowo-technicznego.* Rewitalizacja rzeki Nidy (górnej Wkry) w powiecie nidzickim w 2009 roku, finansowanego przez WFOŚ i GW w Olsztynie na podstawie umowy nr 00029/09/62011/OP-PO/D (maszynopis), UWM w Olsztynie.

STARMACH K. 1955. *Metody badania planktonu,* PWRiL, Warszawa.

STREBLE H., KRAUTER D. 1978. *Das Leben im Wassertropfen. Mikroflora und Mikrofauna des Süßwassers*. Kosmos. Franckh'sche Varlagshandlung, W. Keller & Co., Stuttgart 1978.

SUTELA T., HUUSKO A. 2000. *Varying resistance of zooplankton prey to digestion: implications for quantifying larval fish diets*. Transactions of the American Fisheries Society 129, 545–551.
VAUGHN C.C., HAKENKAMP C.C. 2001. *The functional role of burrowing bivalves in freshwater ecosystems*. Freshwater Biology, 46: 1431–1446.
ZARFDJIAN M.H., MICHALOUDI E., BOBORI D.C., MOURELATOS S. 2000. *Zooplankton abundance in the Aliakmon River, Greece*. Belg. J. Zool., 130 (suppl. 1): 29–33.
ŻELAZO J., POPEK Z. 2002. *Podstawy renaturyzacji rzek*. Wyd. SGGW, Warszawa.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 401–414, Y. 2010

DOI 10.2478/v10020-010-0037-3

ECOLOGICAL STATUS AND PHYTOCOENOTIC DIVERSITY OF MACROPHYTES OF LAKE SZELĄG WIELKI (NORTH – EAST POLAND)*

***Mirosław Grzybowski*[1], *Józef Szarek*[2], *Krystyna A. Skibniewska*[3], *Janusz Guziur*[4]**

[1] Chair of Applied Ecology
[2] Chair of Pathophysiology, Forensic Veterinary Medicine and Administration
[3] Chair of the Foundations of Safety
[4] Department of Fish Breeding and Rearing
University of Warmia and Mazury in Olsztyn

K e y w o r d s: ESMI, phytocoenotic diversity, Lake Szeląg Wielki.

A b s t r a c t

This study was aimed at determining whether a pesticide tomb affects the ecological status of Lake Szeląg Wielki and to recognize how it affects phytocoenotic diversity of its vegetation. In addition, the results obtained will contribute to a database update and to the evaluation of the usability of a new Polish macrophytic method for the assessment of the ecological status of lakes. Analyses conducted in Lake Szeląg Wielki demonstrated that its ecological status was good, though the pesticide tomb had a modifying effect on the phytocoenotic diversity of aquatic and rush plants.

STAN EKOLOGICZNY I RÓŻNORODNOŚĆ FITOCENOTYCZNA MAKROFITÓW JEZIORA SZELĄG WIELKI (PÓŁNOCNO-WSCHODNIA POLSKA)

***Mirosław Grzybowski*[1], *Józef Szarek*[2], *Krystyna A. Skibniewska*[3], *Janusz Guziur*[4]**

[1] Katedra Ekologii Stosowanej
[2] Katedra Patofizjologii, Weterynarii Sądowej i Administracji
[3] Katedra Podstaw Bezpieczeństwa
[4] Katedra Biologii i Hodowli Ryb
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: ESMI, różnorodność fitocenotyczna, Jezioro Szeląg Wielki.

Address: Mirosław Grzybowski, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (89) 523 37 42, e-mail: grzybomi@uwm.edu.pl
* Research granted from dcientific sources as a research project in 2006–2009 MniSW no: N305 006 31/0326

Abstrakt

Przeprowadzone badania miały na celu stwierdzenie czy mogilnik pestycydowy wpływa na stan ekologiczny jeziora Szeląg Wielki i rozpoznać jak wpływa na różnorodność fitocenotyczną roślinności tego zbiornika. Uzyskane wyniki przyczyniają się do aktualizacji danych i oceny przydatności nowej polskiej metody makrofitowej – oceny stanu ekologicznego jezior. Przeprowadzone analizy wykazały, że stan ekologiczny jeziora Szeląg Wielki był dobry, mimo że mogilnik pestycydowy wywierał wpływ na zmianę różnorodności fitocenotycznej roślinności wodnej i szuwarowej jeziora.

Introduction

Lakes are an inherent element of environment, they constitute living--spaces for a variety of flora and fauna species and are an important element of a balanced landscape persistence. Phytocoenoses are subject to cyclic alterations, fluctuations and succession which, in the classic perspective, constitutes an oriented process of development, i.e. substitution of phytocoenoses over time (FALIŃSKA 1996, SCHEFFER 1998). These natural processes are accompanied by anthropogenic effects, of which especially hazardous appear to be landfills of overdue pesticides, the so-called "pesticide tombs". Lake Szeląg Wielki (Figure 1) is located in close proximity to a closed pesticide tomb (< 1 km). Pesticide tombs pose one of the most severe threats to the natural environment in Poland. Since 2003, complex analyses of edaphic and aquatic habitats have been carried out on their surrounding areas (GRZYBOWSKI et al. 2003, 2004, 2005 a,b, 2006, 2007 a,b, SAWICKA-KAPUSTA et al. 2005, SKIBNIEWSKA et al. 2003, SZAREK et al. 2004, 2006, 2007 a,b,c, ZAKRZEWSKA et al. 2005, ZMYSŁOWSKA et al. 2005).

This study was aimed at determining whether a pesticide tomb affects the ecological status of Lake Szeląg Wielki and to recognize how it affects phytocoenotic diversity of its vegetation. In addition, the results obtained will contribute to a database update and to the evaluation of the usability of a new Polish macrophytic method for the assessment of the ecological status of lakes.

Characteristics of study area

Lake Szeląg Wielki is located in the Ostróda Commune of the Iławski Lake District, belonging to the Warmia and Mazury Province. The aquifer is a typical ribbon-like narrow and very elongated lake (Table 1). It entries three small watercourses, two of which bring water from neighboring lakes: Szeląg Mały and Tabórz. Runoff from the aquifer proceeds south-west from

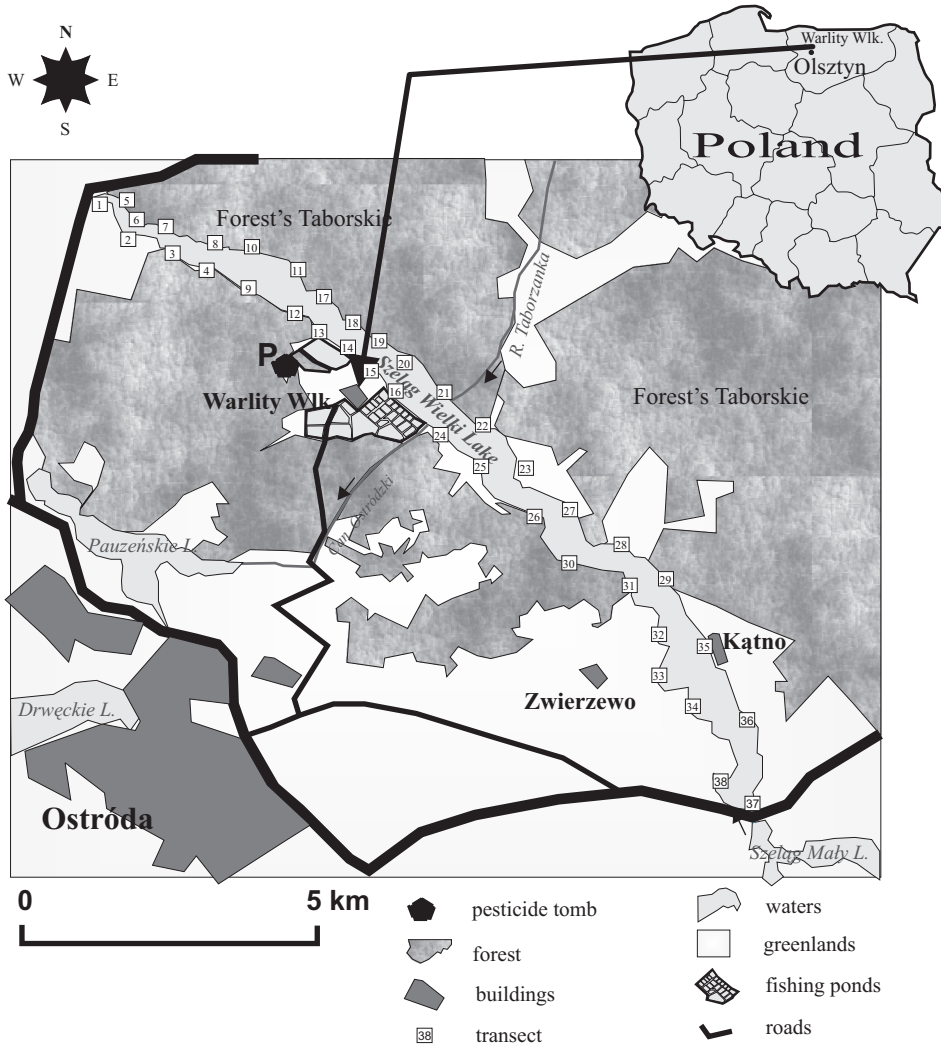

Fig. 1. Location of study area

Pauzeńskie Lake and Drwęckie Lake (Figure 1). The lake possesses a poorly developed shoreline (Table 1). The zone of littoral and sublittoral is narrow. The littoral occupies ca. 15% of bottom area.

Table 1

Morphometric data of the Lake Szeląg Wielki

Morphometric data (IIF, 1964)	
Area [ha]	599.0
Area of littoral [ha]	89.85
Area restricted by 2.5 m izobath [ha]	144.25
Volume [thousand m^3]	81 111.2
Maximal depth [m]	35.5
Mean depth [m]	13.5
Maximal length [m]	12 500
Maximal width [m]	900
Shoreline length [m]	28 000
Shoreline development	3.2
Exposure index	25.1

A pesticide tomb (PT) was in operation in the years 1968–2004 on a hill to the south-west of the lake, covering an area of 0.7 ha. The tomb was used as a landfill of 54 tonnes of toxic substances disposed in 36 silos and 2 unprotected cavities. The pesticide tomb under study was situated in sandy formations and even a small leakage of the chambers poses a threat to contamination of ground waters and the neighboring ecosystems of Lake Szeląg Wielki (Figure 1).

Following abiotic typology (KOLADA et al. 2005), Szeląg Wielki was classified as a stratified, charophyte deep lake. During summer stagnation, epilimnion reaches to a depth of 8 m and constitutes 36% of lake surface area and it is characterized by good aerobic conditions. In the stratum of metalimnion, there is a distinct reduction of oxygen concentration that intensifies gradually in the stratum of hypolimnion – which constitutes 44% of the lake bottom area. Oxygen depletion at the bottom does not occur and the mean oxygen saturation of lake hypolimnion exceeds 25% (CYDZIK et al. 1995, GRZYBOWSKI et al. 2005a, *Morfometric...* 1964).

Indices of the primary production, including the concentration of chlorophyl, dry matter of seston and water transparency, indicate its moderate trophy (CYDZIK et al. 1995). Bacteriological analyses of the lake demonstrated its very good sanitary status. In addition, water concentrations of the pesticides examined were within the binding reference values, yet these are mostly trace amounts (CYDZIK et al. 1995).

Lake Szeląg Wielki is characterized by high resistance to degradation – I category (CYDZIK et al. 1995). Worthy of notice is its small annual water

exchange that prevents the introduction of contaminants from the basin area to the lake. The prevalence of forests and relatively low recreational pressure exert an additional beneficial effect on the lake.

Methods

The ecological status of Lake Szeląg Wielki was evaluated with the use of an Ecological Status Macrophyte Index (ESMI) calculated in transects of lake phytolittoral. Methods of macrophytic assessment of the ecological status of lakes based on transects are applied in the monitoring system of European countries, including Germany (SCHAUMBURG et al. 2004a,b), Austria (CIECIERSKA et al. 2006), and Finland (LEKA et al. 2002, LEKA 2005), are recommended by the CEN standard (CEN 2003). The method consists of:

– plotting transects 20–30 in width perpendicularly to the shoreline;

– determining the maximum depth of plant occurrence (depth reach of macrophytes) which defines transect's length and the area the study is conducted on;

– estimating total percentage coverage of transects with plants;

– identifying all plant communities occurring in a transect and estimating their percentage coverage as compared to the total area occupied by plants converted in the scale of BRAUN-BLANQUET (1964).

For analyses of plants, the number of transects was determined from the formula *MLT* – minimal required number of transects (JENSÉN 1977, KESKITALO, SALONEN 1994).

Next, calculations were performed for the Ecological Status Macrophyte Index (CIECIERSKA et al. 2006). The ESMI is a multimetrix constructed from indices of species composition and macrophytes abundance (CIECIERSKA et al. 2006):

$$ESMI = 1 - \mathrm{ep}\left[-\frac{H}{H_{\max}} \cdot Z \cdot \exp\left(\frac{N}{P}\right)\right]$$

where:

The index of biocenotic diversity [H] (SAMOSIEJ 1987) has been calculated from Shannon-Wiener formula (SHANNON, WEAVER 1949):

$$H = -\sum \frac{n_i}{N} \ln \frac{n_i}{N},$$

where:
H – the phytocoenotic diversity index,
n_i – mean coverage of each phytocenosis (i) within phytolittoral is an arithmetic mean of its coverage in particular transects, after conversion of degrees of the BRAUN-BLANQUET scale into mean percentage coverage according to Table 2.
N – the phytolittoral area (100%).

Table 2

Scale of coverage degree used in the ESMI method (CIECIERSKA et al. 2006)

Range of coverage classes [%]	Braun-Blanquet Scale	Mean coverage [%]
75–100	5	86
50–75	4	61
25–50	3	34
5–25	2	15
1–5	1	3
0.1–1	+	0.5
0.1	r	0.1

The maximum value of phytocoenotic diversity (H_{max}) was determined from the following formula:

$$H_{max} = \ln S,$$

where:
S – the number of plant communities forming the phytolittoral,
Z – colonization index $Z = \frac{N}{izob.2{,}5}$,
izob.2.5 – littoral area restricted with 2.5 izobath (ha).

The obtained value of the ESMI index enables determining the ecological status of a lake (Table 3).

Table 3

Boundaries of classes of the ecological status for charophyte deep lakes based on macrophytes (CIECIERSKA et al. 2006)

Ecological status	Range of ESMI values
	charophyte deep lakes
Very good	0.680–1.000
Good	0.340–0.679
Moderate	0.170–0.339
Poor	0.090–0.169
Bad	< 0.090
	lack of submersed plants

The method of transects was also used in the evaluation of β-diversity according to WHITTAKER (1977) as the change among various phytocoenoses within one type of landscape.

