UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal Of Natural **Sciences** (4/2009) 24



EDITORIAL BOARD

Janusz Falkowski (Editor-in-chief), Eugeniusz Biesiadka, Jan Glogowski, Ryszard Zadernowski, Hans Harms (Germany), Vaclav Matoušek (Czech Republic), Juraj Mlynek (Slovak Republic)

Executive editor Agnieszka Orłowska-Rachwał

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

© Copyright by Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego Olsztyn 2009

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. 6,8, ark. druk. 5,5, nakład 110 egz. Druk – Zakład Poligraficzny UWM w Olsztynie zam. nr 649

TABLE OF CONTENTS

Agriculture

М.	MARKS, P. MAKOW	yski, K. Orz	ech – Assessme	nt of Energy R	equirements	for
	Different Methods of	of Two-Year l	Fallow Keeping o	and Cultivation	of Winter Wh	eat 187

Animal Breeding and Husbandry

K.	KARPIESIUK,	J.	FALKOWSKI	- 7	The 1	Effect	of	Feeding	and	Ho	usin	g (Condition	s	
	of Growing-H	Fin	ishing Pigs	on	Pork	e Qua	lity	,						1	98

Environmental Protection

Z. MAŁECKI – Impact of Wastewater Treated in Saczyn Wastewater Treatment Plant	
upon Quality of the Water of the Pokrzywnica River – Right Tributary of the	
Prosna River	207

Fishery

A.	HAKUĆ-BŁAŻOWSKA, K. KUPREN, K. TURKOWSKI, K. TARGOŃSKA, M. JAMRÓZ,	
	S. KREJSZEFF, M. KWIATKOWSKI, D. ŻARSKI, D. KUCHARCZYK – Comparison	
	of Economic Effectiveness of Applying Different Hormonal Preparations for	
	Reophile Cyprinid Fish Reproduction Stimulation Based on the Example of Asp	
	Aspius Aspius (L.) and Ide Leuciscus Idus (L.)	224
D.	Żarski, D. Kucharczyk, K. Targońska, M. Jamróz, S. Krejszeff, A. Mamcarz	
	- Application of Ovopel and Ovaprim and Their Combinations in Controlled	
	Reproduction of Two Reophilic Cyprinid Fish Species	235

Food and Nutrition Sciences

A.	MIKOŁAJCZYK – The Survival of Salmonella spp. in Relation to Exposure to Lactic	
	Acid and the Storage Time of Turkey Carcasses	245
M.	MODZELEWSKA-KAPITUŁA, L. KŁĘBUKOWSKA, K. KORNACKI, W. ŁUKASZUK – The	
	Evaluation of Usefulness of Potentially Probiotic Lactobacillus Strains	
	as Components of Industrial Starter Cultures	254
Т.	ŻMIJEWSKI, A. KWIATKOWSKA, M. CIERACH – The Effect of Cold Storage	
	on the Color of Venison	263

SPIS TREŚCI

Rolnictwo

М.	MARKS,	Ρ.	Makowski,	K.	Orzech	—	Ocena	energetyczna	różnych	sposobów	
	dwuletni	iego	ugorowania	ı i	uprawy j	psz	enicy c	zimej			187

Chów i Hodowla Zwierząt

K.	KARPIESIUK, J. FALKOWSKI – Jakość mięsa w zależności od sposobu utrzymania	
	i żywienia tuczników	198

Ochrona Środowiska

Z. Małecki –	Wpływ ścieków	oczyszczonych	w oczyszczaln	i Saczyn i	na jakość wód	ł
rzeki Pokrz	zywnicy – prau	obrzeżnego do	pływu Prosny			207

Rybactwo

A.	HAKUĆ-BŁAŻOWSKA, K. KUPREN, K. TURKOWSKI, K. TARGOŃSKA, M. JAMRÓZ,	
	S. KREJSZEFF, M. KWIATKOWSKI, D. ŻARSKI, D. KUCHARCZYK – Porównanie	
	opłacalności stosowania różnych preparatów hormonalnych do symulacji roz-	
	rodu karpiowatych ryb reofilnych na przykładzie bolenia Aspius Aspius (L.)	
	i jazia Leuciscus Idus (L.)	224
D.	ŻARSKI, D. KUCHARCZYK, K. TARGOŃSKA, M. JAMRÓZ, S. KREJSZEFF, A. MAMCARZ	
	- Zastosowanie preparatów Ovopel i Ovaprim oraz ich kombinacji w kon-	
	trolowanym rozrodzie dwóch gatunków karpiowatych ryb reofinych	235

Nauka o żywieniu i żywności

A. MIKOŁAJCZYK – Wpływ kwasu mlekowego i czasu składowania tuszek indyczy	ch
na przeżywalność pałeczek Salmonella	245
M. MODZELEWSKA-KAPITUŁA, L. KŁĘBUKOWSKA, K. KORNACKI, W. ŁUKASZ	UK
– Ocena przydatności potencjalnie probiotycznych pałeczek z rodzaju Lactobac	il-
lus jako składników szczepionek przemysłowych	254
T. ŻMIJEWSKI, A. KWIATKOWSKA, M. CIERACH – Wpływ chłodniczego przechowywan	ia
na barwę mięsa jelenia	263

DOI 10.2478/v10020-009-0018-6

ASSESSMENT OF ENERGY REQUIREMENTS FOR DIFFERENT METHODS OF TWO-YEAR FALLOW KEEPING AND CULTIVATION OF WINTER WHEAT

Marek Marks, Przemysław Makowski, Krzysztof Orzech

Chair of Agricultural Systems University of Warmia and Mazury in Olsztyn

Key words: fallow land, previous crops, winter wheat, energy effectiveness index.

Abstract

During exclusion of the land from market production for two vegetation seasons the highest energy outlays were incurred for maintaining the black fallow and the lowest for the herbicide fallow.

In winter wheat production technique the highest energy outlays (15 800 MJ ha⁻¹) were incurred in materials. In the group of outlays for materials, 66.4% were fertilizers, 31.4% the sowing material and just 2.2% the plants protection media. The second largest flow of energy outlays consisted of energy carriers at 1754.1 MJ ha⁻¹, among which the largest shares were those of soil cultivation 54.3%, harvest 17.9% and crops cultivation 13.2%. Tractors and machines were the third largest energy consumers group (731.1 MJ ha⁻¹), where the demand structure was as follows: 35.5% harvest, 34.5% soil cultivation and 15% crops cultivation, fertilization 9.2% and sowing 5.8%. Among the agricultural technique components, fertilization and sowing had the largest share representing respectively 58.0% and 27.8% of all outlays.

Among the compared three-field rotation system components, the highest energy efficiency coefficient of 9.9 was achieved while cultivating winter wheat after two-years of red clover, which was coupled with the lowest energy outlays for production of 1 cereal unit. The lowest energy efficiency coefficient (5.4) was achieved in the crops rotation system component of black fallow – black fallow – winter wheat.

OCENA ENERGETYCZNA RÓŻNYCH SPOSOBÓW DWULETNIEGO UGOROWANIA I UPRAWY PSZENICY OZIMEJ

Marek Marks, Przemysław Makowski, Krzysztof Orzech

Katedra Systemów Rolniczych Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: ugór, przedplon, pszenica ozima, wskaźnik efektywności energetycznej.

Address: Marek Marks, University of Warmia and Mazury, pl. Łódzki 3, phone: +48 (89) 523 48 85, e-mail: marek.marks@uwm.edu.pl

Abstrakt

Podczas wyłączenia gruntów z produkcji towarowej na dwa sezony wegetacyjne najwyższe nakłady energetyczne poniesiono na utrzymanie ugoru czarnego, a najniższe – ugoru herbicydowego.

W technologii produkcji pszenicy ozimej największe nakłady energii (15 800 MJ ha⁻¹) pochłonęły użyte materiały. W grupie nakładów materiałowych 66,4% stanowiły nawozy, 31,4% – materiał siewny, a jedynie 2,2% – środki ochrony roślin. Drugim w kolejności strumieniem nakładów energii były nośniki energii – 1754,1 MJ ha⁻¹, a największy ich udział przypadał na uprawę roli – 54,3%, zbiór – 17,9% i pielęgnację – 13,2%. Trzecim w kolejności odbiorcą energii były ciągniki i maszyny (731,1 MJ ha⁻¹), gdzie struktura zapotrzebowania rozkładała się następująco: 35,5% przypadało na zbiór, 34,5% – na uprawę roli, 15% – na pielęgnację, nawożenie – 9,2% i siew – 5,8%. Wśród ogniw agrotechniki największy udział miało nawożenie i siew, osiągając odpowiednio 58,0 i 27,8% wszystkich nakładów.

Z porównywanych trójpolowych członów zmianowania najwyższy wskaźnik efektywności energetycznej, wynoszący 9,9, stwierdzono uprawiając pszenicę ozimą po dwuletniej koniczynie czerwonej, odnotowano przy tym najmniejsze nakłady energii na produkcję 1 jednostki zbożowej. Najniższy wskaźnik efektywności energetycznej (5,4) uzyskano w ogniwie zmianowania: ugór czarny – ugór czarny – pszenica ozima.

Introduction

In view of many centuries of agricultural science and practice, the fallow had and still has a well-established position. Already in primitive farming systems it formed an inseparable component of crops rotation system (NOWICKI et al. 2007). After accession of Poland to the European Union Structures and introduction of direct subsidies, agricultural producers are required to observe the minimum conservation requirements for agricultural land (Rozporzadzenie Ministra... 2004). According to the regulation by the Minister of Agriculture and Rural Development (2007), the requirements concerning lying arable land fallow are considered satisfied if it was subject to cultivation but was not sown with a crop for a period exceeding 6 months or was covered with vegetation and was subject to at least one mowing before July 30 (Rozporządzenie Ministra... 2007). The method of conservation of soils temporarily excluded from cultivation (fallow) should secure the most favorable conditions of following market production on them (NOWICKI et al. 2007). Optimization of fallow method from the economic perspective is no less important.

This paper aims at assessment of energy efficiency of different methods of two-year fallow use as forecrop for winter wheat in three-fields system of rotation fallow – fallow – winter wheat.

Material and Methods

The results of studies on the basis of which the appropriate computations were made come from a closed field experiment conducted during the years 2003–2006 at the Experimental-Production Farm in Bałcyny. The field experiment was established on gray-brown podzolic, medium dusty soil, with gley underneath. The base is light clays without structure containing up to 26% of floatable fraction and up to 17% of dust fractions. The surface level of mollic type contained around 23% of floatable parts and 12% of dust fractions. The soil was characterized by slightly acid reaction (pH_{KCL} 5.2–6.2) and medium content of organic substance (1.3–1.9%). As concerns agricultural suitability it was classified to use class IIIa and goof wheat complex 2.

In the experiment established every year on the field after winter wheat, six three-field rotation system components were analyzed encompassing the following plants and sequence of crops:

- spring rape pea winter wheat (control field),
- black fallow black fallow winter wheat,
- herbicide fallow herbicide fallow winter wheat,
- fallow sown with red clover winter wheat,
- fallow sown with Italian ryegrass winter wheat,
- fallow sown with the mix of red clover and Italian ryegrass winter wheat.

The black fallow was maintained using the soil miller. The milling was done at the time when weeds reached the height of around 10–15 cm or covered a significant part of soil surface. The herbicide fallow was maintained on a similar principle as the black fallow, Instead of milling spraying with Roundup 300 SL herbicide at 3 dm³ ha⁻¹ was applied. During the second year of use the last mowing of red clover, Italian ryegrass and the mix of red clover and Italian ryegrass was ploughed as green fertilizer.

Zyta cultivar winter wheat was sown during the second decade of September at quantities assuring the number of plants after seedling development at 500 plants m⁻², i.e. around 275–280 kg ha⁻¹.

Aiming at determination of forecrop value of the fields for winter wheat the decreased nitrogen fertilization was applied that totaled 120 kg N ha⁻¹. Fertilizer dose was divided into two parts applying the first one at 80 kg N ha⁻¹ in the spring at the time when vegetation started in the form of 46% urea and the second one at 40 kg N ha⁻¹ in the form of 34% ammonium nitrate at full tillering of the wheat.

For winter wheat protection against agrophages during individual years of studies spraying with preparations available in the market was applied according to the recommendations by the Institute of Plant Protection in Poznań. The conducted energy balance for fallow and winter wheat cultivation encompassed: energy outlays according to flows: labor, materials (fertilizers, means of plants protection, sowing material), machines and tractors, energy carriers (fuel), energy outlays according to stages of agricultural technique (soil cultivation, sowing, fertilization, cultivation, harvest), energetic value of production, unit energy outlays and energy efficiency.

Aiming at elimination of year-to-year variation the average yields of seeds of spring rape and pea, winter wheat grain as well as green mass of red clover and Italian ryegrass obtained during the years 2003–2006 were assumed for the basis of economic assessment. The energy efficiency analysis was carried out by applying the method described by WIELICKI (1989), and recommended by FAO. In determining the energetic value of the harvest it was assumed that 1 kg of green mass is equivalent to 0.71 MJ or 1 kg of dry mass to – 18.36 MJ (WÓJCICKI 1981). The volume of outlays accumulated in means of production (materials) was determined according to the actual consumption of fertilizer, sowing material and plant protection preparations.

The level of energy outlays resulting from use of tractors and machines in the production process was determined by multiplying the unit material consumption of the equipment piece by the equivalent set at 112 MJ kg⁻¹. Human labor was calculated assuming after PAWLAK (1989) the standard of 40 MJ work h⁻¹. The volume of fuel and other materials and means of production consumed were converted to MJ using for that purpose the applicable accumulated energy coefficients applied in energy balance of plant production (WóJCICKI 1981, MACIEJKO 1984, WIELICKI 1986, ANUSZEWSKI 1987, Goć, MUZALEWSKI 1997, WIELICKI 1989): nitrogen fertilizers (1 kg N) – 77 MJ, phosphorus fertilizers (1 kg P₂O₅) – 15 MJ, potassium fertilizers (1 kg K₂O) – 10 MJ, plant protection media (1 kg of active substance) – 300 MJ, seeds of small seed legume plants and grasses (1 kg) – 30 MJ, seeds of oil and leguminous plants (1 kg) – 24 MJ, sowing seeds of winter wheat (1 kg) – 16.0 MJ, liquid fuel (1 kg) – 48 MJ.

The energy efficiency index (E_e) was computed according to the formula:

$$E_e = \frac{P_e}{N_e}$$

where:

 P_e – energetic value of the obtained crop yield per ha, MJ,

 N_e – energy outlays incurred to obtain the yield per ha.

Results

The highest energy outlays were made for keeping the control object where sowing pea was the element of rotation in the production cycle and winter rape during the second year (Table 1). In total they were 30 533.5 MJ ha⁻¹, and materials used for production in the form of fertilizers, plant protection mean and sowing material were the largest component according to the energy flows (79.5%). Among the compared variants of two-year fallow use the black fallow was the most energy intensive variant where the energy outlays were 9979.7 MJ. They were, however, lower by 67.3% as compared to the control object and in the structure of expenditures energy carriers were the largest component (76.6%) followed by tractors and machines 16.0% and labor 7.7%. Objects after Italian ryegrass and the mix of Italian ryegrass and red clover followed with almost identical energy outlays. The structure of energy flows was similar and energy carriers had the largest share in them. During two years of fallow use, the lowest energy outlays were incurred for maintaining the herbicide fallow. Those outlays represented only 12.5% of those on cultivation of pea and winter rape on the control object and energy carriers (50% of all outlays) and materials -34.0% were the main flows.

As concerns the operations of agricultural technique the largest energy outlays required for cultivation of pea and winter rape (control object) where those on fertilization 59.8% while soil cultivation represented 12.3% only. The remaining operations (sowing, cultivation and harvest) together represented 27.9% of the outlays (Table 2).

Among the compared fallow use methods, black fallow was the most energy intensive one where 100% of energy outlays were those on soil cultivation (the group of post-harvest operations following the harvest of forecrop and several milling operations), of which 60.1% of outlays were made during the first and 39.9% during the second year of fallow use. In all variants of two-year green fallow use cultivation of soil was also the dominating component of agricultural technique in he overall balance of energy outlays because in different variants of maintaining the soil in effective condition no fertilizers were applied. In the structure of energy outlays it ranged from 54.1% on objects after Italian ryegrass and the mix of red clover with Italian ryegrass to 57.6% in case of keeping the fallow with red clover. In case of the least energy intensive fallow use, the herbicide fallow the highest share in energy outlays was also that on soil cultivation at 52.8%.

In winter wheat production technology the highest energy outlays $(15\ 800\ \text{MJ}\ \text{ha}^{-1})$ were those on materials used (Table 3). In the group of material outlays fertilizers represented 66.4%, sowing material 31.4% and plant protection means 2.2% only. The second highest flow of energy

						Af	ter hold	ing land f	allow for	r two year	rs		
		Spring	rape-							green	fallow		
Time of fallow use	Item	pea-w whe (control	inter eat field)	black 1	fallow	herbi treated	icide fallow	red ci	lover	Italian r	yegrass	mix of re and It ryeg	d clover alian rass
		MJ ha ⁻¹	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%
	labor	243.9	0.80	434.9	4.7	161.6	4.2	262.6	6.1	269.9	5.7	269.8	5.7
Rivet woon	machines and tractors	712.3	2.3	941.4	9.4	356.5	9.3	595.5	13.8	610.7	12.8	610.7	12.8
of fallow use	energy carriers	2382.0	7.8	4621.4	46.3	$1 \ 751.9$	45.9	$2 \ 337.0$	54.3	2410.3	50.7	$2\ 410.3$	50.7
	materials	10277.5	33.7	0.0	0.0	648.0	17.0	540.0	12.6	900.0	18.9	900.0	18.9
	total a	13615.7	44.6	5997.7	60.1	$2\ 918.0$	76.4	3 735.1	86.8	$4\ 190.9$	88.1	4 190.8	88.1
	labor	216.3	0.7	300.0	3.0	26.7	0.7	53.3	1.2	53.3	1.1	53.3	1.1
Coord woon	machines and tractors	780.9	2.6	658.0	6.6	73.0	1.9	146.9	3.4	146.9	3.1	146.9	3.1
of fallow use	energy carriers	$1\ 924.9$	6.3	$3\ 024.0$	30.3	154.6	4.1	366.2	8.5	366.2	7.7	366.2	7.7
	materials	13995.6	45.8	0.0	0.0	648.0	17.0	0.0	0.0	0.0	0.0	0.0	0.0
	total b	16917.8	55.4	3982.0	39.9	902.3	23.6	566.4	13.2	566.4	11.9	566.4	11.9
Tota	$1 \operatorname{sum} a + b$	30533.5	100	9 979.7	100	$3\ 820.3$	100	$4 \ 301.5$	100	$4\ 757.3$	100	4 757.2	100

Energy outlays according to flows for variants of two-year follow use

Table 1

Marek Marks et al.

						Af	ter holdi	ng land f	allow for	r two year	rs		
		Spring	rape-							green	fallow		
Time of fallow use	Agricultural element	pea-w whe (control	inter eat field)	black f	fallow	herbi treated	icide fallow	red cl	lover	Italian r	yegrass	mix of re and It ryeg	d clover alian tass
		MJ ha ⁻¹	%	$MJ ha^{-1}$	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%	MJ ha ⁻¹	%
	soil cultivation	$2\ 129.1$	7.0	5.997.7	60.1	2015.7	52.8	$2\ 280.1$	53.0	2375.9	49.9	$2\ 375.9$	49.9
	fertilization	$4\ 238$	13.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rivet woon	sowing	6298.5	20.6	0.0	0.0	0.0	0.0	718.5	16.7	$1\ 078.5$	22.7	$1\ 078.5$	22.7
of fallow use	crop cultivation	516	1.7	0.0	0.0	902.3	23.6	0.0	0.0	0.0	0.0	0.0	0.0
	harvest	434.1	1.4	0.0	0.0	0.0	0.0	736.5	17.1	736.5	15.5	736.5	15.5
	total a	$1\ 3615.7$	44.6	5.997.7	60.1	2918.0	76.4	$3 \ 735.1$	86.8	$4\ 190.9$	88.1	$4\ 190.9$	88,1
	soil cultivation	1611.1	5.3	3982	39.9	0.0	0.0	198.2	4.6	198.2	4.2	198.2	4.2
	fertilization	$14\ 029.3$	45.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Second wear	sowing	298.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
of fallow use	crop cultivation	387.0	1.3	0.0	0.0	902.3	23.6	0.0	0.0	0.0	0.0	0.0	0.0
	harvest	592.0	1.9	0.0	0.0	0.0	0.0	368.2	8.6	368.2	7.7	368.2	7.7
	total b	16917.8	55.4	3982	39.9	902.3	23.6	566.4	13.2	566.4	11.9	566.4	11.9
Tota	$1 \operatorname{sum} a + b$	30533.5	100	7.676 6	100	3820.3	100	$4 \ 301.5$	100	4757.3	100	4 757.2	100

Energy outlays according to agricultural elements for maintenance of two-year fallow land

Table 2

Assessment of Energy Requirements...

ultivation of winter wheat	Materials Total	MJ ha ⁻¹ % MJ ha ⁻¹ %	3 0.0 0.0 1 299.4 7.0) 10 484.0 66.4 10 714.6 58.0	3 4 968.0 31.4 5 146.4 27.8	2 348.0 2.2 729.4 3.9	0.0 0.0 592.0 3.2	15 800 100 1848 1.8 100
Energy outlays according to flows and ag	Machines and trac	6 MJ ha ⁻¹ %	.1 251.9 34.	.2 67.5 9.5	.2 42.5 5.8	.3 109.6 15.0	.3 259.6 35.1	0 731.1 100
	Labor	element MJ ha ⁻¹ %	on 94.5 48.1	24.0 12.2	20.0 10.2	ion 39.9 20.3	18.2 9.3	196.6 100
	-	Agricultural	Soil cultivatio	Fertilization	Sowing	Crop cultivati	Harvest	Total

Table 3

Marek Marks et al.

Energy balance for rotation fallow land - fallow land - winter wheat

Table 4

			Cro	op rotation segme	nt	
Item	Spring rape-pea- -winter wheat (control field)	2 years black fallow – winter wheat	2 years herbicide treated fallow – winter wheat	2 years red clover – winter wheat	2 years Italian ryegrass – winter wheat	2 years mix of red clover and Italian ryegrass - winter wheat
Outlays (MJ ha ⁻¹ 1)	49015.3	28461.6	22302.0	$22\ 783.3$	$23\ 239.1$	23239.1
Yield value (MJ ha ⁻¹ 1)	290.640	$152 \ 460$	$140\ 040.0$	225320.0	$130\ 800.0$	201 600.0
Cumulate energy gain (MJ ha ⁻¹ 1)	241624.7	123998.4	$117\ 738.0$	202536.7	107560.9	178360.9
Production energy intensity	0.169	0.187	0.159	0.101	0.178	0.115
Production effectiveness	5.9	5.4	6.3	9.6	5.6	8.7
Production in cereal units z 1 ha	174.0	84.7	77.8	229.1	5.96	191.6
Energy outlay for1 JZ production (MJ JZ ⁻¹)	281.7	336.0	286.7	99.45	234.3	121.3

outlays was that consisting of energy carriers at 1754.1 MJ ha⁻¹, and the highest share among them was that of soil cultivation 54.3%, harvest 17.9% and crop cultivation 13.2%). The third group of energy consumers were tractors and machines (731.1 MJ ha⁻¹), where the demand structure was as follows: harvest 35.5%, soil cultivation 34.5%, crop cultivation 15%, fertilization 9.2% and sowing 5.8%. Labor is the last flow of energy outlays. In case of winter wheat production it was computed at the level of 196.6 MJ ha⁻¹.

It was established that fertilization consuming 58% of total energy outlays was the most energy intensive operation in winter wheat production consuming 58% of the total energy outlays. Sowing ranks second at 27.8% of outlays (because of the sowing material used) and they are followed by soil cultivation at 7%, crop cultivation at 3.9% and harvest at 3.2%.

Energy efficiency is a synthetic indicator as the ratio of energy accumulated in the yield to the energy input. In average in the studied segments of crop rotation system that indicator was at a very high level (Table 4). The highest production efficiency index at 9.9 was found in the rotation system of red clover – red clover – winter wheat. It was found at the same time that this system was characterized by the lowest energy outlays for production of 1 cereal unit.

The second place in the ranking based on production effectiveness was taken by the rotation system involving two years of red clover cultivation in the mix with Italian ryegrass – winter wheat. The third place was taken by herbicide fallow (for two years) – winter wheat where the energy efficiency index was 6.3. The energy outlays for production of 1 cereal unit were the highest in the system of two years of black fallow followed by winter wheat at 336.0 MJ cereal unit⁻¹.

Discussion

In market economy every production undertaking (agricultural or nonagricultural) can be considered rational on condition that it was verified through economic calculation and subjected to assessment in the aspect of expected negative and positive consequences for the surrounding environment (Woś 1992).

Plant production technologies currently applied in practice differ in the level of consumption of yield generating means, i.e. production intensity, and making the energy balance allows the optimal choice of such technologies. The assessment of production technologies applied in most cases is limited to production-economic criteria to which the energy balance could be a complement (MACIEJKO 1984). High comparability of results indifferent of the

relations of prices is its particular advantage. It can be applied for assessment of energy intensity of individual agricultural technology operations, e.g. soil cultivation (GONET, ZAORSKI 1988, GONET 1991, KORDAS 1999), fertilization (NASALSKI 2001), technique of cultivation of selected crops (WIELICKI 1986), crops rotation systems, and even entire farms (ORLIŃSKI 1986, PAWLAK 1989).

Among agricultural technology operation, the highest energy outlays were related to fertilization. According to NASALSKI (2001) fertilization is the basic factor determining the economic effectiveness of agricultural production. It is a significant yield-generating factor and at the same time it has a significant share in the structure of outlays and costs of production. High-energy outlays related to soil cultivation were reported by GONET and ZAORSKI (1988), GONET (1991) and KORDAS (1999),

Energy efficiency characterized by the index expressing the relation between the energy contained in the harvest and the energy outlays made in the process of production is also an important criterion of economic nature applied in analysis and assessment of production. WIELICKI (1989) reports that under average conditions of farming four units of energy of product produced should be generated per one unit of energy outlays. In the tested two-field segments of rotation system that indicator exceeded those value in every case.

Conclusions

1. In two-year cycle of fallow maintenance the highest energy outlays were incurred for maintenance of black fallow and the lower in case of herbicide fallow.

2. The energy outlays for winter wheat cultivation were dominated by materials (fertilizers, means of plants protection, sowing material) representing as much as 85.5% of the total. As concerns the agricultural technique operations the highest share was that of fertilization and sowing representing 58.0% and 27.8% of all the outlays respectively.

3. Among the six compared three-field elements of crops rotation the highest energy efficiency index (9.9) was characteristic for the system red clover – red clover – winter wheat offering at the same time the lowest energy outlays for production of 1 cereal unit. The lowest energy efficiency index (5.4) was recorded for the rotation system of black fallow – black fallow – winter wheat.

