

CZECH ACADEMY OF AGRICULTURAL SCIENCES

*Czech Journal of*  
**ANIMAL SCIENCE**

ŽIVOČIŠNÁ VÝROBA



INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

**4**

VOLUME 48  
PRAGUE 2003  
ISSN 1212-1819

# CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the auspices of the Czech Academy of Agricultural Sciences and financed by the Ministry of Agriculture of the Czech Republic

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**Periodicity:** The journal is published monthly (12 issues per year). Volume 48 appearing in 2003.

**Acceptance of manuscripts:** Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: +420 227 010 352, fax: +420 227 010 116, e-mail: [edit@uzpi.cz](mailto:edit@uzpi.cz).

**Subscription information:** Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic. Subscription price for 2003 is 1176 CZK or 214 USD.

## CONTENTS

## ORIGINAL PAPERS

## Physiology and Reproduction

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

BRZUSKA E.: Artificial propagation of European catfish (*Silurus glanis*): application of a single dose of pellets containing D-Ala<sup>6</sup>, Pro<sup>9</sup>NET-mGnRH and dopamine inhibitor metoclopramide to stimulate ovulation in females of different body weight ..... 152

## Genetics and Breeding

BOBČEK B., ŘEHÁČEK P., MATOUŠEK V.: Analysis of reproduction and production parameters in dam populations of pigs and genealogical sire populations by means of M BLUP-AM method in Slovakia ..... 164

## Nutrition and Feeding

ŠUSTALA M., TRINÁCTÝ J., KUDRNA V., ILLEK J., ŠUSTOVÁ K.: The effect of iodine supplementation on its output and thyroid gland status in dairy cows on a diet containing rapeseed meal ..... 170

## OBSAH

## PŮVODNÍ PRÁCE

## Fyziologie a reprodukce

BRZUSKA E.: Umělý výtěr kapra (*Cyprinus carpio*): výsledky dvouleté reprodukce jikernaček maďarské linie W a polské linie 6 po stimulaci ovulace kapří hypofýzou a přípravkem obsahujícím mGnRH-a a dopaminergní inhibitor ..... 139

BRZUSKA E.: Umělý výtěr sumce velkého (*Silurus glanis*): jednorázové podání přípravku obsahujícího analog GnRH a dopaminergní inhibitor pro stimulaci ovulace jikernaček různé hmotnosti ..... 152

## Genetika a šlechtění

BOBČEK B., ŘEHÁČEK P., MATOUŠEK V.: Analýza reprodukčních a produkčních vlastností materských populací prasát a genealogických linií otcovských populací pomocí metody M BLUP-AM na Slovensku ..... 164

## Výživa a krmení

ŠUSTALA M., TRINÁCTÝ J., KUDRNA V., ILLEK J., ŠUSTOVÁ K.: Vliv přídatku jodu na jeho výdej a činnost štítné žlázy u dojnic krměných dávkou s řepkovým extrahovaným šrotem ..... 170

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Kaláb J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): *Lactation in Cattle*. Academic Press, London. 876–888.

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# Artificial propagation of the carp (*Cyprinus carpio*): two-year reproduction results of females of Hungarian line W and Polish line 6 after ovulation stimulation with carp pituitary or mGnRH-a and dopaminergic inhibitor

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**ABSTRACT:** Effects of propagation were investigated in females of Hungarian line W and Polish line 6 during two successive years. In females of these provenances ovulation was stimulated with carp pituitary (dose I of 0.3 mg/kg and after 12 h dose II of 2.7 mg/kg body weight) or with Ovopel – preparation containing mGnRH-a and metoclopramide (dose I of 1/5 pellet/kg and after 12 h dose II of 1 pellet/kg). Irrespective of the ovulation stimulator higher percentages of females both of Hungarian and Polish origin spawned in the first year of the investigation. In the two years of the investigation in the two fish provenances eggs were obtained from a higher percentage of females treated with Ovopel than after hypophysation. The effect of year on the weight of eggs was statistically significant both in grams and in percentage of female body weight ( $P \leq 0.01$  and  $P \leq 0.05$ , respectively), being insignificant with respect to traits characterising the quality of eggs. The ovulation stimulator, provenance of females, and the interaction between the stimulator and provenance did not significantly affect the weight and quality of eggs. The interaction between the year and ovulation stimulator and between the year and provenance of females was statistically significant ( $P \leq 0.01$  and  $P \leq 0.05$ , respectively) only with respect to traits characterising the weight of eggs.