In order to determine whether the pesticide tomb affects the ecological status of the lake, statistically significant differences were sought in phytocoenotic abundance, diversity index and phytocoenoses contribution in transects located in proximity to, and at a distance from, the pesticide tomb between the analyzed transects plotted for the phytolittoral of Lake Szeląg Wielki. The Pielous Evenness Index [J] was also computed (MAGURRAN 1988):

$$J = \frac{H_{obs}}{\log s}$$

where:
s – the number of phytocoenoses of a community in a transect.

The first stage of the statistical analysis involved verification of the distribution of results with the normal distribution by means of a Shapiro-Wilk test [W]. In the case of finding the normal distribution, use was made of a parametric Student's t-test (significance level of $\alpha = 0.05$), which assumes the normal distribution of results or differences between results. For the evaluation of significance of differences between mean values whose distribution was not consistent with the normal distribution, a U Mann-Whitney test (significance level of $\alpha = 0.05$) was used. The above-mentioned statistical analyses were carried out using Statistica 7.1 software (StatSoft, Inc. 2005). As transects remote from PT those located at least 2 km from the perpendicular line plotted so as to cut through two points were arbitrarily adopted: a point on the Lake Szeląg Wielki shoreline being the closest to the pesticide tomb and a point marked by the centre of a source of xenobiotics. Diversity computations were performed with Multi Variate Statistical Package (MVSP) ver. 3.1 software.

Results

Analyses carried out in the study enabled determining the number of transects in the phytolittoral of Lake Szeląg Wielki necessary for surveying, MLT = 38 transects. Analyses of transects demonstrated the presence of 22 communities (Table 4). The greatest range of plant occurrence in Lake Szeląg Wielki, i.e. 4.8 m, was noted for phytocenosis with *Fontinalis antipyretica*.

Community	Transect															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	mean coverage [%]															
Scirpetum lacustris (Allorge 1922) Chouard 1924	3	·	·	3	·	15	·	·	·	·	·	·	·	34	·	.
Phragmitetum australis (Gams 1927) Schmale 1939	15	61	34	61	61	61	34	86	3	86	3	·	·	61	·	.
Glycerietum maximae Hueck 1931	·	·	15	·	15	·	·	·	3	·	·	·	·	·	·	.
Equisetetum fluviatilis Steffen 1931	·	·	15	·	·	·	·	·	·	·	·	61	·	·	·	.
Typhaetum latifoliae Soó 1927	61	·	·	·	·	·	·	·	·	·	·	·	·	·	·	.
Typhaetum angustifoliae (Allorge 1922) Chouard 1924	·	·	·	0.1	·	·	3	·	·	·	·	·	·	·	·	.
Acoretum calami Kobendza 1948	·	·	·	·	·	·	·	·	34	·	·	·	·	·	·	.
Sparganietum erecti Roll. 1938	·	·	·	·	·	·	·	·	3	·	·	34	·	·	·	.
Eleocharitetum palustris Sennikov 1919	·	·	·	·	·	·	·	·	·	·	·	·	61	·	·	.
Sagittario-Sparganietum emersi R.Tx. 1953	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	.
Caricetum rostratae Ruebel 1912	·	·	·	·	·	·	3	·	·	·	·	·	·	·	·	.
Caricetum acutiformis Sauer 1937	·	·	·	·	·	·	·	·	·	1.5	·	·	·	·	15	.
Comm. with *Buttomus umbellatus*	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	15
Iridetum pseudoacori Eggler 1933	·	·	·	·	·	·	·	·	15	·	·	·	·	·	86	.
Lemno-Spirodeletum Soó 1927	·	·	·	·	·	·	·	0	0.1	·	·	·	·	·	·	0.5
Potametum perfoliati Koch 1926	·	·	0.5	·	·	·	3	·	15	·	34	·	·	·	·	.
Potametum natantis Soó 1927	·	·	·	·	·	·	·	·	·	·	0	·	·	·	·	.
Myriophylletum spicati Soó 1927	·	·	15	·	3	·	·	·	·	·	·	·	·	·	·	86
Elodeetum canadensis (Pign. 1953) Pass. 1964	·	·	·	·	·	·	·	·	3	·	·	·	·	·	·	.
Ceratophylletum demersi Hild 1956	·	34	3	34	3	·	15	15	·	·	·	·	34	·	·	.
Ranunculetum circinati (Bennema et West. 1943) Segal 1965	·	·	15	·	·	·	3	·	·	·	61	·	·	·	·	.
Fontinaletum antipyreticae Hub. 1957	·	15	·	·	·	15	34	·	15	·	·	·	·	·	·	.
Depth of plants occurrence in a transect	2	3.5	3.2	4.5	2	4	6	1.6	4.8	1.9	2.6	1.2	1.8	2.2	0.8	2
Percentage coverage of transect wth plants	90	90	100	90	90	80	100	90	100	95	80	90	90	100	90	90
Shanon-Wiener Index [*H*]	1.41	1.39	2.40	1.12	1.56	1.24	2.15	0.61	2.60	0.61	1.11	0.94	0.94	0.94	0.61	0.65
Evenness [*J*] Pielou	0.70	0.88	0.80	0.56	0.67	0.79	0.72	0.61	0.78	0.61	0.70	0.94	0.94	0.94	0.61	0.41
Num.Syntax.	4	3	8	4	5	3	8	2	10	2	3	2	2	2	2	3
Group	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1

Group: differentiates transects into those located in its proximity at a distance < 2 km from pesticide tomb (1)

Table 4

transects of Lake Szeląg Wielki

Transect																				
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
mean coverage [%]																					
.	.	.	.	15	.	.	.	.	.	.	.	34	.	.	3	.	.	.	.	.	.
.	86	86	61	61	61	15	.	.	.	.	34	61	61	61	34	.	61	34	3	15	61
.	.	.	.	.	15	.	.	.	.	.	34	.	.	.	.	.	.	.	3	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.
.	.	.	.	.	.	.	.	.	.	61	.	.	.	.	.	.	.	.	.	15	.
.	.	.	.	.	.	.	.	.	.	.	.	.	34	34	61	.	.	.	.	.	.
.	.	.	34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	64	.	.	3	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
86	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	3	.	.	.	3	.	86	.	.	3	.	.	.	.	.	.	.	.	.	.
.	.	.	.	3	.	.	86	.	.	.	.	.	.	.	.	34	.	15	.	61	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	34	.	.	.	.	.	.	.	.	.	15	.	.
.	.	.	0.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.1	.	.
.	15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	15	.	.
.	.	.	.	15	.	.	.	.	.	0	34	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	15	.	.	.	61	.	.	.	.	.	.	.	.	15	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34	3	.	3
.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	0.5	0.1	.	.	3	15
.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.1	.	.	.	.
.	.	.	.	.	3	61	.	.	.	.	.	.	.	.	.	.	34	.	15	.	3
1.6	2.4	3	2.6	2.8	4	5.4	0.4	0.6	1.8	2	2.2	2.4	2.6	2.6	2.9	3.4	4.8	3.4	4.8	3.2	4.2
90	100	100	90	100	90	95	100	100	100	90	100	100	100	100	100	100	100	90	100	100	100
0.61	0.61	0.78	0.99	1.41	1.41	1.41	0.00	0.00	0.94	0.94	1.73	0.94	0.94	0.94	1.11	0.97	1.13	2.03	2.60	1.41	1.56
0.61	0.61	0.34	0.50	0.70	0.70	0.70	0.00	0.00	0.94	0.94	0.86	0.94	0.94	0.94	0.70	0.61	0.49	0.87	0.78	0.70	0.67
2	2	5	4	4	4	4	1	1	2	2	4	2	2	2	3	3	5	5	10	4	5
1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

and those remote at a distance > 2 km from pesticide tomb (2)

The ecological status of the lake determined based on the ESMI was rated as good (Table 5). Results of analyses carried out for the normality of distribution of phytocoenotic diversity variables were collated in Table 5. The normal distribution was demonstrated for the variable: Shannon-Wiener diversity index. A lack of consistency with the normal distribution was observed in the case of the two other variables, i.e.: Pielou Evenness index [*J*] and the number of phytocoenoses in a transect (Table 6).

Table 5

Szeląg Wielki and reference lakes' phytocoenotic parameters

Lake name	Area of 2.5 m izobath	Area of phyto-littoral (N) [ha]	Number of phyto-littoral communities (*S*)	Index of phyto-coenotic diversity (*H*)	Index of colo-nization (*Z*)	Index of max. phytoco-enotic diversity (H_{max})	Syna-ntthro-pization index (*Ws*)	Ecological Status Macro-phyte Index (*ESMI*)
Szeląg Wielki	144.25	141.9	22	1.94	1.02	3.14	0.53	0.47

Table 6

Shapiro-Wilk's test for variables of phytocoenotic diversity

	N	*W*	*P*	Distribution
Index Shannon-Wiener	38	0.945448	0.063080	*N*
Pielou Evenness	38	0.856309	0.000185	n.n
Num_Syntax	38	0.828293	0.000042	n.n

W – value of Shapiro-Wilk coefficient; p – level of significance, n – normal distribution $W>W(\alpha;n)$; n.n – not normal distribution $W<W(\alpha;n)$; $W_{max}(0.05;38) = 0.863$

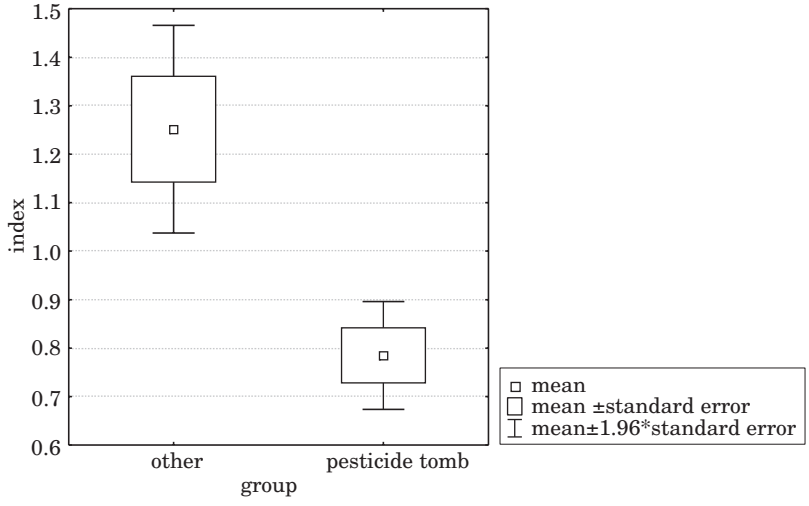

Fig. 2. Differences between mean values of Shannon-Wiener index for phytocoenoses located in the proximity of the pesticide tomb and those remote from it (Student's t-test, $\alpha = 0.05$; $p < 0.03$)

In analyses of the Shannon-Wiener index use was made of the Student's t-test. The results obtained were statistically significant ($\alpha = 0.05$; $p < 0.03$) and indicated significant differences between phytocoenoses of transects located close to the pesticide tomb (> 2 km) or remote from it (< 2 km) – Figure 2.

The analysis of the number of phytocoenoses and phytocoenotic diversity index was carried out based on a Mann-Whitney U test. The results obtained were statistically significant for the number of phytocoenoses: ($U = 101.5$; $p = 0.3194$) and for the Pielou phytocoenotic diversity index ($U = 87.5$, $p = 0.1398$).

Discussion

Aquatic and rush vegetation is of key significance for the proper functioning of the entire lacustrine ecosystem, in addition it displays high susceptibility to changes in all habitat factors of an aquatic ecosystem (BAATTRUP-PEDERSEN et al. 2001, SMOLDERS et al. 2001, CIECIERSKA 2004, SCHAUMBURG et al. 2004a,b). The higher the taxonomic and syntaxonomic diversity of phytolittoral, the higher the ecological status of a lake (ENDLER et al. 1999, GRZYBOWSKI, ENDLER 2003, CIECIERSKA et al. 2006). The mean value of the Shannon-Wiener diversity index (H) for aquatic and rush plants in Lake Szeląg Wielki (Table 4), that of Pielou Evenness index (J) (Table 4), as well as the mean number of phytocoenoses (Table 4) was typical of poorly eutrophic lakes (ENDLER et al. 1999, MURPHY 2002). The presence of 22 communities observed in the examined transects indicates that the method of transects fully reflects the phytocoenotic diversity of a lake; the number of macrophytic phytocoenoses observed during phytosociological assays of Lake Szeląg Wielki was identical (GRZYBOWSKI et al. 2007b). A good ecological status of Lake Szeląg Wielki, determined based on the ESMI index, was the same as in the case of 39 lakes out of the 153 ones examined with this method (CIECIERSKA et al. 2006), which were also characterized by a similar phytocoenotic composition, but differed in terms of the presence of green algae (*Charophyceae*) whose communities are an important indicator of high macrophytic evaluation of the ecological status of lakes (FORSBERG 1964, KRAUSE 1981). They were not detected in Lake Szeląg Wielki.

The total number of plant communities in a lake results, among other things, from diversity of habitat conditions of littoral (JENSÉN 1977, ROONEY, KALFF 2000). The size of a lake, its length and development of its shoreline affect the number of microhabitats for macrophytes. Lake Szeląg Wielki is characterized by favorable habitat conditions. The average number of plant communities among 153 reference lakes originating from a data base of lakes

selected for requiring lake surveillance in Poland following the Directive 2000/60/EC (*Directive* 2000/60/EC...) ranges from 21 to 23 depending on the type of lake (CIECIERSKA et al. 2006). In Lake Szeląg Wielki there were 22 communities detected, which indicates considerable phytocoenotic diversity of its phytolittoral.

Conclusions

Analyses conducted in Lake Szeląg Wielki demonstrated that:
– its ecological status was good;
– the pesticide tomb affected its ecological status;
– the pesticide tomb had a modifying effect on the phytocoenotic diversity of aquatic and rush plants.

Translated by JOANNA JENSEN

Accepted for print 16.02.2010

References

BAATTRUP-PEDERSEN A., ANDERSSON B., BRANDRUD T.E., KARTTUNEN K., RIIS T., TOIVONEN H. 2001. *Macrophytes*. [In:] *Biological monitoring in Nordic rivers and lakes.* Ed. J. Skriver. National Environmental Research Institute, Denmark, Temanord, 513: 53–60.

BRAUN-BLANQUET J. 1964. *Pflanzsoziologie. Grundzuge der Vegetationskunde.* – 3 Aufl. Springer, Wien-New York.

CEN 2003: EN-14184, *Guidance standard for the surveying of macrophytes in lakes.* CEN/TC230/WG2/TG3:N72, Comite Europeen de Normalisation.

CIECIERSKA H., 2004. *Różnorodność fitocenotyczna litoralu jezior Mazurskiego Parku Krajobrazowego – stan obecny i zmiany*. Teka Komitetu Ochrony Kształtowania Środowiska Przyrodniczego, Lublin, 1: 32–38.

CIECIERSKA H., KOLADA A., SOSZKA H. GOŁUB M. 2006. *Opracowanie podstaw metodycznych dla monitoringu biologicznego wód powierzchniowych w zakresie makrofitów i pilotowe ich zastosowanie dla części wód reprezentujących wybrane kategorie i typy.* T. II *Jeziora.* IOŚ, UWM. Warszawa-Poznań-Olsztyn.