Translated by JERZY GOZDEK

Accepted for print 9.09.2009

References

- ANUSZEWSKI R. 1987. Metoda oceny energochłonności produktów rolniczych (MET). Zag. Ekon. Rol., 4: 16–26.
- Goć E., MUZALEWSKI A. 1997. Wskaźniki eksploatacyjno-ekonomiczne maszyn i ciągników rolniczych stosowanych w gospodarstwach indywidualnych. IBMER, Warszawa.
- GONET Z. 1991. Metoda i niektóre wyniki badań energochłonności systemów uprawy roli. Fragm. Agron., 2: 7–18.
- GONET Z., ZAORSKI T. 1988. Energochłonność orki w różnych warunkach glebowych. Pam. Puł., 91: 137–152.
- KORDAS L. 1999. Energochlonność i efektywność różnych systemów uprawy roli w zmianowaniu. Fol. Univ. Agric. Stetin., Agricultura, 74: 47–52.
- MACIEJKO W. 1984. Rachunek energetyczny w rolnictwie. Zag. Ekon. Rol., 2: 85-96.
- NASALSKI Z. 2001. Effectiveness of financial means invested in vegetable production. Economic Sci., 4: 247–260.
- Nowicki J., MARKS M., MAKOWSKI P. 2007. Ugór jako element współczesnego krajobrazu rolniczego. Fragm. Agron., 4: 48–57.
- ORLIŃSKI J. 1986. Analizy energochłonności skumulowanej w gospodarstwach indywidualnych. Rocz. Nauk. Rol., ser. C, Ekonomika, 3: 11–20.
- PAWLAK J. 1989. Analiza energochłonności produkcji roślinnej. [W:] Organizacyjne i ekonomiczne aspekty mechanizacji produkcji roślinnej w indywidualnych gospodarstwach rolnych. PWRiL, Warszawa.
- Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 7 kwietnia 2004 r. w sprawie minimalnych wymagań utrzymywania gruntów rolnych w dobrej kulturze rolnej. Dz.U. 2004, nr 65, poz. 600.
- Rozporządzenie ministra Rolnictwa i Rozwoju Wsi z dnia 12 marca 2007 r. w sprawie minimalnych norm. Dz.U. 2007, nr 46, poz. 306.
- WIELICKI W. 1986. Analiza porównawcza energochłonności roślin rolniczych. Rocz. Nauk Rol., ser. C, 77(3): 183–190.
- WIELICKI W. 1989. Analiza efektywności energetycznej w rolnictwie. Post. Nauk Rol., 1: 69-86.

Woś A. 1992. Rolnictwo zrównoważone. Zag. Ekon. Rol., 1-3: 9-21.

Wójcicki Z. 1981. Energochłonność produkcji rolniczej. Rocz. Nauk Rol., ser. C, 75(1): 165–198.

DOI 10.2478/v10020-009-0019-5

THE EFFECT OF FEEDING AND HOUSING CONDITIONS OF GROWING-FINISHING PIGS ON PORK QUALITY

Krzysztof Karpiesiuk, Janusz Falkowski

Department of Pig Breeding University of Warmia and Mazury in Olsztyn

Key words: pork, proximate chemical composition, physicochemical properties.

Abstract

The objective of this study was to determine the quality of meat from pigs housed on litter and in litterless pens and fed during the fattening period (from 48 kg to 105 kg bw) complete diets and complete diets supplemented with green forage. Hybrid growing-finishing pigs [Q(QPO) handrace x \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping of \circ Polish Large White) x \circ (QP is a mapping White) x \circ (QP is a mapping White) x \circ (QP

JAKOŚĆ MIĘSA W ZALEŻNOŚCI OD SPOSOBU UTRZYMANIA I ŻYWIENIA TUCZNIKÓW

Krzysztof Karpiesiuk, Janusz Falkowski

Katedra Hodowli Trzody Chlewnej Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: mięso wieprzowe, podstawowy skład chemiczny, ocena fizykochemiczna.

Abstrakt

Badano jakość mięsa świń utrzymywanych w kojcach ściołowych lub bezściołowych i żywionych w okresie tuczu (od 48 kg do 105 kg) mieszanką pełnoporcjową lub mieszanką pełnoporcjową i dodatkowo podawaną zielonką. Tuczniki mieszańcowe [Q(Q) polska biała zwisłoucha x \checkmark wielka

Address: Krzysztof Karpiesiuk, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: +48 (089) 523 38 43, e-mail: krzysztof.karpiesiuk@uwm.edu.pl

biała polska) x σ (\mathfrak{P} pietrain x σ duroc)] podzielono na 4 grupy doświadczalne po 12 sztuk w każdej. Z tusz tuczników pobrano próbki mięśnia najdłuższego grzbietu (*m. longissimus dorsi*) do analizy podstawowego składu chemicznego i oceny fizykochemicznej. Nie stwierdzono istotnego wpływu zastosowanych sposobów żywienia i utrzymania na skład chemiczny oraz jakość mięsa mięśnia najdłuższego grzbietu (*m. longissimus dorsi*) tuczników.

Introduction

Meat quality is affected by both genetic and environmental factors. The main indicators of meat quality, which determine its processing suitability and eating attributes, are acidity, color (including uniformity and stability), water-holding capacity, water-binding capacity, emulsifying and gelling properties, shelf-life, production yield, appearance (color and marbling), texture (tenderness and juiciness) and palatability (KOĆWIN-PODSIADŁA, KRZĘCIO 2005). Genetic factors are believed to be responsible for meat quality in 30%, whereas environmental factors, i.e. management conditions, pre-slaughter handling, slaughter and post-slaughter procedures – in 70% (KOĆWIN-PODSIADŁA 1993). The quality of meat and meat products has become an important consideration in recent years. Efforts to improve production results through raising welfare standards (access to bedding and roughage) are appreciated by consumers who are ready to pay more for organic pork.

Available Polish literature provides information on the impact of different genotypes on the quality and processing suitability of meat (FLORKOWSKI et al. 2007, FLORKOWSKI et al. 2008), as well as on the influence of feeding schemes on the rate of changes in blood biochemical parameters (FALKOWSKI, RAUBO 2007, KLUCZEK, KLUCZEK 2008, MIGDAŁ et al. 2003, REKIEL 2008). However, only a few studies have investigated the combined effects of roughage (green forage, hay-silage, alfalfa silage), housing systems and feeding regimes on fattening results, carcass quality and animal welfare (KAPELAŃSKI et al. 2004, KOZERA 2007, KARPIESIUK, FALKOWSKI 2008a, 2008b). Therefore, the objective of the present study was to analyze the chemical composition and physicochemical properties of meat from hybrid growing-finishing pigs as dependent on the applied housing and feeding system.

Materials and Methods

Samples were collected from the carcasses of 48 hybrid growing-finishing pigs produced by simple four-breed crossing [$\Im(\Im Polish Landrace x \circ Polish Large White) x \circ (\Im Pietrain x \circ Duroc)$]. Experimental animals were selected by the analogue method, based on body weight, age and sex. Pigs were

divided into 4 experimental groups, each of 12 animals, and were placed in pens (4.2 m x 3.6 m), according to the following design: group I (control) – litterless housing (solid floor), a complete diet offered *ad libitum*; group II – litterless housing (solid floor), a complete diet offered *ad libitum*, supplemented with alfalfa meal; group III – shallow litter, a complete diet offered *ad libitum*, supplemented with alfalfa meal. Group HII – shallow litter, a complete diet offered *ad libitum*, supplemented with alfalfa meal. All pigs were fed complete balanced cerealsoybean diets, in accordance with *Normy żywienia świń…* 1993, offered *ad libitum* from self-feeders. Pigs of two experimental groups received also alfalfa meal in the amount of approximately 5 kg, off the floor, in the morning and evening.

Slaughter and a carcass analysis were carried out in accordance with the relevant provisions. Meat content was determined on hot hanging right half-carcasses, using UltraFom 300. Carcasses were graded under the EUROP classification system (Polish Standard Mieso w tuszach... PN-91/A-82001/A1/1995). A fragment of *m. longissimus dorsi* was taken for a physicochemical analysis. LD samples were collected at the level of the 1st -3^{rd} lumbar vertebra. pH₄₅ and pH₂₄ were measured using a pH-meter (POL--EKO-APARATURA) and a Double Pore glass-combination electrode (Hamilton). Pork color parameters, i.e.: L^* – lightness, a^* – redness, b^* - yellowness, were determined with the use of a Minolta CR colorimeter. Measurements were performed on fresh samples collected together with samples for a physicochemical analysis. Pork samples were assayed for the content of: dry matter (*Mieso...* PN-ISO 1442:2000), total protein – by the Kjeldahl method (Produkty... PN-75/A-04018/Az3:2002), crude fat - by the Soxhlet method (Mieso... PN-ISO 1444:2000) and crude ash (Mieso... PN-ISO 936:2000). The water-holding capacity of meat (i.e. the ability to retain own water) was determined by the method proposed by GRAU and HAMM (1952), modified by POHJ and NINIVARA (1957). The analyses were conducted at the Department of Commodity Science and Animal Raw Material Processing, University of Warmia and Mazury in Olsztyn.

The results were validated statistically. The significance of differences between the mean values of the analyzed parameters in experimental groups was estimated by a two-factorial analysis of variance and Duncan's test. The relationships between selected quality attributes of *m. longissimus dorsi* were determined by a cluster analysis, as described by STRZELECKI (2004). Calculations were performed using STATISTICA PL ver. 7.0 software.

Results and Discussion

The results of a physicochemical analysis of pork are presented in Table 1. There were no statistically significant differences between groups as regards the chemical composition of meat contained in *m. longissimus dorsi*. The content of meat components (except for fat) was at a similar level in all experimental groups. Dry matter content ranged from 25.98% in group 4 to 26.16% in group I (control), while total protein content – from 23.44% in group I to 23.66% in group II. Pork fat content was affected by the housing system. Meat from pigs kept on litter contained less crude fat (1.40% in group IV and 1.29% in group II). The fat content of meat from pigs placed in litterless pens

Proximate chemical composition and physicochemical properties of *m. longissimus dorsi* of experimental pigs

			Litterle	ess housing	Shallow-litter housing				
Specification	Unit	Statistical measure	complete diet	complete diet supplemented with green forage	complete diet	complete diet supplemented with green forage			
			group I (control)	group II	group III	group IV			
Drv matter	%	\bar{x}	26.16	26.01	25.99	25.98			
	,	S	0.48	0.60	0.49	0.29			
Total protein	%	x	23.44	23.66	23.52 23.64				
10tal protoin	70	S	0.41	0.48	0.87	0.55			
Crude fat	%	x	1.68	1.58	1.29	1.40			
or uuc iut	70	S	0.54	0.55	0.20	0.42			
Ash	01.	x	1.087	1.092	1.082	1.096			
ASII	-70	S	0.025	0.014	0.035	0.081			
лU		\bar{x}	5.99	5.95	5.89	5.97			
p11 ₄₅		S	0.18	0.22	0.29	0.21			
		x	5.29	5.22	5.27	5.22			
рп ₂₄		S	0.19	0.09	0.16	0.12			
W-+	2	-	8.20	7.99	7.74	8.03			
water-noiding capacity	cm-	8	0.80	0.47	0.64	0.43			
	1	-	55.90^b	56.93^{Aa}	55.60^{B}	55.33^{B}			
Color lightness L^*	1	s	2.69	2.39	2.39	2.81			
	1	x	0.119^{B}	0.299^{b}	0.677^{Aa}	0.165^b			
a*		s	1.188	1.076	1.323	0.838			
1.*	1	-	10.41	10.31	10.34	10.14			
0.	L L	S	0.851	1.003	0.968	0.972			

A, $B - P \le 0.01$ a, $b - P \le 0.05$ Table 1

was 1.58% in group II and 1.68% in group I (control). However, the above differences were non-significant. According to KOŁACZ et al. (2004), differences in the content of dry matter, intramuscular fat and protein in pork may be affected by, among others, carcass tissue composition. Scandinavian researchers (JOHANSSON et al. 2002), who investigated the effect of genotype and red clover silage as a feed supplement on the fat content and fatty acid composition of pork loin, reported similar values of dry matter content (from 25.9% in the group fed red clover silage to 26.2% in the group fed a complete diet), and lower values of total protein content (20.3–20.6), in comparison with the present study. The cited authors noted a higher fat content of pork, ranging from 1.7% (pigs fed a diet supplemented with red clover silage) to 2.2% (pigs fed a standard diet). The value in the group receiving a roughage-supplemented diet was highly significantly lower. It should be stressed that in this group the energy content of the ration was by around 2% lower than in the control group.

Based on active acidity measurements (pH₄₅ from 5.89 to 5.99), none of the samples was identified as PSE or partially PSE (Table 1). The measurement of pH₂₄ enables to eliminate DFD pork whose pH is higher than 6.2. Normal-quality meat has pH in the range of 5.5–5.8 to 6.0 within 24 hours post mortem (KORTZ 2001). In this experiment pH₂₄ ranged from 5.22 in group 2 to 5.29 in group 1, thus indicating the absence of DFD pork.

Water-holding capacity (WHC) is defined as the ability to retain the water contained in meat, mostly by proteins and tissue fibrillar structures. WHC is also an indicator of the processing suitability of meat. A low WHC may result in high weight loss due to drip loss during storage and distribution. In our study, pork from group 3 pigs had the highest WHC (7.74 cm²), while the lowest WHC (8.20 cm²) was noted in pork from control group pigs fed a complete diet and kept in litterless pens. RASSMUSSEN et al. (1996), as cited in KSOBIAK et al. (2005), demonstrated that an unacceptably high drip loss may be caused by muscle protein denaturation, chill shrinkage and low pH levels.

KOZERA (2007) studied the influence of two different housing (indoor and free-range) and feeding (a complete diet and a silage-supplemented diet) systems on fattening results, pig behavior and carcass quality. The above systems were found to have no significant effect on the majority of the analyzed parameters. A trend towards a lower fat content of pork from free-range pigs was only observed, although the values noted in most of the experimental groups were higher than those obtained in our experiment. The percentage protein content of pork was by approximately 2% lower than in the present study. In the cited experiment, the lean meat content of carcasses was high (approx. 54%) in all experimental groups. The values of WHC were also higher in experimental groups in the above study. The highest WHC

 (8.90 cm^2) was observed in the group of pigs kept indoor and fed a diet supplemented with alfalfa silage, and the lowest – in the group kept indoor and fed a standard diet (8.56 cm^2) .

Meat color is an important quality attribute, which affects consumer preferences and shows significant correlations with other meat traits (KORTZ et al. 2000). Color is characterized by three physical parameters, i.e. the predominant wavelength, saturation and lightness. Exudative meat with a low water-holding capacity is lighter in color, because its texture does not permit light penetration into deeper layers which results in a low percentage of light reflection at the surface. Color lightness (L^*) of pork in the present study was relatively high, ranging from 55.33 in group IV to 55.93 in group II. Highly significant differences were noted with respect to this parameter between groups IV and III and group II, and significant differences were observed between group II and group I. As regards the contribution of redness (a^*) to pork color, highly significant differences were reported between group III and group I, and significant differences – between group III and groups IV and II. The average values of vellowness (b^*) ranged from 10.14 in group IV to 10.41 in group I, and they did not differ significantly. Similar values of color lightness (L^*) were obtained by KOZERA (2007): from 55.68 to 56.44 in experiment 1 and from 55.07 to 56.22 in experiment 2.

The lean meat content of carcasses was similar in all groups, at 55.70% on average. Table 2 shows the percentage of carcasses classified into different grades under the EUROP system in each group. Grade E carcasses dominated in all experimental groups (77.1%), followed by grade U carcasses (20.8%). Only 2.1% carcasses were classified to grade R. None of the carcasses belonged to grades O and P. A gradual increase in the percentage content of lean meat in pork carcasses observed in Poland (by 6.4% over 10 years) has resulted in a higher percentage of high-grade carcasses in the EUROP system. In 2006, the structure of pig purchase according to the EUROP classification system was as follows: E - 39.6%, U - 36.4%, R - 17.2%, O - 5.5%, P - 1.3%. This shows that pigs slaughtered in Poland during the period covered by this study were characterized by a lower percentage of high-grade carcasses (E and U), compared with the animals used in our experiment (*Zintegrowany...* 2007).

PRZYBYLSKI et al. (2008) found that a cluster analysis allows to discriminate between meat samples differing in quality, and to select those with the most desirable traits and best eating quality. Figure 1, illustrating the technological properties of *m. longissimus dorsi*, shows three clusters of the investigated parameters. The first cluster comprises the values of pH_{45} , pH_{24} and WHC, the second cluster – the content of fat and ash, and the third cluster – the percentage levels of dry matter and protein in pork. Color lightness (L^*) in the CIE Lab system proved to be a quality indicator of lesser significance. Pork samples collected in all experimental groups were characterized by comparable quality and technological properties. Similar hierarchical relationships between the analyzed quality attributes, i.e. the pH_{45} and WHC of normal pork, were reported by STRZELECKI (2004). The presented computational procedures pertain to selected quality indicators, but they also adequately describe the processing suitability of meat. Similar results of clustering the values of pH_{45} , pH_{24} and WHC were noted by CHWASTOWSKA (2006).

Table 2

		Litterl	ess housing	Shallow-lit	ter housing			
Class	Range (%)	complete diet	complete diet supplemented with green forage	complete diet supplemented with green forage	complete diet supplemented with green forage	Number of carcasses in the class	Percentage of carcasses in the class	
		group I (control)	group II	group III	group IV			
Е	> 55	10	10	9	9 8		77.1	
U	50 - 54.9	2	2	3	3 3		20.8	
R	45-49.9	-	-	-	1	1	2.1	
0	40-44.9			-	-	-	-	
Р	< 40	-	-	-			-	

Carcass classification into grades under the EUROP system and the percentage content of lean meat in the carcass



Fig. 1. Cluster analysis of the quality attributes of pork

Conclusions

The applied feeding and housing systems had no significant effect on the chemical composition of *m. longissimus dorsi* samples collected from growing-finishing pigs.

The values of color lightness and active acidity, as well as a cluster analysis show that pork in all experimental groups was characterized by the desired quality attributes and could be used for both processing and consumption.

Translated by Aleksandra Poprawska

Accepted for print 25.11.2009

References

- CHWASTOWSKA I. 2006. Jakość i przydatność przetwórcza mrożonej polędwicy wieprzowej w zależności od czasu chłodniczego przechowywania i metody rozmrażania (rozprawa doktorska, UWM w Olsztynie).
- GRAU R., HAMM R. 1952. Eine einfache Methode zur Bestimmung der Wassebindung Fleisch. Fleischwirtschaft, 4: 295–297.
- FALKOWSKI J., RAUBO B. 2007. Tempo wzrostu, parametry krwi i mięsność tusz tuczników w zależności od warunków chowu. Roczniki Naukowe PTZ, 3: 39–45.
- FLORKOWSKI T., PISULA A., KAMYCZEK M. 2007. Ocena wpływu wysokiej mięsności na jakość mięsa świń wolnych od genu RYRT. Medycyna Wet., 63 (3): 326–329.
- FLORKOWSKI T., PISULA A., ROLA M. 2008. Porównanie mięsności i jakości technologicznej mięsa świń rasy puławskiej i jej krzyżówek z rasami wielka biała polska i polska biała zwisłoucha. Med. Wet., 64 (5): 673–676.
- JOHANSSON L., LUNDSTRÖM K., JONSÄLL A. 2002. Effects of RN genotype and silage feed on fat content and fatty acid composition of fresh and cooked pork lion. Meat Sci., 60: 17–24.
- KAPELAŃSKI W., JANKOWIAK H., KSOBIAK S., BIEGNIEWSKA M. 2004. Produkcyjność i przejawy zachowań tuczników utrzymywanych systemem wolnowybiegowym. Zesz. Nauk. AR Wrocław, Zoot., LI(501): 99–105.
- KARPIESIUK K., FALKOWSKI J. 2008a. Effect of the feeding and housing system on pig Fattening Results. Pol. J. Natur. Sc., 23(4): 769–778.
- KARPIESIUK K., FALKOWSKI J. 2008b. Reakcja tuczników na zróżnicowane warunki chowu. Roczniki Naukowe PTZ, 4: 65–75.
- KLUCZEK Sz., KLUCZEK P.J. 2008. Wpływ warunków tuczu na aktywność wybranych enzymów krwi tuczników. Med. Wet., 64(4): 335–338.
- Koćwin-Podsiadła M. 1993. Metoda wykrywania mięsa wadliwego u świń (Rozp. hab., WSRP Siedlce).
- KOĆWIN-PODSIADŁA M., KRZĘCIO E. 2005. Jakość wieprzowiny i metody jej doskonalenia. I. Stan jakościowy surowca wieprzowego w zakresie umięśnienia oraz jakość mięsa i jej odchylenia. Przeg. Hod., 73(4): 13–20.
- KOŁACZ R., KORNIEWICZ A., DOBRZAŃSKI Z., BYKOWSKI P., KOŁACZ D., KORNIEWICZ D. 2004. Effect of dietary fish and rapeseed oils on sensory and physicochemical charakteristies of pigs M. longisimus dorsi and fatty acid composition. J. Anim. Feed Sci. 13: 143–152.
- KORTZ J., KAPELAŃSKI W., GRAJEWSKA S., KURYŁ J., BOCIAN M., RYBARCZYK A. 2000. Meat quantity to meat quality relations when the RYR1 gene effect is eliminated. [In:] Quality of meat and fat in pigs as affected by genetics and nutrition. Eds. C. Wenk, J.A. Fernandez, M. Dupuis, Proc. 51st EAAP (100): 143–146.
- KORTZ J. 2001. The chief defects of meat and methods of defection. Pol. J. Food Nutr. Sc., 10(51), S, 1(3): 5–10.

- KOZERA W. 2007. Efektywność tuczu i zachowanie się tuczników w zależności od systemu utrzymania i żywienia (rozp. hab., UWM Olsztyn).
- KSOBIAK S., RAK B., JANKOWIAK H. 2005. Porównanie cech jakości tusz i mięsa tuczników ras białych i ich mieszańców z udziałem rasy pietrain. Roczniki Naukowe PTZ, 1(2): 367–372.
- Normy żywienia świń. Wartość pokarmowa pasz. 1993. Omnitech Press, Warszawa.
- Mięso i przetwory mięsne. Oznaczenie popiołu całkowitego. PN-ISO 936:2000.
- Mieso i przetwory miesne. Oznaczenie zawartości tłuszczu wolnego. PN-ISO 1444:2000.
- Mięso i przetwory mięsne. Oznaczenie zawartości wody (metoda odwoławcza) PN-ISO 1442:2000.
- Mięso w tuszach, półtuszach i ćwierćtuszach. PN-91/A-82001/A1/1995.
- MIGDAŁ W., SECHMAN A., RZĄSA J., BOROWIEC F., FANDREJEWSKI H., RAJ S., WAREMKO D., SKIBA G. 2003. Zmiany poziomu hormonów tarczycy, lipidów i cholesterolu w surowicy krwi tuczników. Med. Wet., 59(10): 879–883.
- POHJA N.S., NINIVARA F.P. 1957. Die Estimmung der Wasser bindung des Fleiches mittels der Konstadruckmethods. Fleischwirtschaft, 9: 193–195.
- Produkty rolniczo-żywnościowe. Oznaczanie azotu metodą Kjeldahla i przeliczanie na białko. PN--75/A-04018/Az3:2002.
- PRZYBYLSKI W., JAWORSKA D., CZARNIECKA-SKUBINA E., KAJAK-SIEMIASZKO K. 2008. Ocena możliwości wyodrębnienia mięsa kulinarnego o wysokiej jakości z uwzględnieniem mięsności tuczników, pomiaru barwy i pH z zastosowaniem analizy skupień. Żywność. Nauka, Technologia, Jakość, 2008, 4(59): 43–51.
- STRZELECKI J. 2004. Badania nad anatomiczno-przestrzennym rozkładem wad jakościowych mięsa w tuszach wieprzowych uwarunkowanych szybkością istopniem zakwaszenia (rozp. hab., Roczn. Inst. Przem. Mięs. i Tł. 41(2)).
- REKIEL A. 2008. Wpływ probiotyków na wskaźniki biochemiczne krwi tuczników. Med. Wet., 64(1): 110–112.
- Statistica for Windows, wersja 7.0.
- WICKE M., MAAK S., VON LENGERKEN G. 1998. Structural and function traits of the skeletal muscle for the improvement of pork quality. Pol. J. Food Nutr. Sci., Supl., 7/48 (4): 21–31.
- Zintegrowany system rolniczej informacji rynkowej. Rynek mięsa wieprzowego. 22/2007. www.minrol.gov.pl; 30.10.2009.

DOI 10.2478/v10020-009-0020-z

IMPACT OF WASTEWATER TREATED IN SACZYN WASTEWATER TREATMENT PLANT UPON QUALITY OF THE WATER OF THE POKRZYWNICA RIVER – RIGHT TRIBUTARY OF THE PROSNA RIVER

Zdzisław Małecki

Polish Society of Ecological Engineering Kalisz Region

K e y w o r d s: basin, river, wastewater treatment plant, raw and treated wastewater, surface water quality.

Abstract

The purpose of this study was to identify the impact of wastewater treated in the mechanical & biological wastewater treatment plant in Saczyn upon quality of the water in the Pokrzywnica and recommend actions to be taken to improve cleanness of the surface waters. The analysis embraced identification of characteristics of the Pokrzywnica basin and an analysis of concentrations of selected physical & chemical components of its water and of wastewater treated in Saczyn in 2006-2007. The study also includes a description of treatment technologies applied in the said treatment plant along with photo records. The analysis of selected results of physical & chemical composition tests of the water in the Pokrzywnica was performed in reference to the measurement cross-section of the Porwita 8.0 km based on the criteria contained in the decree by the Minister of Environment (Rozporzadzenie Ministra Środowiska z dnia 11 lutego 2004... Dz.U. z 2004 r., nr 32, poz. 284) on classification of surface and underground waters (due to absence of a new one, the analysis and evaluation of the water quality was carried out in accordance with the "cancelled" decree). An analysis of quality of the treated wastewater was performed according to the standards contemporaneously in effect: $BZT_5 - Jakość$ wody... PN-EN 1899-1:2002; ChZT-Cr - Oznaczanie... PN-74/C-04578:03, suspension - PB/ZAW-1/05 rev. 1 from 01.10.2005. In 2006-2007 significant exceedance was recorded in relation to the parameters specified in the water permit for the concentration of chemical components and oxygen index in treated wastewater (in 2006 the following parameters were exceeded – BZT_5 by 25%, ChZT-Cr by 70.2%, general suspension by 66.0%, general nitrogen by 75.0%, general phosphorous by 118.0%). The water in the Pokrzywnica during the analysis period (2006-2007) belonged to quality class IV, i.e. water contaminated beyond standard (BZT5 2.93 and 2.40 mgO₂ dm⁻³, dissolved oxygen 8.18 and 8.28 mgO₂ dm⁻³, ChZT-Cr 27.3 mgO₂ dm⁻³ (2006), Kjeldahl nitrogen 1.265 and 1.07 mgN dm⁻³, general nitrogen 5.65 and 6.16 mgN dm⁻³, general phosphorous 0.201 and 0.89 mgP dm^{-3}). It was found out that the wastewater treated in Saczyn exerted significant impact upon the quality of water in the Pokrzywnica. Modernization of the wastewater treatment plant in Saczyn should be continued taking advantage of the phosphorous precipitation process. Furthermore, the water and wastewater management should be improved accompanied by extension of biological filters along the Pokrzywnica.