**Keywords:** artificial propagation; carp; pituitary; Ovopel (mGnRH-a and metoclopramide); origin of females; effect of year

The presented study is another paper in a series concerning the effects of controlled reproduction of different breeding lines of the carp and its crosses. In these fish ovulation was stimulated with natural stimulator (carp pituitary, bream pituitary, HCG) or synthetic stimulator (Gn-RH-analogues or the following preparations: Ovaprim, Ovopel, Dagin, Aquaspawn).

The females investigated in experiments described in preceding papers (Brzuska, 1990, 1991, 2000a,b, 2001a,b; Brzuska and Adamek, 1989; Brzuska and Grzywaczewski, 1999) and these used in the present study were selected from 18 lines which were bred in the Gołysz Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences and were the property of this Institute. The results

of long-term studies conducted on this genetically differentiated material permit to draw a conclusion that different effects can be obtained in various lines of this fish species even though the time and conditions of the treatment with a given ovulation stimulator were identical (Brzuska and Adamek, 1997; Brzuska, 2000a; Brzuska and Białowąs, 2002).

The aim of the present study was to show the effects of controlled reproduction in two genetically distant lines of the carp, i.e. of Hungarian and Polish origin, after treatment with natural or synthetic ovulation stimulator in two-year experiments. The effect of the time of ovulation after the hormonal treatment on the results of controlled fish reproduction was also investigated.

## MATERIAL AND METHODS

The data used in the calculation concerned the results of controlled propagation of the carp and were obtained in two experiments conducted in May 1998 and 1999. The carp females used in the reproduction belonged to Hungarian line W (Irnazarow and Białowas, 1995; Białowas, 2000) and Polish line 6 (Irnazarow and Białowas, 1994; Białowas, 1998), their numbers being given in Table 1. The applied ovulation stimulators were carp pituitary or Ovopel (Horváth and Szabó, 1996; Horváth *et al.*, 1997) (the preparation containing DesGlyNH<sub>2</sub><sup>10</sup>[D-Ala<sup>6</sup>, ProNHet<sup>9</sup>]mGnRH and the water soluble dopamine receptor antagonist, metoclopramide). The doses of the two stimulators are given in Table 1 and the statistical characteristics of the body weight of females used for reproduction in Table 2. In each experiment on May 12 of 1998 and May 9 of 1999, fish caught in the pond were transferred to concrete tanks 2.5 m<sup>3</sup> in volume at the hatchery, in water of 21–22°C. After a two-day adaptation period the stimulation of ovulation started by the application of carp pituitary in group I and of Ovopel in group II (Table 1).

Eggs yielded by individual fish were weighed and fertilized with mixed milt taken from several males previously hypophysectomized with carp pituitary at the dose of 2 mg/kg body weight. The incubation of fertilized eggs from each female separately was conducted in a Weiss glass at 21–22°C. The percentage of fertilization was calculated after 12-hour incubation and the percentage of live embryos after 24-hour incubation. Statistical characteristics were calculated for the numerical data obtained (Table 2).

Analysis of variance was carried out using the least-squares method (Harvey, 1960, 1987) to

estimate the effect of year, ovulation stimulator, provenance of females, and the interaction between the ovulation stimulator and provenance on the investigated traits. The investigated traits were: weight of eggs in grams, weight of eggs expressed as a percentage of female body weight, percentage of egg fertilization after 12-hour incubation and percentage of live embryos after 24 hours. Two additional interactions (between the year of the investigation and the provenance of females and between the year and the ovulation stimulator) were introduced into a linear model in order to answer the question if significant differences in the effects of spawning were the result of these interactions.