CYDZIK D., KUDELSKA D., SOSZKA H. 1995. *Atlas stanu czystości jezior Polski badanych w latach 1989–1993.* Biblioteka Monitoringu Środowiska PIOŚ, Warszawa.

ENDLER Z., GRZYBOWSKI M., JUŚKIEWICZ B. 1999. *Przegląd fitocenoz wodnych i szuwarowych Pojezierza Mazurskiego.* Zeszyty WSP, Prace biologiczne, Zeszyt II: 35–43.

FALIŃSKA K. 1996. *Ekologia roślin. Podstawy teoretyczne, populacja, zbiorowisko, procesy.* PWN, Warszawa, 21–97.

FORSBERG C. 1964. *Phosphorus, a maximum factor in the growth of Characeae*. Nature, 201: 517–518.

GRZYBOWSKI M., ENDLER Z. 2003. *Zmiany zbiorowisk makrofitów podmiejskiego Jeziora Trackiego pod wpływem antropopresji.* Acta Sci. Pol., Formatio Circumiectus, 2(2): 149–156.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K.A., SAWICKA-KAPUSTA K., GUZIUR J., ENDLER Z. 2003. *Assessment of flora development in the Iława district Lake Szeląg Wielki.* 1st Workshop *''Persistent Toxic Substances Contamination of the European Region.''* Brno, Czech Republic, 10–12: 161–164.

GRZYBOWSKI M., SZAREK J., ZMYSŁOWSKA I., GUZIUR J., ENDLER Z., SKIBNIEWSKA K. 2004. *Ecological*

characteristic of pesticide tomb in the Warmia region on the basis of index numbers of vascular plants. Pol. J. Environ. Stud., 13(6): 683–692.

GRZYBOWSKI M., SKIBNIEWSKA K., GUZIUR J., SZAREK J., ENDLER Z., ZMYSŁOWSKA I. 2005a. *Zawartość Pb i Cd w wybranych gatunkach roślin wokół mogilnika pestycydowego*. J. Elementol., 10(2): 289–293.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K., SAWICKA-KAPUSTA K., GUZIUR J., ENDLER Z. 2005b. *The characteristics of plants in the littoral zone of Lake Szeląg Wielki in the Iława Lake District threatened by a pesticide tomb*. Fresenius Environ. Bull., 14(5): 357–362.

GRZYBOWSKI M., SKIBNIEWSKA K.A., SZAREK J., GUZIUR J., ENDLER Z., ZMYSŁOWSKA I., SAWICKA--KAPUSTA K., ZAKRZEWSKA M. 2006. *The ecological characteristics of antropogenic forest phytocoenoses threatened by a pesticide tomb*. Pol. J. Natur. Sc., 21(2): 769–791.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K.A., GUZIUR J. 2007a. *Evaluation of diversity of submerged and emerged flora of Lake Szeląg Wielki as threatened by a pesticide tomb*. Pol. J. Nat. Sc., 25(2): 154–172.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K.A., GUZIUR J., LEW S., MIESZCZYŃSKI T., GESEK M. 2007b. *Evaluation of alpha diversity of an anthropogenic forest threatened by pesticide tomb*. Pol. J. Nat. Sc., 25(1): 63–80..

Morfometric data of Szeląg Wielki Lake. 1964. Institute of Inland Fishery, Olsztyn.

JENSÉN S. 1977. *An objective method for sampling the macrophyte vegetation in lakes*. Vegetatio 33: 107–118.

KESKITALO J., SALONEN K. 1994. *Manual for integrated monitoring. Subprogramme Hydrobiology of Lakes*. National Board of Waters and the Environment, Helsinki, Vesi-Ja Ymparistohallinnon Julkasuja, Seria B, 16: 28–30.

KOLADA A., SZOSZKA H., CYDZIK D., GOŁUB M. 2005. *Abiotic typology of Polish lakes*. Limnologica, 35(3): 145–150.

KRAUSE W. 1981. *Characeen als Bioindikatoren für den Gewässerzustand*. Limnologica, 13(2): 399–418.

LEKA J. 2005. *Macrophytes as a tool to assess the ecological status of lakes*. [In:] *Sampling. Presentations of three training seminars on Quality Assurance (QA). Biological methods of Water Framework Directive and Waste water sampling techniques*. Ed. H. Lääne, W. Heinonen. Suomen ympäristökeskuksen moniste. 328: 60–64.

LEKA J., YALTA-HULKKONEN K., KANNINEN A., AIRAKSINEN O. 2002. *Aquatic macrophytes in the dassification of ecological status of boreal lakes: Testing field study methods and aerial photographing as tool for monitoring*. [In:] *Typology and ecological dassification of lakes and rivers*. Eds. M. Ruoppa, K. Karttunen. TemaNord., 566: 93–96

MAGURRAN A. 1988. *Ecological diversity and its measurement*. Princeton, New Jersey: Princeton University Press.

MURPHY K.J. 2002. *Plant communities and plant diversity in softwater lakes of northern Europe*. Aquatic Botany, 73: 287–324.

Directive 2000/60/EC of the European Parliament and of the Council of the European Union 23 October, 2000 establishing a framework for Communities in the field of water policy. Official Journal of the European Union, issue L., 327 pp.

ROONEY N., KALFF J. 2000. *Inter-annual variation in submerged macrophyte community biomass and distribution: the influence of temperature and lake morphometry*. Aquat. Bot., 68: 321–335.

SAMOSIEJ L. 1987. *Wpływ antropopresji na roślinność litoralu jezior w krajobrazie rolniczym południowych Kujaw*. Lodz University [unpublished work].

SAWICKA-KAPUSTA M., ZAKRZEWSKA M., KUTRZEBA L., SKIBNIEWSKA K.A., SZAREK J., GUZIUR J., GRZYBOWSKI M., ZMYSŁOWSKA I. 2005. *Zawartość metali ciężkich w narządach myszy leśnej (Apodemus flavicollis) z lasów w pobliżu mogilnika pestycydowego*. J. Elementol, 10(3): 781–786.

SHANNON C.E., WEAVER W. 1949. *The mathematical theory of communication*. Urbana, University of Illinois Press.

SCHAUMBURG J., SCHRANZ CH., HOFMANN G., STELZER D., SCHNEIDER S., SCHMEDTJE U. 2004a. *Handlungsanweisung für die ökologische Bewertung von Seen żur Umsetzung der EU-Wasserrahmenrichtlinie. Makrophyten und Phytobenthos*. Bayerisches Landesamt für Wasserwirtschaft, München.

SCHAUMBURG J., SCHRANZ CH., HOFMANN G., STELZER D., SCHNEIDER S., SCHMEDTJE U. 2004b. *Macrophytes and phytobenthos as indicators of ecological status in German lakes – a contribution of the implementation of the Water Framework Directive*. Limnologica, 34: 302–314.

SCHEFFER M. 1998. *Ekology of Shallow Lakes*. Chapman and Hall, London.

SKIBNIEWSKA K.A., GUZIUR J., GRZYBOWSKI M., SZAREK J. 2003. *DDT in the soil of a pesticide tomb and surrounding ecosystems*. [In:] *On Analitical and Environmental Problems*. Ed. Z. Gallbacs.

SMOLDERS A.J.P., LAMERS L.M.P., ROELOFS J.G.M. 2001. *Aquatic macrophytes in assessment and monitoring of ecological status of aquatic environments*. TemaNord, 563: 23–31.

StatSoft, Inc. 2005. STATISTICA (data analysis software system), version 7.1. www.statsoft.com.

SZAREK J., SKIBNIEWSKA K.A., LIPIŃSKA J., GUZIUR J., BABIŃSKA I., ANDRZEJEWSKA A., GRZYBOWSKI M., KAPUSTA-SAWICKA K., ZMYSŁOWSKA I., ZAKRZEWSKA M. 2004. *Zagrożenie ekosystemów wodnych na przykładzie mogilnika pestycydowego w Warlitach Wielkich*. [In:] *Ochrona zdrowia ryb – aktualne problemy*. Eds. A.K. Siwicki, J. Antychowicz, W. Szweda. Wyd. IRŚ, Olsztyn, 203–210.

SZAREK J., SKIBNIEWSKA K.A., GUZIUR J., WOJTACKA J., GRZYBOWSKI M., BABIŃSKA I., ANDRZEJEWSKA A., SAWICKA-KAPUSTA K., ZMYSŁOWSKA I., ZAKRZEWSKA M., CHEŁSTOWSKA D. 2006. *Pathomorphological pattern of hepatopancreas in carp (Cyprinus carpio L.) originating from a pond near a pesticide tomb*. [In:] *Biotechnology 2006*, Environmental biotechnology. Ed. V. Řehout. Scientific Pedagogical Publishing, Česke Budějovice, Czech Republic, pp. 899–901.

SZAREK J., SKIBNIEWSKA K.A., GUZIUR J., WOJTACKA J., GRZYBOWSKI M., BABIŃSKA I., ANDRZEJEWSKA A., SAWICKA-KAPUSTA K., FELSMANN M., ZAKRZEWSKA M., ZMYSLOWSKA I. 2007a. *Pathomorphological pattern of the hepatopancreas and kidneys in carp (Cyprinus carpio Linnaeus, 1758) bred in a pond near a pesticide tomb in Ilawskie Lake District in the years 2004 and 2006*. Proceedings of the III International Conference *"Fishery"*, Ideja Print, Loznica, Republic of Serbia. pp. 198–206.

SZAREK J., SKIBNIEWSKA K.A., GRZYBOWSKI M., GUZIUR J., SAWICKA-KAPUSTA K., ZAKRZEWSKA M., ANDRZEJEWSKA A., ZMYSLOWSKA I., FELSMANN M. 2007b. *Pathomorphology as a tool in searching for sources of environmental pollution*. Proceedings of the International Conference on Environmental Management, Engineering, Planning and Economics. Skiathos, GRAFIMA Publ., Thessaloniki, Greece, 7–12.

SZAREK J., SKIBNIEWSKA K.A., WOJTACKA J., GUZIUR J., BABIŃSKA I., ANDRZEJEWSKA A., SAWICKA-KAPUSTA K., GRZYBOWSKI M., ZAKRZEWSKA M., ZMYSŁOWSKA I., FELSMANN M. 2007c. *Effect of the pesticide tomb in Ilawskie Lake District on the morphological pattern of the liver and kidneys of the black-striped field mouse (Apodemus agrarius, Pallas 1771) in the year 2004 and 2006*. Pol. J. Pathol., 58(2): 147.

WHITTAKER R.H. 1977. *Evolution of species diversity in land communities*. Evol. Biol., 10: 1–67.

ZAKRZEWSKA M., SAWICKA-KAPUSTA M., ALEKSANDROWICZ P., STOCHMA M., SZAREK J., GRZYBOWSKI M., SKIBNIEWSKA K.A., GUZIUR J., ZMYSŁOWSKA I. 2005. *Poziom metali ciężkich w tkankach nornicy rudej (Clethrionomys glareolus) z terenu oddziaływania mogilnika pestycydowego*. J. Elementol., 10(3): 851–856.

ZMYSŁOWSKA I., SZAREK J., GOLAŚ I., GRZYBOWSKI M., GUZIUR J., SKIBNIEWSKA K.A. 2005. *Oddzialywanie mogilnika pestycydowego na mikroflorę promieniowców i grzybów w środowisku wodnym i glebowym.*. Zesz. Probl. Post. Nauk Roln., 505: 529–543.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 415–426, Y. 2010

DOI 10.2478/v10020-010-0038-2

DIFFERENCES BETWEEN THE EFFECTS ON REPRODUCTION IN CARP *CYPRINUS CARPIO* (L.) OF YUGOSLAVIAN STRAIN J AND HUNGARIAN STRAIN 0 AFTER STIMULATION OF OVULATION WITH CARP PITUITARY HOMOGENATE OR OVOPEL

Elżbieta Brzuska

Institute of Ichthyobiology and Aquaculture in Gołysz
Polish Academy of Sciences

K e y w o r d s: stimulation of ovulation, carp's strains, carp pituitary homogenate (CPH), Ovopel.

A b s t r a c t

The effects on reproduction were investigated in carp of the Hungarian strain 0 and the Yugoslavian strain J after CPH or Ovopel tratment. After hypophysation eggs were obtained from a similar percentage (~85%) of fish in both strains while after the Ovopel treatment from 100% of females in strain 0 and 50% in strain J. Interaction between the preparation and the provenance of the fish was statistically significant ($P \leq 0.05$) for the weight of eggs (in grams and in percentage of female body weight). The highest weight of eggs was obtained after Ovopel treatment of females of strain J and the lowest after CPH application to fish of the same strain (1042.98 g, 15.60% and 746.49 g, 10.97%, respectively). The statistical significance ($P \leq 0.01$) of the interaction was noted too for the fertilization percentage; the lowest quality characterized eggs obtained from females of strain J treated with CPH and the highest for eggs of the same strain treated with Ovopel (95.80% and 98.20%, respectively). The effect of latency as an important factor of quality of the eggs obtained after ovulation induction with CPH and with Ovopel from females of the strain 0 was determined. The comparison of reproduction effects of the investigated breeding strains of carp showed that these strains differently respond to the ovulation stimulators applied.

RÓŻNICE W EFEKTACH ROZRODU KARPIA *CYPRINUS CARPIO* (L.) JUGOSŁOWIAŃSKIEJ LINII J ORAZ WĘGIERSKIEJ LINII 0 PO STYMULOWANIU OWULACJI HOMOGENATEM PRZYSADKI KARPIA LUB OVOPELEM

Elżbieta Brzuska

Zakład Ichtiobiologii i Gospodarki Rybackiej w Gołyszu
Polska Akademia Nauk

S ł o w a k l u c z o w e: stymulowanie owulacji, linie karpia, homogenat przysadki karpia (CPH), Ovopel.

Address: Elżbieta Brzuska, Polish Academy of Sciences, Institute of Ichthyobiology and Aquaculture in Gołysz, ul. Kalinowa 2, 43-520 Chybie, Poland, phone: +48 (33) 853 37 78,
e-mail: urszula.karosek@fish.edu.pl

Abstrakt

Badano wyniki rozrodu karpia węgierskiej linii 0 oraz jugosłowiańskiej linii J po podaniu samicom CPH lub Ovopelu. Po hypofizacji pozyskano ikrę od podobnego procentu (~85%) ryb z obu linii, natomiast po podaniu Ovopelu od 100% samic z linii 0 i 50% z linii J. Interakcja między podawanym preparatem a pochodzeniem samic była statystycznie istotna ($P \leq 0.05$) dla masy ikry (wyrażonej w gramach i w procencie masy ciała samic). Najwyższą masę jaj pozyskano po podaniu Ovopelu samicom linii J, a najniższą po zastosowaniu homogenatu przysadki mózgowej u ryb z tej samej linii (odpowiednio 1042,98 g, 15,60% i 746,49 g, 10,97%). Statystyczną istotność ($P \leq 0,01$) badanej interakcji odnotowano dla procentu zapłodnienia; najniższą jakością charakteryzowały się jaja pozyskane od samic z linii J, którym podano CPH, a najwyższą od samic z tej samej linii, którym aplikowano Ovopel (odpowiednio 95,80% i 98,20%). Odnotowano wpływ czasu latencji na jakość ikry otrzymanej po stymulowaniu owulacji CPH i Ovopelem u samic z linii 0. Porównanie wyników rozrodu badanych linii hodowlanych karpia pozwoliło wykazać, że linie te różnie reagują na podanie użytych stymulatorów owulacji.