Address: Zdzisław Małecki, Polish Society of Ecological Engineering, Kalisz Region, ul. Łódzka 218, 62-800 Kalisz, phone: +48 (062) 767 01 79, e-mail: zdzisław.malecki@euroexbud.com. pl

WPŁYW ŚCIEKÓW OCZYSZCZONYCH W OCZYSZCZALNI SACZYN NA JAKOŚĆ WÓD RZEKI POKRZYWNICY – PRAWOBRZEŻNEGO DOPŁYWU PROSNY

Zdzisław Małecki

Polskie Towarzystwo Inżynierii Ekologicznej Oddział Ziemi Kaliskiej

Słowa kluczowe: zlewnia, rzeka, oczyszczalnia ścieków, ścieki surowe i oczyszczone, jakość wód powierzchniowych.

Abstrakt

Celem pracy było: rozpoznanie wpływu ścieków oczyszczonych w oczyszczalni mechaniczno--biologicznej w Saczynie na jakość wód rzeki Pokrzywnicy oraz wskazanie kierunków działań zmierzających do poprawy czystości wód powierzchniowych. Badania obejmowały rozpoznanie charakterystyki zlewni rzeki Pokrzywnicy oraz analize steżeń wybranych składników fizycznych i chemicznych jej wód, a także ścieków oczyszczonych w oczyszczalni w Saczynie w latach 2006-2007. W pracy zamieszczono także opis technologii oczyszczania ścieków w wymienionej oczyszczalni wraz z dokumentacją fotograficzną. Analize wybranych wyników badań składu fizykochemicznego wód w rzece Pokrzywnica wykonano w odniesieniu do przekroju pomiarowego Porwity km 8.0 w oparciu o kryteria zawarte w rozporządzeniu Ministra Środowiska z dnia 11 lutego 2004 r. ... Dz.U. z 2004 r., nr 32, poz. 284 (badania i ocenę jakości wód wykonano wg przedmiotowego rozporządzenia "anulowanego" z dniem 01.01.2005 r. z powodu braku nowego). Badanie jakości ścieków oczyszczonych przeprowadzano natomiast wg obowiazujących ówcześnie norm: BZT₅ – Jakość wody... PN-EN 1899-1:2002; ChZT-Cr - Oznaczanie... PN-74/C-04578:03, zawiesiny - PB/ZAW-1/05 wyd. 1 z 1.10.2005. W latach 2006-2007 stwierdzono znaczne przekroczenie parametrów określonych w pozwoleniu wodnoprawnym – wielkości stężeń składników chemicznych i wskaźników tlenowych w ściekach oczyszczonych (w 2006 r. przekroczono parametry dla: – BZT₅ o 25%, ChZT-Cr o 70,2%, zawiesiny ogólnej o 66,0%, azotu ogólnego o 75,0%, fosforu ogólnego o 118,0%). Wody w rzece Pokrzywnicy w okresie badawczym (2006-2007) należały do IV klasy jakości - jako wody ponadnormatywnie zanieczyszczone (stężenia odpowiednio wynosiły dla: BZT₅ 2,93 i 2,40 mgO₂ dm³, tlenu rozpuszczonego 8,18 i 8,28 mgO2 dm3, ChZT-Cr 27,3 mgO2 dm3 (2006), azotu Kjeldahla 1,265 i 1,07 mgN dm⁻³, azotu ogólnego 5,65 i 6,16 mgN dm⁻³, fosforu ogólnego 0,201 i 0,189 mgP dm⁻³). Stwierdzono w oparciu o wyniki badań, że istotny wpływ (znaczny) na jakość wody w rzece Pokrzywnicy mają ścieki oczyszczone w oczyszczalni Saczyn. Wobec tego należy kontynuować modernizacje oczyszczalni ścieków w Saczynie, wraz z zastosowaniem w technologii oczyszczania procesu wytracania fosforu, oraz uporzadkować gospodarke wodnościekowa w zlewni z równoczesna rozbudowa wzdłuż rzeki Pokrzywnicy tzw. filtrów biologicznych.

Introduction

The environment constitutes the whole of surrounding interrelated components influence by natural, atmospheric, hydrological, lithological as well as social, cultural and economic circumstances, which result from the human activity. The present scientific and technical progress and related social and economic transformations cause development unbalance between the nature and the man. While predicting future issues related to the environment protection one can notice a kind of a vicious circle, which the man is unwilling to abandon due to benefits only to adversely impact the environment with their whole existence. There is a justified concern that trespassing the border (barrier) of the environmental "capacity" may lead to disturbing ecological risks on a global scale (KOZŁOWSKI 1997).

Contamination from site sources gets into surface waters as a result of infiltration, surface flow, water and wind erosion. The anthropogenic increase of contamination, including biogenic components, includes primarily increased load of wastewater, intensified fertilization in farming and increased erosion within the basin. Quite frequently farming is the first to blame for water contamination (BARTOSIEWICZ 1990).

Taking into account a group of the contamination components one can see that farming areas basically supply waters with elements which cause eutrophication. The basic rule when eliminating vast area contamination is to slow down and close the water and matter loop in various parts of the basin. In case of farming there is significant risk of pesticides permeating into water, particularly those with long decomposition time.

Specific risks are also present at arable lands, which make use of wastewater. The highest concentration of biogenic compounds in the basin is found in water courses (rivers) which are loaded with social wastewater. A significant factor which affect water quality in small basins is poor water and wastewater management (PIJANOWSKI, KANOWNIK 1997). If we treat wastewater insufficiently, which can considerably affect quality of surface and underground waters, this may lead shortage of both drinking water and industrial water (impeding economy development). Composition of atmospheric precipitation also impacts quality of waters in the basin. Atmospheric precipitation can bring 6.0–14.4 nitrogen and 0.2–4.28 phosphorous per 1 hectare of the basin (THORNTON, DISE 1998). The factors which deteriorate quality of water include changes of the area use (urbanization, cutting down forests, excessive dehydration of waterlogged areas), insufficient (or lack of) treatment of social and industrial wastewater, intensified farming, power supply (emission of gas pollutants, heated water discharges), transportation, chemical, metallurgical, farming and food industries (KOWAL, ŚWIDERSKA-BRÓŹ 1998). Contamination coming from spot focuses permeates water circulation in nature which can be substantially neutralized (reduced) by erecting wastewater treatment plants.

Farming contamination, remains of natural and artificial fertilizers (fertilizer ingredients delivered to the soil surface are sorbed within the surface layer aeration zone), pesticides containing heavy metals and toxic organic compounds, which permeate into water, cause mass death rate among water organisms and classless water. Gas and dust contamination, which can travel long distances and settle on the soil surface, also reduces pH of surface and underground waters thereby putting biological life in rivers at risk. Afforested basins are characterized by relatively low biogenic concentration (PIJANOWSKI, KANOWNIK 1997).

One of the key goals that our country needs to meet according to the Framework Water Directive is the quality of surface waters. This can be achieved by reducing amounts of discharged contamination.

Examination scope and methodology

Examination of the impact of the two-stage mechanical & biological wastewater treatment plant in Saczyn (municipality of Godziesze, country of Kalisz) upon the quality of the water in the Pokrzywnica were carried out in 2006–2007.

The scope of examination covered characteristics of the Pokrzywnica basin, an analysis of concentrations of chemical compositions and physical indicators of selected test results in the Pokrzywnica measuring point before the wastewater discharge – Porwita 8.0 km and below the wastewater discharge point into the river in Saczyn – 12.0 km. The examination and evaluation of the surface water quality in the Pokrzywnica were conducted against the criteria included in the Minister of Environment (Rozporządzenie Ministra Środowiska z dnia 11 lutego 2004... Dz.U. z 2004 r., nr 32, poz. 284) on classification of surface and underground waters, which became ineffective as of 1 January 2005. However, due to absence of a new one, the examination and evaluation of the surface water quality in 2006–2007 was based on the "cancelled" decree. The output of the treated wastewater quality (WIOŚ Agency in Kalisz, Municipality Office in Godziesze) was based on the then standards: BZT₅ – Jakość wody... PN-EN 1899-1:2002; ChZT-Cr – Oznaczanie... PN-74/C-04578:03, general suspension – PB/ZAW-1/05 rev.1 from 1 October 2005.

Examined objects

The Pokrzywnica basin is located in the south-eastern Wielkopolska and belongs of the area of the poorest water resources in Poland and Europe, characterized by the smallest atmospheric precipitation reaching 450–650 mm on average annually and in dry years even less than 350 mm.

The basic morphometric parameters of the Prosna and Pokrzywnica basins are shown in Table 1. The Pokrzywnica is supplied with water coming directly from precipitation and melting snow. Water flows are characterized by quick

	Wotow	mater quality (2007)	above norm contamina- tion							
Basic morphometric parameters of the rivers Prosna and Pokrzywnica	tamination	area	 farming municipal untreated waste 	farmingmunicipaluntreated waste						
	Con	punctual /(purifie. plant)	Kuchary near Kalisz	Brzeziny Saczyn						
		Catchment use	agricultur industr.	agricultur industr.						
	Catabmont	area (km²)	A = 4924.7	$A_{Pokrz} = 234.4$						
	Flow corton	Kalisz (m ³ s ⁻¹)	SNQ = 3.18 $SSQ = 1 1.5$	SNQ = 0.26 SSQ = 2.03						
	1 (km)	region Kalisz	$L_r = 53.0$	$L_{r} = 36.1$						
	Length	total	$L_{r} = 217.0$	$L_{r} = 36.1$						
		Notes	the left Warta inflow	the right Prosna inflow						
		River	Prosna	Pokrzywnica						

p ŕ

Table 1

transformation from culminations to the low water states which generally start in June and last till October (the end of hydrologic year). The average unit run-off for the Prosna river in Kalisz is 4.1 dm³ s⁻¹ km⁻² (the European average is 9.6 dm³ s⁻¹ km⁻²), whereas the total annual outflow varies from 401–550 mln m³. The structure of Pokrzywnica basin is as follows: arable land approximately 72%, forests – approximately 20.0%, meadows – 6.5%, dispersed development and compact settlement – approximately 1.5%.

The examined area is located in the mesothermal climate zone with considerable influence of Atlantic climate. The area is, above all, under the influence of advecting maritime-polar air with diversified temperature, which causes weather instability. One can observe days with frosty weather which appear there relatively more often (about 78 days). More frequently than in other parts of the country, one can observe sunny days with transparent sky cover (approximately 118 days), and less often with opaque sky cover (almost 42 days). Whereas average annual temperatures in Kalisz were between 9°C (1987) up to 10.0°C (2000), which gave an average annual temperature from the multi-year period of 8.7°C (IMGW branch office in Kalisz).

In the Pokrzywnica River basin, there is a Brzeziny district and partially, Godziesze Wielkie. The basin is of a typically agricultural character. Mineral fertilizers (particularly nitrogen fertilizers) flowing on the soil surface permeate into the river, dumped pesticides, domestic sewage and industrial wastes usually coming from the meat processing industry, treated in district wastewater mechanical-biological treatment plants located in Brzeziny and Saczyn for Godziesze Wielkie (in the Saczyn wastewater treatment plant, wastewater is not subject to phosphorus precipitation process). In the Pokrzywnica river basin, in Czempisz village, there is a non-cultivated, closed rubbish (waste) dump. In the basin, there are also fish ponds with the total area of about 150 ha (Dzikie Nowe, Wróbel, Brzeziny, Saczyn).

Characteristic physical and geographical parameters of the Pokrzywnica river basin are as follows (Figure 1): the total area of the basin is $A_{Pokrz} = 234.4 \text{ km}^2$, river length $L_r = 36.1 \text{ km}$, (Table 1), basin length $L_{cat} = 32.9 \text{ km}$, average basin width $B_{cat} = 7.12 \text{ km}$, average basin slope $i_{avg.cat} = 1.9\%$, average longitudinal river decline $i_{avg.cat} = 0.29\%$ (MALECKI 2005).

A two stage wastewater treatment plant (Figure 2) is 800 metres away from the Pokrzywnica river, it is located in the forest, in Saczyn village, district of Godziesze. In the study there are photos which show the Pokrzywnica river in the Porwita measuring cross-section – km 8.0 (Figure 3), secondary, vertical settling tank and aerated pond (Figure 4), stabilizing-sedimentation pond (Figure 5) and reed plot (Figure 6). Treated domestic wastewater from the wastewater treatment plant is carried away by means of melioration ditches to the Pokrzywnica river (km 12.0). Currently, the wastewater treatment plant



Fig. 1. Map of Pokrzywnica (Szałe) dam reservoir 1:300 000



Zdzisław Małecki

is supplied with wastewater from Godziesze, Skrzatki and wastewater delivered by vehicles. Wastewater is subject to mechanical and biological treatment.

Pursuant to the water permit issued by the County authorities in Kalisz dated December 30, 2005 regarding requirements for carrying away treated wastewater from the wastewater treatment plant, the following parameters have been established, namely:

 $\begin{array}{l} Qd_{avg} = 250.0 \ m^3 \ d^{\text{-1}} \\ Q_{dmax} = 275 \ m^3 \ d^{\text{-1}} \\ Q_{hmax} = 15.6 \ m^3 \ h^{\text{-1}} \\ Q_y = 91.250 \ m^3/\text{annum} \\ BZT_5 = 25.0 \ mgO_2 \ dm^{\text{-3}} \\ ChZT = 125.0 \ mgO_2 \ dm^{\text{-3}} \\ General \ suspended \ matter = 35.0 \ mg \ dm^{\text{-3}} \\ pH \ reaction = 6.5-9 \end{array}$



Fig. 3. Pokrzywnica river

Technological process

Wastewater from the Godziesze district area is delivered to the drainage basin of the mechanical and biological centre wastewater treatment plant in Saczyn by means of waste removing vehicles. Then they are gravitationally carried away to the pumping plant and then they are forced, by means of a pipe line, to the sand trap (Figure 1). Upon leaving the sand trap, the wastewater flows to a collective intermediate pumping station where it is forced, by means



Fig. 4. Wastewater treatment plant in Saczyn - secondary sedimentation tank



Fig. 5. Wastewater treatment plant in Saczyn - overhead pond

of pumps, to oxygen-free reactor. Wastewater which is subcleaned in the oxygen-free reactor flows to the oxygen reactor. Next, wastewater is supplied to the vertical secondary settling tank from where they gravitationally flow to aerated ponds I° and II° . Produced deposits are re-circulated to the collective



Fig. 6. Wastewater treatment plant in Saczyn - reed plot

chamber of wastewater intermediate pumping station. From the aerated ponds I° and II°, the wastewater gravitationally flows through stabilization and sedimentation pond (Figure 5) and reed plot (Figure 6) to the measuring chamber, from which wastewater is directed to the collector. Ponds I° and II° are aerated by means of aerating unit with the use of jet equipment. The air supplied to the ponds ensures appropriate mixture of wastewater and supply of the right level of oxygen for biochemical processes proceeding in ponds. The ponds consist of oxygen and oxygen-free zones which are responsible for nitrification and de-nitrification processes. At the same time, the secondary deposit accumulating on the ponds' bottom undergoes mineralization process.

Results

In 2006, concentrations and indexes (Table 2) of wastewater treated in mechanical-biological wastewater plant in Saczyn and of those dumped to the Pokrzywnica river (Saczyn km 12.0) were in case of: $BZT_5 - (6.3-88.0)$ avg. $31.2 \text{ mgO}_2 \text{ dm}^{-3}$ (exceeded by 25%); ChZT-Cr - (61.7-252.0) avg. 144.5 mgO₂ dm⁻³ (exceedance by 16.4%); general suspended matter - (21.0-104.0) 56.4 mg dm⁻³ (exceedance by 61.1%).

Zdzisław Małecki

Table 2 Physical & chemical composition of treated waste waster (selected parameters) in the waste water treatment plant in Saczyn in 2006–2007 (Raport... 2006, 2007)

	tal	otal phorus		dm ⁻³	treated	I	Ι	Ι	Ι	Ι	-	I	-	10.92	-	Ι	10.92			
	tot	phosp		MgP	Wri	I	-	-	-	-	-	I	-	-	-	-	-			
Indices	tal	lahle		dm ⁻³	treated	I	I	I	I	I	I	I	I	71.7	I	I	71.7			
	to.	Kjeld		MgM	ąraw	I	I	I	I	I	I	I	I	I	I	I	I			
	tal	ogen		dm ⁻³	treated	I	I	I	I	I	I	I	I	52.5	I	I	52.5			
	to.	nitr		NgM	WBY	I	I	I	I	I	I	I	I	I	I	I	I			
	rate	ogen	ngen	0 ₃ dm ⁻³	pətsərt	I	I	I	I	I	I	I	I	<0.1	I	I	<0.1			
	niti	nitr	nitr		mgNN	WBT	I	I	I	I	I	I	I	I	I	I	I	I		
	rite	ogen	nit	$0_2 \mathrm{dm}^{-3}$	treated	I	I	I	I	I	I	I	I	<0.001	I	I	<0.001			
	nit	nitr	n	mgNN	WBY	I	I	I	I	I	I	I	I	I	I	I	I			
	tion	nit		Н	bətsərt	7.5	I	I	I	I	I	I	7.5	I	I	I	I			
	reac	m		d	WB1	7.5	I	I	I	I	I	I	7.5	I	I	I	I			
	tal	nsion		dm ⁻³	treated	LL	27	68	104	61	16	21	174	106	$\overline{56}$	12	54.4			
	to	adsns		Mg	VBY	151	I	313	265	264	274	398	277.7	I	I	I	I			
	(J.)	D ₅ COD (Cr)		e dm ⁻³	treated	252	120	250	127	96.6	104	61.7	144.5	314	250	74.0	212.7			
				COD		COI	mg0	WBT	405	I	814	703	668	725	849	694	I	I	I	I
				D		2 dm ⁻³	treated	88.0	17.0	34.5	40.0	20.3	6.3	12.0	31.2	243.0	97.0	15.0	118.3	
	ЪВС	BOI			WBY	158.0	I	337.0	391.0	260.0	389.0	443.0	329.7	I	I	I	I			
Sampling date					01.03.06	11 - 17.05.06	25 - 30.05.06	19-20.09.06	16-17.10.06	29 - 30.11.06	13-14.12.06	Avg 2006	26.06.07	22-23.08.07	18-19.10.07	Avg 2007				
Whereas in 2007, average concentrations and indicators of treated wastewater and dumped wastewater to the Pokrzywnica river were respectively: BZT_5 (15.0–243.0) avg. 118.3 mgO₂ dm⁻³ (exceedance by 373.2%); ChZT-Cr – (74.0–314.0) avg. 212.7 mgO₂ dm⁻³ (exceedance by 66.0%); general nitrogen – 52.5 mgN dm⁻³ (exceedance for generally accepted standards, by 75.0%); general phosphorus – 10.92 mgP dm⁻³ (exceedance for generally accepted standards, by 118.0%).

In reference to the results of analysis (Table 2) conducted in 2006–2008, a considerable exceedance of analysed chemical components concentrations and the rate of oxygen indexes for treated wastewater was found in comparison with the parameters determined in the water permit requirements.

The water in the Pokrzywnica river (Table 3), in the Porwity measuring point, km 8.0 between 2006 and 2007 was characterized with relatively high level of oxidation (the quantity of oxygen dissolved in water was 8.18 and 8.28 mgO₂ dm⁻³; the quantity of oxygen indicators: BZT₅ – 2.93 and 2.40 mgO₂ dm⁻³, ChZT-Cr – 27.3 mgO₂ dm⁻³), a scanty quantity of general deposit matter (8.5 and 6.03 mg dm⁻³), a considerable quantity of biogenes and, above all general nitrogen (5.647 and 6.16 mgN dm⁻³). The water in the Pokrzywnica river in the analysed period (2006–2007) belonged to the IV quality class (the water was excessively polluted). In 2007, in comparison to 2006, an increase of concentrations was observed for: general nitrogen by 9.1%, phosphates by 10.5% and concentration decrease was observed in case of: Kjeldahle nitrogen by 27.6% and general phosphorus by o 6.4%.

Table 3

Water physical & chemical composition of	of the Pokrzywnica river at the sampling	point Porwity km 8.0 in
the year 2	2006–2007 (Raport 2006, 2007)	

		Sampling point Porwity km 8.0					
		year					
Indices of water quality	Unit	2006		2007			
		avg. concentration	class	avg. concentration	class		
Reaction	pH	8.0	Ι	7.4	Ι		
Oxygen dissolved	$mgO_2 \ dm^{-3}$	8.18	III	8.28	III		
BOD_5	$mgO^2 dm^{-3}$	2.93	III	2.40	III		
COD (Cr)	${ m mgO_2~dm^{-3}}$	27.3	IV	-	-		
Total Kjeldahle	MgN dm ⁻³	1.365	III	1.07	III		
Nitrate nitrogen	$mgN-NO_3 \ dm^{-3}$	4.25	-	-	-		
Nitrite nitrogen	$mgN-NO_2 \ dm^{-3}$	0.0367	-	-	-		
Total nitrogen	MgN dm ⁻³	5.647	III	6.16	IV		
Phosphorus	mgP–PO₄ dm ⁻³	0.201	Π	0.222	II		
Total phosphorus	MgP dm ⁻³	0.201	II	0.189	II		
Total suspension	Mg dm ⁻³	8.5	Ι	6.03	Ι		

Discussion

As a consequence of the conducted results analysis from the period between 2006–2007, it was found out that the following concentrations of chemical components and oxidation indexes were exceeded in the wastewater treated in mechanical – biological wastewater treatment plant in Saczyn (2006 for: – BZT₅ by 25%, ChZT-Cr by 16.4%, general deposit matter by 61.1%; 2007 for: – BZT₅ – by 373.2%, ChZT-Cr by 70.2%, general deposit matter by 66.0%, general nitrogen by 75.0%, general phosphorus by 118.0%).

The water in the Pokrzywnica river (Porwity km 8.0) during the analysis period (2006–2007) belonged to the IV quality class (2006–2007 concentrations and indexes were respectively: dissolved oxygen 8.18 and 8.28 mgO₂ dm⁻³, BZT₅ – 2.93 and 2.40 mgO₂ dm⁻³, ChZT-Cr – 27.3 mgO₂ dm⁻³, general nitrogen 5.647 and 6.16 mgN dm⁻³, general phosphorus 0.201 and 0.189 mgP dm⁻³). The above results indicate that the quality (cleanliness) of water is mainly determined by the not "completely" treated wastewater in Saczyn and by the wastewater dumped in the distance of 4 km (Saczyn km 12.0) from the measuring point (Porwity km 8.0). By pointwise dumping of poor quality water (not "completely" treated in Saczyn wastewater treatment plant), there is reduced self purification ability of the river (dilution and mixing, suspension sedimentation, sorption processes and biodegradation).

Apart from the water and wastewater management, in the Pokrzywnica river basin there is a closed waste dump in Czempisz, as well as fish ponds (with the area of approximately 150 ha (MAŁECKI 2006)) water management in Brzeziny and Godziesze districts is not organized, the water of which is highly eutrophicated (2007 r. - general nitrogen avg. 16.75 mgN dm⁻³, general phosphorus avg. 0.99 P dm⁻³). Poor water and wastewater management in the Pokrzywnica river basin, (illegal waste dumps, leaky septic tanks, illegal beaches, lack of manure plates etc.) agricultural pollutions, increase of erosion in the basin, refluxes from closed waste dump (Czempisz km 23–24), "dumpings" of water from fish ponds (Dzikie Nowe km 24.0 of the Pokrzywnica river, Brzeziny km 22.0, Grzymaczewski Channel km 15.0 – inflow of water from Wróbel fish ponds etc.) cause, with high probability, the increase of contamination of water in the Pokrzywnica river above the point, where not completely treated water from Saczyn wastewater treatment plant is dumped, with organic substances (a noticeable increase of oxygen indexes, Porwity km 8.0 of the river – BZT₅ average indexes 2.7 mgO₂ dm⁻³, ChZT-Cr 27.3 mgO₂ dm⁻³) and increase of biogenes concentration (e.g. average concentration of general nitrogen was 6.0 mgN dm⁻³), which was also confirmed on other objects of the scientific literature (Hus 1994, PIJANOWSKI et al. 1997). In the Pokrzywnica river basin includes also a farming areas where liquid manure is used (there

is a high probability that the acceptable manuring limit is exceeded), which undoubtedly has effect on the increase of water contamination (MOSIEJ 1999). Forest area is approximately 20% of the Pokrzywnica river basin area (free from pointwise contaminations from farmsteads) and it indicates low biogenes concentrations within those areas, which is confirmed in the literature (TAYLOR et al. 1997, MUSCUTT, WHITHERS 1996, PEKESOVA et al. 1999, CLARK et al. 2004).

Conclusions

The thesis presents achieved results of the study regarding protection of the surface waters in the Pokrzywnica river in the measurement cross section (Porwity km 8.0) below mechanical – chemical wastewater treatment plant in Saczyn and results of "not completely" treated water (wastewater) which is dumped to the river above the wastewater treatment plant. Based on the study results analysis, a quality state analysis was performed: of the treated wastewater flowing into the Pokrzywnica river (Saczyn km 12.0) and of the river waters (Porwity km 8.0). Treated wastewater quality analysis helped to make the evaluation of the wastewater treatment plant influence on the quality of water in the Pokrzywnica river (Porwity km 8.0). The quality of water in the Pokrzywnica river, with relatively low flows within one year (SNQ = $0.26 \text{ m}^3 \text{ s}^{-1}$), deteriorates due to dumping of "not completely" treated wastewater from the mechanical - chemical wastewater treatment plant in Saczyn. Based on the analysis results (2006-2007) it was noted that in order to limit biogenes quantity, which are "introduced" from the wastewater treatment plant to the river, quality of treated wastewater must be improved (improvement of wastewater treatment technology), with simultaneous development of coastal zones of graminoids (so called biological filters) enriched with bushes and trees (e.g. purple willow and alder). Also, one should head towards reasonable shaping of basin use structure and mount small swelling cascades and surface run-off retardants in the meliorating ditches. An essential enterprise to improve water purification indexes in the Pokrzywnica river includes organization of proper water and wastewater management within the Pokrzywnica river basin (e.g. reasonable management of water in fish ponds and further restoration of the closed waste dump in Czempisz). The continuation of further modernization of the mechanical – chemical wastewater treatment plant in Saczyn (km. 12.0) should be the priority. Furthermore, an analysis of wastewater quality dumped from the mechanical – chemical wastewater treatment plant in Brzeziny (km 22.0) should be done (there is a high probability that wastewater of "not the best quality" is dumped to the Pokrzywnica river).