Analysis of variance was conducted according to the following linear model:

$$Y_{ijkl} = \alpha + r_i + g_j + p_k + (gp)_{jk} + (rg)_{ij} + (rp)_{ik} + bW_{ijkl} + e_{ijkl} \quad (1)$$

- where:  $\alpha$  = theoretical general mean with the assumption that  $W_{ijkl} = 0$   
 $r_i$  = effect of year ( $i = 1 \dots 2$ )  
 $g_j$  = effect of ovulation stimulator ( $j = 1 \dots 2$ )  
 $p_k$  = effect of the provenance of females ( $k = 1 \dots 2$ )  
 $(gp)_{jk}$  = interaction between the ovulation stimulator and the provenance of the female  
 $(rg)_{ij}$  = interaction between the year and the ovulation stimulator  
 $(rp)_{ik}$  = interaction between the year and the provenance of the female  
 $b$  = regression on female body weight  
 $W_{ijkl}$  = body weight of a female  
 $e_{ijkl}$  = random error connected with observation  $l$

Table 1. Substances and doses used as ovulation stimulators, the method of application and the number of females in groups

Group	No. of females				Ovulation stimulator	Doses/kg body weight of females	Method of application
	year 1998		year 1999				
	strain W	strain 6	strain W	strain 6			
I	5	5	6	6	carp pituitary	0.3 mg and 2.7 mg after 12 h	intraperitoneal injections
II	5	4	6	6	Ovopel*	1/5 pellet and 1 pellet after 12 h	intraperitoneal injections
$\Sigma$	10	9	12	12			

\*1 pellet of Ovopel contains 18–20 µg of mGnRH-a and 8–10 mg of metoclopramide

Table 2. Statistical characteristics of the data

Variable	Descriptive statistics					
	<i>n</i>	$\bar{x}$	S	min	max	SD
Weight of females (kg)						
year 1998	16	8.65	0.78	5.40	15.70	3.11
year 1999	14	11.47	0.38	9.50	14.50	1.42
strain W	16	11.04	0.40	8.50	15.70	1.59
strain 6	14	8.74	0.92	5.40	14.50	3.43
carp pituitary	12	9.83	0.94	5.60	15.70	3.26
Ovopel	18	10.05	0.61	5.40	14.50	2.59
Weight of eggs (g)						
year 1998	16	920.63	134.57	170.00	2 045.00	538.26
year 1999	14	1 468.21	107.52	855.00	1 970.00	406.03
strain W	16	1 284.06	131.80	170.00	2 045.00	527.19
strain 6	14	1 052.86	151.19	280.00	1 970.00	565.68
carp pituitary	12	1 167.50	140.23	670.00	2 045.00	485.75
Ovopel	18	1 181.94	141.55	170.00	1 970.00	600.54
Weight of eggs (% of female body weight)						
year 1998	16	10.65	1.11	1.45	19.85	4.44
year 1999	14	13.02	1.07	6.84	18.48	4.01
strain W	16	11.76	1.26	1.45	19.85	5.05
strain 6	14	11.75	0.95	5.09	17.28	3.55
carp pituitary	12	12.20	1.15	6.84	19.85	3.99
Ovopel	18	11.46	1.10	1.45	18.48	4.66
Fertilized eggs after 12-h incubation						
year 1998	16	95.69	0.84	86.00	99.00	3.36
year 1999	14	93.64	0.95	88.00	98.00	3.54
strain W	16	93.75	0.98	86.00	98.00	3.92
strain 6	14	95.86	0.74	92.00	99.00	2.77
carp pituitary	12	95.83	1.02	88.00	99.00	3.54
Ovopel	18	94.00	0.81	86.00	98.00	3.45
Live embryos after 24-h incubation						
year 1998	16	93.19	1.10	82.00	98.00	4.40
year 1999	14	91.79	0.86	86.00	96.00	3.21
strain W	16	91.75	1.13	82.00	98.00	4.51
strain 6	14	93.43	0.79	89.00	98.00	2.95
carp pituitary	12	94.08	1.13	86.00	98.00	3.92
Ovopel	18	91.50	0.85	82.00	98.00	3.62