Introduction

The effects of different breeding strains on the reproduction of common carp have been investigated in extensive studies in the Institute of Ichthyobiology and Aquaculture at Gołysz (Polish Academy of Sciences) (BRZUSKA 2006a). The investigations presented in this paper document the progress in evaluating the results of reproduction of different carp strains after ovulation stimulation with Ovopel – a commercial preparation frequently used in the stimulation of ovulation and spermation in various fish species, not only from the family of Cyprinidae. This preparation (containing in 1 pellet 18–20 μg of D-Ala^6, Pro^{9}NEt-mGnRH and 8–10 mg of dopaminergic inhibitor – metoclopramide) was applied for ovulation stimulation in common carp by HORVÁTH et al. (1997), KŁODZIŃSKA, OKONIEWSKI (1998), KOUŘIL at al. (2003) and MIKOŁAJCZYK at al. (2004). In the investigation conducted in the Gołysz Institute, Ovopel was applied to females of the following breeding strains: Hungarian strain W (BRZUSKA 2000, 2003a, 2005, BRZUSKA, BIAŁOWĄS 2002), Hungarian strain 7 (BRZUSKA 2006c), Polish strain 6 (BRZUSKA 2000, 2003a, 2005), Polish strain 3 (BRZUSKA 2003b), Lithuanian strain B (BRZUSKA 2006b), Israeli strain Dor-70 (BRZUSKA 2001, BRZUSKA, GRZYWACZEWSKI 1999), and French strain F (BRZUSKA, BIAŁOWĄS 2002). The effects of ovulation stimulation with Ovopel in females of ten breeding strains of carp from Gołysz on the quality of eggs was given by CEJKO (2007).

In the present study the experiment involved fish of the Hungarian strain 0 and Yugoslavian strain J. The Hungarian strain 0 was produced in the Szarvas Institute of Fish Culture in the 1960s as the cross of two Hungarian breeding strains e.g. strain 7 and strain 8 (BIAŁOWĄS 1999). For many years strain 0 was regarded as being of the highest consumption value and the

highest productivity. Fish of this strain are characterized by very rapid growth and high survival rates during the first production season. In 1973 the spawners of this strain were brought from Szarvas to Gołysz. Females of this strain are most frequently crossed with males from the Polish strain 3 or the Polish strain 6 to obtain stocking material of good quality for commercial production. The initial material for the Yugoslavian strain J was a batch of two-year-old carp brought from Yugoslavia to the Gołysz Institute in 1975. The parents of successive generations were spawners characterized by the highest growth rate (BIAŁOWĄS 2000). The effects of reproduction after ovulation stimulation with CPH show that the females of strain J yielded eggs of the lowest weight and of strain 0 of the highest weight in a stock of 13 investigated strains (BRZUSKA 1991). The aim of the reseach presented in this paper was to compare the propagation effects in strain J and strain 0 after treatment with CPH or with Ovopel. It was also attempted to determine if the latent period affected the weight and parameters characterizing the quality of eggs obtained from fish of the two genetically distant investigated strains.

Material and Methods

The experiment was carried out in May on 28 females aged 8 years and 4.65–8.30 kg body weight. Of this number, 12 fish were of the Yugoslavian strain J and 16 of the Hungarian strain 0. The females were selected on the basis of external maturity signs from a greater population of spawners kept in one pond. The fish were transferred to the hatchery and divided into two groups of 14 individuals each. Each group consisted of six females of the Yugoslavian strain J and eight of the Hungarian strain 0. The fish were kept in four tanks of 3 m^3 with seven individuals per tank. During the whole period of the experiment the temperature of water was maintained at 21–22°C. The adaptation period was 24 h, then the stimulation of ovulation was started. In group I the ovulation stimulator was carp pituitary homogenate (CPH: 0.3 mg after 12 h 2.7 mg kg^{-1}) and in group II – Ovopel (1/5 pellet after 12 h 1 pellet kg^{-1}). CPH and Ovopel were applied to the females in the form of intraperitoneal injections. The gonadotrophic activity of carp hypophyses (derived from the own resources of the Gołysz Institute after a 6-year storage period) were determined according to JACÓ et al. (1989).

After stripping, eggs from every female of the two groups collected in separate containers were weighed and fertilized with pooled milt from three males of each strain which also were hormonally stimulated (one intraperitoneal dose of Ovopel; 1 pellet kg^{-1}). After the elimination of stickiness (according to Woynarovich, Woynarovich, 1980) the fertilized eggs from each

female were separately incubated in Weiss glasses (7 L) in water at 21–22°C during the incubation period. After 12 h incubation the percentage of egg fertilization and after 24, 36, and 48 h the percentages of live embryos were calculated for each female separately.

In order to estimate the effect of the main classification factors (ovulation stimulator, the provenance of females, and the interaction between the ovulation stimulator and the provenance of females) on the investigated parameters (i.e. the weight of eggs expressed in grams and as a percentage of female body weight, percentage of fertilization, and percentage of living embryos after 24, 36 and 48 h incubation of the eggs) analysis of variance was carried out using the least-squares method (HARVEY 1987), according to the following linear model:

$$Y_{ijk} = \alpha + g_i + p_j + (gp)_{ij} + bW_{ijk} + e_{ijk}$$

where:

α – the theoretical general mean with the assumption that $W_{ijk} = 0$;
g_i – the effect of an ovulation stimulator ($i = 1...2$);
p_j – the effect of female's provenance ($j = 1...2$);
gp_{ij} – the interaction between the ovulation stimulator and the provenance of the female; b – the regression on the body weight of a female k;
b – the regresion on the body weight of a female k;
W_{ijk} – body weight of a female k;
e_{ijk} – the random error connected with observation k.

The F-test was used in checking the significance of the main classification factors on the investigated parameters. The estimated constants and least-squares means for the investigated parameters within main classification factors are given in Table 1.

With the aim of showing the effect of latency on the investigated parameters of reproduction seven analyses of variance were carried out using the least-squares method. The effect of latency, stimulator of ovulation and provenance of females were entered into the respective linear model, according to which these analyses were carried out. Statistical analyses permitted the estimation of constants and the least-squares means characterizing the effects of reproduction associated with latency. These data are given in Table 2.

Table 1

Constants (LSC) and least-squares means (LSM) estimated for investigated parameters and results of F-test (*$P \leq 0.05$; **$P \leq 0.01$)

Classification factor	Parameters																							
	Weight of eggs [g]				Weight of eggs [% of female body weight]				Percentage of fertilized eggs after 12 h incubation				percentage of living embryos after											
													24 h incubation				36 h incubation				48 h incubation			
	α = 924.31				α = 13.80				α = 96.99				α = 93.29				α = 90.08				α = 86.27			
	LSC	LSM	SE	F	LSC	LSM	SE	F	LSC	LSM	SE	F	LSC	LSM	SE	F	LSC	LSM	SE	F	LSC	LSM	SE	F
Ovulation stimulator				*				*				–				–				–				–
CPH (group I)	-75.40	848.91	81.59	–	-1.20	12.60	1.16	–	-0.34	96.65	0.41		1.21	94.50	2.93		1.53	91.61	3.41		1.11	87.38	3.55	
Ovopel (group II)	75.40	999.71	78.74		1.20	15.00	1.12		0.34	97.33	0.48		-1.21	92.09	2.83		-1.53	88.55	3.29		-1.11	85.64	3.43	
Origin of the females				–				–				–				–				–				–
strain J	-29.58	894.74	91.38		0.51	13.29	1.30		-0.09	96.90	0.46		1.42	94.71	2.28		2.07	92.15	3.82		1.92	88.20	3.98	
strain 0	29.58	953.89	74.79		-0.51	14.32	1.07		0.09	97.09	0.38		-1.42	91.87	3.28		-2.07	88.01	3.13		-1.92	84.35	3.25	
Interaction stimulator x origin of females				*				*				**				–				–				–
CPH x J	-72.85	746.49	116.26		-1.11	10.97	1.66		-0.73	95.83	0.59		1.21	94.50	2.93		0.17	93.85	4.86		-0.69	88.61	5.06	
CPH x 0	72.85	951.34	100.54		1.11	14.23	1.43		0.73	97.48	0.51		-1.21	92.09	2.83		-017	89.37	4.21		0.69	86.15	4.37	
Ovopel x J	72.85	1042.99	126.72		1.11	15.60	1.81		0.73	98.00	0.64		1.42	94.71	3.28		-0.17	90.45	5.30		0.69	87.78	5.51	
Ovopel x 0	-72.85	959.44	88.03		-1.11	14.40	1.25		-0.73	96.69	0.44		-1.42	91.87	2.69		0.17	86.67	3.68		-0.69	82.55	3.83	
Regression/body weight	136.82	136.82	55.21	*	-0.07	-0.07	0.79	–	-0.83	-0.83	0.28	–	-1.52	-1.52	1.98	–	-1.27	-1.27	2.31	–	-1.33	-1.33	2.40	–

SE, standard error of LSM; α, the theoretical general means; LSC, least-squares constants

Table 2

Constants (LSC) and least-squares means (LSM) characterizing the effect of propagation associated with the latency[L] and results of the F-test (*$P \leq 0.05$)

Classification factor	Weight of eggs [g]				Weight of eggs [% of female body weight]				Percentage of fertilized eggs after 12 h incubation				Percentage of living embryos after											
													24 h incubation				36 h incubation				48 h incubation			
	α	LSC	LSM	F	α	LSC	LSM	F	α	LSC	LSM	F	α	LSC	LSM	F	α	LSC	LSM	F	α	LSC	LSM	F
CPH (group I)																								
strain 0	1090.83				14.88				97.72				86.55				82.66				77.17			
Latency 9 h		-66.73	1024.10	–		-0.95	13.93	–		0.82	98.54	–		14.49	99.98	*		15.76	98.43	*		20.28	97.44	*
Latency 11 h		66.73	1157.56			0.95	15.82			-0.82	96.90			-14.49	72.14			-15.76	66.89			-20.28	56.89	
Ovopel (group II)																								
strain 0	973.67				14.16				97.16				91.55				88.40				84.45			
Latency 9 h		-50.68	922.99	–		-0.47	13.70	–		0.52	97.68	–		4.17	95.71	*		4.49	92.89	*		5.83	90.28	*
Latency 11 h		50.68	1024.35			0.47	14.64			-0.52	96.64			-4.17	87.39			-4.49	83.91			-5.83	78.62	
Strain 0																								
Latency 9 h	935.28				14.02				97.78				95.21				91.97				89.03			
CPH		23.98	959.27	–		0.16	14.17	–		0.13	97.91	–		-0.85	94.36	–		-1.50	90.47	–		-1.73	83.70	*
Ovopel		-23.98	911.30			-0.16	13.86			-0.13	97.64			0.85	96.06			1.50	93.47			1.73	90.77	
Strain 0																								
Latency 11 h	1131.17				14.99				97.11				82.83				79.00				72.50			
CPH		35.36	1166.53	–		0.49	15.49	–		-0.4	97.07	–		-5.79	77.04	–		-5.77	73.23	–		-8.45	64.04	–
Ovopel		-35.36	1095.88			-0.49	14.50			0.4	97.15			5.79	88.62			5.77	84.76			8.45	80.95	
Strain J																								
Latency 11 h	770.02				12.75				97.66				94.98				92.37				89.22			
CPH		-210.15	559.87	*		-3.25	9.51	*		-1.00	96.66	*		1.08	96.07	–		2.08	94.45	*		1.09	90.31	–
Ovopel		210.15	980.17			3.25	16.00			1.00	98.66			-1.08	93.91			-2.08	90.28			-1.09	88.14	
CPH (group I)																								
Latency 11 h	888.14				13.03				97.34				86.32				83.50				77.61			
strain J		-359.96	528.18	*		-5.12	7.91	*		-1.03	96.30			14.74	99.98	*		16.45	99.95	*		18.42	96.02	*
strain 0		359.96	1248.09			5.12	18.15			1.03	98.37			-14.74	71.58			-16.45	67.06			-18.42	59.19	
Ovopel (group II)																								
Latency 11 h	1018.33				14.99				97.50				90.67				86.94				83.17			
strain J		84.20	1102.53	–		1.36	16.35	–		1.13	98.62	*		3.79	94.46	–		3.41	90.35	–		6.12	89.29	*
strain 0		-84.20	934.14			-1.36	13.62			-1.13	96.38			-3.79	86.87			-3.41	83.53			-6.12	77.04	

α, the theoretical general means; LSC, least-square constants. [L]Latency = hours elapsed from the resolving injection of GnRH or the resolving injection of CPH and initial eggs release

Results

Percentage of spawning females after hormonal stimulation

After the hypophysation, spawning occurred in 83.33% females of strain J and in 87.50% fish of strain 0. In the case of Ovopel treatment, eggs were obtained from 50% of fish of strain J and from all the females of strain 0.

Ovulation time

After hypophysation in four females of strain J, ovulation took place 11 h after the second CPH injection and in one fish after a further two hours. In this group one female of strain 0 spawned 6h after the hypophysation, three females after 9 h, and three after 11 h. After Ovopel treatment latency for all ovulated fish of strain J was 11 h. In this group two latent periods were recorded for females of strain 0; four fish yielding eggs 9 h after Ovopel injection while the remaining four females spawned 2 h later.

The effect of ovulation stimulators on the weight and quality of eggs obtained

Analysis of variance and the F-test showed a significant ($P \leq 0.05$) effect of the stimulator on the weight of eggs expressed in grams and on percentage of female body weight. The means of the least-squares estimated for these parameters show that a greater weight of eggs was obtained from females treated with Ovopel, than from fish treated with CPH (999.71 g, 15.00% and 848.91 g, 12.60%, respectively; Table 1). The effects of the ovulation stimulator was statistically insignificant with respect to the four investigated parameters of egg quality (Table 1).

The effect of strains' on the weight and quality of eggs obtained

The provenance of females did not significantly affect any of the investigated parameters. Although, it should be stressed that a greater weight of eggs was obtained from fish of strain 0; the least-squares means for these females being 953.89 g, 14.32% and for fish of strain J 894.74 g, 13.29% (Table 1).