An analysis of the results obtained in the period 2006–2007 helped to reach the following conclusions:

1. Anthropogenic inflow of contaminants (biogenes) to the Pokrzywnica river includes, first of all, the increase of load connected with domestic sewage inflows, fertilization in agriculture and increase of erosion within the basin.

2. Chemical components concentration and values of oxygen indexes considerably exceeded the permitted limits in the wastewater which is treated in the mechanical – chemical wastewater treatment plant in Saczyn and dumped to the Pokrzywnica river (further modernization of the wastewater treatment plant is required).

3. Probably, in the nearest future, unless "not completely" treated wastewater dumping to the Pokrzywnica river from wastewater treatment plant (Saczyn km 12.0) is reduced, the ability of the Pokrzywnica river to "self purify" will be substantially reduced.

4. Phosphorus compounds dumped with the treated wastewater from the wastewater treatment plant in Saczyn to the Pokrzywnica river should be quickly and radically reduced (apply the process of phosphorus precipitation in the treatment technology) and further modernization of the wastewater treatment plant should be continued. If the aforementioned steps are not taken and water and wastewater management is not improved in Pokrzywnica river basin, then the river will be in danger of degradation.

5. It is strongly recommended that further quality issues identification in the Pokrzywnica river basin and performance of scientific – engineering and development studies regarding possible "support" of existing mechanical – biological wastewater treatment plants by treatment of wastewater in the soil-plant environment, in the soil aeration zone (biologically active layer of the soil) should be continued.

Translated by JACEK MAJEWSKI

Accepted for print 29.07.2009

References

BARTOSIEWICZ A. 1990. Chemizm wód gruntowych w zlewni użytkowanej rolniczo w warunkach glebowo-klimatycznych Równiny Kościańskiej. [W:] Obieg wody i bariery biogeochemiczne w krajobrazie rolniczym. Wyd. Nauk. UAM w Poznaniu, 127–142.

CLARK M.J., CRESSER M.S, SMART R., CHAPMAN P.J., EDWARDS A.C. 2004. The influence of catchment charactenstics on the seansonality of carbon and nitrogen species concentrations in upland rivers of Northern Scotland. Biogeochemistry, 68:

Kozłowski S. 1997. W drodze do ekorozwoju. PWN, Warszawa 1997.

Hus S. 1994. Ocena wpływu gospodarki wodno-ściekowej wsi górskiej na jakość wód powierzchniowych. Zagosp. Ziem Górskich, Kraków, 37: 217–230.

Jakość wody. Oznaczanie biochemicznego zapotrzebowania tlenu po n dniach (BZTn). Część 1: Metoda rozcieńczania i szczepienia z dodatkiem allilotiomocznika. PN-EN 1899-1:2002.

- KRZEMIEŃ E., KURZBAUER, PAWLIK-DOBROWOLSKI J. 2003. Znaczenie badań składu chemicznego opadów w problematyce rolniczych zanieczyszczeń obszarowych. Materiały Seminaryjne. IMUZ Falenty, 26: 45–52.
- KOWAL A.L, ŚWIDERSKA-BRÓŹ M. 1998. Oczyszczalnie wody. PWN, Warszawa-Wrocław.
- MALECKI Z. 2005. Zbiorniki retencyjne w powiecie kaliskim. Pokrzywnica (Szałe) k. Kalisza. Wyd. Nauk. Gabriel Borowski, Lublin.
- MAŁECKI Z. 2006. Wody stojące w powiecie kaliskim. Zlewnia rzeki Pokrzywnicy. Wyd. Nauk. Gabriel Borowski, Lublin.
- MAŁECKI Z. 2008. Ocena wpływu wybranych zbiorników retencyjnych na środowisko w zlewni Prosny. Wyd. Nauk. Gabriel Borowski, Lublin.
- Mosiej J. 1999. Przyrodniczo-techniczne uwarunkowania gospodarowania wodą w dolinie rzeki Ner. Rozp. Nauk. i Monogr., 222, Wydaw. SGGW w Warszawie.
- MUSCUTT A.D, WHITHERS J.A. 1996. The phosphorus content of rivers in England and Wales Water. Res, 30: 5.
- Oznaczanie chemicznego zapotrzebowania tlenu (ChZT) metodą dwuchromianową. Metoda miareczkowa. Zakres metody: powyżej 10 mg O₂/l. PN-74/C-04578:03.
- PEKASOVA P., MIKLANEK P., KONICEK A., PEKAR J. Water quality in experimental basin, National report 1999 of the IHP UNESCO Project. 1,1 FRIEND and of the project European Reference Basins. Inst. Of Hydrology SAS, Bratyslava.
- Raport z badań wody i ścieków. Delegatura w Kaliszu, Urząd Gminy w Godzieszach, Kalisz 2006, 2007. PIJANOWSKI Z., KANOWNIK W. 1997. Zmienność stężeń wybranych substancji chemicznych w wodach powierzchniowych przepływających przez tereny wiejskie o różnym zagospodarowaniu. Rocz. AR Poznań, CCXCIV, Melior. Inż. Środ., 19, cz. 2, 347–358.
- Rozporządzenie Ministra Środowiska 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. z 2004 r., nr 32, poz. 284.
- TAYLOR A.W., EDWARDS W.M., SIMPSON E.C. 1997. Nutrients in streams draining woodland and farmland near Coshoton. Ohio, Water Resour, Res. 7,1.
- THORNTON G.J.P., DISE N.B. 1998. The influence of catchments characteristics, agricultural activities and atmospheric deposition on the chemistry of small stream the English Lake District. The Science on the Total Environment, 63–75: 216.

DOI 10.2478/v10020-009-0021-y

COMPARISON OF ECONOMIC EFFECTIVENESS OF APPLYING DIFFERENT HORMONAL PREPARATIONS FOR REOPHILE CYPRINID FISH REPRODUCTION STIMULATION BASED ON THE EXAMPLE OF ASP ASPIUS ASPIUS (L.) AND IDE LEUCISCUS IDUS (L.)*

Anna Hakuć-Błażowska, Krzysztof Kupren, Konrad Turkowski, Katarzyna Targońska, Marta Jamróz, Sławomir Krejszeff, Maciej Kwiatkowski, Daniel Żarski, Dariusz Kucharczyk

Department of Lake and River Fisheries University of Warmia and Mazury in Olsztyn

Key words: asp, ide, hormonal stimulation, economic effectiveness.

Abstract

Assessment of the economic effectiveness of hormonal stimulation application in reophile cyprinid fish reproduction based on the example of asp *Aspius aspius* (L.) and ide *Leuciscus idus* (L.) was the goal of the studies presented in this paper. Three hormonal preparations: Ovopel, Ovaprim and carp pituitary were tested during this study. Economic effectiveness of application of those hormonal preparations for asp and ide reproduction stimulation considering the relative working fertility of asp and ide, effectiveness of the selected hormonal preparations and their price were assessed. The cost of hormonal injection per 1000 spawn grains and per 1000 spawn grains in eyed stage was assumed for the measure of economic effectiveness. In case of both studied species injections with Ovaprim and Ovopel were more economic than stimulation with carp pituitary. This is linked to the high application effectiveness of GnRH analogues with dopamine inhibitors found in controlled reproduction of asp and ide. The effectiveness of those hormonal preparations is confirmed by a high percentage of ovulations as well as the high survival rate of the embryos to the eyed stage.

Address: Anna Hakuć-Błażowska, University of Warmia and Mazury, ul. Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: +48 (089) 523 44 36, e-mail: hakuc.blazowska@uwm.edu.pl

^{*} The study was financed within the frameworks of the Project Optimization of reophile cyprinid fish stocking material production under controlled conditions; Sectoral Operational Program Fisheries and fish processing 2004–2006; (00040-61535-OR1400009/07)

PORÓWNANIE OPŁACALNOŚCI STOSOWANIA RÓŻNYCH PREPARATÓW HORMONALNYCH DO SYMULACJI ROZRODU KARPIOWATYCH RYB REOFILNYCH NA PRZYKŁADZIE BOLENIA ASPIUS ASPIUS (L.) I JAZIA LEUCISCUS IDUS (L.)

Anna Hakuć-Błażowska, Krzysztof Kupren, Konrad Turkowski, Katarzyna Targońska, Marta Jamróz, Sławomir Krejszeff, Maciej Kwiatkowski, Daniel Żarski, Dariusz Kucharczyk

Katedra Rybactwa Jeziorowego i Rzecznego Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: boleń, jaź, stymulacja hormonalna, efektywność ekonomiczna.

Abstrakt

Celem badań była ocena ekonomicznej efektywności stosowania stymulacji hormonalnej w rozrodzie karpiowatych ryb reofilnych na przykładzie bolenia *Aspius aspius* (L.) i jazia *Leuciscus idus* (L.). Do badań zastosowano trzy środki hormonalne: Ovopel, Ovaprim i przysadkę mózgową karpia. Oceniono efektywność ekonomiczną zastosowania tych preparatów do stymulacji rozrodu bolenia i jazia, biorąc pod uwagę płodność roboczą względną bolenia i jazia, skuteczność wybranych preparatów hormonalnych i ich cenę. Jako miarę efektywności ekonomicznej przyjęto koszt iniekcji hormonalnej w przeliczeniu na uzyskane 1000 ziaren ikry i na 1000 ziaren ikry w stadium zaoczkowania. W przypadku obu badanych gatunków iniekcje hormonalne z użyciem Ovaprimu i Ovopelu w porównaniu z stymulacją przysadką mózgową karpia są bardziej opłacalne. Ma to związek ze stwierdzoną wysoką skutecznością stosowania analogów GnRH wraz z inhibitorami dopaminy w kontrolowanym rozrodzie bolenia i jazia. O skuteczności tych środków hormonalnych świadczy zarówno wysoki odsetek owulacji, jak i wysoki odsetek przeżywalności embrionów do stadium zaoczkowania.

Introduction

Reophile cyprinid fish are an inseparable element of the diversity of Polish rivers so every action to the benefit of that group is important to the environment as a whole strengthening it, making it more sustainable and immune to stimuli disturbing its equilibrium (BŁACHUTA 1998, PENCZAK et al. 2000). Decreasing presence of reophile cyprinid fish, or even their disappearance, that is observed forces undertaking of protective activities. The study of different aspects of reproductive biotechnology of wild cyprinids were done (GLOGOWSKI et al. 1997, 1999, KUCHARCZYK et al. 1997b,c,d, 1998b, 2005, 2008, KUJAWA et al. 1997, BABIAK et al. 1998, CIERESZKO et al. 1999, WOJTCZAK et al. 2003, KOWALSKI et al. 2003, 2004, KREJSZEFF et al. 2008, 2009, CEJKO et al. 2009, ŻARSKI et al. 2009). Stocking with appropriately selected stocking material, also that obtained from artificial reproduction under controlled conditions (KUCHARCZYK 2002, JAMRÓZ et al. 2008a, KREJSZEFF et al. 2008, ZARSKI et al. 2008b), is the best active method of fish protection (AUGUSTYN 2002, KUJAWA 2004, WOLNICKI 2005). Choice of the appropriate hormonal preparation and its appropriate dose are important elements of successful controlled reproduction. Stimulation is applied at the level of the: hypothalamus, pituitary and gonads (BIENIARZ, EPLER 1991). In reophile cyprinid fish controlled reproduction practice the homogenate of the carp or bream pituitary (CIEŚLA 1998, KUCHARCZYK 2002), human chorionic gonadotrophin (hCG) and gonadotrophin release hormone (GnRH) and its analogues, frequently in combination with a dopamine antagonist (KUCHARCZYK 2002, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, ZARSKI et al. 2008b) are applied. In the literature few results concerning economic aspects of reproduction and seeing of reophile cyprinid fish under controlled conditions are available (KUPREN et al. 2008b, TURKOWSKI et al. 2008). The usefulness of those studies is supported by few existing publications on the subject (TLUSTY 2002). Also data on costs of hormonal stimulation application in reophile fish reproduction can rarely be found in the literature (KŁODZIŃSKA, OKONIEWSKI 1998). That is why this study aimed at assessment of economic effectiveness of different preparations use for reproduction stimulation in asp Aspius aspius (L.) and ide Leuciscus idus (L.).

Materials and Methods

Asp spawners originated from Pierzchały Dam Reservoir on the Pasłęka River while the ide spawners originated from Janowo Fishery Farm near Szczytno. Both asp and ide spawners caught were transported to the Department of Lake and River Fisheries hatchery where they were separated according to sex and placed in 1000 l basins with controlled water temperature and photoperiod (KUJAWA et al. 1999). The entire reproduction technology starting with the hormonal injection through spawn incubation was carried out according to the methodology by KUCHARCZYK et al. (1997a) and ŻARSKI et al. (2008a). Spawn survival was determined during the eyed stage.

Hormonal injections

Three hormonal preparations were chosen for stimulation of ide and asp maturing. The fish were divided into four groups. In group one ovulation was stimulated by application of the Hungarian preparation Ovopel containing 18–20 μ g of mammal GnRH analogue and 8–10 mg of metoclopramide in a single granule (HORVATH et al. 1997, manufacturer Unic-trade, Hungary).

In the second group of the females were obtained the Canadian preparation Ovaprim (manufacturer Syndel, Canada), containing salmon GnRH-a and domperidone (PETER et al. 1993). The females in group three were treated with carp pituitary homogenate (Argent, USA). In case of the controls group physiological liquid was applied. Ovopel was administered at 2 granules kg⁻¹ body weight of the female, Ovaprim at 0.5 ml kg⁻¹ body weight of the female and the pituitary at 3.0 mg kg⁻¹ body weight of the female. Prior to pituitary or Ovopel administration they were homogenized and dissolved in sterile 0.9% solution of NaCl. Fish were anesthetized in the solution of 2-phenoxyethanol (at 0.5 ml l⁻¹ of water, manufacturer Sigma-Aldrich, Germany), and next the preparation was administered in a single dose, intraperitoneally under the caudal fin (KUCHARCZYK 2002). The mass of the fish in individual groups and the results obtained (such as the relative working fertility and survival of embryos until the eyed stage) were subjected to variance analysis (ANOVA) and *post-hoc* Tukey;s test at the significance level $\alpha = 0.05$.

Economic effectiveness of hormonal stimulation

Economic effectiveness was assesses considering the relative working fertility and preparation prices. The relative working fertility was assumed according to the literature data. In case of asp it was in average 66 spawn grains g^{-1} body weight of the female (VOSTRADOVSKY, VASA 1981), and in case of ide – in average 84 spawn grains g^{-1} body weight of the female (WITKOWSKI et al. 1997). The cost of hormonal injection per 1000 spawn grains obtained and per 1000 spawn grains at eyed stage were assumed as the measure of economic effectiveness.

Purchase prices of individual hormonal preparations converted to Polish zlotys according to the exchange rate of 12 12 2007 for Ovopel and on 8 11 2007 for Ovaprim and pituitary were assumed for the computations. The purchase price per 1 g of carp pituitary was USD 339, 10 ml of Ovaprim – USD 25.50, and 1granule of Ovopel – EUR 0.4. The cost of the single dose of Ovopel, Ovaprim and carp pituitary per 1 kilogram of fish body weight was computed. Next the total cost of individual hormonal preparations applied for hormonal stimulation was computed considering the percentage of ovulations in individual groups. In the computations those artificial reproduction cost elements were disregarded which remained at the same level indifferent of the preparations applies (equipment depreciation, lighting, labor).

Results

Hormonal stimulation of asp

Hormonal preparations application had a significant influence on the asp reproduction effects studied (Table 1). In the control group no final maturing of oocytes was found in any of the females. Following the Ovopel injection, the percentage of ovulating females was 83. To stimulation with Ovaprim the females reacted with 100% ovulation while following pituitary application ovulation was confirmed in 33% of the females. The average body weights of females in the individual groups were similar and ranged from 3900 g to 4100 g. The effectiveness of different hormonal preparations application expressed as the percentage of eyed spawn was: for Ovopel – 55; for Ovaprim – 68 and for the pituitary – 49 (Table 1).

Table 1

		A	Ide					
	control	ovopel	ovaprim	pituitary	$\operatorname{control}$	ovopel	ovaprim	pituitary
Number of females (individuals)	6	6	6	6	10	10	10	10
Average body weight of females (g)*	3 980±130	4 120±132	4 000±130	$3970{\pm}135$	489±36	502±45	504±42	498±41
Percentage of ovulations	0	83	100	33	0	100	100	90
Quantity of spawn obtained (K eggs)	0	1354.2	1 584.0	508.3	0	421.3	423.0	376.3
Embryos survival to the eyed stage (%)**	0	54.6 ± 2.3^{b}	$68.4{\pm}1.5^{a}$	48.7 ± 2.0^{c}	0	56.2 ± 1.8^{b}	64.7 ± 1.9^{a}	37.6 ± 2.2^{c}

Results obtained during artificial reproduction of asp and ide

* no statistical differences between the weight of fish in individual groups of the two studied species were found

 ** the results of embryos survival for both species marked with different letter index differ statistically

Hormonal stimulation of ide

The application of hormonal preparations had similar effects (as in case of asp) to the studied effects of ide reproduction (Table 1). In the control group no female ovulated. Following the injection with Ovopel and Ovaprim 100% of the females reacted with ovulation while following application of the pituitary ovulation was confirmed for 90% of the females. The body weight of the females in individual groups ranged from 489 g to 504 g. At the eyed stage the percentage of live spawn in the group injected with Ovopel was 56; with Ovaprim 65 and after application of the carp pituitary the embryos survival rate to the eyed stage was 38% (Table 1).

Economic effectiveness of hormonal stimulation

The analyzed costs of individual hormonal preparations application for reproduction stimulation of asp and ide are presented in Table 2. The costs of a single dose of hormonal preparations per kilogram of female body weight was for: Ovopel – PLN 2.88, Ovaprim – PLN 3.19 and pituitary – PLN 2.54. The total cost of Ovopel and the total cost of Ovaprim used for hormonal infections in case of asp females was similar while the total cost of applying the pituitary was lower. In case of hormonal injections administered to ide females the total cost of individual preparations application was similar.

Table 2

		Asp		Ide			
	ovopel	ovaprim	pituitary	ovopel	ovaprim	pituitary	
Hormonal preparation single dose average cost per 1 kg body weight of a female (PLN)	2.88	3.19	2.54	2.88	3.19	2.54	
Hormonal preparations costs (PLN)*	71.19	76.56	59.28	14.45	16.06	12.64	
Hormonal preparations costs per 1000 spawn grains (PLN)	0.05	0.05	0.12	0.03	0.04	0.03	
Hormonal preparations costs per 1000 spawn grains at eyed stage (PLN)	0.10	0.07	0.24	0.06	0.06	0.09	

Costs of hormonal preparations applied for hormonal stimulation of asp and ide

* the cost of hormonal preparations incurred in total in the individual groups of females of the two species separately

Table 2 presents the asp and ide hormonal stimulation cost per 1000 grains of spawn obtained and per 1000 grains of spawn in eyed stage. The hormonal stimulation cost per 1000 grains of asp spawn obtained was the lowest when applying Ovopel and Ovaprim at PLN 0.05. In case of ide Ovopel and pituitary were the most economical (PLN 0.03). Larger differences in profitability of applying different hormonal preparations were recorded in case of hormonal injection costs per 1000 spawn grains at the eyed stage.

Discussion

During the last decade the number publications concerning artificial reproduction of reophile cyprinid fish, including asp and ide has been increasing (CIEŚLA 1998, KUCHARCZYK et al. 1998a, ŚLIWIŃSKI 1998, TARGOŃSKA-DIETRICH et al. 2004, KUCHARCZYK et al. 2007, JAMRÓZ et al. 2008a,b, ŻARSKI et al. 2008a). This is caused by both the fear of the possibility of disappearance of those species from our waters and the increased interest in the stocking material of that group of fish.

Choice of the appropriate hormonal preparation for ovulation stimulation is an important element in artificial reproduction of fish. In case of reophile fish the carp pituitary preparation used to be used (VOSTRADOVSKY, VASA 1981, DVORAK 1982, KOURIL et al. 1988, CIEŚLA 1998, ŚLIWIŃSKI 1998, KUCHARCZYK 2002, TARGOŃSKA-DIETRICH et al. 2004). During the recent years, however, synthetic GnRH analogues with the addition of dopamine inhibitors such as Ovopel or Ovaprim have enjoyed an increasing interest. Suitability of Ovopel for reproduction of fish has already found confirmation in numerous scientific works. Many researchers obtained very good results in spawning stimulation of actually all studies species of reophile cyprinid fish after application of Ovopel (CIEŚLA 1998, KŁODZIŃSKA, OKONIEWSKI 1998, KUCHARCZYK et al. 1998a, ŚLIWIŃSKI 1998, KUCHARCZYK et al. 1999, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, ZARSKI et al. 2008b). Less numerous are the reports on suitability of Ovaprim in reproduction of fish in Poland (KUCHARCZYK et al. 2007, JAMRÓZ et al. 2008a,b, ZARSKI et al. 2008a,b), while it has been applied with success in Asian countries since 1990 (DAS 2004).

The studies conducted confirm high effectiveness of Ovopel and Ovaprim in controlled asp and ide reproduction. As compared to synthetic GnRH analogues used in the studies the effectiveness of the pituitary is lower. In case of asp only 33% of females ovulated after pituitary homogenate application while after application of Ovaprim 100% of females produced spawn and after application of Ovopel – 83%. Also the results of ide hormonal injections confirm better suitability of Ovopel and Ovaprim as compared to the pituitary. Following stimulation with GnRH synthetic analogues 100% of females ovulated while after treatment with the pituitary – 90%. Those results match those published by, among others, KUCHARCZYK 2002, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, ŻARSKI et al. 2008a.

Following application of Ovaprim and Ovopel, higher survival rates of embryos to the eyed stage were also recorded than after pituitary application. The percentage of eyed live spawn after treatment with Ovopel was 54.6% in case of asp and 56.2% in case of ide, after Ovaprim – 68.4% and 64.7%

respectively while after treatment with carp pituitary the survival of embryos to the eyed stage was 48.7% for asp and 37.6% for ide.

The dynamic increase in production of reophile cyprinid fish stocking material production influenced an increase in the economic aspects of that production (KUPREN et al. 2008b, TURKOWSKI et al. 2008). As concerns economic effectiveness better results were obtained applying Ovopel and Ovaprim than applying the carp pituitary. In difference to the results given by KŁODZIŃSKA and OKONIEWSKI (1998), the cost of a single pituitary dose is lower than the cost of Ovopel and Ovaprim, however, considering the effectiveness of artificial spawning presented as the percentage of ovulating females, in case of the asp the cost of hormonal stimulation with the pituitary per 1000 grains of spawn obtained was the highest and in case of ide at the level of Ovopel application cost. In the study the relative working fertility was assumed on the base of the literature data. The fertility obtained during the study was very close to those values and no differences between groups were detected.

Much larger differences in economic effectiveness of individual hormonal preparations application are visible in the costs of hormonal injections per 1000 spawn grains at eyed stage. Similar results were obtained during studies on the hormonal stimulation effectiveness and economic effectiveness of pikeperch *Stizostedion lucioperca* (L.) production (HAKUĆ-BŁAŻOWSKA et al. 2008, KUPREN et al. 2008a).

Stimulation with the carp pituitary proved to be the least economic in case of both studied species, the application costs of which was at the level of PLN 0.09 per 1000 grains of ide spawn at eyed stage and in case of asp at as much as PLN 0.24 per 1000 grains of spawn at eyed stage. Hormonal stimulation with Ovopel and Ovaprim proved more economic. In case of asp the cost of Ovopel injection was PLN 0.10 per 1000 grains of spawn at eyed stage and in case of Ovaprim – PLN 0.07 per 1000 grains of spawn at eyed stage. The costs of applying both Ovopel and Ovaprim in case of ide are PLN 0.06 per 1000 grains of spawn at eyed stage.

Conclusions

1. The results obtained in the studies confirm high effectiveness of Ovopel and Ovaprim in controlled reproduction of asp and ide.

2. In the studies conducted the effectiveness of Ovopel and Ovaprim is confirmed by both the high percentage of ovulations and high embryos survival rates to the eyed stage, particularly as compared to the results obtained after application of the carp pituitary in reproduction of ide. 3. Better economic effectiveness results were obtained after applying Ovopel and Ovaprim than the carp pituitary for reproduction stimulation in case of both those species.

Translated by JERZY GOZDEK

Accepted for print 8.09.2009

References

- AUGUSTYN L. 2002. Sztuczny rozród świnki, Chondrostoma nasus (L.) i brzany, Barbus barbus (L.) z Popradu. [W:] Wylęgarnia 2001–2002. Red. Z. Okoniewski, E. Brzuska. Wyd. IRŚ, Olsztyn: 29–36.
- BABIAK I., GLOGOWSKI J., KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1998. Cryopreservation of sperm from asp Aspius aspius. Progr. Fish-Cult., 60: 146–148.
- BIENIARZ K., EPLER P. 1991. Rozród ryb. Wyd. Lettra, Kraków, 1-202.
- BLACHUTA J. 1998. Rola i znaczenie rodzimych gatunków karpiowatych ryb reofilnych w ekosystemach rzek. [W:] Karpiowate ryby reofilne. Red. H. Jakucewicz, R. Wojda. Wyd. PZW, Warszawa, 17–21.
- CEJKO B.I., KOWALSKI R.K., KUCHARCZYK D., TARGOŃSKA K., KREJSZEFF S., ŻARSKI D., GLOGOWSKI J. 2009. Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus L. Aquacult. Res. [in press].
- CIERSZKO A., DABROWSKI K., KUCHARCZYK D., DOBOSZ S., GORYCZKO K., GLOGOWSKI J. 1999. The presence of uric acid, an antioxidantive substance, in fish seminal plasma. Fish Physiol. Biochem., 21(4): 313–315.
- CIESLA M. 1998. Wyniki badań nad opracowaniem metodyki sztucznego rozrodu jazia, Leuciscus idus. [In:] Karpiowate ryby reofilne. Red. H. Jakucewicz, R. Wojda. Wyd. PZW, Warszawa, 41–49.
- DAS S.K. 2004. Evaluation of a New Spawning Agent, Ovopel in Induced Breeding of Indian Carps. Asian Fish. Sci., 17: 313–322.
- DVORAK J. 1982. Umely vyter a podchov pludku Parmy. Ryb., 1: 53-54.
- GLOGOWSKI J., BABIAK I., KUCHARCZYK D., ŁUCZYŃSKI M. 1997. The effect of individual male variability on cryopreservation of bream (Abramis brama (L.)) sperm. Pol. Arch. Hydrobiol., 44: 281–285.
- GLOGOWSKI J., BABIAK I., KUCHARCZYK D., ŁUCZYŃSKI M., PIROS B. 1999. Some properties of bream Abramis brama L. sperm and its cryopreservation. Aquacult. Res., 30: 765–772.
- HAKUĆ-BŁAŻOWSKA A., KUCHARCZYK D., KUPREN K., TARGOŃSKA K. 2008. Comparison of economic effectiveness of pikeperch summer fry production independence of reproduction methods. [In:] Percid Fish Culture For Research to Production. Eds. P. Fontaine, P. Kestemont, F. Teletcheta, N. Wang. Presses Universitaires de Namur, Namur (Belgium), 110–111.
- HORVATH L., SZABO T., BURKE J. 1997. Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. Pol. Arch. Hydrobiol., 44: 281–292.
- JAMRÓZ M., HAKUĆ-BŁAŻOWSKA A., KUCHARCZYK D., KWIATKOWSKI M., TARGOŃSKA K., ZARSKI D., MAMCARZ A. 2008a. Wpływ stosowania preparatu Ovaprim na efekty pozasezonowego oraz sezonowego rozrodu jazia (Leuciscus idus L.) w warunkach kontrolowanych. [W:] Biotechnologia w akwakulturze. Red. Z. Zakęś, J. Wolnicki, K. Demska-Zakęś, R. Kamiński, D. Ulikowski. Wyd. IRS, Olsztyn, 159–164.
- JAMRÓZ M., KUCHARCZYK D., HAKUĆ-BŁAŻOWSKA A., KREJSZEFF S., KUJAWA R., KUPREN K., KWIATKOWSKI M., TARGOŃSKA K., ŻARSKI D., CEJKO B.I., GLOGOWSKI J. 2008b. Comparing the effectiveness of Ovopel, Ovaprim and LH-RH analogue used in the controlled reproduction of ide, Leuciscus idus (L.). Arch. Pol. Fish., 16(4): 363–370.
- KLODZIŃSKA H., OKONIEWSKI Z. J. 1998. Ovopel nowy środek do stymulacji rozrodu ryb. [W:] Wylęgarnia 1997–1998. Red. J. Waluga. Wyd. IRS, Olsztyn, 45–49.
- KOURIL J., PRIKRYL I. 1988. Pracovni plodnost jikernacek bolena draveho (Apius aspius L.) z udolni nadrze Żelivka pri umelem vyteru. Bul. VURH, Vodnany, 4: 16–19.