$\bar{x}$  = arithmetical mean; S = standard error of the mean; SD = standard deviation

Table 3. Constants (LSC) and least-squares means (LSM) estimated for the investigated traits (A) and characterising the effects of propagation associated with the time of ovulation (B)

Classification factor	Weight of eggs (g)			Weight of eggs (% of female body weight)			Percentage of fertilized eggs after 12 h incubation			Percentage of live embryos after 24 h incubation		
	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE
<b>A</b>												
$\alpha$	1 195.00			11.96			94.74			92.59		
Year												
1998	-279.04	915.96	111.97	-2.58	9.38	1.11	1.93	94.74	1.07	1.63	94.22	1.17
1999	279.04	1474.05	131.06	2.58	14.56	1.30	-1.93	92.81	1.26	-1.63	90.96	1.37
Ovulation stimulator												
carp pituitary (group I)	15.12	1 210.13	106.70	0.47	12.41	1.06	0.64	95.39	1.02	1.04	93.64	1.11
Ovopel (group II)	-15.12	1 179.88	84.54	-0.47	11.50	0.84	-0.64	94.10	0.81	-1.40	91.55	0.88
Provenance of the females												
Hungarian line W	166.28	1 361.29	110.06	1.55	13.53	1.09	-1.62	93.12	1.06	-1.40	91.19	1.15
Polish line 6	-166.28	1 028.72	107.45	-1.55	10.41	1.07	1.62	96.37	1.03	1.40	93.99	1.12
Regression/body weight	-41.53	-41.53	53.02	-1.33	-1.38	0.53	0.68	0.69	0.51	0.65	0.65	0.55
<b>B</b>												
$\alpha$	1 655.54			14.48			93.48			91.38		
Ovopel: time of ovulation												
12 h after first dose	-41.60	1 613.94	250.24	-0.72	13.76	2.14	0.40	93.87	2.71	0.99	92.86	2.09
11 h after second dose	-69.56	1 585.98	203.98	-0.49	13.99	1.74	-2.55	90.93	2.21	-2.16	89.71	1.70
14 h after second dose	111.17	1 766.71	207.38	1.21	15.69	1.77	2.15	95.63	2.24	1.18	93.05	1.73
Regression/body weight	-91.33	-91.33	94.55	-1.92	-1.92	0.81	-0.10	-0.10	-1.02	-0.03	-0.03	0.79

SE = standard error of least-squares means;  $\alpha$  = theoretical general means

The significance of the main factors of the classification for the investigated traits was checked using the *F*-test. The analysis enabled to evaluate the constants and least-squares means representing the values of the individual factors within the main effects investigated (Harvey, 1960, 1987). The estimated least-squares means for the investigated traits are given in Table 3A. The values of interactions between the ovulation stimulator and provenance of the female, between the year and the ovulation stimulator and between the year and provenance of the females, are presented in a graphic form in Figures 1, 2, 3.

In the second year not all the females yielded eggs at the same time. This suggested a question whether the weight and quality of eggs obtained earlier differed from the weight and quality eggs obtained later. The solution of the question was approached by conducting analysis of variance according to the following linear model:

$$Y_{ijk} = \alpha + c_i + p_j + bW_{ijk} + e_{ijk} \quad (2)$$

where:  $\alpha$  = theoretical general mean with the assumption that  $W_{ijk} = 0$

$c_i$  = effect of ovulation time ( $i = 1 \dots 3$ )