Interaction

The interaction between the ovulation stimulator and the provenance of fish was statistically significant ($P \leq 0.05$) for the weight of eggs both in grams and in percentage of female body weight. The highest weight of eggs was obtained in the case of Ovopel applied to females of strain J and the lowest from fish of strain J after hypophysation (1042.99 g, 15.60% and 746.49 g, 10.97%, respectively; Table 1). A statistically significant ($P \leq 0.01$) interaction was also found for the percentage of egg fertilization; the lowest quality characterizing eggs from females of strain J after the CPH treatment (95.83%) and the highest from fish of the same strain after Ovopel (98%) (Table 1). The investigated interaction was not significant for the percentage of living embryos after 24, 36 and 48h of egg incubation. The values of the least-squares means for the percentage of living embryos after 48h incubation showed the highest quality of eggs from females of strain J treated with CPH and also from these treated with Ovopel and the lowest from fish of strain 0 after Ovopel treatment (88.61%, 87.78% and 82.55%; respectively, Table 1).

Latency and the weight and quality of eggs

The latent period had no statistically significant effect on the weight of eggs obtained from females of strain 0 after CPH and after Ovopel treatment. Although it should be stressed that a higher weight of eggs (both in grams and in percentage of female body weight) was obtained from fish of this strain 11 h after CPH and Ovopel treatments compared with fish whose ovulation occurred two hours earlier (Table 2). Within both preparations the latency has a statistically significant ($P \leq 0.05$) effect on the percentage of living embryos after 24, 36, and 48 h incubation of eggs obtained from females of strain 0 and higher values of the least-squares means for these traits being noted for the latency of 9 h (Table 2).

The least-squares means calculated for the two ovulation stimulators (within strain 0 and the latency of 9 h and 11 h) did not differ significantly with respect to the two parameters characterizing the weight of eggs as well as the percentage of living embryos after 24 h and 36 h incubation of eggs (Table 2). In the case of 9 h latency the percentage of living embryos after 48h incubation was significantly ($P \leq 0.05$) higher after the application of Ovopel in comparison with the percentage of living embryos in eggs obtained from fish treated with CPH (90.77% and 83.70%, respectively; Table 2).

In fish of strain J and 11 h latency statistically significant ($P \leq 0.05$) differences were found between the mean weight of eggs obtained from

hypophysed females and from these stimulated with Ovopel (559.87 g, 9.51% and 980.17 g, 16%, respectively; Table 2). Within this classification the ovulation stimulator significantly ($P \leq 0.05$) determined the percentage of living embryos after 36 h incubation of eggs and a higher value of the mean was noted for eggs yielded by females after CPH (Table 2).

In hypophysed fish and with the latency of 11 h a statistically significant ($P \leq 0.05$) difference was found between the weight of eggs obtained from females of both strain. The least-squares means characterizing the weight of eggs (in grams and in percentage of female body weight) showed higher values for strain 0 compared with these for strain J (1248.09 g, 18.15% and 528.18 g, 7.91%, respectively; Table 2). The origin of the females significantly ($P \leq 0.05$) affected the percentage of living embryos after 24, 36, and 48 h incubation of eggs while the least-squares means for these parameters showed much higher values for strain J (Table 2). In the Ovopel treated group and with the latency of 11 h, a statistically significant ($P \leq 0.05$) effect of the provenance of fish was noted for the percentage of fertilization and living embryos after 48 h incubation of eggs; a better quality of eggs being found for strain J (Table 2).

Discussion

The obtained results indicate that after the application of Ovopel to females of strain 0 a higher percentage of fish yielded eggs compared with the group treated with CPH. On the other hand, in fish of strain J treated with Ovopel, the ovulation was recorded in a lower percentage of females in comparison with the hypophysed fish. The lower percentage of ovulating females of strain J after Ovopel treatment compared with CPH treated fish was also reported by CEJKO (2007). The results of the investigation carried out so far in the Gołysz Institute (which included various breeding strains and inter-strain crosses of carp) most frequently showed that after the ovulation induction with Ovopel, eggs were obtained from a higher (or equal) percentage of females compared with the percentage of fish spawning after hypophysation. HORVÁTH et al. (1997) reported that, in a Hungarian hatchery, the mean ovulation ratio was similar in the CPH treated carp females and in the Ovopel treated ones (82.25% and 80.75% respectively). KŁODZIŃSKA and OKONIEWSKI (1998) complied data which included the results of controlled spawning of carp in two Polish hatcheries and showed that after Ovopel stimulation in one of these hatcheries the mean percentage of spawning females was approximately the same as the average percentage of females giving eggs after the treatment with CPH (~72%). In another hatchery, after the Ovopel stimulation, eggs were obtained from 86.60% of females but after the CPH treatment from only

66.67%. In reporting the results of carp propagation with the use of the above-mentioned preparations, KOUŘIL et al. (2003) reports that after Ovopel application ovulation occurred in 88% of females and after CPH in 41% only. MIKOŁAJCZYK et al. (2004) noted 91% and 85% of spawning carp females after Ovopel stimulation.

The results of the presented experiment distinctly show that generally after Ovopel stimulation a higher weight of eggs was obtained both in grams and in percentage of female body weight. This agrees with the data obtained in previous investigation (BRZUSKA 2003b, 2005, 2006b,c, BRZUSKA, BIAŁOWĄS 2002). KOUŘIL et al. (2003) reported a higher weight of carp eggs (expressed as the percentage of female body weight) after Ovopel treatment in comparison with the weight of eggs recorded after hypophysation (5.07% and 3.02%, respectively). However, KŁODZIŃSKA and OKONIEWSKI (1998) noted that the females treated with CPH were characterized by a higher number of yielded eggs (per 1 kg of female body weight) compared with fish stimulated with Ovopel (94.233 and 89.297, respectively).

The answer to the question of whether, in the present experiment, differences were noted in the reproduction effects of females of the two investigated strains, can be found in the results of interaction between the ovulation stimulator and the provenance of females. The least squares means for this interaction distinctly show that the females of the investigated strains responded differently to the application of the stimulators. After the CPH stimulation females of strain J yielded eggs of a much lower weight (both in grams and in percentage of body weight) compared with fish of the same strain stimulated with Ovopel (above 290 g and above 4.50% on the average). The quality of eggs (after 48h incubation) from females of this strain stimulated with the applied preparations was similar. The mean weight of eggs after CPH and after Ovopel application to females of strain O was similar whether expressed in grams or in percentage of fish body weight. On the other hand the quality of eggs expressed in percentage of living embryos after 48 h incubation was different, being higher for CPH treated fish by more than 4.50%. The results of the investigation carried out by Cejko (2007) show that the quality of eggs (expressed as a percentage of living embryos after 36 h incubation) obtained from females of strain J was higher after Ovopel treatment and from fish of strain 0 after hypophysation. The quality of carp eggs after Ovopel treatment (expressed by the fertilization percentage and the percentage of eyed-stage eggs) was observed by MIKOŁAJCZYK et al. (2004); in one experiment the mean values of these percentages were 88.5% and 91.0% and in another one 87.3% and 86.0%.

The dependence of propagation effects on latency also seem very important for hatchery practice. This dependence was already taken into consideration in earlier studies on the propagation effects of carp of different provenance (see

Introduction for references). The currently presented results showed that in strain 0 (both after the stimulation with CPH or with Ovopel) the quality of eggs after 24, 36 and 48 h was much better (although the weight of eggs was slightly lower) in the case of fish which spawned earlier (latency of 9 h) in comparison with eggs obtained from females ovulating 2 h later (latency of 11 h). It is also worth noting that in the group of hypophysed fish (with latency of 11 h) statistically significant ($P \leq 0.05$) differences between the means for strain J and strain O were found in the case of such traits as the weight of eggs (in grams and in percentage of female body weight) and the percentage of live embryos after 24, 36, and 48 h incubation. In the group of fish treated with Ovopel (with latency of 11 h) statistically significant ($P \leq 0.05$) differences between the means for these strains were only noted in the case of the fertilization percentage and live embryos after 48 h incubation.

In summary, after the hypophysation, ovulation occurred in a similar percentage of females from the two investigated strains while after the Ovopel treatment this percentage was twice that in fish of strain 0 compared with fish from strain J. It is striking that the highest weight of eggs of very good quality was obtained from females of strain J treated with Ovopel, however, in the case of hypophysation, fish of this strain yielded eggs of the lowest weight but of the best quality after 24, 36 sand 48 h incubation. The weight of eggs yielded by fish of strain 0 was similar after both Ovopel treatment and hypophysation, however, in the case of 48 h incubation the quality of eggs was much higher after hypophysation. In general, the best effects were recorded in the case of strain 0 fish after CPH and after Ovopel treatments; the percentage of ovulating fish was high and females yielded eggs of high weight and of satisfactory quality It is striking that synchronization of ovulation was observed only in fish of strain J.

The results of investigations carried out with females of the strains 0 and J subjected to ovulation stimulation with CPH or Ovopel confirm previous our observations that in the case of different provenances of the females (even if they are of the same age and are reproduced at the same time), similar results of induced propagation can not be expected.

Translated by Elżbieta Wróblewska

Accepted for print 29.10.2010

References

Białowąs H. 1999. *Carp breeding lines in Poland. Hungarian line 0*. Przeg. Ryb., 3: 61–62.

Białowąs H. 2000. *Carp breeding lines in Poland. Yugoslavian line J*. Przeg. Ryb., 5: 47.

Brzuska E. 1991. *Estimation of controlled spawning in carp (Cyprinus carpio L.) and an attempt of the polyoptimization of its effects.* Zeszyty Naukowe Akademii Rolniczej im. H. Kołłątaja, Kraków. Rozpr. Hab., 153: 1–75.

BRZUSKA E. 2000. *Artificial spawning of the carp Cyprinus carpio L; differences between the effects of reproduction in females of Hungarian and Polish provenance treated with carp pituitary and Ovopel*. Proc. of the 6th Internat. Symp. on the Reproductive Physiology of Fish. Eds. B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, S.O. Stefansson. Bergen, Norway 1999.

BRZUSKA E. 2001. *Ovulation stimulation with carp pituitary and Ovopel in carp (Cyprinus carpio L.) females: further studies on the reproduction effects of Israeli strain Dor-70 and its cross-breed with Hungarian line 8*. [In:] Hatchery 1999–2000. Ed. Z. Okoniewski. Institute of Inland Fisheries, Olsztyn, Poland, pp. 67–70.

BRZUSKA E. 2003a. *Artificial propagation of the carp (Cyprinus carpio): two-year reproduction results of females of Hungarian line W and Polish line 6 after ovulation stimulation with carp pituitary or mGnRH-a and dopaminergic inhibitor*. Czech J. Animal Sci., 48: 139–151.

BRZUSKA E. 2003b. *Artificial spawning of female Polish line 3 carp (Cyprinus carpio L.) after treatment with pituitary homogenate and/or Ovopel*. Aquacult. Res., 34: 1321–1327

BRZUSKA E. 2005. *Artificial spawning of carp (Cyprinus carpio L.): differences between females of Polish strain 6 and Hungarian strain W treated with carp pituitary homogenate, Ovopel or Dagin*. Aquacult. Res., 36**:** 1015–1025.

BRZUSKA E. 2006a. *The investigation on effects of carp Cyprinus carpio (L.) reproduction conducted in Gołysz Institute of Ichthyobiology and Aquaculture (1995-2005)*. [In:] Reproduction, Rearing and Profilactics of Cyprinidae and another fish species. Eds. Z. Zakęś, K. Demska-Zakęś, J. Wolnicki. Institute of Inland Fisheries Olsztyn, Poland, pp. 42–59.

BRZUSKA E. 2006b. *Artificial spawning of female Lithuanian strain B carp (Cyprinus carpio L.) after treatment with carp pituitary homogenate, Ovopel or [D-Tle^{6},ProNHEt^{9}]GnRH-a (Lecirelin)*. Aquacult. Res., 37: 264–271.

BRZUSKA E. 2006c. *Artificial propagation of female Hungarian strain 7 carp (Cyprinus carpio) after treatment with carp pituitary homogenate, Ovopel or Dagin*. Czech J. Anim. Sci., 51: 132–141.

BRZUSKA E., GRZYWACZEWSKI R. 1999. *Artificial spawning of carp Cyprinus carpio L; differences between the effects on reproduction in females of Israeli strain Dor-70 and its cross-breed treated with CPH and Ovopel*. Aquacult. Res., 30: 559–570.

BRZUSKA E., BIAŁOWĄS H. 2002. *Artificial spawning of carp, Cyprinus carpio (L.)*. Aquacult. Res., 33: 753–765.

CEJKO B. 2007. *Effect of hormonal stimulation of females from different strains of carp (C. carpio L.) on the quality of eggs*. (doctoral theses). Uniwersytet Warmińsko-Mazurski, Wydział Ochrony Środowiska i Rybactwa, Olsztyn, Poland, pp. 1–139.

HARVEY W.R. 1987. *User's guide for LSMLMW PC-1 Version. Mixed Model Least-Squares and Maximum Likelihood Computer Program*. Copyright W.R. Harvey U.S.A., p. 59.

HORVÁTH L., SZABÓ T., BURKE J. 1997. *Hatchery testing of GnRH analogue – containing pellets on ovulation in four cyprinid species*. Polish Arch. Hydrobiol., 44: 221–226.

JACÓ Z., EPLER P., BIENIARZ K. 1989. *Effect of the time of storage on gonadotropic activity of carp hypophysis fixed in acetone*. Pol. Arch. Hydrobiol., 36(3): 373–383.

KŁODZIŃSKA H., OKONIEWSKI Z. 1998. *Ovopel – new preparation for stimulation of fish reproduction*. [In:] *Hatchery 1997–1998*. Ed. J. Waluga. Institute of Inland Fisheries, Olsztyn, Poland, pp. 45–49.

KOUŘIL J., HAMÁČKOVÁ J., KOZÁK P., BARTH T. 2003. *Application of combined preparations containing analogue GnRH and dopaminergic inhibitor for hormonally induced artificial propagation of carp (Cyprinus carpio) and grass carp (Ctenopharyngodon idella) females*. Proc. of the 6th Czech Con. of Ichthyology. Ed. M. ŠVATORA. Agriculture University of Prague, Czech Republic 2003, pp. 35–40.

MIKOŁAJCZYK T., CHYB J., SZCZERBIK P., SOKOŁOWSKA-MIKOŁAJCZYK M., EPLER P., ENRIHT W.J., FILIPIAK M., BRETON B. 2004. *Evaluation of the potency of azagly – nafarelin (GnRH analogue), administered in combination with different formulation of pimozide, on LH secretion, ovulation and egg quality in common carp (Cyprinus carpio L.) under laboratory, commercial hatchery and natural conditions*. Aquaculture, 234: 447–460.

WOYNAROVICH E., WOYNAROVICH V. 1980. *Modified technology for elimination of stickness of common carp (Cyprinus carpio L.) eggs*. Aquacultura Hungarica (Szarvas), 11: 19–21.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 427–437, Y. 2010

DOI 10.2478/v10020-010-0039-1

THE EFFECT OF FREEZING AND PRESSURE OF 50 MPA AND 100 MPA ON THE PROTEOLYTIC ACTIVITY OF ENZYMES IN EDAM CHEESE

Maria Wachowska

Chair of Food Biotechnology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: cheese, frozen, high pressure, ripening, proteolytic activity.