- KOWALSKI R., GLOGOWSKI J., KUCHARCZYK D., GORYCZKO K., DOBOSZ S., CIERESZKO A. 2003. Proteolytic activity and electrophoretic profiles of proteases from seminal plasma of teleosts. J. Fish Biol., 63: 1008–1019.
- KOWALSKI R., GLOGOWSKI J., KUCHARCZYK D., MAK M., DOBOSZ S., ZAKĘŚ Z., CIERESZKO A. 2004. Characterization of gelatinolytic activity in seminal plasma of some teleost fish. Aquacult. Int., 12: 57–68.
- KREJSZEFF S., KUCHARCZYK D., KUPREN K., TARGOŃSKA K., MAMCARZ A., KUJAWA R., KACZKOWSKI Z., RATAJSKI S. 2008. Reproduction of chub, Leuciscus cephalus L., under controlled conditions. Aquacult. Res., 39: 907–912.
- KREJSZEFF S., TARGOŃSKA K., ŻARSKI D., KUCHARCZYK D. 2009. Domestication affects spawning of the ide (Leuciscus idus) – preliminary study. Aquaculture, 295: 145–147.
- KUCHARCZYK D. 2002. Rozród kontrolowany i androgeneza wybranych ryb karpiowatych. UWM, Olsztyn, Rozpr. Monogr., 63: 1–80.
- KUCHARCZYK D., KUJAWA R., ŁUCZYŃSKI M., GLOGOWSKI J., BABIAK I., WYSZOMIRSKA E. 1997a. Induced spawning in bream, Abramis brama (L.), using carp pituitary extract and hCG. Aquacult. Res., 28: 139–144.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997b. Artificial spawning in bream (Abramis brama (L.)). Pol. Arch. Hydrobiol., 44: 203–207.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997c. Induced spawning in rudd (Scardinius erythrophthalmus L.). Pol. Arch. Hydrobiol., 44: 209–213.
- KUCHARCZYK D., ŁUCZYŃSKI M., KUJAWA R., CZERKIES P. 1997d. Effect of temperature on embryonic and larval development of bream (Abramis brama L.). Aquat. Sci., 59: 214–224.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., SKRZYPCZAK A., WYSZOMIRSKA E. 1998a. Rozród bolenia (Aspius aspius), jazia (Leuciscus idus L.) i klenia (Leuciscus cephalus L.) w warunkach kontrolowanych poza okresem tarła naturalnego. [W:] Karpiowate ryby reofilne. Red. H. Jakucewicz, R. Wojda. Wyd. PZW, Warszawa, 57–64.
- KUCHARCZYK D., ŁUCZYŃSKI M., KUJAWA R., KAMIŃSKI R., ULIKOWSKI D., BRZUZAN P. 1998b. Influences of temperature and food on early development of bream (Abramis Brama L.). Arch. Hydrobiol., 141: 243–256.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E., ULIKOWSKI D. 1999. Artificial spawning of ide Leuciscus idus under controlled conditions. EJPAU 2(2): 05 (www.ejpau.media.pl).
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., TARGOŃSKA-DIETRICH K., WYSZOMIRSKA E., GLOGOWSKI J., BABIAK I., SZABO T. 2005. Induced spawning in bream (Abramis brama L.) using pellets containing GnRH. Czech J. Anim. Sci., 50: 89–95.
- KUCHARCZYK D., BOREJKO A., TARGOŃSKA K., ROŻEK W., CHWALUCZYK R., KOWALSKI R., GLOGOWSKI J. 2007. Wpływ Ovaprimu na efekty rozrodu jazia (Leuciscus idus). [W:] Rozród, podchów, profilaktyka ryb jeziorowych i innych gatunków. Red. J. Wolnicki, Z. Zakęś, R. Kamiński. Wyd. IRS, Olsztyn, 31–35.
- KUCHARCZYK D., TARGOŃSKA K., HLIWA P., GOMUŁKA P., KWIATKOWSKI M., KREJSZEFF S., PERKOWSKI J. 2008. Reproductive parameters of common carp (Cyprinus carpio L) spawners during natural season and out-of-season spawning. Reprod. Biol., 8 (3): 285–289.
- KUJAWA R. 2004. Biologiczne podstawy podchowu larw reofilnych ryb karpiowatych w warunkach kontrolowanych. UWM, Olsztyn, Rozpr. Monogr., 88: 1–88.
- KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1997. Effect of temperature on embryonic development of asp (Aspius aspius L.). Pol. Arch. Hydrobiol., 44: 139–143.
- KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1999. A model system for keeping spawners of wild and domestic fish before artificial spawning. Aquacult. Eng., 20: 85–89.
- KUPREN K., BŁAŻOWSKA-HAKUĆ A., TARGOŃSKA K., KUCHARCZYK D., TURKOWSKI K. 2008a. Applications of chosen hormonal agents in pikeperch reproduction under controlled conditions – some economic aspects. [In:] Percid Fish Culture For Research to Production. Eds. P. Fontaine, P. Kestemont, F. Teletcheta, N. Wang. Presses Universitaires de Namur, Namur (Belgium), 120–121.
- KUPREN K., TURKOWSKI K., KUCHARCZYK D., KREJSZEFF S., ŻARSKI D., HAKUĆ-BŁAŻOWSKA A., TARGOŃSKA K., KWIATKOWSKI M., JAMRÓZ M., CZARKOWSKI T. 2008b. Economic aspects of rearing larval asp, Aspius aspius (L.) and ide, Leuciscus idus (L.) in closed recirculating systems. Arch. Pol. Fish., 16(4): 413–420.

- PENCZAK T., KRUK A., KOSZALIŃSKI H., ZIĘBA G. 2000. Ichtiofauna rzeki Bzury. Rocz. Nauk. PZW, 13: 23–33.
- PETER R.E., LIN H.R., KRAAK G. VAN DER, LITTLE M. 1993. Releasing hormones, dopamine antagonists and induce spawning. [In:] Recent Advances in Aquaculture. Eds. Muir J.F., Roberts R.J. Blackwell Sci. Publ. Ltd, Oxford, 25–30.
- ŚLIWIŃSKI J. 1998. Sztuczne tarło bolenia (Aspius aspius L.). [W:] Karpiowate ryby reofilne. Red. H. Jakucewicz, R. Wojda. Wyd. PZW, Warszawa, 57–64.
- TARGOŃSKA-DIETRICH K., ZIELAZNY T., KUCHARCZYK D., MAMCARZ A., KUJAWA R. 2004. Out-of-season spawning of cultured ide (Leuciscus idus L.) under controlled conditions. EJPAU, 7(2): 02 (www.ejpau.media.pl).
- TLUSTY M. 2002. The benefits and risks of aquacultural production for the aquarium trade. Aquaculture, 205: 203–219.
- TURKOWSKI K., KUCHARCZYK D., KUPREN K, HAKUĆ-BŁAŻOWSKA A., TARGOŃSKA K., ŻARSKI D., KWIATKOWSKI M. 2008. Economic aspects of the experimental rearing of asp, Aspius aspius (L.), ide, Leuciscus idus (L.), and dace Leuciscus leuciscus (L.) under controlled conditions. Arch. Pol. Fish., 16(4): 397–411.
- VOSTRADOVSKY J., VASA J. 1981. Bolen dravy (Aspius aspius L.) nowy obiekt umeleho chovu. Bul. VURH Vodany, 3: 10–13.
- WITKOWSKI A., CIEŚLA M., NAPORA K. 1997. Jaź. Wyd. IRS, Olsztyn, 1–158.
- WOJTCZAK M., GLOGOWSKI J., KOŁDRAS M., KUCHARCZYK D., CIERESZKO A. 2003. Characterization of protease inhibitors of seminal plasma of cyprinids. Aquat. Liv. Res., 16(5): 461–465.
- WOLNICKI J. 2005. Intensive rearing of early stages of cyprinid fishes under controlled conditions. Arch. Pol. Fish. 13(1): 5–87.
- ŻARSKI D., KUCHARCZYK D., TARGOŃSKA K., CHWALUCZYK R., KREJSZEFF S., KWIATKOWSKI M., JAMRÓZ M. 2008a. Wpływ środka hormonalnego Ovaprim na efektywność rozrodu bolenia (Aspius aspius) w warunkach kontrolowanych. [In:] Biotechnologia w akwakulturze. Red. Z. Zakęś, J. Wolnicki, K. Demska-Zakęś, R. Kamiński, D. Ulikowski. Wyd. IRS, Olsztyn, 153–158.
- ŻARSKI D., TARGOŃSKA K., RATAJSKI S., KACZKOWSKI Z., KUCHARCZYK D. 2008b. Reproduction of nase, Chondrostoma nasus (L.), under controlled conditions. Arch. Pol. Fish., 16(4): 355–362.
- ŻARSKI D., KUCHARCZYK D., TARGOŃSKA K., JAMRÓZ M., KREJSZEFF S., MAMCARZ A. 2009. Application of Ovopel, Ovaprim and their combination in controlled reproduction of two rheophilic cyprinids. Pol. J. Nat. Sc., 24(4): 235–244.

DOI 10.2478/v10020-009-0022-x

APPLICATION OF OVOPEL AND OVAPRIM AND THEIR COMBINATIONS IN CONTROLLED REPRODUCTION OF TWO REOPHILIC CYPRINID FISH SPECIES*

Daniel Żarski, Dariusz Kucharczyk, Katarzyna Targońska, Marta Jamróz, Sławomir Krejszeff, Andrzej Mamcarz

Department of Lake and River Fisheries University of Warmia and Mazury in Olsztyn

Key words: ide, dace, hormonal stimulation, GnRHa, unsticking eggs.

Abstract

The aim of the study was to compare the female dace *Leuciscus leuciscus* (L.) and ide *Leuciscus idus* (L.) hormonal stimulation effectiveness with two preparations (Ovopel and Ovaprim) separately and in combination. The experiment was conducted under controlled conditions. During the experiment the percentage of ovulating females, ovulation time and survival of embryos to the eyed stage were recorded. Stimulation with Ovopel resulted in shorter ovulation appearance time in females of both species (48–50 h for dace and 36–38 h for ide) while stimulation with Ovaprim in a higher percentage of live embryos in the eyed stage (almost 80 and 60% for dace and ide respectively). The highest hormonal stimulation effectiveness was recorded in the group where a combination of those two hormonal substances was applied as shortening of the time for achievement of spawning readiness (48 and 36 hours for dace and ide respectively), a significant ovulation synchronization and the highest embryos survival rates (almost 80 and 70% for dace and ide respectively) were recorded.

ZASTOSOWANIE PREPARATÓW OVOPEL I OVAPRIM ORAZ ICH KOMBINACJI W KONTROLOWANYM ROZRODZIE DWÓCH GATUNKÓW KARPIOWATYCH RYB REOFINYCH

Daniel Żarski, Dariusz Kucharczyk, Katarzyna Targońska, Marta Jamróz, Sławomir Krejszeff, Andrzej Mamcarz

Katedra Rybactwa Jeziorowego i Rzecznego Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: jaź, jelec, stymulacja hormonalna, GnRHa, odklejanie ikry.

Address: Daniel Żarski, University of Warmia and Mazury, ul. Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: +48 (089) 523 44 36, e-mail: danielzarski@poczta.interia.pl

^{*} The study was financed within the frameworks of the Project Optimization of reophile cyprinid fish stocking material production under controlled conditions; Sectoral Operational Program Fisheries and fish processing 2004–2006; (00040-61535-OR1400009/07)

Abstrakt

Celem pracy było porównanie efektywności stymulacji hormonalnej samic jelca *Leuciscus leuciscus* (L.) i jazia *Leuciscus idus* (L.) po zastosowaniu dwóch preparatów (Ovopel i Ovaprim) osobno oraz ich kombinacji. Eksperyment przeprowadzono w warunkach kontrolowanych. W trakcie jego trwania notowano odsetek owulujących samic, czas owulacji oraz przeżywalność embrionów do stadium zaoczkowania. Stymulacja Ovopelem poskutkowała krótszym okresem wystąpienia owulacji u samic obu gatunków (48–50 h dla jelca i 36–38 h dla jazia), natomiast Ovaprimem – wyższym odsetkiem żywych embrionów w stadium zaoczkowania (blisko 80 i 60% odpowiednio dla jelca i jazia). Najlepszy efekt stymulacji hormonalnej stwierdzono w grupie, w której zastosowano kombinację obydwu środków hormonalnych, gdzie odnotowano skrócenie czasu uzyskania przez ryby gotowości tarłowej (48 i 36 odpowiednio dla jelca i jazia), znaczną synchronizację owulacji oraz najwyższy odsetek przeżywalności embrionów (blisko 80 i 70% odpowiednio w przypadku jelca i jazia).

Introduction

For many years artificial reproduction of non-commercial reophilic cyprinid fish species was outside the range of interests of both the fish culturists and the scientists (KUCHARCZYK 2002). During the recent years, however, a significant increase in the scientific activities concerning wild cyprinid fish can be observed (GLOGOWSKI et al. 1997, 1999, KUCHARCZYK et al. 1997a, b, c, 2005, BABIAK et al. 1998, KUCHARCZYK 1999, 2001, WOJTCZAK et al. 2003, KOWALSKI et al. 2003, 2004, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, 2009, ŻARSKI et al. 2008a,b). Predictability of fish production results under controlled conditions caused that during the recent years controlled reproduction and rearing have become an intensively developing segment in aquaculture (PHILIPPART 1995).

Hormonal preparations applied in fish aquaculture allow improving artificial reproduction techniques both during and outside the spawning season (BRZUSKA, ADAMEK 1999, KUCHARCZYK et al. 1999, 2000, 2008, KUCHARCZYK 2002, TARGOŃSKA-DIETRICH et al. 2004, ULIKOWSKI 2004, TARGOŃSKA et al. 2005, 2008, CEJKO et al. 2008a,b, 2009). Also, the economic analysis of hormonal stimulation in wild fishes were done (KUPREN et al. 2008, TUR-KOWSKI et al. 2008, HAKUĆ-BŁAŻOWSKA et al. 2009). This allows conducting restitution programs and studies in the area (BABIAK et al. 1998, KUCHARCZYK 1999, 2001). During the last several years numerous new hormonal preparations that can be applied in controlled fish reproduction appeared in the market (YARON 1995, YARON et al. 2009, BRZUSKA 2000, SZABO 2003, BRZUSKA 2005). Determining their suitability, however, requires many studies aiming at defining the optimum conditions for conducting the treatment and the type of hormonal preparation applied as well as its quantity and dose size. In case of cyprinid fish, the analogues of gonadoliberine, the effectiveness of which is frequently conditioned by applying them jointly with a dopamine antagonist,

proved successful (SZABO et al. 2002, BRZUSKA 2005, JAMRÓZ et al. 2008b, ŻARSKI et al. 2008a,b). As concerns the commonly available preparations, numerous studies concerning Ovopel have been published so far. The number of reports on application of Ovaprim in reophile cyprinid fish, however, is small (KUCHARCZYK et al. 2007, JAMRÓZ et al. 2008a, b, ŻARSKI et al. 2008a, b).

This study aimed at comparison of the effectiveness of female dace *Leuciscus leuciscus* (L.) and ide *Leuciscus idus* (L.) hormonal stimulation with Ovopel and Ovaprim separately and in selected combination of those two preparations.

Materials and Methods

Spawners of dace (average body weight 167 ± 9 g) were obtained from the Marózka River (north-eastern Poland), and the spawners of ide (average body weight 411 ± 21 g) originated from the Fish Farm Janowo near Szczytno. After transporting the fish to the hatchery of the Department of Lake and River Fisheries the males and females were placed separately in 1000 dm³ basins (KUJAWA et al. 1999). Samples of oocytes were collected from the females using catheters and their stage of maturity was determined on the base of the nucleus position in the cell according to the scale proposed by BRZUSKA, BIENIARZ (1977):

I stage – germinal vesicle in a central position

II stage – early germinal vesicle migration (less than half of the radius)

III stage - late germinal vesicle migration (more than half of the radius)

IV stage – periphery germinal vesicle or germinal vesicle breakdown (GVBD)

Hormonal stimulation was conducted when the oocytes were in stadium II-II/III of maturity. Water temperature during 3 day acclimation was 10° C (±0.1). The photoperiod during keeping the fish in the hatchery was constant (12L : 12 D). The light intensity was not measured. Following application of the first injection, water temperature was increased to 11°C, and after application of the releasing injection to 12°C. In case of ide, 24 hours after the last injection, water temperature was increased to 13.5°C.

Before commencement of the injections, the fish were divided into four groups: control and three experimental. The control group consisted of the fish that were treated with the injection of sterile saline solution (0.9% NaCl). The following preparations were used for hormonal stimulation of fish in the experimental groups: Ovopel (Unic-Trade, Hungary) (preparation containing a mammal analogue of GnRH [(D-Ala⁶, Pro⁹-Net)-mGnRH] and dopamine antagonist – metoclopramide) (HORVATH et al. 1997) and Ovaprim (Syndel,

Canada) (complex of the analogue of salmon hypothalamic hormone [(D-Arg⁶, Pro⁹-Net) sGnRH] and domperidone – dopamine antagonist) (BRZUSKA, ADA-MEK 1999). In the third experimental group the injection of Ovaprim was preceded by an injection of Ovopel. In case of males only the second injection was applied as in the groups of the females respectively for each group. All the injections were applied intraperitoneally under the caudal fin in the dose of 0.5 cm³ kg⁻¹. The doses and types of hormones applied are specified in Table 1. During the manipulation the spawners were anesthetized in the solution of 2-phenoxyethanol (Sigma-Aldrich, Germany) at 0.5 cm³ dm⁻³. All manipulations, including obtaining gametes were conducted according to the methodology described by TARGOŃSKA et al. (2008) for ide. During the study the number of females producing spawn, time between the releasing injection and ovulation and survival of embryos to the eyed stage were recorded.

Table 1

Hormonal agents and their doses used for induction of ovulation in controlled reproduction of dace and ide. Time betwen injections amount 24 hours

Group	I injection	II injection
Control	1 cm ³ kg ⁻¹ (0.9 % NaCl)	1 cm ³ kg ⁻¹ (0.9 % NaCl)
Experimental 1	$\begin{array}{c} 0.2 \hspace{0.1 cm} \text{pellet} \hspace{0.1 cm} \text{kg}^{\text{-}1} \\ (\text{Ovopel}) \end{array}$	1 pellet kg ⁻¹ (Ovopel)
Experimental 2	-	0.5 cm ³ kg ⁻¹ (Ovaprim)
Experimental 3	$\begin{array}{c} 0.2 \text{ pellet } \mathrm{kg}^{-1} \\ \mathrm{(Ovopel)} \end{array}$	0.5 cm ³ kg ⁻¹ (Ovaprim)

The spawn obtained during spawning was fertilized using "dry method". Unsticking the eggs was achieved by baths in Wojnarowicz solution (40 g urea + 35 g NaCl in 10 dm³ water) and short baths in tannin solution (7g 10 dm⁻³ water). The unstuck eggs were placed in Weiss' jars so that the spawn from each group could be incubated separately. Additionally, the spawn from each female (ca. 150 eggs in three repetitions) was incubated on Petri dishes. Water temperature during incubation was 12°C. Statistical differences between groups concerning survival of embryos to the eyed stage were analyzed by applying the one-way analysis of variance (ANOVA) and using the *post hoc* Tukey's test (P < 0.05).

Results

In the experimental groups 90–100% spawned. No ovulation was detected in any of the fish in the control group indifferent of the species (Tables 2 and 3). The time of ovulation in the presented study after treatment with Ovaprim was the longest comparing to the other groups at 48–72 h for dace (Table 2) and 38–48 h for ide (Table 3). That long time of latency was defined as lack of synchronization in achievement of readiness for spawning by the fish. In the group stimulated with the combination of Ovopel and Ovaprim, 100% ovulation synchronization was detected and the latency time was 48 and 36 h respectively for dace (Table 2) and ide (Table 3). The survival rates of dace embryos to the eyed stage in experimental groups 2 and 3 were significantly higher than after treatment with Ovopel reaching almost 80% (Table 2). In case of ide the significantly highest percentage of live embryos (almost 70%) was recorded in the group 3 (Table 3). Additionally, similar values concerning the survival rates of embryos depending on the incubation method were recorded for both species.

Table 2

D 1/	1, 1, 1	1 .	1.0 1	1	c	1	C:	1.00	1 1		1
Regults	contained	during	artiticial	reproduction	of (ace	atter	different	hormonal	agente	annlication
resulu	obtanica	uuiing	arunnar	reproduction	01 0	auce	anuci	uniciciti	monu	agenus	application

Specification	Control	Experimental 1	Experimental 2	Experimental 3
Number of females	9	10	10	10
Percentage of ovulated females (%)	0	90	100	100
Latency time (hours)	Ι	48–50	48-72	48
Synchronization of ovulation	Ι	yes	no	yes
Survival of embryos in the eyed stage (%)	-	58.6 ± 5.6^a	77.5 ± 2.6^{b}	78.6 ± 3.2^b
Survival of embryos in the eyed stage in Weiss' jars (%)	_	60.3 ± 3.4^a	80.2 ± 3.1^b	81.9 ± 3.4^b

* data in rows indicated with the same letter did not differ statistically (P < 0.05).

Table 3

Results obtained during artificial reproduction of ide after different hormonal agents application

Specification	Control	Experimental 1	Experimental 2	Experimental 3
Number of females	10	10	10	10
Percentage of ovulated females (%)	0	90	100	100
Latency time (hours)	-	36–38	38–48	36
Synchronization of ovulation	-	yes	no	yes
Survival of embryos in the eyed stage (%)	I	52.4 ± 3.1^a	60.5 ± 2.3^b	$69.5\pm2.8^{\circ}$
Survival of embryos in the eyed stage in Weiss' jars (%)	_	50.2 ± 2.7^{a}	61.2 ± 2.4^b	$71.7\pm3.3^{\circ}$

* data in rows indicated with the same letter did not differ statistically (P < 0.05).

Discussion

This study confirmed the current reports on the effectiveness of hormonal preparations in reproduction of reophile cyprinid fish concerning both the dace and the ide (KUCHARCZYK et al. 1999, 2007, KUCHARCZYK 2002, TARGOŃSKA-DETRICH et al. 2004, CEJKO et al. 2008b, 2009, JAMRÓZ et al. 2008a,b, KREJSZEFF et al. 2009). The positive effect of stimulation with those preparations was also observed in case of the nase *Chondrostoma nasus* (L.) (SZABO et al. 2008a). In this experiment ovulation was not obtained only in case of the control group, which confirms the earlier reports concerning reproduction of reophile cyprinid fish under controlled conditions (e.g.: KUCHARCZYK 2002, SZABO et al. 2002, ŻARSKI et al. 2008, ŻARSKI et al. 2008a).

Different latency times after treatment with hormonal preparations were described already earlier (YARON 1995, BRZUSKA 2000, 2005). This frequently results from the differences in the fish body reaction time to the pituitary homogenate (CPH) as compared to preparations containing GnRH analogues. In the experiment described two different GnRH analogues that have the same influence and contain different substances that are dopamine antagonists were used. The difference in the time of reaching spawning maturity by the females between groups stimulated with Ovopel and Ovaprim can result from the different compositions of those preparations. The differences in ovulation time resulting from treatment with different GnRH analogues have been recorded. among others, in case of the carp (BRZUSKA 2006). In case of the ide a similar situation was also observed by JAMRÓZ et al. (2008b), who recorded the highest ovulation synchronization after treatment with Ovopel (36 h) as opposed to the group stimulated with Ovaprim, in which ovulation was recorded between 36 and 44 hours after the releasing injection. The longer latency time after treatment with Ovaprim as compared to Ovopel was also found in case of the asp Aspius aspius (L.) (ZARSKI et al. 2008a, TARGOŃSKA et al. - unpublished data). In case of stimulation with Ovaprim, application of the preceding injection of Ovopel allowed shortening the time of ovulation to 48 hours in case of the dace and 36 hours in case of the ide and synchronize it significantly. A compatible effect of applying combined hormonal stimulation has been recorded so far in case of the ide (JAMRÓZ et al. 2008a) and the asp (ZARSKI et al. 2008a). That effect can also depend on the different GnRH analogues applied in this variant for the initiating and releasing injections. The combination in which Ovaprim would be applied as the initiating preparation was not tested as according to the manufacturer's recommendations that preparation should be used for single injection only.

Biological quality of obtained gametes, expressed as the percentage of live eyed stage embryos is one of the major parameters of effectiveness in case of controlled reproduction with hormonal stimulation application. In this work, in case of both species that parameter proved the best in the group where the combination of hormonal preparations was applied (group 3). However, in case of the dace it did not differ statistically significantly from that for the group stimulated with Ovaprim. The same relation of results after application of the same preparations is also given by JAMRÓZ et al. (2008b) for ide and ŻARSKI et al. (2008a) for asp. This also applies to desynchronization in achievement of readiness for spawning by the females following stimulation with Ovaprim, which was also recorded in case of this study.

A positive influence of stimulation with that preparation was also recorded in case of asp semen quality analysis (CEJKO et al. 2008b) and determining the mobility of ide sperms (JAMRÓZ et al. 2008b). As a consequence, the higher survival rates of embryos in groups where Ovaprim was applied could be the consequence of a much better semen obtained in those experimental groups.