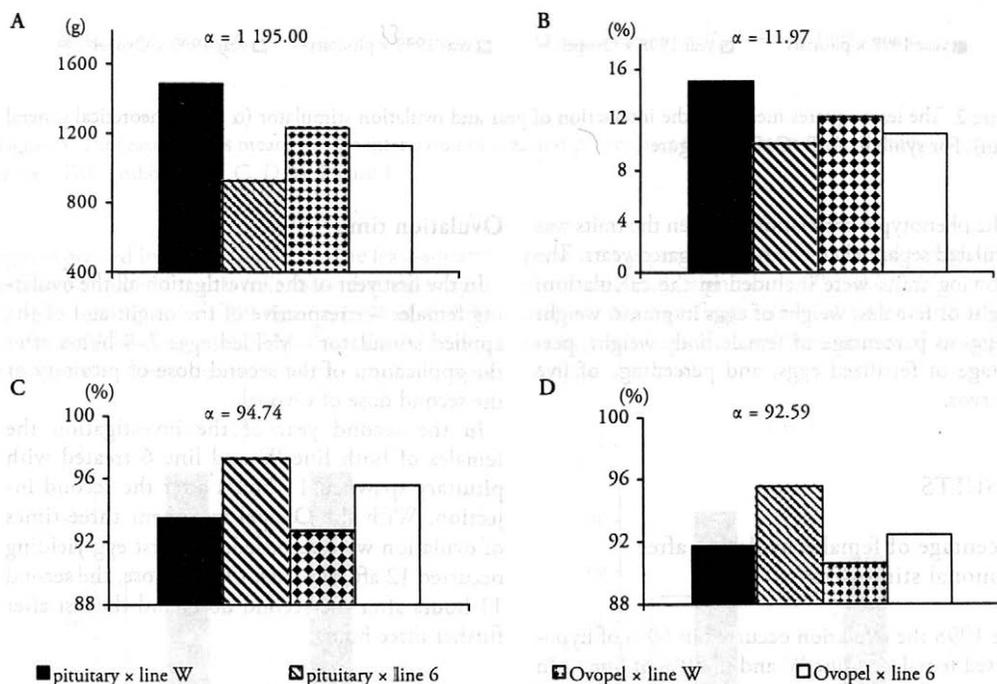
$p_j$  = effect of the provenance of females ( $j = 1 \dots 2$ )

$b$  = regression on female body weight

$W_{ijk}$  = body weight of a female  $k$

$e_{ijk}$  = random error connected with observation  $k$

Analysis of variance conducted according to linear model (2) allowed to estimate the constants and least-squares means describing the effects of reproduction associated with the time of ovulation in fish of the investigated origin treated with Ovopel in the second year of the investigation. The constants and the least-squares means are given in Table 3B.



A – weight of eggs in grams; B – weight of eggs as percentage of female body weight; C – percentage fertilization within 12 h of incubation; D – percentage of live embryos within 24 h of incubation

Figure 1. The least-squares means for the interaction of the ovulation stimulator and provenance of females ( $\alpha$  is the theoretical general mean)