A b s t r a c t

This study examined the effects of pressure treatment at 50 MPa / 0.5 h and 100 MPa / 0.5 h, at 18 ± 2°C, on the proteolytic activity of edam cheese subject to freezing after week 1, 4, 6 and 8 of ripening. The aim of the study was to test the utility of high pressures in cheese ripening under model conditions.

Sensory and chemical analysis was performed on Edam cheeses after varied periods of ripening. The chemical analysis involved determination of active acidity of cheese, water and salt content and the content of different fractions of nitrogen compounds. Subsequently, the cheeses were frozen in a freezer (freezing rate 1 cm h^{-1}) or in an alcohol bath (freezing rate 0.1 cm h^{-1}), in order to speed up the lysis of starter cells. Extracts of frozen cheeses were subjected to high pressure treatment. Proteolytic activity of enzymes was determined by the Westhoff method in frozen, frozen and pressurised and control cheese samples after 1 and 2 weeks of incubation at 30°C.

The results of the sensory and chemical analysis displayed the normal course of the ripening process. The proteolytic activity of enzymes increased as the cheese ripened. No significant difference was found between the proteolytic activity in extracts from control cheese and in those from frozen and pressurised cheese. Freezing the cheese after 8 weeks of ripening lowered the activity of proteolytic enzymes. The proteolytic activity of enzymes in extracts from frozen cheeses after 8 weeks of ripening, pressurised at 50 MPa / 0.5 h was higher than in those subjected to the pressure of 100 MPa / 0.5 h. Pressure treatment of extracts from frozen cheeses at 50 MPa or 100 MPa did not result in expected acceleration of proteolysis.

WPŁYW MROŻENIA ORAZ CIŚNIENIA 50 MPA I 100 MPA NA AKTYWNOŚĆ PROTEOLITYCZNĄ ENZYMÓW W SERZE EDAMSKIM

Maria Wachowska

Katedra Biotechnologii Żywności
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: ser, mrożenie, wysokie ciśnienie, aktywność proteolityczna.

Address: Maria Wachowska, University of Warmia and Mazury, ul. Jana Heweliusza 1, 10-724 Olsztyn, Poland, phone: +48 (89) 523 47 74, e-mail: maria.iwanczak@uwm.edu.pl

A b s t r a k t

Badano wpływ ciśnienia 50 MPa / 0,5 h i 100 MPa / 0,5 h, w temperaturze 18 ± 2°C, na aktywność proteolityczną enzymów sera edamskiego, poddanego mrożeniu po 1, 4, 6 i 8 tygodniach dojrzewania. Celem było sprawdzenie, w warunkach modelowych, możliwości zastosowania wysokich ciśnień do przyspieszenia procesu dojrzewania serów.

Sery edamskie o zróżnicowanym stopniu dojrzałości poddawano ocenie sensorycznej i analizie chemicznej. Analiza chemiczna obejmowała oznaczenie kwasowości czynnej sera, zawartości wody, soli i poszczególnych frakcji związków azotowych. Następnie sery mrożono w zamrażalniku lodówki (prędkość zamrażania 1cm h^{-1}) lub w łaźni alkoholowej (prędkość zamrażania 0,1 cm h^{-1}), w celu przyspieszenia lizy komórek starterowych. Ekstrakty z serów mrożonych poddawano działaniu wysokiego ciśnienia. W ekstraktach z serów mrożonych, mrożonych i poddanych ciśnieniu oraz kontrolnych (niemrożonych) oznaczano aktywność proteolityczną enzymów met. Westhoffa po 1 i 2 tygodniach inkubacji w temp. 30°C.

Wyniki oceny sensorycznej i analizy chemicznej serów wykazały prawidłowy przebieg procesu dojrzewania. Aktywność enzymów proteolitycznych wzrastała w miarę dojrzewania sera. Nie stwierdzono statystycznie istotnych różnic między aktywnością proteolityczną w ekstraktach z sera kontrolnego i ekstraktach z serów mrożonych poddanych wysokiemu ciśnieniu. Mrożenie sera po 8 tygodniach dojrzewania spowodowało obniżenie aktywności enzymów proteolitycznych. Aktywność proteolityczna enzymów w ekstraktach z serów mrożonych po 8 tygodniach dojrzewania, poddanych ciśnieniu 50 MPa / 0,5 h była wyższa niż poddanych ciśnieniu 100 MPa / 0,5 h. Poddanie ekstraktów z mrożonych serów ciśnieniu 50 MPa lub 100 MPa nie spowodowało oczekiwanego przyspieszenia procesu proteolizy.

Introduction

High-pressure technology is a non-thermal method of food preserving and processing, consisting in applying, at room temperature, pressure of 100–1 000 MPa. This method has aroused much interest, since the application of high pressure makes it possible to adjust the speed of enzymatic reactions in food and to eliminate undesirable microorganisms. The application of high pressure also facilitates the creation of new products with much more favourable organoleptic characteristics and nutritive value. High pressure technology has already been applied in the production of jams and fruit jellies, juices, desserts, yogurts and sauces. High pressure is also used for fish preparations, seafood, as well as meat products (JANKOWSKA 2001).

Abundant research has been conducted on the possibility of applying high pressure technology in cheese making (CAPELLAS et al., 2001, KOŁAKOWSKI et al. 1998, MESSENS et al. 1999, 2000, 2001, SALDO et al. 2002, TRUJILLO et al. 2002). YOKOYAMA et al. (1993) found that exposure of cheddar cheese to pressure of 50 MPa at 25°C reduced its ripening time from 6 months to 3 days. In addition, MESSENS et al. (2000, 2001) treating smear-ripened cheese and paillardin cheese at pressure of 50 MPa / 8 h, found a more intense course of the proteolytic process in comparison with traditionally ripening cheese, particularly in the external part of cheese. On the other hand, they did not find

any significant changes in the course of the ripening process in gouda cheese subjected to pressure of 50 MPa / 3 days, in comparison to the control cheese (MESSENS et al. 1999). These results are consistent with the results of research conducted by KOŁAKOWSKI et al. (1998), who applied pressure in the range of 50–500 MPa / 4 h. Similarly, SHEEHAN et al. (2005) did not prove any influence of pressure treatment at 400 MPa / 5 min at 21°C on the course of proteolysis and rheological properties of mozzarella cheese. According to O'REILLY et al. (2002, 2003), the course of the ripening process in cheese subjected to pressure depends on the type of cheese, value and duration of the pressure applied, as well as the temperature. On the other hand, JUAN et al. (2008), while examining sheep's milk cheese pressure-treated at 300 MPa / 10 min, observed that the course of proteolysis depended on the day of cheese ripening process on which high pressure was applied. The application of high pressure at earlier stages of ripening resulted in higher changes in the course of the cheese ripening process.

In view of the possibility of using high pressure technology to accelerate the process of cheese ripening, the aim of the research undertaken was to determine the effect of freezing and subsequent pressurizing at 50 MPa and 100 MPa on changes in the activity of proteolytic enzymes of edam cheese.

Materials and Methods

Edam cheese, of varied ripening time – 1, 4, 6, 8 weeks – was divided into cuboid pieces with dimensions of 5 cm x 10 cm x 12 cm, packaged into freezing bags and frozen at -35°C by two methods. Slow freezing was applied for 24 hours in a freezer (freezing rate 1 cm h^{-1}) and fast freezing in an alcohol bath – for 2.5 h (freezing rate 0.1 cm h^{-1}). Cheese samples were subsequently defrosted at 18 ± 2°C. Water extracts obtained from cheese were pressurized at 50 MPa / 0.5 h or 100 MPa / 0.5 h using high-pressure apparatus produced by Unipress Equipment.

Cheese was subjected to chemical analysis and sensory evaluation. Chemical analysis included determination of water content, active acidity of cheese, salt content, total content of nitrogen compounds (KREŁOWSKA-KUŁAS 1993), content of nitrogen compounds soluble in water extract of cheese, nitrogen compounds soluble at 4.6 pH (SODE-MOGENSEN 1948), amino acid nitrogen compounds (STADHOUDERS 1960) and non-protein nitrogen compounds (SCHOBER et al. 1961).

A sensory evaluation of the cheeses was carried out by the scoring method by a team of 6 people trained in making sensory evaluations of edam cheese by a competent person (internal training). The following attributes were taken

into account: taste, flavour, texture, colour and eye formation. A 5-point scale was used.

Proteolytic activity of enzymes (2% TCA and 12% TCA) was determined after 1 and 2 weeks of incubation at 30°C in aqueous extracts from frozen cheese, in pressurised extracts from frozen cheese and in control extracts (from non-frozen cheese), according to a modified method of WESTHOFF et al. (1993). An activity unit (a.u.) was assumed to be the amount of the enzyme which under established conditions of reaction resulted in the growth in absorbance by 0.01.

In order to determine the effect of ripening time and the type of extract on proteolytic activity of enzymes, the results were analysed statistically with the use of two-factorial analysis of variance without repetitions, at the level of significance of $p = 0.05$. Moreover, the results were analysed statistically after 8 weeks of ripening with the use of single-factorial analysis of variance at the level of significance of $p = 0.05$. The mean values and standard deviations were also calculated. All the analyses were made in 3 repetitions, except for determinations of nitrogen compounds, which were made in 2 repetitions. The statistical analysis was performed with the use of Microsoft Excel 2003 for Windows 2003.

Results and Discussion

On the basis of the chemical analysis performed, it was found that water content in cheese amounted to about 41%, which was consistent with the standard. The acidity of cheese after week 1 of ripening was 5.18 pH; it decreased in the course of the ripening process to reach the value of 5.42 pH after 8 weeks (Table 1).

Table 1

Chemical analysis of cheese

Time of ripening cheese (weeks)	Moisture (%)	Acidity (pH)	NaCl (%)	Total nitrogen compounds (%)
1	41.38 ± 0.12	5.18 ± 0.01	1.21 ± 0.02	4.64 ± 0.03
4	40.94 ± 0.40	5.30 ± 0.01	1.18 ± 0.01	4.75 ± 0.04
6	41.02 ± 0.31	5.35 ± 0.01	1.14 ± 0.01	4.54 ± 0.04
8	41.48 ± 0.18	5.42 ± 0.01	1.16 ± 0.01	4.48 ± 0.06

A low content of NaCl in cheese, amounting to about 1.2 %, was very favourable for health reasons, since a high consumption of NaCl is one of most frequently listed factors for hypertension.

Total nitrogen content in cheese was at the level of about 4.6% (Table 1). During the ripening process, a growth of the nitrogen compound forms was observed, namely of nitrogen compounds soluble in water extract of cheese, nitrogen compounds soluble at 4.6 pH, amino acid nitrogen compounds and non-protein nitrogen compounds, which demonstrated the proper course of the cheese proteolysis process (Table 2).

Table 2

Analysis of nitrogen compounds in cheese

Time of ripening cheese (weeks)	Non-protein nitrogen compounds (% $N_{og.}$)	Nitrogen compounds soluble at 4,6 pH (% $N_{og.}$)	Amino acid nitrogen compounds (% $N_{og.}$)	Nitrogen compounds in cheese extract (% $N_{og.}$)
1	4.09 ± 0.11	6.47 ± 0.14	2.95 ± 0.42	8.19 ± 0.58
4	5.02 ± 0.58	10.32 ± 1.46	4.31 ± 0.41	16.42 ± 0.65
6	8.21 ± 0.49	13.22 ± 0.28	7.05 ± 0.57	24.23 ± 1.09
8	11.97 ± 0.35	14.51 ± 0.72	11.16 ± 0.72	34.42 ± 0.38

The sensory evaluation of cheese indicated that it was characterized by a typical, clear taste and smell, elastic consistency and proper holes (Table 3).

Table 3

Sensory evaluation of cheeses

Quality factors	Severity coefficient (a)	Time of ripening cheese (weeks)					
		4		6		8	
		mean evaluation score (b)	product ($a \cdot b$)	mean evaluation score (b)	product ($a \cdot b$)	mean evaluation score (b)	product ($a \cdot b$)
Flavour	0.25	3.67 ± 0.52	0.92	4.33 ± 0.52	1.08	4.67 ± 0.52	1.17
Taste	0.30	3.83 ± 0.75	1.15	4.33 ± 0.52	1.30	4.83 ± 0.41	1.45
Texture	0.15	3.33 ± 0.52	0.50	4.17 ± 0.75	0.63	4.50 ± 0.53	0.68
Colour	0.10	4.17 ± 0.75	0.42	4.67 ± 0.52	0.47	4.67 ± 0.52	0.47
Eye formation	0.20	3.33 ± 0.81	0.67	4.67 ± 0.52	0.93	4.67 ± 0.52	0.93
Sum total	1.00	–	3.66	–	4.14	–	4.70

The statistical analysis with two-factorial variance without repetitions, at the significance level of $p = 0.05$, revealed a significant effect of cheese ripening time on the proteolytic activity of enzymes in all the extracts under examination, except for the cheese frozen in an alcohol bath, in which no statistically significant differences were found between the proteolytic activity of enzymes after 6 and 8 weeks of ripening (Table. 4–11).