Stickiness of the spawn is a characteristic causing many problems during mass production of cyprinid fish stocking material and removing it is a necessary condition for positive effects of incubation (LINHART et al. 2006, TARGOŃSKA et al. – unpublished data). The method of unsticking the eggs applied in this study did not influence the embryos survival as compared to spawn samples incubated without that treatment. This confirms the effective-ness of that method and the method of experimental determination of controlled reproduction effects by incubation of the spawn on Petri dishes.

The results obtained in this study indicate the possibility of increasing the effectiveness of controlled reproduction in case of the species studied by applying the appropriate type of hormonal stimulation. It also confirms suitability of Ovaprim in fish reproduction stimulation (LEU, CHOU 1996, VIVEIROS et al. 2002, OLSEN et al. 2006, KUCHARCZYK et al. 2007, KUJAWA et al. 2007). The fact, that treatment of the fish with injections of two different preparations with different compositions caused achievement of the best results is also an important aspect. The higher percentage of ovulating females recorded and obtaining the gametes of higher biological quality after application of the selected combination of hormonal preparations allows producing larger volumes of stocking material in the future.

Translated by JERZY GOZDEK

Accepted for print 14.09.2009

References

- BABIAK I., GLOGOWSKI J., KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1998. Cryopreservation of sperm from asp Aspius aspius. Progr. Fish-Cult., 60: 146–148.
- BRZUSKA E., BIENIARZ K. 1977. Metoda przeżyciowego określania dojrzałości płciowej samic karpia w związku z iniekcjami homogenatu przysadki mózgowej karpia. Broszura IRS, Olsztyn, nr 105.
- BRZUSKA E, ADAMEK J. 1999. Artificial spawning of European catfish, Silurus glanis L.: stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary extract. Aquacult. Res., 30: 59–64.
- BRZUSKA E. 2000. Artificial spawning of carp Cyprinus carpio L.: differences between the effects on reproduction in females of Polish and Hungarian provenance treated with carp pituitary and (D-Ala⁶) GnRH ProNHEt (Kobarelin). Aquacult. Res., 31: 457–465.
- 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. 2006. Artificial spawning of female Lithuanian strain B carp (Cyprinus carpio L.) after treatment with carp pituitary homogenate, Ovopel or [D-Tle⁶, ProNHEt⁹] GnRH-a (Lecirelin). Aquacult. Res., 37: 264–271.
- CEJKO B.I., GLOGOWSKI J., KOWALSKI R.K., KUCHARCZYK D., TARGOŃSKA K. 2008a. Description of pikeperch, Sander Lucioperca L., semen obtained from males held under different rearing conditions. Arch. Pol. Fish., 16(1): 93–100.
- CEJKO B.I., KUCHARCZYK D., TARGOŃSKA K., KUBIAK D., STAROSIEK B., GLOGOWSKI J. 2008b. Quality parameters and selected biochemical markers of asp, Aspius aspius (L.), semen obtained after hormonal stimulation with Ovaprim or Ovopel. Arch. Pol. Fish., 16(2): 179–188.
- CEJKO B.I., KOWALSKI R.K., KUCHARCZYK D., TARGOŃSKA K., KREJSZEFF S., ŻARSKI D., GLOGOWSKI J. 2009. Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus L. Aquacult. Res. (in press).
- CIESLA M. 1998. Wyniki badań nad opracowaniem metodyki sztucznego rozrodu jazia, Leuciscus idus.
 [W:] Karpiowate ryby reofilne. H. Jakucewicz, R. Wojda, Wyd. PZW, Warszawa, 41–49.
- GLOGOWSKI J., BABIAK I., KUCHARCZYK D., ŁUCZYŃSKI M. 1997. The effect of individual male variability on cryopreservation of bream (Abramis brama (L.)) sperm. Pol. Arch. Hydrobiol., 44: 281–285.
- GLOGOWSKI J., BABIAK I., KUCHARCZYK D., ŁUCZYŃSKI M., PIROS B. 1999. Some properties of bream Abramis brama L. sperm and its cryopreservation. Aquacult. Res., 30: 765–772.
- HAKUĆ-BŁAŻOWSKA A., KUPREN K., TURKOWSKI K., TARGOŃSKA K., JAMRÓZ M., KREJSZEFF S., KWIATKOWSKI M., ŻARSKI D., KUCHARCZYK D. 2009. Comparison of economic effectiveness of applying different hormonal preparations for reophile cyprinid fish reproduction stimulation based on the example of asp Aspius aspius (L.) and ide Leuciscus idus (L.). Pol. J. Natur. Sc., 24(4): 224–234.
- HORVATH L., SZABO T., BURKE J. 1997. Hatchery testing of GnRH analogue. containing pellets on ovulation in four cyprinid species. Pol. Arch. Hydrobiol., 44: 281–292.
- JAMRÓZ M., HAKUĆ-BŁAŻOWSKA A., KUCHARCZYK D., KWIATKOWSKI M., TARGOŃSKA K., ŻARSKI D., MAMCARZ A. 2008a. Wpływ stosowania preparatu Ovaprim na efekty pozasezonowego oraz sezonowego rozrodu jazia (Leuciscus idus) w warunkach kontrolowanych. [W:] Biotechnologia w akwakulturze Red. Z. Zakęś, J. Wolnicki, K. Demska-Zakęś, R. Kamiński, D. Ulikowski. Wyd. IRS, Olsztyn, 159–164.
- JAMRÓZ M., KUCHARCZYK D., HAKUĆ-BŁAŻOWSKA A., KREJSZEFF S., KUJAWA R., KUPREN K., KWIATKOWSKI M., TARGOŃSKA K., ŻARSKI D., CEJKO B.I., GLOGOWSKI J. 2008b. Comparison of Ovopel, Ovaprim and LH-RH analogue application effectiveness in controlled reproduction of ide Leuciscus idus (L.). Arch. Pol. Fish., 16(4): 363–370.
- KOWALSKI R., GLOGOWSKI J., KUCHARCZYK D., GORYCZKO K., DOBOSZ S., CIERESZKO A. 2003. Proteolytic activity and electrophoretic profiles of proteases from seminal plasma of teleosts. J. Fish Biol., 63: 1008–1019.
- KOWALSKI R., GLOGOWSKI J., KUCHARCZYK D., MAK M., DOBOSZ S., ZAKĘŚ Z., CIERESZKO A. 2004. Characterization of gelatinolytic activity in seminal plasma of some teleost fish. Aquacult. Int., 12: 57–68.
- KREJSZEFF S., KUCHARCZYK D., KUPREN K., TARGOŃSKA K., MAMCARZ A., KUJAWA R., KACZKOWSKI Z., RATAJSKI S. 2008. Reproduction of chub, Leuciscus cephalus L., under controlled conditions. Aquacult. Res., 39: 907–912.

- KREJSZEFF S., TARGOŃSKA K., ŻARSKI D., KUCHARCZYK D. 2009. Domestication affects spawning of the ide (Leuciscus idus)-preliminary study. Aquaculture, 295: 145–147.
- KUCHARCZYK D. 1999. Genetic inactivation of ide (Leuciscus idus L.) sperm using UV irradiation. Cytobios, 392: 149–158.
- KUCHARCZYK D. 2001. Genetic inactivation of Leuciscus idus L. (ide) oocytes using UV irradiation. Cytobios, 407: 189–195.
- KUCHARCZYK D. 2002. Rozród kontrolowany i androgeneza wybranych gatunków ryb karpiowatych. Rozpr. i monogr., 63, p. 81. Wyd. UWM, Olsztyn.
- KUCHARCZYK D., JANKUN M., ŁUCZYŃSKI M. 1997a. Ploidy level determination in genetically manipulated bream, Abramis brama, based on the number of active nucleoli per cell. J. Appl. Aquacult., 7: 13–21.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997b. Artificial spawning in bream (Abramis brama (L.)). Pol. Arch. Hydrobiol., 44: 203–207.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E. 1997c. Induced spawning in rudd (Scardinius erythrophthalmus L.). Pol. Arch. Hydrobiol., 44: 209–213.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E., ULIKOWSKI D. 1999. Artificial spawning of ide Leuciscus idus under controlled conditions. EJPAU 2(2): 05 (www.ejpau.media.pl).
- KUCHARCZYK D., KRÓL R., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E., SZCZERBOWSKI A., ŁUCZYŃSKI M.J. 2000. Out of season spawning of ide (Leuciscus idus L.) under controlled conditions. Europ. Aquacult. Soc., Spec. Publ. 28, 353–353.
- KUCHARCZYK D., KUJAWA R., MAMCARZ A., TARGOŃSKA-DIETRICH K., WYSZOMIRSKA E., GLOGOWSKI J., BABIAK I., SZABO T. 2005. Induced spawning in bream (Abramis brama L.) using pellets containing GnRH. Czech J. Anim. Sci., 50: 89–95.
- KUCHARCZYK D., BOREJKO A., TARGOŃSKA K., ROŻEK W., CHWALCZYK R., KOWALSKI R., GLOGOWSKI J. 2007. Wpływ Ovaprimu na efekty rozrodu jazia (Leuciscus idus). [W:] Rozród, podchów, profilaktyka ryb jeziorowych i innych gatunków Red. J. Wolnicki, Z. Zakęś, R. Kamiński, Wyd. IRS, Olsztyn, 31–35.
- KUCHARCZYK D., TARGOŃSKA K., HLIWA P., GOMUŁKA P., KWIATKOWSKI M., KREJSZEFF S., PERKOWSKI J. 2008. Reproductive parameters of common carp (Cyprinus carpio L) spawners during natural season and out-of-season spawning. Reprod. Biol., 8(3): 285–289.
- KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1999. A model system for keeping spawners of wild and domestic fish before artificial spawning. Aquacult. Eng., 20: 85–89.
- KUJAWA R., JAMRÓZ M., MAMCARZ A., KUCHARCZYK D. 2007. Rozród płoci w warunkach kontrolowanych. In: Rozród, podchów, profilaktyka ryb jeziorowych i innych gatunków Red. J. Wolnicki, Z. Zakęś, R. Kamiński. Wyd. IRS, Olsztyn, 15–22.
- KUPREN K., TURKOWSKI K., KUCHARCZYK D., KREJSZFF S., ŻARSKI D., HAKUĆ-BŁAŻOWSKA A., TARGOŃSKA K., KWIATKOWSKI M., JAMRÓZ M., CZARKOWSKI T. 2008. Economic aspects of rearing larval asp, Aspius aspius (L.), and ide, Leuciscus idus (L.), in closed recirculating systems. Arch. Pol. Fish., 16(4): 413–420.
- LEU M.-Y, CHOU Y.-H. 1996. Induced spawning and larval rearing of captive yellowfin porgy, Acanthopagrus latus (Houttuyun). Aquaculture, 143: 155–166,
- LINHART O., RODINA M., KOCOUR M., GELA D. 2006. Insemination, fertilization and gamete management in tench, Tinca tinca (L.). Aquacult. Int., 14: 61–73.
- OLSEN K.H., SAWISKY G.R., STACEY N.E. 2006. Endocrine and milt responses of male crucian carp (Carassius carassius L.) to periovulatory females under field conditions. Gen. Comp. Endocrinol., 149: 294–302.
- PHILIPPART J.C. 1995. Is captive breeding an effective solution for the preservation of endemic species? Biol. Conserv., 72: 281–295.
- SZABO T. 2003. Ovulation induction in northern pike Esox lucius L. using different GnRH analogues, Ovaprim, Dagin and carp pituitary. Aquacult. Res., 34: 479–486.
- SZABO T., MEDGYASSZAY C., HORVÁTH L. 2002. Ovulation induction in nase (Chondrostoma nasus, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone. Aquaculture, 203: 389–395.
- TARGOŃSKA-DIETRICH K., ZIELAZNY T., KUCHARCZYK D., MAMCARZ A., KUJAWA R. 2004. Out-of-season spawning of cultured ide (Leuciscus idus L.) under controlled conditions. EJPAU 7(2): 02 (www.ejpau.media.pl).

- TARGOŃSKA K., KUCHARCZYK D., KRASUCKA A., MAMCARZ A. 2005. Artificial reproduction of minnow (Phoxinus phoxinus) in captivity. Aquaculture Europe 2005 Conference "Optimizing the Future", Trondheim, Norway, 5–8.
- TARGOŃSKA K., KUCHARCZYK D., MAMCARZ A., GLOGOWSKI J., KREJSZEFF S., PRUSIŃSKA M., KUPREN K. 2008. Influence of individual variability in the percentage of motile spermatozoa and motility time on the survival of embryos of chosen fish species. Pol. J. Natur. Sc., 23(1): 178–187.
- TURKOWSKI K., KUCHARCZYK D., KUPREN K., HAKUĆ-BŁAŻOWSKA A., TARGOŃSKA K., ŻARSKI D., KWIAT-KOWSKI M. 2008. Economic aspects of the experimental rearing of asp, Aspius aspius (L.), ide, Leuciscus idus (L.), and dace, Leuciscus leuciscus (L.), under controlled conditions. Arch. Pol. Fish., 16(4): 397–411.
- ULIKOWSKI D. 2004. European catfish (Silurus glanis L.) reproduction outside of the spawning season. Arch. Pol. Fish., 12(2): 121–131.
- VIVEIROS A.T.M., FESSEHAYE Y., TER VELD M., SZULTZ R.W., KOMEN J. 2002. Hand-stripping of semen and semen quality after maturational hormone treatments, in African catfish Clarias gariepinus. Aquaculture, 213: 373–386.
- WOJTCZAK M., GLOGOWSKI J., KOŁDRAS M., KUCHARCZYK D., CIERESZKO A. 2003. Characterization of protease inhibitors of seminal plasma of cyprinids. Aquat. Liv. Res., 16 (5): 461–465.
- YARON Z. 1995. Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture, 129: 49–73.
- YARON Z., BOGOMOLNAYA A., DRORI S., BITON I., AIZEN J., KULIKOVSKY Z., LEVAVI-SIVAN B. 2009. Spawning Induction in the Carp. Past Experience and Future Prospects – A Review. Israeli J. Aquacult. – Bamidgeh, 61(1): 5–26.
- ŻARSKI D., KUCHARCZYK D., TARGOŃSKA K., CHWALUCZYK R., KREJSZEFF S., KWIATKOWSKI M., JAMRÓZ M. 2008a. Wpływ środka hormonalnego Ovaprim na efektywność rozrodu bolenia (Aspius aspius) w warunkach kontrolowanych. [W:] Biotechnologia w akwakulturze Red. Z. Zakęś, J. Wolnicki, K. Demska-Zakęś, R. Kamiński, D. Ulikowski, Wyd. IRS, Olsztyn, 153–158.
- ŻARSKI D., TARGOŃSKA K., RATAJSKI S., KACZKOWSKI Z., KUCHARCZYK D. 2008b. Artificial reproduction of nase, Chondrostoma nasus (L.), in captivity. Arch. Pol. Fish., 16(4): 355–362.

DOI 10.2478/v10020-009-0023-9

THE SURVIVAL OF SALMONELLA SPP. IN RELATION TO EXPOSURE TO LACTIC ACID AND THE STORAGE TIME OF TURKEY CARCASSES

Anita Mikołajczyk

Division of Neurobiology and Human Anatomy University of Warmia and Mazury in Olsztyn

Key words: Salmonella, lactic acid, storage, turkey carcasses.

Abstract

The studies aimed at determining the influence of lactic acid on Salmonella spp. during storage of turkey carcasses' samples for 2, 4 and 6 days. The initial average contamination of turkey carcasses' elements with Salmonella spp. was $2.4 \cdot 10^3$ bacteria. Following the immersion in water in average $4.3 \cdot 10^2$ Salmonella spp. cfu was recovered and that number was assumed as the inoculum. The number of Salmonella spp. decreases during storage of turkey carcasses' samples in the refrigerator at 4°C. Compared to elements of carcasses immersed in sterile water the largest reduction, by two logarithmic cycles was recorded after 2 days of storage of samples treated with 1% lactic acid. In case of the other variants of the experiment when 1% solution of lactic acid was applied S. Enteritidis grew in numbers within the same logarithmic range. Compared to the samples immersed in sterile water, 2% lactic acid caused reduction in the number of Salmonella spp. on elements of poultry carcasses by one logarithmic cycle both immediately after contamination and after 2 and 6 days of storage; unfortunately after 4 days of storage S. Enteritidis grew in numbers that were within the same logarithmic range.

During storage of the turkey samples tested at 4°C for 2, 4 and 6 days, the numbers of *Salmonella* spp. decreased. That decrease compared to samples immersed in sterile water was the largest after 2 days of storage after application of 1% lactic acid.

WPŁYW KWASU MLEKOWEGO I CZASU SKŁADOWANIA TUSZEK INDYCZYCH NA PRZEŻYWALNOŚĆ PAŁECZEK SALMONELLA

Anita Mikołajczyk

Zakład Neurobilogii i Anatomii Człowieka Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: Salmonella, kwas mlekowy, przechowywanie, tuszki indycze.

Address: Anita Mikołajczyk, University of Warmia and Mazury, ul. Warszawska 30, 10-082 Olsztyn, Poland, phone: +48 (089) 523 34 91, e-mail: anita.mikolajczyk@uwm.edu.pl

Abstrakt

Celem badań było określenie wpływu kwasu mlekowego na pałeczki Salmonella podczas przechowywania próbek tuszek indyczych przez 2, 4 i 6 dni. Średnie wyjściowe zanieczyszczenie bakteriami elementów tuszek wynosiło 2.4 · 10³. Po zanurzeniu w wodzie izolowano średnio 4.3 · 10² pałeczek Salmonella – liczbę tę przyjęto za inoculum. Zmniejszała się ona podczas przechowywania próbek w lodówce w temperaturze 4°C. Największy spadek o dwa cykle logarytmiczne, względem elementów tuszek zanurzonych w jałowej wodzie, odnotowano po 2 dniach przechowywania próbek potraktowanych 1% kwasem mlekowym. W pozostałych wariantach doświadczenia po zastosowaniu 1% roztworu kwasu mlekowego liczba S. Enteritidis rosła w liczbach mieszczących się w tym samym przedziale logarytmicznym. W porównaniu z próbkami zanurzonymi w jałowej wodzie 2% kwas mlekowy powodował redukcje pałeczek Salmonella na elementach tuszek drobiowych o jeden cykl logarytmiczny zarówno bezpośrednio po kontaminacji, jak i po 2 i 6 dniach składowania. Niestety po 4 dniach przechowywania liczba S. Enteritidis rosła w liczbach mieszczacych sie w tym samym przedziale logarytmicznym. W trakcie przechowywania badanych próbek indyczych w temperaturze 4°C przez 2, 4 i 6 dni zmniejszyła się liczba pałeczek Salmonella. Spadek ten, w porównaniu z próbkami zanurzonymi w jałowej wodzie, był największy po 2 dniach składowania, po zastosowaniu 1% kwasu mlekowego.

Introduction

Salmonellosis is a serious epizootic and epidemiological problem. Detailed analyses of disease cases show that next to hen's eggs, poultry meat is the main cause of food poisoning cases. Slaughter poultry is the main reservoir of Salmonella spp. In slaughter chicken and turkey immediately after stunning contamination with Salmonella spp. bacteria involving up to 7% of them was found while before chilling it increased to even as much as 48% in case of chicken (MIKOŁAJCZYK, RADKOWSKI 2001a, MIKOŁAJCZYK, RADKOWSKI 2001b, MIKOŁAJCZYK, RADKOWSKI 2002b, MIKOŁAJCZYK, RADKOWSKI 2002c). The above results obtained in Poland are correlated with the studies conducted by the USDA Food Safety and Inspection Service that indicate that 4–5% of broilers brought for slaughter were infected with *Salmonella* spp. while poultry leaving the slaughterhouse was contaminated in 35–36% (LILLARD 1990). This is the consequence of post-slaughter contamination, which occurs at every processing step. As a consequence, there is high probability that poultry reaching the consumer will be contaminated with *Salmonella* spp. to an even higher extent. In 55% of poultry carcasses from shop shelves tested by BYSTRON at al. (2004) Salmonella spp. was recovered. S. Enteritidis was the dominating serological strain. The high percentage of poultry carcasses in retail outlets contaminated with Salmonella spp. indicates the direct risk to the consumers.

The number of *Salmonella* spp. on poultry carcass purchased is from 1 to 20 cells (FEHLHABER 1996). Although that number is low there is always the risk of *Salmonella* spp. growth in the meat. Whether symptoms of disease appear and what the development of the disease would be depends on the number of *Salmonella* spp.

Disease development in humans requires infecting the alimentary system with a larger number of *Salmonella* spp. cells than in case of the typhoid fever or paratyphoid fever, e.g.: S. Newport $1.0 \cdot 10^5$, S. Bareilly $1.0 \cdot 10^5$, S. Enteritidis $1.0 \cdot 10^5$, S. Derby $1.5 \cdot 10^6$, S. Anatum $5.0 \cdot 10^6$, S. Meleagridis $5.0 \cdot 10^7$, S. Gallinarum $1.3 \cdot 10^9$ (DUGID et al. 1991). It should be added that in frequent cases low numbers of *Salmonella* spp. were recovered from the food that was the cause of poisoning. This applied mainly to cases in small children and the elderly. The number of *Salmonella* spp. cells in a poultry carcass that is a threat to human health depends on the person's age and health condition, i.e. the individual biological immunity.

Despite numerous attempts at avoiding secondary contaminations with *Salmonella* spp. on the slaughter line and during processing the search for methods of eliminating the bacteria is still searched for. For many years, in the USA, which is the largest in the world chicken producer, sodium hypochlorite has been added to water immediately prior to the chilling process to destroy *Salmonella* spp. cells. Sodium hypochlorite is considered safe and as a consequence chicken subjected to treatment with it are also found in shops within the European Union. However, as sodium hypochlorite at higher concentrations causes colour and flavour change, search for another method of eliminating those bacteria continues.

The number of chemical additives applied in food processing is limited as a consequence of their negative influence on human body and difficulties with solubility and direct application. Additionally, eco-focused lifestyle of the consumers forces use of only those chemical substances that appear in the environment naturally in food processing.

In search for methods of eliminating *Salmonella* spp. attention has been focused on a large group of organic acids and their salts that are commonly considered safe. Polish food industry allows use of the following organic acids as food additives: lactic, acetic, citric, tartaric and their salts (Rozporządzenie Ministra Zdrowia... 2008).

Lactic acid is a natural component of many food products such as sauerkraut, pickled cucumbers, cured sausages or yoghurt. It finds wide applications in food industry for achievement of diverse processing effects. It is among the food additives allowed for application without limitation and according to the applicable legislation (Rozporządzenie Ministra Zdrowia... 2008) the maximum lactic acid dose added to is defined by the liberal term "quantum satis". According to the European Union numeric identification system lactic acid is identified as E 270.

In the literature available there is a vast volume of data on widely understood lactic acid application in, e.g. pharmaceutical, cosmetic, and food industries, in meet processing and little information on lactic acid influence on Salmonella spp. present in turkey carcasses (MIKOŁAJCZYK, RADKOWSKI 2002a); there is no reports, however, on the influence of lactic acid on Salmonella spp. during storage of carcasses.

As a consequence, it seems that undertaking studies aiming at determination of lactic acid influence on *Salmonella* spp. cells present in samples from turkey carcasses stored for the period of 6 days, as that is the usual maximum time before the carcass reaches the consumer, could contribute to improvement in food hygiene level and human health protection.

Materials and Methods

Studies were conducted on 192 samples of turkey breast purchased from poultry plants. Each sample was divided into two parts after weighing and marking. One part was checked for natural presence of *Salmonella* spp. while the other was purposefully contaminated with the test strain.

S. Enteritidis no. 33/66 obtained from the Museum of Bacterial Strains of the National Veterinary Research Institute in Puławy was used for the studies.

The strain was maintained on agar slopes in a refrigerator at 4°C. The above strain was inoculated on nutritive broth and incubated at 37°C for 24 hours. Following incubation, 10 ml of broth was transferred to 4 l of the liquid for dilutions consisting of: peptone 1 g, sodium chloride NaCl 8.5 g, distilled water 1000 ml (Mięso i przetwory mięsne... PN-A-82055-3 1994), in which turkey breast samples were immersed. After 5 minutes the samples were taken out, drained for 2 minutes, placed on specially prepared sterile trays with dripper and held in the refrigerator at 4°C for 20 minutes. For each series of tests the initial contamination level of control samples was determined.

Next the samples were transferred to sterile beakers with 250 ml of 1 and 2% solution of lactic acid ($C_3H_6O_3$) pure for analyses for 15 minutes.

The controls consisted of breast samples artificially contaminated with *Salmonella* and samples immersed in sterile water for 15 minutes that were tested directly without decontamination (the number assumed as inoculum).

Swabs were collected from the external and internal turkey breast surface using sterile tampons and patterns. The pattern of stainless steel with window area of 25 cm² was placed on each surface tested. Two swabs were collected, one from the external surface and one from the internal surface, representing in total the area of 50 cm². The tampons with swabs were placed in beakers with pearls containing 50 ml of the liquid for dilutions and shaken for ca. 2 minutes. In this way the initial dilution was obtained in which 1 ml of the liquid corresponded to 1 cm² of tested surface. Next 10-times dilution was prepared from that liquid and the number of *Salmonella* spp. colony forming units was determined by the Most Probable Number (MPN) method (Microbiology of food... ISO 7218:1996). For that purpose from the initial suspension and its consecutive 10-times dilutions 1 ml was inoculated to three parallel tubes containing buffered peptone water (BPW, CM 509, Oxoid Basingstoke Hampshire, UK), that were incubated at 37°C for 20 hours and next transferred on the S.C. medium (SC, 0 687-17-1, Difco Laboratories Detroit MI, USA), on Müller-Kauffman medium (MK, CM 343, Oxoid Basingstoke Hampshire, UK) and Rappaport-Vassiliadis medium (RV, CM 669, Oxoid Basingstoke Hampshire, UK). After 24 hours of incubation at 41.5°C (RV) and 37°C (MK and SF) transfer on brilliantine green agar (BGA, CM 329, Oxoid Basingstoke Hampshire, UK), BSA medium (BSA, 00 73-01-1, Difco Laboratories Detroit MI, USA) and XLD medium (XLD, CM 469 Oxoid Basingstoke Hampshire, UK) was performed. The most probable numbers of *Salmonella* spp. was read from tables by Hoskins.

The tests were conducted according to the methodologies specified in regulations (Mikrobiologia... PN-ISO 6579: 1998, Microbiology... ISO 6579: 1993, Microbiology of food... ISO 7218:1996).

Each turkey breast was tested for determination of *Salmonella* spp. numbers immediately as well as 2, 4 and 6 days of keeping in a refrigerator at 4° C. The tests for each variant were carried out in six repetitions.

The experimental data was processed statistically using the T-Student test and correlation analysis. The correlation analysis was conducted on log numbers.