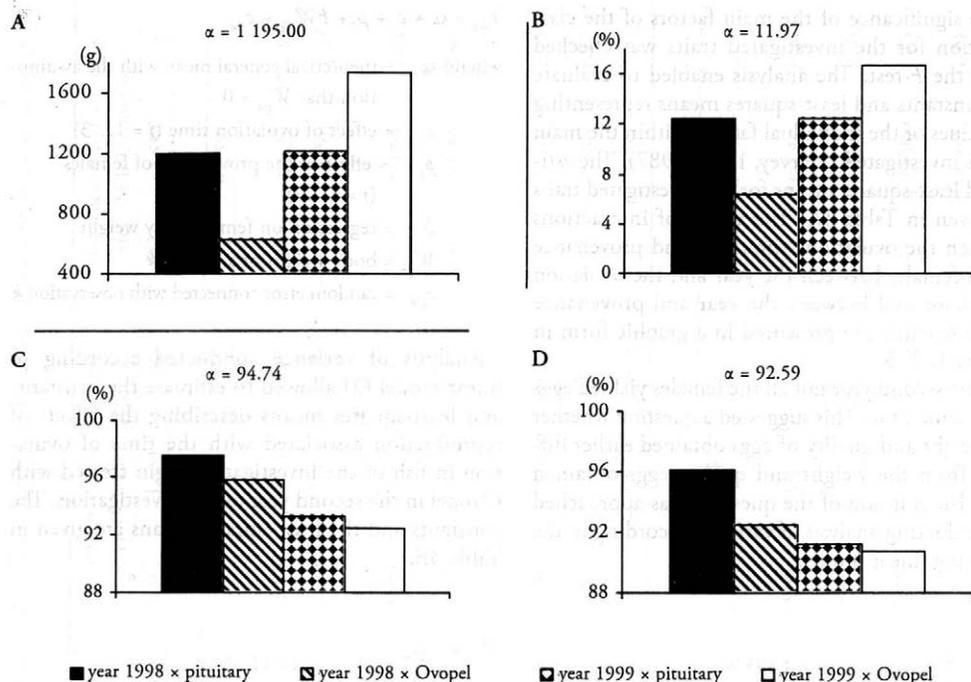


Figure 2. The least-squares means for the interaction of year and ovulation stimulator ( $\alpha$  is the theoretical general mean). For symbols A, B, C, D see Figure 1

The phenotypic correlation between the traits was calculated separately for two investigated years. The following traits were included in the calculation: weight of females, weight of eggs in grams, weight of eggs as percentage of female body weight, percentage of fertilized eggs, and percentage of live embryos.

## RESULTS

### Percentage of females ovulating after hormonal stimulation

In 1998 the ovulation occurred in 60% of hypophysed females of line W and in 80% of line 6. In the case of Ovopel treatment eggs were obtained from all the females of the investigated lines. In 1999 hypophysation induced the ovulation in 50% females of line W and in 33.33% of those of line 6. After Ovopel stimulation the spawning occurred in 83.33% of females of line W and in 66.67% of line 6 (Figure 4).

### Ovulation time

In the first year of the investigation all the ovulating females – irrespective of the origin and of the applied stimulator – yielded eggs 7–8 hours after the application of the second dose of pituitary or the second dose of Ovopel.

In the second year of the investigation the females of both line W and line 6 treated with pituitary spawned 11 hours after the second injection. With the Ovopel treatment three times of ovulation were recorded. The first egg yielding occurred 12 after the first Ovopel dose, the second 11 hours after the second dose, and the last after further three hours.

### Effect of year on the weight and quality of eggs

The results of analysis of variance and *F*-test showed a statistically significant ( $P \leq 0.01$ ) effect of the year of propagation on the weight of obtained

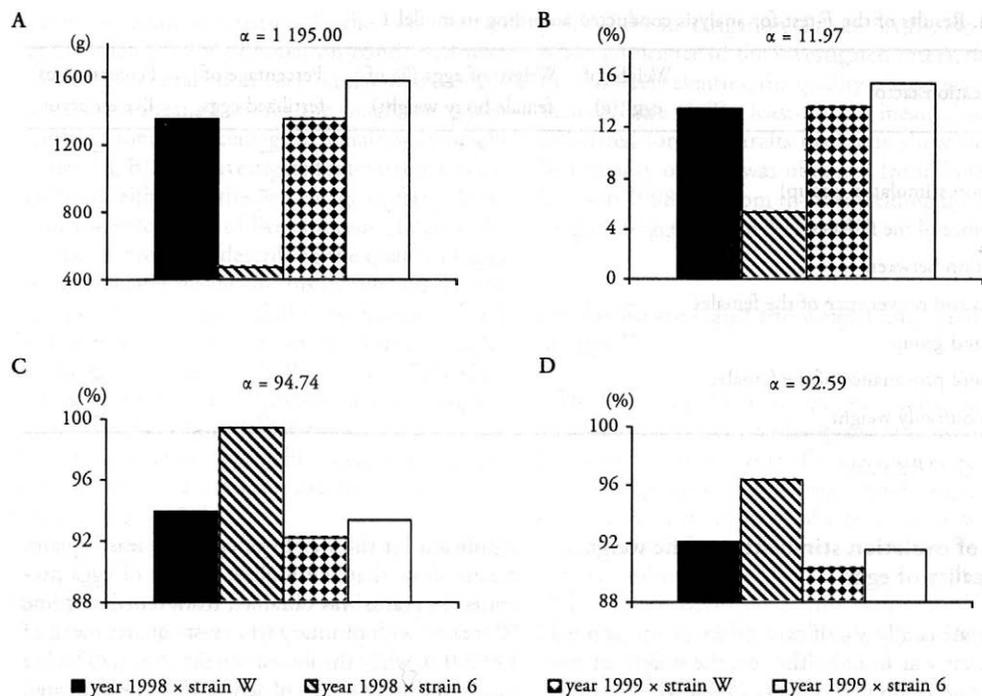


Figure 3. The least-squares means for the interaction of year and provenance of females ( $\alpha$  is the theoretical general mean). For symbols A, B, C, D see Figure 1