Table 4
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (2%TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$19.58^{ac} \pm 0.86$	$28.85^{df} \pm 0.23$	$41.56^{gi} \pm 0.32$	$42.80^{jl} \pm 0.46$
m	$20.61^{ab} \pm 0.26$	$32.84^{de} \pm 0.28$	$39.89^{gh} \pm 0.22$	$37.62^{ik} \pm 0.62$
m + 50 MPa	$23.02^{e} \pm 0.60$	$33.66^{f} \pm 0.21$	$39.95^{i} \pm 0.62$	$38.06^{l} \pm 0.41$
m + 100 MPa	$22.86^{ac} \pm 0.31$	$31.58^{df} \pm 0.29$	$46.45^{gi} \pm 0.35$	$36.93^{jl} \pm 0.41$

k – control extract from unfrozen and unpressurised cheese
m – extracts from frozen cheeses
m + 50 MPa – extracts from frozen cheeses, pressurised at 50 MPa
m + 100 MPa – extracts from frozen cheeses, pressurised at 100 MPa
The same letters denote absence of statistical differences at $p = 0.05$

Table 5
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (2%TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$25.62^{a} \pm 0.38$	$42.98^{b} \pm 0.52$	$78.04^{c} \pm 0.52$	$86.54^{d} \pm 0.12$
m	$26.61^{a} \pm 0.12$	$47.17^{b} \pm 0.51$	$73.15^{c} \pm 0.37$	$72.88^{d} \pm 1.02$
m + 50 MPa	$26.03^{a} \pm 0.44$	$46.57^{b} \pm 0.30$	$69.00^{c} \pm 0.68$	$78.73^{d} \pm 0.17$
m + 100 MPa	$25.98^{a} \pm 0.44$	$47.99^{b} \pm 0.20$	$72.25^{c} \pm 0.28$	$74.92^{d} \pm 0.10$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 6
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (2% TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$19.58^{ad} \pm 0.86$	$28.85^{eh} \pm 0.23$	$41.56^{il} \pm 0.32$	$42.80^{lo} \pm 0.46$
m	$19.57^{ac} \pm 0.71$	$33.10^{eg} \pm 0.65$	$45.11^{ik} \pm 0.66$	$39.06^{lm} \pm 0.02$
m + 50 MPa	$20.85^{bc} \pm 0.24$	$31.82^{gf} \pm 0.11$	$46.65^{jk} \pm 0.77$	$44.68^{mo} \pm 0.30$
m + 100 MPa	$19.71^{bd} \pm 0.30$	$33.48^{fh} \pm 0.45$	$43.31^{jk} \pm 0.44$	$40.25^{mo} \pm 0.71$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 7
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (2% TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$25.62^{ab} \pm 0.38$	$42.98^{cd} \pm 0.52$	$78.04^{ef} \pm 0.52$	$86.54^{gh} \pm 0.12$
m	$25.25^{ab} \pm 0.05$	$43.08^{cd} \pm 0.83$	$74.39^{ef} \pm 0.29$	$79.50^{gh} \pm 0.49$
m + 50 MPa	$29.22^{a} \pm 0.43$	$50.94^{c} \pm 0.38$	$73.23^{e} \pm 0.69$	$81.54^{g} \pm 0.04$
m + 100 MPa	$29.05^{b} \pm 0.39$	$43.45^{d} \pm 0.28$	$73.26^{f} \pm 0.05$	$72.76^{h} \pm 0.63$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 8
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (12%TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$21.70^{ab} \pm 0.28$	$26.98^{cd} \pm 0.42$	$38.51^{ef} \pm 0.49$	$42.50^{gh} \pm 0.46$
m	$23.81^{ab} \pm 0.26$	$29.22^{cd} \pm 0.06$	$37.09^{ef} \pm 0.49$	$32.35^{gi} \pm 0.15$
m + 50 MPa	$22.37^{a} \pm 0.42$	$29.79^{c} \pm 0.65$	$41.37^{e} \pm 0.35$	$38.15^{g} \pm 0.07$
m + 100 MPa	$22.04^{b} \pm 0.17$	$27.38^{d} \pm 0.35$	$38.27^{f} \pm 0.14$	$36.81^{h} \pm 0.37$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 9
Proteolytic activity of enzymes in extract from the cheese frozen in a freezer (12%TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$24.96^{a} \pm 0.83$	$36.81^{b} \pm 0.29$	$66.27^{c} \pm 0.76$	$80.99^{d} \pm 0.45$
m	$26.79^{a} \pm 0.46$	$34.73^{b} \pm 0.57$	$69.67^{c} \pm 0.15$	$70.38^{d} \pm 0.30$
m + 50 MPa	$29.68^{a} \pm 1.12$	$40.62^{b} \pm 1.22$	$65.48^{c} \pm 0.24$	$73.28^{d} \pm 0.30$
m + 100 MPa	$29.97^{a} \pm 0.38$	$37.03^{b} \pm 0.44$	$68.28^{c} \pm 0.84$	$70.39^{d} \pm 0.57$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 10
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (12% TCA). Time of incubation – 1 week

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$21.70^{ab} \pm 0.28$	$26.98^{de} \pm 0.42$	$38.51^{gh} \pm 0.49$	$42.50^{gh} \pm 0.46$
m	$21.60^{ac} \pm 0.26$	$31.10^{df} \pm 0.64$	$39.22^{gi} \pm 0.22$	$38.23^{gi} \pm 0.09$
m + 50 MPa	$20.08^{ab} \pm 0.51$	$34.37^{de} \pm 0.43$	$44.23^{gh} \pm 0.47$	$38.58^{gh} \pm 0.29$
m + 100 MPa	$19.76^{b} \pm 0.51$	$27.81^{e} \pm 0.56$	$37.86^{h} \pm 0.12$	$32.99^{h} \pm 0.62$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

Table 11
Proteolytic activity of enzymes in extract from the cheese frozen in an alcohol bath (12% TCA). Time of incubation – 2 weeks

Extract type	Time of ripening cheese (weeks)			
	1	4	6	8
	proteolytic activity (a.u./g cheese)			
k	$24.96^{a} \pm 0.83$	$36.81^{b} \pm 0.29$	$66.27^{c} \pm 0.76$	$80.99^{c} \pm 0.45$
m	$22.61^{a} \pm 0.46$	$37.91^{b} \pm 0.27$	$73.38^{c} \pm 0.60$	$73.20^{c} \pm 1.06$
m ± 50 MPa	$23.13^{a} \pm 0.43$	$37.39^{b} \pm 0.68$	$72.14^{c} \pm 0.73$	$72.27^{c} \pm 0.39$
m ± 100 MPa	$23.03^{a} \pm 0.63$	$31.43^{b} \pm 0.80$	$69.69^{c} \pm 1.07$	$71.50^{c} \pm 0.63$

Explanations as in Table 4
The same letters denote absence of statistical differences at $p = 0.05$

An analysis of nitrogen compounds soluble in 2% TCA, and in 12% TCA revealed that the proteolytic activity of enzymes in aqueous cheese extracts increased during the 6-week period of cheese ripening (Table 4–11).

It was also observed that in cheese ripening for 6 and 8 weeks, the increase of proteolytic activity of enzymes determined after 2 weeks of incubation was more rapid.

For example, in week 2 of incubation, the proteolytic activity of enzymes in the extract from control cheese after 4 weeks of ripening increased by 14.13 a.u./g of cheese, after 6 week of ripening – by 36.48 a.u./g of cheese, and after 8 weeks of ripening – by 43.74 a.u./g of cheese (Table 4, 5).

In the cheese ripening process, due to the lack of an easily available source of sugar-derived carbon, bacteria of cheese starters are subjected to autolysis and release intracellular peptidases. The presence of aminopeptidases, as well as di-, tri- and sometimes carbopeptidases were established in cells of *Lactoccoccus* strains (CICHOSZ 2004). The aim of freezing cheese in the experiment

was to facilitate lysis of cheese starter bacteria, which resulted in higher susceptibility of released enzymes to high pressure.

However, the two-factorial analysis of variance without repetitions at the significance level of $p = 0.05$ did not reveal any significant differences between the proteolytic activity of enzymes in extracts from the control cheese and extracts from pressurised frozen cheeses (Table 4–11). A statistically significant difference was only found between the proteolytic activity in the extract from control cheese and the extract from frozen cheese pressurised at 50 MPa, after 1 week of incubation (Table 6).

Moreover, in order to examine the effect of the type of extract on the proteolytic activity of enzymes after 8 weeks of cheese ripening, a single-factorial analysis of variance was carried out. It revealed statistically significant differences at the level of $p = 0.05$ between the proteolytic activity of enzymes in frozen cheese extracts after 8 weeks of ripening, pressurised at 50 MPa, and the proteolytic activity of enzymes in extracts from the same cheese pressurised at 100 MPa. The proteolytic activity was higher in extracts pressurised at 50 MPa than in those pressurised at 100 MPa. The tendency was observed in the cheeses frozen in both an alcohol bath and a freezer (Table 4–11). No statistically significant difference at the significance level of $p = 0.05$ was found between the proteolytic activity of enzymes determined after 2 weeks of incubation, in extracts from cheeses frozen in an alcohol bath, pressurised at 50 MPa and the proteolytic activity of enzymes pressurised at 100 MPa (Table 11). These observations are consistent with the research conducted by KOŁAKOWSKI et al. (1998), who found a higher proteolytic activity of enzymes in camembert cheese subjected to a pressure of 50 MPa in comparison with cheese pressurized at 100 MPa. Additionally, O'RELLY et al. (2002, 2003) established that a more intensive course of the proteolysis process in cheddar cheese subjected to the pressure of 50 MPa / 72 h / 25°C could result from improved proteolytic activity of enzymes caused by high pressure.

Statistically significant differences at the significance level of $p = 0.05$ were also found between the proteolytic activity of enzymes in extract from 8-week control cheese and the proteolytic activity of enzymes in extracts from frozen cheeses after 8 weeks of ripening and subsequently pressurised (Table 4–11).

Nevertheless, the activity of proteolytic enzymes in extracts from frozen cheeses subjected afterwards to high pressure was lower than the activity of enzymes in the extract from the control cheese, which suggests an unfavourable effect of low temperature on the activity of enzymes in cheese after 8 weeks of ripening. The freezing applied in the study probably "damaged" the proteolytic enzymes. An analysis of compounds soluble in 2% TCA and 12% TCA, after 8 weeks of cheese ripening also showed significant differences at the level of $p = 0.05$ between the proteolytic activity of enzymes in extracts from control cheeses and the enzyme activity in extracts from frozen cheeses, with the

proteolytic enzyme activity in frozen cheese extract depending on the cheese freezing method applied. Smaller changes in the enzyme activity were caused by rapid freezing. The proteolytic activity of enzymes in cheeses subjected to slow freezing in a freezer, determined after 1 week of incubation, decreased by 12.1%, and that determined after 2 weeks of incubation – by 15.78%, whereas the proteolytic activity of enzymes in cheeses subjected to fast freezing in an alcohol bath decreased by 8.74% and 8.13%, respectively (Table 4–7).

Summing up, exposing cheese to freezing in order to induce lysis of starter culture cells and afterwards applying a high pressure of 50 MPa / 30 min and 100 MPa / 30 min did not result in the expected growth of activity of proteolytic enzymes.

Numerous studies indicate that high pressure technology provides great prospects for improving cheese production. However, the development of optimal parameters requires further research.

Conclusions

1. An increase in the activity of proteolytic enzymes was noted during the cheese ripening process.

2. Pressure treatment of extracts from frozen cheese at 50 MPa / 0.5 h and 100 MPa / 0.5 h had no significant effect on the growth activity of proteolytic enzymes.

Translated by Joanna Jensen

Accepted for print 16.07.2010

References

Capellas M., Mor-Mur M., Sendra E., Guamis B. 2001. *Effect of high pressure processing on physico – chemical characteristics of fresh goats' milk cheese (Mató)*. Int. Dairy J., 11: 165–173.

Cichosz G. 2004. *Stabilność przechowalnicza serów dojrzewających*. Prz. Mlecz., 5: 4–7.

Jankowska B. 2001. *Pod właściwym ciśnieniem*. Prz. Gastr., 55(10): 3–8.

Janowicz M., Kaczmarski Ł. 2004. *Zastosowanie wysokich ciśnień w przetwórstwie żywności*. Przem. Spoż., 58(7): 31–32.

Juan B., Ferragut V., Guamis B., Trujillo A.J. 2008. *The effect of high – pressure treatment at 300 MPa on ripening of ewes' milk cheese*. Int. Dairy J., 18: 129–138.

Kołakowaski P., Reps A., Babuchowski A. 1998. *Characteristics of pressurized ripened cheeses*. Pol. J. Food Nutr. Sci., 7(48): 473–483.

Krełowska-Kułas M. 1993. *Badanie jakości produktów spożywczych*. PWE, Warszawa.

Messens W., Estepar-Garcia J., Dewettinck K., Huyghebaert A. 1999. *Proteolysis of high pressure treated gouda cheese*. Int. Dairy J., 9: 775–782.

Messens W., Foubert I., Dewettinck K., Huyghebaert A. 2000. *Proteolysis of a high pressure treated smear – ripened cheese*. Milchwiss., 55: 328–332.

Messens W., Foubert I., Dewettinck K., Huyghebaert A. 2001. *Proteolysis of a high – pressure – treated mould – ripened cheese*. Milchwiss., 56: 201–203.

Mleko i przetwory mleczarskie. Sery podpuszczkowe dojrzewające. PN-68/A-86230

O'REILLY C.E., MURPHY P.M., KELLY A.L., GUINEE T.P., AUTY M.A.E., BERESFORD T.P. 2002. *The effect of high pressure treatment on the functional and rheological properties of mozzarella cheese*. Innovat. Food Sci. Emerg. Tech., 3: 3–9.

O'REILLY C.E., KELLY A.L., OLIVEIRA J.C., MURPHY P.M., AUTY M.A.E., BERESFORD T.P. 2003. *Effect of varying high-pressure treatment conditions on acceleration of ripening of cheddar cheese*. Innovat. Food Sci. Emerg. Tech., 4: 277–284.

SALDO J., MCSWEENEY P.L.H., SENDRA E., KELLY A.L., GUAMIS B. 2002. *Proteolysis in caprine milk cheese treated by high pressure to accelerate cheese ripening*. Int. Dairy J., 12: 35–44.

SCHOBER R., NICLAUS W., CHRIST W. 1961. *Anwendung der „Finger – Abdruck – Methode" auf die Kennzeichnung von Kasesorten durch ihre proteolytischen Inhaltsstoffe*. Milchwiss., 16: 140–148.

SHEEHAN J.J., HUPPERTZ T., HAYES M.G., KELLY A.L., BERESFORD T.P., GUINEE T.P. 2005. *High pressure treatment of reduced – fat mozzarella cheese: effects on functional and rheological properties*. Innovat. Food Sci. Emerg. Tech., 6: 73–81.

SODE-MOGENSEN T. 1948. *Determination of the degree of proteolytic decomposition in cheese with special reference to the formal titration*. Meddelande. 21, Fran Statens Mejeriförsôk, Malmo, 279–436.

STADHOUDERS J. 1960. *The hydrolysis of protein during the ripening of Dutch cheese. The enzyme and bacteria involved*. Neth. Milk & Dairy J., 14(2): 83–110.

TRUJILLO A.J., BUFFA M., CASALS I., FERNÁNDEZ P., GUAMIS B. 2002. *Proteolysis in goat cheese made from raw, pasteurized or pressure treated milk*. Innovat. Food Sci. Emerg. Tech., 3: 309–319.

WESTHOFF D.C., COWMAN R.A., SPECK M.L. 1971. *Isolation and partial characterization of a particulate proteinase from a slow acid producing mutant of Streptococcus lactis*. J. Dairy Sci., 54: 1253–1258.

YOKOYAMA H., SAWAMURA N., MOTOBAYA-SHI N. 1993. USA, patent US005 180 596A.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 25(4): 438–443, Y. 2010

DOI 10.2478/v10020-010-0040-8

CHARACTERISTICS OF THE BLACK CARROT (*DAUCUS CAROTA* SSP. *SATIVUS* VAR. ATRORUBENS ALEF)

Ryszard Zadernowski, Beata Piłat, Sylwester Czaplicki, Dorota Ogrodowska

Chair of Food Plant Chemistry and Processing
University of Warmia and Mazury in Olsztyn

K e y w o r d s: purple carrot, black carrot, chemical compounds, anthocyanins.

A b s t r a c t

The aim of this study was to characterize chemical composition of the black carrot and to assess the effects of processing methods and storage conditions of the carrot root on anthocyanin stability.The black carrot is characterized by a large content of phenolic compounds, including acylated anthocyanins, which ensure colour stability. The process of carrot freezing reduces anthocyanin stability. The degree of fragmentation, as well as the pH value determine the intensity of changes in the content of anthocyanins present in the black carrot.