Results and Discussion

Table 1 and Figure 1 present the changing numbers of Salmonella spp. during storage of turkey carcasses at 4°C. The average initial contamination of turkey carcasses' elements with Salmonella spp. was $2.4 \cdot 10^3$ of Salmonella bacteria. Following immersion in water the average of $4.3 \cdot 10^2$ Salmonella spp. cfu were recovered and that number was assumed as the inoculum. The initial inoculum of Salmonella enteritidis was 10^2 cfu per 1 cm² of turkey carcass surface. The number of Salmonella spp. cfu decreases during storage of turkey carcasses in the refrigerator at 4°C.

As compared to elements immersed in sterile water, the largest decrease, by 2 log cycles, was recorded after 2 days of storage for samples treated with 1% lactic acid (Table 1, Figure 1). In other experimental variants where 1% lactic acid was applied, S. Enteritidis grew in numbers that were within the same log range. As compared to samples immersed in sterile water, 2% lactic acid resulted in reduction of *Salmonella* spp. on elements of turkey carcasses by one

Table 1

Influence of lactic acid on surviv	al of <i>Salmonella</i>	I Enteritidis on	elements o	of turkey	carcasses	stored
	at 4°C for 2, 4 a	and 6 days $(n =$	= 6)			

Test substance	Concentration (%)	I	Correlation between the number of cfu and storage time				
		immediately after contamination	2	4	6	correla- tion coefficient	signifi- cance level
			r	p			
Control	0	$2.4\cdot 10^3$	$4.3\cdot10^2$	$4.3\cdot 10^2$	$4.3\cdot10^2$	- 0.77	> 0.10
Water	0	$4.3\cdot 10^2$	$2.4\cdot 10^2$	$9.3\cdot10^{1}$	$4.3\cdot10^1$	- 0.99	0.01
Lastia agid	1	$2.4\cdot 10^2$	$2.3\cdot 10^{0}$	$9.3\cdot10^{1}$	$4.3\cdot10^{1}$	- 0.09	> 0.10
Lactic acid	2	$2.3\cdot 10^1$	$2.3\cdot 10^1$	$2.4\cdot 10^1$	$9.3\cdot 10^{0}$	- 0.75	> 0.10



Fig. 1. Influence of lactic acid on survival of *Salmonella* Enteritidis on elements of turkey carcasses stored at 4°C for 2, 4 and 6 days

log cycle immediately after contamination as well as after 2 and 4 days of storage; unfortunately after 4 days of storage S. Entertiidis grew in numbers within the same log range.

After slaughter poultry carcasses the temperature within the muscles of which is ca. 40°C, must be chilled down to 4°C to increase shelf life of the meat and inhibit microorganic growth.

If turkey carcasses are contaminated with *Salmonella* spp. during processing, currently applied processes do not contribute to eliminating them. Air temperatures of 0° C to -1° C do not cause elimination of *Salmonella* and carcasses contaminated can be released for sale. The time between chilling and getting the carcass to the consumer varies and depends on numerous factors. The producer defines on the basis of shelf life tests the shelf life of fresh poultry.

The eliminating mechanism of treatment with organic acids in relation to *Salmonella* spp. is related to the presence of dissociated molecules and low pH of the acids (CONNER, BILGILI 1994). The acids applied are dissociated to a different extent and probably that entire combination of hydrogen ions with acid residues creates more unfavourable conditions for *Salmonella* than is the case in case of individual acids.

In own studies the influence of lactic acid solution on *Salmonella* spp. under conditions nearest to the natural, i.e. poultry carcasses coming directly from poultry processing plants not subjected at the laboratory to any processes aimed at liquidation of accompanying microflora and stored under conditions of constant temperature of 4°C was investigated.

BENEDICT et al. (1991) found that high level of bacterial suspension adhesion to skin is achieved through immersion of carcasses in bacterial suspension. The serotype of *Salmonella* spp. and bacterial suspension temperature do not influence adhesion of microorganisms to the skin (CONNER, BILGILI 1994). Cellular structures such as fimbriae and cilia are important in the mechanism of adhesion to the skin (DICKSON 1992, GRAFT-HANSON de, HEATH 1990). The contact time of bacterial suspension with the skin plays a very important role (CONNER, BILGILI 1994). Immediately after inoculation of the studied bacterial culture on the skin the samples should be kept for an appropriate time to obtain better adhesion of the rods to the skin of carcasses. According to CONNER, BAGGILY (1994), 10 minutes is the optimal time required for settlement and adhesion of *Salmonella* spp. to the skin if the inoculum of 10^4 is applied. In case a lower concentration inoculum is applied the holding time should be extended, e.g. for inoculum $10^3 - 20$ minutes, for $10^2 - 30$ minutes.

Salmonella cells firmly attached to the skin are much more resistant to chemical media than those that did not have the time to attach strongly and that are loosely attached to the skin (LILLARD 1989a, 1989b, TAMBLYN et al. 1997). BAILEY et al. (1986) observed 90–96% reduction of S. Typhimurium caused by 3.5 second spray using sodium hypochlorite at 20–40 ppm. Methods of that type frequently decrease the number of, but rarely eliminate Salmonella spp. from poultry carcasses as they are ineffective for bacterial cells set or firmly attached to the skin. As a consequence there is need for

testing media eliminating *Salmonella* spp. firmly attached to the skin (TAM-BLYN, CONNER 1997, CONNER, BILGILI 1994).

The critical point in studies on eliminating *Salmonella* spp. is that the bacteria can be firmly attached to the skin, particularly when the carcasses are at the initial stages of processing. It may even happen that *Salmonella* spp. cells are irreversibly attached to the skin. As a consequence it should be considered that no potential medium would be fully effective (CONNER, BILGILI 1994).

Another problem in effectiveness assessment of *Salmonella* spp. eliminating media is the fact that in spite of applying a variety of carcasses washing, washing-out and multiple rinsing techniques attempts at recovering all rods are unsuccessful (IZAT et al. 1991). LILLARD (1989b) concluded that recovery of bacteria attached to poultry skin is highly difficult and secondary rinsing can lead to recovering high numbers of them. In many works (CONNER, BILGILI 1994, IZAT et al. 1991) it is reported that recovery of *Salmonella* spp. is based on multiple washing and even then not all of them can be recovered.

As shown by the literature discussed and own studies, elimination of *Salmonella* spp. from poultry carcass is a complex process that requires further studies.

Conclusions

1. Lactic acid applied for elimination of *Salmonella* spp. from elements of turkey carcasses stored at 4° C for 2, 4 and 6 days showed a relatively strong influence at concentrations of both 1% and 2%, which depended on the time of storage.

2. During storage of test turkey samples at 4°C for 2, 4 and 6 days the number of *Salmonella* spp. cells decreased.

3. The largest decrease in the number of *Salmonella* cells as compared to samples immersed in sterile water, was recorded after 2 days of storage after application of 1% lactic acid.

Translated by JERZY GOZDEK

Accepted for print 27.07.2009

References

BAILEY J.S., THOMSON J.E., COX N.A., SHACKELFORD A.D. 1986. Chlorine spray washing to reduce bacterial contamination of poultry processing equipment. Poult. Sci., 65(6): 1120–1123.

BENEDICT R.C., SCHULTZ F.J., JONES S.B. 1991. Attachment and removal of Salmonella spp. on meat and poultry tissues. J. Food Safety, 11(2): 135–148.

- BYSTROŃ J., KOSEK-PASZKOWSKA K., MOLENDA J., CZERW M. 2004. Occurrence of Salmonella spp. in chicken carcasses. Med. Wet., 60(3): 225–336.
- CONNER D.E., BILGILI S.F. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against Salmonella attached to broiler skin. J. Food Prot., 57(8): 684–688.
- DICKSON J.S., ANDERSON M.E. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. J. Food Prot., 55(2): 133–140.
- DUGID J.P., NORTH R.A. 1991. Eggs and Salmonella food-poisoning: an evaluation., J. Med. Microbiol., 34(2): 65–72.
- FEHLHABER K. 1996. Problemy mikrobiologiczne u drobiu rzeźnego. Med. Wet., 52(12): 758-762.
- GRAFT-HANSON J. DE, HEATH J.L. 1990. Effect of d-mannose on fimbriae of bacterial isolates from chicken carcasses. Poult. Sci., 69(9): 1582–1589.
- IZAT A.L., YAMAGUCHI W., KANIAWATI S., McGINUIS J.P., RAYMOND S.G., HIERHOLZER R.E., KOPEK J.M. 1991. Research note: use of consecutive carcasses rinses and a most probable number procedure to estimate salmonellae contamination of inoculated carcasses. Poult. Sc., 70(6): 1448–1451.
- LILLARD H.S. 1989a. Factors affecting the persistence of Salmonella during the processing of poultry. J. Food Prot., 52(11): 829–831.
- LILLARD H.S. 1989b. Incidence and recovery of salmonellae and other bacteria from commercially processed poultry carcasses at selected pre- and post-evisceration steps. J. Food Prot., 52(11): 888–891.
- LILLARD H.S. 1990. The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. J. Food Prot., 53(3): 202–204.
- Microbiology. General guidance on methods for the detection of Salmonella. ISO 6579: 1993(E).
- Microbiology of food and animal feeding stuffs General rules for microbiological examinations. ISO 7218:1996(E).
- Mięso i przetwory mięsne. Badania mikrobiologiczne. Przygotowanie próbek i rozcieńczeń. PN-A--82055-3 1994.
- MIKOŁAJCZYK A., RADKOWSKI M. 2001a. Contamination of Salmonella spp. in slaughter chickens. Med. Wet., 57: 745–747.
- MIKOŁAJCZYK A., RADKOWSKI M. 2001b. Zanieczyszczenie pałeczkami Salmonella indyków rzeźnych w zakładach drobiarskich. Życie Wet., 7: 376–378.
- MIKOŁAJCZYK A., RADKOWSKI M. 2002a. Elimination of Salmonella spp. by lactic acid. Pol. J. Vet. Sc., 5(3): 139–143.
- MIKOŁAJCZYK A., RADKOWSKI M. 2002b. Salmonella spp. on chicken carcasses in processing plants in Poland. J. Food Prot., 65: 1475–1479.
- MIKOŁAJCZYK A., RADKOWSKI M. 2002c. The occurrence of Salmonella spp. in turkeys investigation results from a slaughter and after-slaughter dressing line in Poland. Fleischwirtschaft, 32: 52–54. Mikrobiologia. Ogólne zasady metod wykrywania pałeczek Salmonella. PN-ISO 6579 1998.
- Rozporządzenie Ministra Zdrowia z dnia 18 września 2008 r. w sprawie dozwolonych substancji dodatkowych. Dz.U. 2008, nr 177, pozycja 1094, z października 2008.
- TAMBLYN K.C., CONNER D.E. 1997. Bactericidal activity of organic acids against Salmonella typhimurium attached to broiler chicken skin. J. Food Prot., 60(6): 629–633.
- TAMBLYN K.C., CONNER D.E., BILGILI S.F., HALL G.S. 1997. Utilization of the skin attachment model (SAM) to determine the antimicrobial activity of potential carcass treatments. Poult. Sci., 76(9): 1318–1323.

DOI 10.2478/v10020-009-0024-8

THE EVALUATION OF USEFULNESS OF POTENTIALLY PROBIOTIC *LACTOBACILLUS* STRAINS AS COMPONENTS OF INDUSTRIAL STARTER CULTURES

Monika Modzelewska-Kapituła¹, Lucyna Kłębukowska², <u>Kazimierz Kornacki</u>², Wioletta Łukaszuk²

¹ Chair of Meat Technology and Chemistry ² Chair of Industrial and Food Microbiology University of Warmia and Mazury in Olsztyn

Key words: probiotics, Lactobacillus, starter culture, prebiotics.

Abstract

The aim of the study was to investigate the possible use of nine *Lactobacillus* strains, previously isolated from infants faeces, as components of industrial starter cultures for yoghurt, soft white cheese and hard cheese production. There were no strong antagonistic interactions between isolates and commercial probiotics and yoghurt strains. None of isolates was able to grow at 3°C, seven grew at 7°C, all of them at 12°C and 37°C and only three at 45°C. In the culture media containing 10.4% NaCl at pH 6.5 growth of four isolates was detected at 12°C, whereas six strains grew at 37°C. Tested isolates did not hydrolyze casein and arginine and did not produce H_2S . Inulins (HD, IQ, TEX, HPX) and maltodextrins (low and medium dextrose equivalent) added to the culture media stimulated the growth of isolates. The properties of all isolates enable their application in soft white and hard cheeses and strains 4a, 4b and 14 in yoghurts.

OCENA PRZYDATNOŚCI POTENCJALNIE PROBIOTYCZNYCH PAŁECZEK Z RODZAJU LACTOBACILLUS JAKO SKŁADNIKÓW SZCZEPIONEK PRZEMYSŁOWYCH

Monika Modzelewska-Kapituła¹, Lucyna Kłębukowska², <mark>Kazimierz Kornacki</mark>², Wioletta Łukaszuk²

¹ Katedra Technologii i Chemii Mięsa
² Katedra Mikrobiologii Przemysłowej i Żywności Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: probiotyki, Lactobacillus, szczepionki przemysłowe, prebiotyki.

Address: Monika Modzelewska-Kapituła, University of Warmia and Mazury, pl. Cieszyński 1, 10-718 Olsztyn, Poland, phone: +48 (089) 523 32 07, e-mail: monika.modzelewska@uwm.edu.pl
Abstrakt

Celem pracy było określenie możliwości zastosowania dziewięciu szczepów z rodzaju *Lactobacillus*, wyizolowanych z kału niemowląt, jako komponentów szczepionek przemysłowych do produkcji jogurtów, serów twarogowych i dojrzewających. Nie odnotowano silnych antagonistycznych interakcji między izolatami, komercyjnymi szczepami probiotycznymi i jogurtowymi. Zaden spośród badanych izolatów nie wykazał zdolności do wzrostu w temperaturze 3°C, siedem było zdolnych do wzrostu w 7°C, wszystkie rosły w 12°C i 37°C, a tylko trzy – w 45°C. W podłożach zawierających 10.4% NaCl i o pH 6,5 odnotowano wzrost czterech szczepów w temperaturze 12°C i sześciu w 37°C. Badane izolaty nie wykazały zdolności do rozkładu kazeiny i argininy oraz produkcji H₂S. Inuliny (HD, IQ, TEX, HPX) i maltodekstryny (nisko- i średnioscukrzona) dodane do podłoży stymulowały wzrost izolatów. Właściwości wszystkich izolatów umożliwiają ich zastosowanie w serach twarogowych i dojrzewających, a szczepów 4a, 4b i 14 w jogurtach.

Introduction

Probiotics are defined as live micro-organisms which when consumed in adequate amounts confer a health benefit on the host (*Probiotics...* 2006). When selecting probiotics safety and functionality aspects, such as: survivability of strains during passage through the gastrointestinal tract, antagonistic activity against pathogens, as well as their technological properties, should be considered at the first place (MORELLI 2007). Before using a probiotic strain as a component of an industrial starter culture the interactions between probiotic strain and starter culture micro-organisms should also be investigated, because of the possibility of occurrence of the negative interaction which may lead to decrease of viability and activity of the micro-organisms (SAARELA et al. 2000).

Probiotics are used in many food products such as different types of cheese, yoghurts and milk beverages (BERGAMINI 2009, OLSON, ARYANA 2008, ONG et al. 2007). These products are characterized by different acidity and consistency. The pH value of fresh milk is about 6.5 to 6.8 and it decreases to about 4.0 to 4.5 in yoghurts, 4.5 to 4.6 in soft white cheeses and 5.2 to 5.4 in hard cheeses (LITWIŃCZUK et al. 2004). From the technological point of view salts play an important role in food products. Salt content in hard cheeses reaches 1.4 to 1.9%. Another parameter which affects the microbial ability to grow is water activity (a_w). Water activity of cheeses ranges from 0.70 to 0.99, for extra hard and soft white cheeses (cottage), respectively, whereas in semi hard cheeses a_w values are around 0.90 (LIU et al. 1998).

The recent trend in food industry is to apply probiotics together with prebiotics. Prebiotics are dietary components that are not digested in the small intestine and reach the colon intact. They influence the gastrointestinal microbiota, affect flavour and texture of products (CASTRO DE et al. 2009, GUGGISBERG et al. 2009, WANG 2009), but also stimulate the growth of probiotics and increase their viability in food products (DONKOR et al. 2007). The knowledge of the ability of the probiotic micro-organisms to grow in the presence of prebiotics is crucial in composition of synbiotics products, in which pro- and prebiotics are used.

In the earlier studies (MODZELEWSKA et al. 2003) *Lactobacillus* strains were isolated from faeces of 24 infants. All isolated strains (20) exhibited strong antimicrobial activity against potentially pathogenic and technological harmful Gram-positive and Gram-negative bacteria. The characteristics of isolates, including chemotherapeutic resistance, survivability at low pH values and elevated concentration of bile salts, enabled selection of 9 strains with the biggest chances to survive in the gastrointestinal tract environment (MODZELEWSKA-KAPITULA et al. 2008). The aim of the present study was to select the isolates, which may be used as components of starter culture for yoghurt, white, soft and hard cheeses. Therefore, the interactions among isolates and some commercial probiotic and yoghurt strains, ability of isolates to grow at conditions present in production and storage of food products (pH value, temperature, NaCl content) and their proteolytic activity were investigated.

Materials and Methods

The test bacteria were *Lactobacillus* strains (*L. plantarum* strains marked as 1, 4b, 14, 18a, 20a, 20b, 22b, 24 and *L. fermentum* 4a). Before the experiments the strains were cultured in MRS broth (Merck, Darmstadt, Germany) at 37° C for 24 h.

The interactions between isolates and commercial strains with probiotic properties: L. rhamnosus 705, L. casei 163, L. acidophilus NCFM, L. acidophilus 74-2, B. lactis 420 (Danisco Biolacta, Olsztyn, Poland) and components of yoghurt starter culture: L. delbrueckii ssp. bulgaricus, Strepto-coccus thermophilus (isolated from natural yoghurt, Bacoma SA, Warsaw, Poland) were studied using well diffusion method (MODZELEWSKA-KAPITUŁA, MARIN-INIESTA 2005). The type of mutual interactions (stimulation or inhibition) that isolates exhibited towards commercial cultures as well as that of commercial cultures towards isolates, were determined.

In order to study the influence of physical and chemical factors on growth of isolates MRS broth at different pH values: 6.5 (pH of fresh milk) and 5.0 (approximate pH of hard cheese) was used. The MRS broth contained 0.0, 3.3, 6.7 and 10.4% of NaCl, which corresponded to water activity 1.0, 0.98, 0.96 and 0.93, respectively (Thermoconstanter, Novasina AG, Zurich, Switzerland). Bacterial cultures (0.01 cm³) were inoculated into media (9 cm³) and incubated at different temperatures: 3°C (cooling temperature), 7 and 12°C (hard cheese

ripening temperatures), 37°C (optimal for bacteria isolated from human organism). The ability of isolates to grow at 45°C, that is used in yoghurt production, were also investigated in MRS broth (Merck). The growth of bacteria was controlled by absorbance measurement (spectrophotometer He λ ios ε , Unicam Ltd. Cambridge, UK) at 650 nm with frequency adjusted to the rate of bacterial growth until two equal results were noted. If there were no differences in optical density of cultures the experiment was terminated after 7 days of incubation. In order to quantitatively determine bacterial population in the cultures the standard curve showing relationship between optical density and cells number was drawn.

The proteolytic properties of isolates were determined as their ability to casein and arginine hydrolysis and H₂S production. Hydrolysis of casein was tested on milk agar plates (BURBIANKA et al. 1983). Bacterial cultures containing 10^9 cfu cm⁻³ were inoculated in the form of stripes on surfaces of the plates and then incubated for 48 h at 37°C at anaerobic conditions (Anaerocult C, Merck). A strain was considered as possessing ability to hydrolyze casein if media around the stripe became transparent. Decomposition of arginine was tested in liquid medium containing arginine (BURBIANKA et al. 1983), which was inoculated with 0.01 cm³ of bacterial cultures. After 24 h incubation at 37°C Nessler reagent was added to determine ammonia presence (ARICI et al. 2004). Ability of strains to produce hydrogen sulphide was tested on Triple Sugar Iron agar (TSI, Oxoid Ltd. Basingstoke, UK). During 14 days of incubation at 30°C the cultures were monitored daily for blackening the media indicating H₂S production (ARICI et al. 2004).

The isolates were tested for their ability to use the following prebiotics as a carbon source: inulin TEX (polymerization degree DP \geq 9), HD (DP \geq 9), IQ (DP = 9 to 12) (Sensus, Roosendaal, Netherlands), HPX (DP \geq 23) (BENEO-Orafti, Tiennen, Belgium) and maltodextrins of different dextrose equivalent (DE): low (DE = 10.9) and medium (DE = 16.2) (Pepes SA, Łomża, Poland). Bullion Standard (Merck) was modified by addition of 0.5 to 2.5% of prebiotic. As controls Bullion Standard (Merck) and bullion with glucose were used. The bacterial cultures containing 10⁴ cfu cm⁻³ were inoculated into media and incubated for 24 h at 37°C and then the counts of bacteria were determined on MRS agar (Merck) incubated for 48 h at 37°C at anaerobic conditions (Anaerocult C, Merck).

Results and Discussion

Commercial probiotic strains L. acidophilus NCFM, 74-2 and B. lactis 420 and yoghurt strains L. delbrueckii sp. bulgaricus and S. thermophilus did not

inhibit the growth of the strains isolated from infant faeces. It was noted that *L. rhamnosus* 705 slightly inhibited the growth of all isolated strains *L. plantarum* and *L. fermentum* (zone of inhibition 5 mm), whereas *L. casei* 163 slightly inhibited only *L. plantarum* 14 (zone of inhibition 5 mm). Isolates did not inhibit the growth of commercial strains except for *L. acidophilus* NCFM, which was slightly inhibited (zone of inhibition 4 mm) by *L. plantarum* 4b and 20a. The combination of strains which interact antagonistically should be avoided in the products in which a couple of probiotic strains or probiotics and starter culture are used (TIMMERMAN et al. 2004). This antagonistic interaction may be caused by acids, hydrogen peroxide and bacteriocins (which have an effect upon closely related bacteria) produced by lactic acid bacteria (TODOROV, DICKS 2005). The lack of strong antimicrobial interactions among tested isolates and commercial strains indicates the possibility of their collective usage in probiotic products.

None of the strains was able to grow at 3°C regardless of pH value of media and NaCl concentration (Table 1), thus during storage of food products in such low temperatures these bacteria will not proliferate. However, this do not disgualify them as a starter culture components as far as in the final product high enough number of probiotic cells will be provided. The isolates showed more diverse growth at 7°C. Strains: 4b, 14, 18a, 20b grew at 7°C at pH 6.5 and 5.0 and NaCl concentration up to 3.3%, whereas strains 4a and 20a did not grow under these conditions. Along with the increase of incubation temperature more intense growth of isolates was noted. All strains were able to grow at 12° C, pH 5.0 and a_{w} 0.96. Such conditions are prevailing during hard cheese ripening and ability of strains to grow under them indicate possible use of isolates in hard cheese production. The proliferation of probiotic bacteria during cheese ripening is highly desirable, because it enables to maintain high population in the final product. In media at pH value 6.5, incubated at 12°C growth of some strains: 1, 4a, 14, 20a was noted even when salt content reached 10.4%, which corresponded to a_w 0.93. In media at pH 6.5 incubated at 37°C eight strains were able to grow at the presence of 6.7% of NaCl, and only 4a did not. It was noted that strains 4a, 4b and 14 were able to grow at 45°C (Table 1), at which yoghurt fermentation traditionally proceeds. Thus, the strains can be used as adjunct cultures, with probiotic properties, without changes in technology parameters of yoghurt production.

Sodium chloride is a substance commonly used in the food industry, playing an important role e.g. in cheese ripening process (REINHEIMER et al. 1997). However it may decrease the growth and viability of bacterial cells (GOMES et al. 1998). PASSOS et al. (1993) and GÄNZLE et al. (1998) showed that higher than 3% addition of NaCl into media caused an inhibition of growth of lactic acid bacteria, whereas lower concentrations of salt (1 to 2%) positively affected

Parameter			Strains								
pH	°C	% NaCl	1	4a	4b	14	18a	20a	20b	22b	24
5.0	3	0-10.4	-	-	-	-	-	-	-	-	-
6.5	3	0 - 10.4	-	-	-	-	-	-	-	-	-
5.0	7	0-3.3	+	-	+	+	+	-	+	-	-
5.0	7	6.7 - 10.4	-	-	-	-	-	-	-	-	-
6.5	7	0	+	-	+	+	+	-	+	+	+
6.5	7	3.3	-	-	+	+	+	-	+	-	-
6.5	7	6.7 - 10.4	-	-	-	-	-	-	-	-	-
5.0	12	0	+++	+++	+++	+++	+ + +	+++	+ + +	+++	+ + +
5.0	12	3.3 - 6.7	++	++	++	++	++	++	++	++	++
5.0	12	10.4	-	-	-	-	-	-	-	-	-
6.5	12	0 - 3.3	+++	+++	+++	+++	+ + +	+++	+++	+++	+ + +
6.5	12	6.7 - 10.4	+	+	-	+	-	+	-	-	-
5.0	37	0	++	++	+++	+++	+ + +	++	+ + +	+++	++
5.0	37	3.3 - 6.7	+++	+++	+++	+++	+++	+++	+++	+++	+ + +
5.0	37	10.4	-	-	-	-	-	-	-	-	-
6.5	37	0 - 3.3	+++	+++	+++	+++	+++	+++	+++	+++	+ + +
6.5	37	6.7	+++	-	+++	+++	+++	+++	+++	+++	+ + +
6.5	37	10.4	+	-	+	++	++	-	+++	+	-
5.7	45	0	-	+++	+++	+++	_	_	_	-	_

The influence of temperature, pH and sodium chloride on the growth of potentially probiotic Lactobacillus strains

+ 10^7 cfu cm⁻³, ++ 10^8 cfu cm⁻³, +++ $\ge 10^9$ cfu cm⁻³, - no growth

bacterial growth. The isolates tested in the present work showed very high tolerance to NaCl. It implies a possibility to use them in the production of hard cheeses which undergoes salting process.

It was noted that isolates showed diverse ability to grow at different temperature and NaCl concentration in spite of the common origin, which was gastrointestinal tract of infants. Thus it could be concluded that the ability to grow at different conditions is a strain, not only species, dependent property.

Tested strains grew similarly in media of pH 5.0 and 6.5, which correlates well with the results reported by MATAGARAS et al. (2003).

The isolates did not show proteolytic activity. None of them was able to hydrolyze casein and arginine and did not produce H₂S. Proteolytic properties of lactic acid bacteria which are used as components of starter or adjunct cultures in unripening fermented dairy products may negatively affect the sensory properties of products during many weeks of storage (KORNACKI et al. 1997). There are strains of lactic acid bacteria belonging to *L. fermentum* and *L. plantarum* species, which may be able to produce hydrogen sulphide (ARICI et al. 2004, LEE, SIMARD 1984). In hard cheeses H₂S produced during ripening contributes to desirable flavour profile (URBACH 1995). In other dairy products,

Table 1

such as yoghurts and soft cheeses, the presence of H_2S indicates the decomposition of sulphur amino acids, and bacteria able to H_2S production are regarded as technologically harmful (ARICI et al. 2004). The fact, that tested isolates did not produce H_2S increased the possibility of their usage in fermented and non-fermented dairy beverages, and did not disqualify their application in hard cheeses production.