CHARAKTERYSTYKA MARCHWI CZARNEJ (*DAUCUS CAROTA* SSP. *SATIVUS* VAR. ATRORUBENS ALEF)

Ryszard Zadernowski, Beata Piłat, Sylwester Czaplicki, Dorota Ogrodowska

Katedra Przetwórstwa i Chemii Surowców Roślinnych
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: czarna marchew, skład chemiczny, antocyjany.

A b s t r a k t

Celem badań była charakterystyka składu chemicznego korzeni czarnej marchwi oraz oszacowanie strat antocyjanów powstałych podczas stosowania różnych metod przechowywania i przetwarzania. Ustalono, że czarna marchew charakteryzuje się dużą zawartością związków fenolowych, w tym antocyjanów acylowanych. Na podstawie otrzymanych wyników wykazano, że proces zamrażania zmniejsza stabilność antocyjanów, a stopień degradacji zależy od pH środowiska oraz obróbki korzeni marchwi.

Address: Ryszard Zadernowski, University of Warmia and Mazury, pl. Cieszyński 1, 10-726 Olsztyn, Poland, phone: +48 (89) 523 34 66, e-mail: ryszard.zadernowski@uwm.edu.pl

Introduction

An increasing number of diseases which are often caused by inappropriate dietary patterns has led to growing interest in food whose composition stands out against basic food products. Currently, there are about 600 natural dyes and 200 synthetic dyes, while about 50 of them can be absorbed and metabolized in the human body (GRAJEK 2007). Colour is a physical property of the product, which definitely affects its positive or negative reception by consumers. It can provide information on the chemical composition of the product, therefore its suitability for processing, storing or transporting (ZAPOTOCZNY, ZIELIŃSKA 2005). Therefore, attempts have been undertaken to find new sources of natural dyes with both attractive colours and durability. The colour of products containing anthocyanin depends mainly on the structure and the content of individual dyes, environmental pH and the occurrence of substances accelerating processes of their degradation (oxygen, metal ions) (SIKORSKI 2002).

The garden carrot *Daucus carota* L., var. *sativus* Hoffm. can be divided into two cultivars: east-Asian and western. Roots of the western cultivar (*Daucus carota* ssp. *sativus* var. *Dativus* Alef.) are orange, yellow, red or white. The eastern cultivar (*Daucus carota* ssp. *sativus* var. Atrorubens Alef.) is characterized by red-purple or yellow colour of the root (DIETMAR et al. 2005).

The orange carrot is a popular, widely-consumed vegetable. The roots of the purple carrots have been cultivated and consumed for millennia in Asia, but in Poland this raw material is little known. The black carrot is an interesting agricultural product due to its colour and high content of anthocyanin dyes, which show very high stability while preserving its red colour in a wide pH range (CZAPSKI et al. 2009). This results from the fact that acylated peonidin and pelargonidin glycosides present in the black carrot ensure stability of the extract obtained from this raw material. This can be explained by an effect of intramolecular co-pigmentation of acylated anthocyanins, which prevents nucleophilic activity of water and the creation of chalcones through hydrolytic disintegration of the aromatic chain, leading to colour loss (KAMMERER et al 2004).

The aim of this study was to characterize chemical composition of the black carrot and to assess the effects of processing methods and storage conditions of the carrot root on anthocyanin stability.

Material and Methods

The subject of the research were roots of the black carrot. The carrot was cultivated on a plot near Olsztyn, and the seeds were brought from Holland,

from Bejo Zaden b.v.. Fresh carrot was cleaned and divided into two parts. One part of the carrot was frozen in the form of 1 × 1 cm cubes, while the other part was ground in colloid mill, and afterwards further divided into two parts – one of which was frozen and the other was acidified before freezing to pH 2 with hydrochloric acid.

Determination of the chemical composition of fresh carrot included an analysis of the dry matter content (*Przetwory owocowe...* PN-90/A-75101/03), total acidity (*Przetwory owocoew...* PN-90/A-75101/04), sugar content (*Przetwory owocowe...* PN-90/A-75101/07), vitamin C content (*Przetwory owocowe...* PN-90/A-75101/011), and total carotenoids (*Przetwory owocowe...* PN-90/A-75101/12). Pectins were determined with the modified Carre-Heynes method (PIJANOWSKI et al. 1973). Total content of phenolic compounds was spectrophotometrically assessed, applying the method with the Folin-Ciocalteau reagent, using D-catechine to draw a calibration curve (AOAC 1974). Total anthocyanins were analysed using the Ronald E. Wrolstad method (AOAC 1974).

Chromatographic analysis of anthocyanins was carried out with the use of a Hewlett Packard 1050 high-performance liquid chromatography unit, with a UV-Vis detector provided by the same manufacturer using the method described by GOIFFON et al. (1999). A mobile phase was a mixture of acetonitrile, water and formic acid. Separation of the examined compounds was performed on a LiChrospher C_{18} column (250 × 4.6 mm). Peak detection was carried out at the wavelength of 520 nm. The volume of the sample collected for injection was 10 μl.

Identification of peaks was carried out on the basis of a comparison of spectra and retention times determined for patterns.

Results and Discussion

The suitability of vegetal raw materials for obtaining natural dyes depends not only on the amount, but also on its durability and the presence of accompanying substances, which can limit dye availability.

On the basis of the research conducted, it was established that the content of chemical compounds in red-orange and black carrots occurs on the same or a similar level (Table 1).

Sugar content in the raw material from which dyes are obtained is important due to the viscosity of extracts. According to various authors, sugar concentration in the roots of the black carrot amounts to 5.9–52% in dry matter (KAMMERER et al. 2004, KIRCA A. et al. 2007). The black carrot root under analysis was particularly rich in sugars – the total content averaged

Table 1

Characteristics of chemical composition of the black carrot

Discriminant		Mean content in fresh carrot	Mean content in dry matter
Reducing sugars [g in 100 g]		4.89 ± 0.06	43.08 ± 0.56
Total sugars [g in 100 g]		7.95 ± 0.04	70.04 ± 0.37
Vitamin C [mg in 100 g]		1.68 ± 0.04	14.80 ± 0.31
Total acidity [g in 100 g]		0.19 ± 0.01	1.67 ± 0.12
Total carotenoids [mg in 100 g]		1.93 ± 0.10	17.00 ± 0.93
Pectin [g in 100 g]	acc. to Morison	0.57 ± 0.03	5.02 ± 0.25
	acc. to Carre-Haynes	1.03 ± 0.06	9.08 ± 0.50
Total phenolic compounds [mg in 100 g]		248.07 ± 4.21	2185.72 ± 37.13
Anthocyanins [mg in 100 g]		44.25 ± 3.90	389.91 ± 34.38
Dry matter [g in 100 g]		11.35 ± 0.03	–

70.04% in dry matter. These differences can be caused by cultivar, climate and soil condition features. Anthocyanin content in the carrot under examination was, on average, 44.25 mg in 100 g, which converted into dry matter corresponds to 389.91 mg. Similar values were obtained by KAMMERER et al. (2004). Considering the aim of this study, the dye content was most important in the carrot under analysis. It was established that fresh black carrot was characterized by a high content of phenolic compounds (2185.72 mg in 100 g d.m.), 17.84% of which were anthocyanins. Anthocyanins identified as a result of quality analyses include: 1 – cya 3-xylglcgal, 2 – cya 3-xylgcgal acylated with caffeic acid, 3 – cya 3-xylgal, 4 – cya 3-xylglcgal acylated with sinapic acid, 5 – cya 3-xylglcgal acylated with ferulic acid, 6- cya 3-xylglcgal acylated with p-cumar acid (Figure 1). Four out of six determined anthocyanins occurred in their acylated forms.

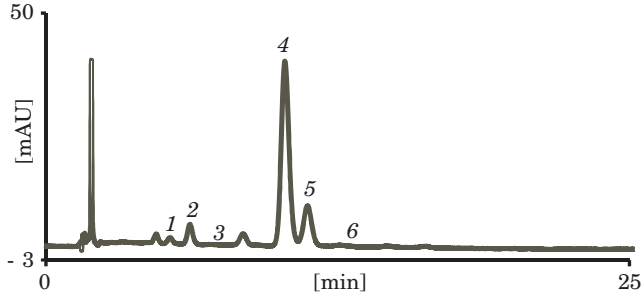

Fig. 1. Characteristics of anthocyanins – fresh root: *1* – cya 3-xylglcgal, *2* – cya 3-xylgcgal acylated with caffeic acid, *3* – cya 3-xylgal, *4* – cya 3-xylglcgal acylated with sinapic acid, *5* – cya 3-xylglcgal acylated with ferulic acid, *6* – cya 3-xylglcgal acylated with p-cumar acid

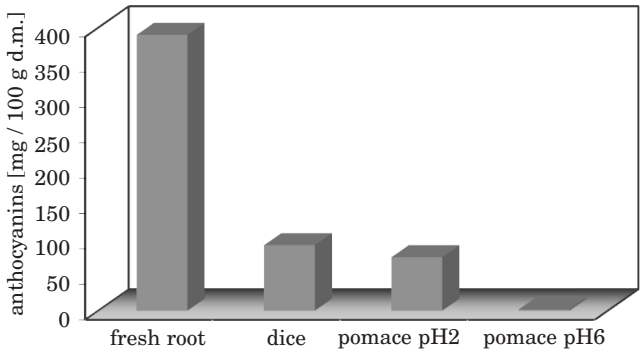

Fig. 2. Total content of anthocyanins during freezer storage

Technological processing of the carrot root and its storage in the frozen state (Figure 2) affected the anthocyanin dyes to a various extant. All samples of frozen carrot revealed a lower content of anthocyanins in comparison to the fresh root. The degree of root fragmenting had a significant effect on changes in anthocyanin content. Better values were obtained for diced carrot as compared to the pomace. Anthocyanins in a non-acidified pomace underwent complete degradation. The effect of acidification of the pomace to pH = 2 on anthocyanin durability proved to be minimal. Anthocyanins present in black carrot stored in the form of non-acidified pomace were completely degraded, while at pH 2 they revealed a slightly higher stability. KIDOŃ and CZAPSKI (2009) analysed the content of anthocyanin dyes in juices during their storage. For juices stored at 4°C for 107 days, they did not notice any visible loses of anthocyanin dyes in comparison to their initial value. Nevertheless, at 35°C the decrease was significant, reaching 45–54%, depending on the cultivar. KIRCA et al. (2007) claim that a significant decrease in anthocyanin stability occurs at a pH value of about 5. Stability of the black carrot under conditions of lowered pH results from acylation of anthocyanins with hydroxycinnamic and hydroxybenzoic acids (KAMMERER et al. 2004).

The most convenient form of storage proved to be freezing carrot in dices in the natural environment. Among the determined anthocyanins, cya 3xylglcgal acylated with ferulic acid, proved most stable in frozen carrot dices.

Conclusions

1. The black carrot is characterized by a large content of phenolic compounds, including acylated anthocyanins, which ensure colour stability.
2. The process of carrot freezing reduces anthocyanin stability.

3. The degree of fragmentation, as well as the pH value determine the intensity of changes in the content of anthocyanins present in the black carrot.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 9.08.2010

References

AOAC (Association of the Official Analytical Chemists). 1974**.** *Official Methods of Analysis*. Washington DC, 9. 110.

Chemia Żywności. 2004. Ed. Z.E. SIKORSKI. Wyd. IV. WNT, Warszawa.

CZAPSKI J., KIDOŃ M., OLEJNIK A., WITROWA-REJCHERT D. *Marchew purpurowa jako surowiec do przetwórstwa owocowo-warzywnego*. Przemysł Fermentacyjny i Owocowo-Warzywny, 1: 31–33.

DIETMAR R., KAMMERER D. 2005. *Black carrots – history, recent findings and perspectives*. Fruit Processing, 10: 302–308.

GOIFFON J.P., MOULY P.P., GAYDOLU E.M. 1999. *Anthocyanic pigment determination in the red fruit juices, concentrated juices and syrups using liquid chromatography*. Analitica Chemica Acta, 382: 39–50.

KAMMERER D., CARLE R., SCHEIBER A. 2004. *Quantification of anthocyanins in black carrot extracts* (*Daucus carota ssp. Sativus var. atrorubens Alef.*) *and evaluation of color properties*. Eur Food Res Technol., 219: 479–284.

KIDOŃ M., CZAPSKI J. 2009. *Ocena zmian zawartości składników bioaktywnych oraz zdolności antyoksydacyjnej soków z marchwi purpurowej podczas przechowywania (Assessment of the changes in the bioactive components and anti-oxidizing properties of black carrot juice during storage)*, Bromat. Chem. Toksykol., 3: 848–853.

KIRCA A., ÖZKAN M., CEMEROĞLU B. 2007. *Effects of temperature, solid content and pH on the stability of black carrot anthocyanins*. Food Chemistry, 101: 212–218.

PIJANOWSKI E. 1973, *Technologia produktów owocowych i warzywnych*. PWRiL, Warszawa.

Przeciwutleniacze w żywności. 2007. Ed. GRAJEK, Wyd. I. WNT, Warszawa 2007, 171–174.

Przetwory owocowe i warzywne. Oznaczanie zawartości cukrów. PN-90/A-75101/07.

Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości suchej masy metodą wagową. PN-90/A-75101/03.

Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości witaminy C. PN-90/A-75101/011.

Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie kwasowości ogólnej. PN-90/A-75101/04.

Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości sumy karotenoidów i β-kartotenu. PN-90/A-75101/12.

ZAPOTOCZNY P., ZIELIŃSKA M. 2005. *Rozważania nad metodyką instrumentalnego pomiaru barwy marchwi*. Żywność. Nauka. Technologia. Jakość, 1(42): 121–132.

Polish Journal of Natural Sciences
Reviewers of Years – book 2010

Ryszard Amarowicz
Irena Bielańska-Grajner
Dorota Bobrecka-Jamro
Piotr Epler
Krzysztof Formicki
Ryszard Gołdyn
Krystyna Grabowska
Eugenia Jacyno
Edmund Kamiński
Sławomir Keszka
Jan Kępczyński
Alicja Krzemińska
Edward Krzywy
Marek Łuczyński
Władysław Migdał
Małgorzata Nogala-Kałucka
Krzysztof Ostrowski
Jan Pikul
Mariusz Piskuła
Gorzysław Poleszczuk
Włodzimierz Popek
Lech Ratajczak
Jacek Rechulicz
Marian Rejewski
Piotr Sablik
Barbara Sawicka
Rajmund Skowron
Stanisław Socha
Danuta Szczerbińska
Andrzej Szczerbowski
Maciej Szkudlarek
Krzysztof Szoszkiewicz
Zofia Tomaszewska
Tadeusz Trziszka
Anna Wąs
Czesław Wołoszyk
Jerzy Wójcik
Sławomir Zduńczyk
Marcin Zych