Application of synbiotics in production of fermented dairy products requires the determination of prebiotics influence on growth and activity of probiotic cultures. Growth of isolates in media containing prebiotics was better than in Bullion Standard and similar or frequently higher than in bullion with glucose (Table 2). After 24 h of incubation the counts of *Lactobacillus* sp. ranged from 10^7 to 10^8 cfu cm⁻³ and from 10^7 to 10^9 cfu cm⁻³, in bullion with glucose and in prebiotic containing media, respectively. Prebiotics used in the study the strongest stimulated the growth of *L. plantarum* 4b, 14, 20a as compared to the growth of the isolates in media without glucose. The results obtained in the study show that lactic acid bacteria are characterized by diverse ability to use prebiotics as a carbon source. The similar findings were reported by BIELECKA et al. (2002) and Su et al. (2007).

Table 2

The growth of potentially probiotic Lactobacillus strains (log cfu cm⁻³) after 24 h of incubation in media containing prebiotics

Medium	Prebiotic	Strains									
	concentration	1	4a	4b	14	18a	20a	20b	22b	24	
BS	0%	8.2	7.8	7.5	7.5	7.8	7.5	8.0	8.6	8.1	
BG	0%	8.8	7.9	8.3	8.0	8.5	8.8	8.3	8.8	8.3	
BS+IQ	0.5 - 2.5%	9.3 - 9.5	9.4 - 9.5	9.5-9.9	9.8	9.4 - 9.7	9.3 - 10.0	9.4-9.6	9.5-9.8	9.4 - 9.7	
BS+TEX	0.5 - 2.5%	8.4-9.0	9.3-9.5	9.8-9.9	9.8-10.0	9.4-9.6	9.3-10.0	9.7-9.8	9.3-9.5	9.4-9.9	
BS+HPX	0.5 - 2.0%	9.2 - 9.3	8.8-9.0	8.7 - 9.2	9.4	9.3-9.5	9.3 - 9.4	8.3 - 9.4	9.4-9.6	9.5 - 9.7	
BS+HD	0.5 - 2.5%	9.0-9.3	7.4-9.0	8.7-9.3	9.4-9.6	9.4-9.6	9.0-9.8	9.2 - 9.7	9.4-9.7	8.8-9.3	
BS+m.m.	0.5 - 2.5%	8.7-9.0	8.7-8.8	9.1	9.4-9.8	9.2 - 9.7	9.3-9.6	9.3-9.8	9.3 - 9.4	9.1 - 9.4	
BS+m.l.	0.5 - 2.5%	9.0 - 9.2	8.3-8.6	9.1	9.8–9.9	9.5 - 9.6	9.8–9.9	9.8–9.9	9.4–9.6	9.3 - 9.4	

 $\rm BS$ – Bullion Standard, BG – bullion with glucose, m.m. – maltodextrin of medium DE, m.l. – maltodextrin of low DE

Conclusions

1. There were no antagonistic interactions among yoghurt strains and L. *plantarum* and L. *fermentum* isolates, and amongst B. *lactis* 420 and isolates, thus they can be used together in yoghurt production.

2. Weak antagonistic interactions were noted among *L. rhamnosus* 705 and all of tested *L. plantarum* and *L. fermentum* strains. The antagonistic

interactions were also found between *L. casei* 163 and *L. plantarum* 14 as well as among *L. acidophilus* NCFM and *L. plantarum* 4b and 20. These interactions should be taken into consideration in composition of starter culture.

3. There is a possibility to use all tested isolates in soft and hard cheeses production because of their ability to proliferate in media of lowered water activity.

4. Strains *L. plantarum* 4b, 14 and *L. fermentum* 4a were able to grow at 45°C. Therefore they can be used in yoghurt production while maintaining the optimal for yoghurt bacteria temperature of fermentation.

5. All isolates grew well in the presence of prebiotics in sugar free media. These findings show the ability of isolates to use inulins: HD, IQ, TEX, HPX and maltodextrins of low and medium dextrose equivalent as a source of carbon.

6. Tested isolates did not show strong proteolytic properties, what enables their use in probiotic food production.

Translated by MONIKA MODZELEWSKA-KAPITUŁA

Accepted for print 24.08.2009

References

- ARICI M., BILGIN B., SAGDIC O., OZDEMIR C. 2004. Some characteristics of Lactobacillus isolates from infant faeces. Food Microbiol., 21: 19–24.
- BERGAMINI C.V., HYNES E.R., PALMA S.B., SABBAG N.G., ZALAZAR C.A. 2009. Proteolytic activity of three probiotic strains in semi-hard cheese as single and mixed cultures: Lactobacillus acidophilus, Lactobacillus paracasei and Bifidobacterium lactis. Int. Dairy J., 19: 467–475.
- BIELECKA M., BIEDRZYCKA E., MAJKOWSKA A. 2002. Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. Food Res. Int., 35: 125–131.
- BURBIANKA M., PLISZKA A., BURZYŃSKA H. 1983. Mikrobiologia żywności. PZWL, wyd. V, Warszawa, 465–515.
- CASTRO F.P. DE, CUNHA T.M., BARRETO P.L.M, M C AMBONI R.D. DE, PRUDÊNCIO E.S. 2009. Effect of oligofructose incorporation on the properties of fermented probiotic lactic beverages. Int. J. Dairy Technol., 62: 68–74.
- DONKOR O.N., NILMINI S.L.I., STOLIC P., VASILJEVIC T., SHAH N.P. 2007. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int. Dairy J., 17: 657–665.
- GÄNZLE M.G., EHRMANN M., HAMMES W.P. 1998. Modelling of growth of Lactobacillus sanfranciscensis and Candida milleri in response to process parameters of sourdough fermentation. Appl. Environ. Microbiol., 64: 2616–2623.
- GOMES A.M.P., TEIXEIRA M.G.M., MALCATA F.X. 1998. Viability of Bifidobacterium lactis and Lactobacillus acidophilus in milk: sodium chloride concentration and storage temperature. J. Food Process. Pres., 22: 221–240.
- GUGGISBERG D., CUTHBERT-STEVEN J., PICCINALI P., BÜTIKOFER U., EBERHARD P. 2009. Rheological, microstructural and sensory characterization of low-fat and whole milk set yoghurt as influenced by inulin addition. Int. Dairy J., 19: 107–115.
- KORNACKI K., ŁANIEWSKA-MOROZ Ł., WARMIŃSKA-RADYKO I. 1997. Podstawy mikrobiologii mleczarskiej. Oficyna Wydawnicza Hoża, Warszawa, 38–39.
- LEE B.H., SIMARD R.E. 1984. Evaluation of methods for detecting the production of H₂S, volatile sulfides, and greening by Lactobacilli. J. Food Sci., 49: 981–983.

- LITWIŃCZUK A., LITWIŃCZUK Z., BARŁOWSKA J., FLOREK M. 2004. Surowce zwierzęce ocena i wykorzystanie. PWRiL, Warszawa, 80–148.
- LIU S.Q., ASMUNDSON R.V., GOPAL P.K., HOLLAND R., CROW V.L. 1998. Influence of reduced water activity on lactose metabolism by Lactococcus lactis subsp. cremoris at different pH values. Appl. Environ. Microbiol., 64(6): 2111–2116.
- MATARAGAS M., METAXOPOLULOS J., GALIOTOU M., DROSINOS E.H. 2003. Influence of pH and temperature on growth and bacteriocin production by Leuconostoc mesenteroides L124 and Lactobacillus curvatus L442. Meat Sci., 64: 265–271.
- MODZELEWSKA M., KORNACKI K., KLEBUKOWSKA L. 2003. Antimicrobial activity of lactic acid bacteria strains isolated from GI tract of infants against Gram positive and Gram negative bacteria. Commun. Agr. Appl. Biol. Sci., 68(2): 437–440.
- MODZELEWSKA-KAPITUŁA M., KŁĘBUKOWSKA L., KORNACKI K., ŁUKASZUK W. 2008. Characterization of probiotic properties of Lactobacillus strains. Pol. J. Natur. Sci., 23(2): 366–373.
- MODZELEWSKA-KAPITUŁA M., MARIN-INIESTA F. 2005. The possibility of using Lactobacillus fermentum strains of human origin as protective cultures in soft cheese. Electron. J. Polish Agric. Univ., Food Sci. Technol., 8:4.
- MORELLI L. 2007. In vitro assessment of probiotic bacteria: From survival to functionality. Int. Dairy J., 17: 1278–1283.
- OLSON D.W., ARYANA K.J. 2008. An excessively high Lactobacillus acidophilus inoculation level in yogurt lowers product quality during storage. LWT, 41: 911–918.
- ONG L., HENRIKSSON A., SHAH N.P. 2007. Proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of Lactobacillus acidophilus, Lb. paracasei, Lb. casei or Bifidobacterium sp. Int. Dairy J., 17: 67–78.
- PASSOS F.V., FLEMMING H.P., OLLIS D.F., HASSANS H.M., FELDER R.M. 1993. Modelling of specific growth rate of Lactobacillus plantarum in cucumber extract. Appl. Microbiol. Biotechnol., 40: 143–150.
- Probiotics in food. Health and nutritional properties and guidelines for evaluation. 2006. FAO Food Nutr. Pap., 85; Rome 2006. ftp://ftp.fao.org/docrep/fao/009/a0512e/a0000000000000
- REINHEIMER J.A., RENZULLI P.M., RUBIOLO A.C., BAILO N.B., BINETTI A.G. 1997. Effect of sodium and potassium chloride on growth and acid production in thermophilic acid bacteria. Microbiologie--Aliments-Nutr., 15: 7–15.
- SAARELA M., MOGEMSEN G., FONDÉN R., MÄTTÖ J., MATTILA-SANDHOLM T. 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197–215.
- SU P., HENRIKSSONA A., MITCHELL H. 2007. Selected prebiotics support the growth of probiotic mono-cultures in vitro. Anaerobe, 13: 134–139.
- TIMMERMAN H.M., KONING C.J.M., MULDER L., ROMBOUTS F.M., BEYNEN A.C. 2004. Monostrain, multistrain and multispecies probiotics – a comparison of functionality and efficacy. Int. J. Food Microbiol., 96: 219–233.
- TODOROV S.D., DICKS L.M.T. 2005. Lactobacillus plantarum isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme Microb. Tech., 36: 318–326.
- URBACH G. 1995. Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. Dairy J., 5: 877–903.
- WANG Y. 2009. Prebiotics: Present and future in food science and technology. Food Res. Int., 42: 8-12.

DOI 10.2478/v10020-009-0025-7

THE EFFECT OF COLD STORAGE ON THE COLOR OF VENISON

Tomasz Żmijewski, Aleksandra Kwiatkowska, Marek Cierach

Chair of Meat Technology and Chemistry University of Warmia and Mazury in Olsztyn

Key words: venison, deer, color, storage.

Abstract

The objective of this study was to evaluate the color of unpackaged and vacuum-packaged venison during storage. Color parameters L^* , a^* and b^* were assessed in vacuum-packaged samples after 72, 144, 216, 288 and 360 h of storage, and in unpackaged samples – after 72, 144 and 216 h. The total change in color ΔE^* in comparison with the color of fresh meat was determined. The results indicate that the storage of unpackaged venison has an adverse effect on color, giving it a darker, grayish hue. The most profound changes were observed after 144 and 216 h of storage. Vacuum packaging of venison minimizes color change and has a long-term stabilizing effect for up to 15 days.

WPŁYW CHŁODNICZEGO PRZECHOWYWANIA NA BARWĘ MIĘSA JELENIA

Tomasz Żmijewski, Aleksandra Kwiatkowska, Marek Cierach

Katedra Technologii i Chemii Mięsa Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: dziczyzna, jeleń, barwa, przechowywanie.

Abstrakt

W pracy oceniano barwę niepakowanego i pakowanego próżniowo mięsa jelenia podczas przechowywania. Oznaczenie parametrów barwy L^* , a^* , b^* wykonano w próbkach pakowanych próżniowo po 72, 144, 216, 288, 360 h przechowywania, zaś w niepakowanych po 72, 144, 216 h. Wyliczono również całkowitą zmianę barwy ΔE^* w stosunku do barwy mięsa świeżego. Wykazano, że przechowywanie mięsa jelenia bez opakowania zmienia niekorzystnie jego barwę, powodując ciemnienie i szarzenie. Zmiany miały największy zakres po 144 i 216 h przechowywania. Przechowywanie mięsa tego gatunku w opakowaniu próżniowym zmniejsza zakres zmian barwy, działając stabilizująco w długim okresie (do 15 dni).

Address: Tomasz Żmijewski, University of Warmia and Mazury, pl. Cieszyński 1, 10-957 Olsztyn, Poland, phone: +48 (089) 523 38 08, e-mail: tomzm@uwm.edu.pl

Introduction

Color is one of the most important parameters in evaluating the quality of raw meat. It is a key criterion for assessing unprocessed meat, as changes in color are often the first noticeable symptoms of the product's deteriorating sensory quality and nutritive value. Adverse changes in color during storage have a negative impact on the consumer evaluation of meat. The extent of such undesirable changes can be minimized through the use of various packaging methods. Vacuum packaging provides the simplest option of modifying the atmosphere surrounding the packaged product. Although an atmosphere composed of 80% O_2 and 20% CO_2 is generally believed to have the most beneficial effect on meat color, some research findings suggest that vacuum packaging contributes to the preservation of high-quality color (CAYUELA et al. 2004, DASZKIEWICZ 2007, INSAUSTI et al. 1999, OLIETE et al. 2005, SØRHEIM et al. 1996). Vacuum packaging is the predominant packaging method at small plants processing game carcasses. Venison is dark meat with a high content of heme pigments and iron, which is why chill storage contributes to adverse changes in its color (DZIERŻYŃSKI-CYBULKO, FRUZIŃSKI 1997, REDE et al. 1986, STRMISKOVA, STRMISKA 1992).

The objective of this study was to evaluate the color parameters of unpackaged and vacuum-packaged venison during cold storage.

Materials and Methods

The study was carried out on samples of *m. longissimus dorsi* of the red deer (Cervus elaphus). Carcasses of five hinds with average weight of 81 kg were used. M. longissimus dorsi was removed from the carcasses 24 h post mortem. Color parameters were evaluated on muscle surface according to CIE (International Commission on Illumination) standards: lightness (L^*) , redness (a^*) and yellowness (b^*) . Measurements were performed using the Dr Lange Spectro-Color device with an 8 mm measuring aperture, D 65 light source, standard colorimetric observer with a 10°C field of view, as well as SPEC-TRAL-QC software. Prior to measurement, the device was calibrated with the use of a black and white calibration template (Dr Lange). The muscle was divided into eight parts, five of which were vacuum packaged, and three were left unpackaged. All samples were stored at a temperature of 4 ± 0.5 °C, relative humidity of $85 \pm 1\%$ and without light access. Color parameters on meat surface were determined in vacuum-packaged samples after 72, 144, 216, 288 and 360 h of storage, by unpacking the samples two hours prior to analysis (meat blooming, i.e. the highest level of oxymyoglobin saturation) (WIKLUND et al.

2001, WIKLUND et al. 2006), and in unpackaged samples – after 72, 144 and 216 h of storage. The results were used to calculate the total change in color ΔE^* after the respective storage time relative to the color determined 24 h *post* mortem. The following calculation formula was applied: $\Delta E^* = ((\Delta L^*)2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$ (BILLER, WIERZBICKA 2003). The results were processed statistically by computing the arithmetic mean and the standard error of the mean (SEM) SEM = s/\sqrt{n} , where s – standard deviation, n – number of samples. The significance of differences was determined with the use of Duncan's test at $p \leq 0.01$ (GAWECKI, WAGNER 1984).

Results and Discussion

The lightness of *m. longissimus dorsi* of deer carcasses reached 27 units 24 h *post mortem*, confirming that venison is dark-colored meat (DASZKIEWICZ 2007, DZIERZYŃSKA-CYBULKO, FRUZIŃSKI 1997, REDE et al. 1986). Significant changes in the above color parameter were noted in unpackaged meat already after 72 hours of storage when lightness decreased by 4.6 units. Further storage resulted in a steady decrease in the investigated color parameter. After 216 h, the value of L^* was only 15 units, i.e. 57% of its initial value, and significant differences were noted in comparison with the data obtained during previous measurements (Figure 1).



Fig. 1. Changes in the L^* color parameter of *m. longissimus dorsi* in the red deer (mean \pm SEM) – the values marked with letters *a*, *b*, *c* on different curves differ significantly at $p \le 0.01$ – the values marked with letters *x*, *y* on different curves after the same storage time differ significantly at $p \le 0.01$

Vacuum-packaged meat was characterized by the following lightness parameters during storage: 72 h post mortem – 30.77, 144 h p.m. – 32.55, 216 h p.m. – 32.40, 288 h p.m. – 34.51 and 360 h p.m. – 35.76. This parameter was stabilized when storage conditions were modified to vacuum packaging. Significant differences were observed between the values determined for 24 h p.m. samples and samples analyzed 144 h p.m. and later. A comparison of the two experimental storage methods showed significantly lower L^* values in unpackaged meat after an identical time of storage.

Color redness (a^*) reached 13 units 24 h *post mortem*, indicating that this parameter significantly affects the color of meat from game animals. After 144 h of storage, color redness decreased significantly in unpackaged meat by 11.6 units to 1.6 units. In vacuum-packaged samples, no significant changes in color parameter a^* were noted after 360 h of storage. A comparison of the redness of unpackaged and packaged muscle samples revealed no significant differences up to 72 h of storage, while significant differences were observed after 144 h and 216 h of storage (Figure 2).



Fig. 2. Changes in the a^* color parameter of *m. longissimus dorsi* in the red deer (mean \pm SEM) – the values marked with letters *a*, *b*, *c* on different curves differ significantly at $p \le 0.01$ – the values marked with letters *x*, *y* on different curves after the same storage time differ significantly at $p \le 0.01$

Contrary to color lightness and redness, color parameter b^* was marked by the least change throughout the experimental period. No differences in this parameter's share were noted during the storage of both unpackaged and vacuum-packaged meat. No significant differences in the share of color parameter b^* were observed subject to the applied storage method (Figure 3).



Fig. 3. Changes in the b^* color parameter of *m. longissimus dorsi* in the red deer (mean \pm SEM) – the values marked with letter *a* on different curves do not differ significantly at $p \leq 0.01$ – the values marked with letter *x* on different curves after the same storage time do not differ significantly at $p \leq 0.01$

The color parameters of raw meat are determined by various factors, such as species, sex, age, diet, muscle type, pH, chemical composition, including the content of heme and other pigments, tissue enzyme activity, water-holding capacity and morphological structure (BOULLIANNE, KING 1998). In addition to the packaging method, the results of this experiment could have also been affected by pH, drip loss and water loss by evaporation from unpackaged meat. The results of a previous study evaluating the water-holding capacity of cold-stored venison did not show significant pH differences throughout the experiment or between unpackaged and vacuum-packaged meat (KWIAT-KOWSKA et al. 2009). In view of the above, the differences in the color of unpackaged and vacuum-packaged meat could be attributed to drip loss and water loss by evaporation from unpackaged samples.

Color lightness results approximate the data characteristic of the meat of 5-6-year-old farm-raised hinds (STEVENSON et al. 1992), and they are below the values noted in the meat from young farm animals where L^* reaches from 32 to 34 (STEVENSON et al. 1992, VOLPELLI et al. 2003, VERGARA et al. 2003, WOODFORD et al. 1996). A review of reference data indicates that the storage of venison under modified atmosphere conditions, with or without oxygen, over a period similar to that in the presented experiment, leads to a slight increase in L^* values by around 0.5–1.4 units (WERGARA et al. 2003).

The share of the redness parameter in venison color varies, reaching 13–14 units (STEVENSON et al. 1992), 15–17 (POLLARD et al. 2002, WERGARA et al. 2003, WOODFORD et al. 1996), 19 (DASZKIEWICZ 2007), and up to 21 units

(WIKLUND et al. 2001, WIKLUND et al. 2006). In the investigated meat samples, the value of a^* reached around 13 units, therefore, it was comparable. In a study by WIKLUND et al. (2006) and POLLARD et al. (2002), the redness parameter in vacuum-packaged red deer meat increased by around 4.5 units after 3 weeks of storage. By comparison, the values of color parameter a^* in vacuum-packaged samples in this study differed by around 3 units, therefore, the differences were statistically non-significant. It can be concluded that regardless of the applied packaging method, the storage of packaged venison always has a stabilizing effect on color redness.

The changes noted in the investigated color parameters are validated by the value of ΔE^* . After 72 h, the total change in the color of unpackaged muscle samples reached 8, after 144 h – 16, and after 216 h – 18. Already after 72 h of storage, ΔE^* was statistically significant, accounting for more than 40% of the maximum change in this parameter. In comparison with the changes observed after 72 h, the indicated changes after 144 h and 216 h were significantly higher (Figure 4). In vacuum-packaged samples, ΔE^* reached from 7.35 to 10.16 throughout the experiment, and no statistically significant differences were noted. A comparison of ΔE^* values between unpackaged and packaged muscle samples revealed significant differences after 216 h of storage.



Fig. 4. Total change in the color of *m. longissimus dorsi* in the red deer (mean \pm SEM) – the values for the same storage method, marked with letters *a*, *b*, differ significantly at $p \leq 0.01$ – the values for the same storage method after the same storage time, marked with letters *x*, *y*, differ significantly at $p \leq 0.01$

Conclusions

1. Cold storage of unpackaged venison has an adverse effect on color by significantly lowering the values of such parameters as lightness and redness.

2. Vacuum packaging stabilizes the color of venison cold-stored for minimum 15 days.

Translated by Aleksandra Poprawska

Accepted for print 6.10.2009

References

BILLER E., WIERZBICKA A. 2003. Wybrane procesy w technologii żywności. SGGW, Warszawa.

- BOULLIANNE M., KING A.J. 1998. Meat color and biochemical characteristics of unacceptable darkcolored broiler chicken carcasses. J. Food Sci., 63: 759–762.
- CAYUELA J.M., GIL M.D., BAÑÓN S., GARRIDO M.D. 2004. Effect of vacuum and modified atmosphere packaging on the quality of pork loin. Eur. Food Res. Technol., 219: 316–320.
- DASZKIEWICZ T. 2007. Charakterystyka mięsa łań jelenia szlachetnego (Cereus elaphus L.) oraz zmian jego jakości w czasie dojrzewania w modyfikowanej atmosferze. Rozpr. monogr. 126, Wyd. UWM, Olsztyn.
- DZIERŻYŃSKA-CYBULKO B., FRUZIŃSKI B. 1997. Dziczyzna jako źródło żywności. PWRiL, Poznań.
- GAWĘCKI J., WAGNER W. 1984. Podstawy metodologii badań doświadczalnych w nauce o żywieniu i żywności. PWN, Warszawa-Poznań.
- INSAUSTI K., BERIAIN M.J., PURROY A., ALBERTI P., LIZASO L., HERNANDEZ B. 1999. Colour stability of beef from different Spanish native cattle breeds stored under vacuum and modified atmosphere. Meat Sci., 53: 241–249.
- KWIATKOWSKA A., ŻMIJEWSKI T., DĄBROWSKA E. 2009. Cechy hydratacyjne chłodniczo przechowywanego mięsa jelenia. Inż. i Ap. Chem. 48(40): 82–83.
- OLIETE B., MORENO T., CARBALLO J.A., VARELA A., MONSERRAT L., SÁNCHEZ L. 2005. Influence of ageing time on the quality of yearling calf meat under vacuum. Euro. Food Res. Technol., 220(5–6): 489–493.
- POLLARD J.C., LITTLEJOHN R.P., ASHER G.W., PEARSE A.J.T., STEVENSON-BARRY J.M., MCGREGOR S.K., MANLEY T.R., DUNCAN S.J., SUTTON C.M. POLLOCK K.L., PRESCOTT J. 2002. A comparison of biochemical and meat quality variables in red deer (Cervus elaphus) following either slaughter at pasture or killing at a deer slaughter plant. Meat Sci., 60: 85–94.
- REDE R., PRIBISCH V., RAHELIĆ S. 1986. Untersuchungen über die Beschaffenheit von Schlachttierkörpern und Fleisch primitiver und hochselektierter Schweinerassen. Fleischwirtschaft, 66: 898–907.
- Sørheim O., Kropf D.H., Hunt M.C., Karwoski M.T., Warren K.E. 1996. Effects of modified gas atmosphere packaging on pork loin colour, display life and drip loss. Meat Sci., 43: 203–212.
- STEVENSON J.M., SEMAN D.L., LITTLEJOHN R.P. 1992. Seasonal variation in venison quality of mature, farmed red deer stags in New Zealand. J. Anim. Sci., 70: 1389–1396.

STRMISKOVA G., STRMISKA F. 1992. Contents of mineral substances in venison. Nahrung, 36: 307–308.

- VERGARA H., GALLEGO L., GARCIA A., LANDETE-CASTILLEJOS T. 2003. Conservation of Cervus elaphus meat in modified atmospheres. Meat Sci., 65: 779–783.
- VOLPELLI L.A., VALUSSO R., MORGANTE M., PITTIA P., PIASENTIER E. 2003. Meat quality in male fallow deer (Dama dama): effects of age and supplementary feeding, Meat Sci., 65: 555–562.
- WIKLUND E., SAMPELS S., MANLEY T.R., PICKOVA J., LITTLEJOHN R.P. 2006. Effects of feeding regimen and chilled storage on water-holding capacity, colour stability, pigment content and oxidation in red deer (Cervus elaphus) meat. J. Sci. Food Agricult., 86: 98–106.

- WIKLUND E., STEVENSON-BARRY J.M., DUNCAN S.J., LITTLEJOHN R.P. 2001. Electrical stimulation of red deer (Cervus elaphus) carcasses – effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity. Meat Sci., 59: 211–220.
- WOODFORD K.B., SHORTHOSE W.R., STARK J.L., JOHNSON G.W. 1996. Carcass Composition and Meat Quality Parameters of Entire and Castrate Farmed Black Antelope (Antilope cervicapra). Meat Sci., 43: 25–36.

Reviewers of Years – book 2009

Włodzimierz Bednarski Dorota Bobrecka-Jamro Danuta Borkowska Zbigniew Dolatowski Wojciech Donderski Zbigniew Endler Piotr Epler Wiesław Fałtynowicz Paweł Gajewczyk Janusz Gołaszewski Jolanta Kempter Leszek Kordas Halina Kozłowska Tomasz P. Kurowski Halina Kurzawińska Zdzisława Libudzisz Marek Łuczyński Ryszard Paczuski Julian Paluch Stanisław Sienkiewicz Andrzej Szczerbowski Renata Tandyrak Zenon Zduńczyk Marek Zin