eggs expressed in grams (Table 4). The least-squares means estimated for this trait show that the weight of eggs was higher in 1999 than in 1998 (1 474.05 and 915.96, respectively) (Table 3A). The difference between the means determining the weight of eggs

presented as percentages of female body weight was also statistically significant ( $P \leq 0.05$ ) (Tables 3A and 4). No significant effect of this classification factor was ascertained with respect to the quality of eggs (Table 4).

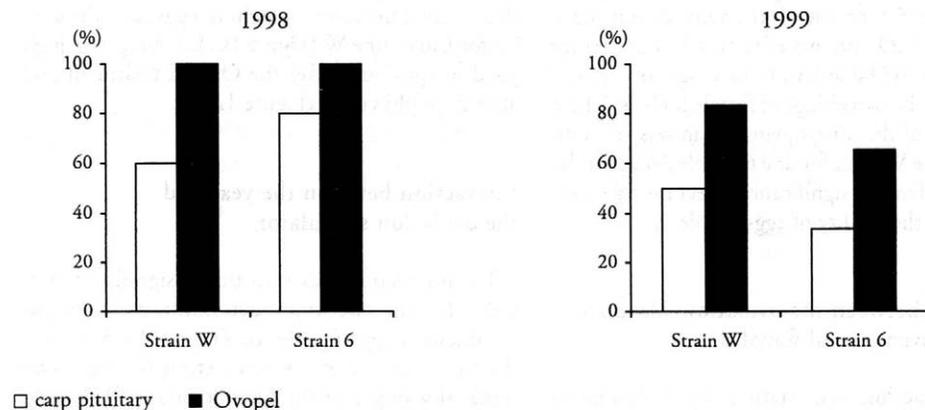


Figure 4. Percentage of ovulating females after hormonal stimulation

Table 4. Results of the *F*-test for analysis conducted according to model 1

Classification factor	Weight of eggs (g)	Weight of eggs (% of female body weight)	Percentage of fertilized eggs	Percentage of live embryos
Year	**	*	–	–
Ovulation stimulator (group)	–	–	–	–
Provenance of the females	–	–	–	–
Interaction between				
group and provenance of the females	–	–	–	–
year and group	**	**	–	–
year and provenance of the females	*	*	–	–
Regression/body weight	–	*	–	–

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$

### Effect of ovulation stimulator on the weight and quality of eggs

No statistically significant effect of the applied stimulator was found either on the weight of obtained eggs or on their quality (Table 4), very similar values of the least-squares means estimated for the four investigated traits being determined in the case of the two stimulators (Table 3A).

### Effect of the origin of females on the weight and quality of eggs

The origin of females used for the propagation did not significantly affect any of the traits investigated (Table 4). However, the values of the least-squares means for the weight of eggs presented in grams show that for fish of line W the value of this mean reached 1 361.29 and exceeded that (1 028.72) for females of line 6 (Table 3A). If the weight of eggs was calculated as the percentage of female body weight, a higher value of the least-squares mean was also estimated for line W than for line 6 (Table 3A). Nor did the origin of females significantly affect the two traits determining the quality of eggs (Table 4).

### Interaction between the ovulation stimulator and the provenance of females

This interaction was statistically insignificant for any of the four investigated traits (Table 4). However, the investigated interaction being in-

significant for the weight of eggs, the least-squares means show that the highest weight of eggs presented in grams was obtained from females of line W treated with pituitary (the least-squares mean of 1 492.00), while the lowest weight of eggs (928.15) was found in the case of females of line 6 treated with the same ovulation stimulator (Figure 1A). The least-squares means of the investigated interaction for the weight of eggs in grams and in percentage of body weight show that in the two lines treated with Ovopel the obtained values did not differ in such a great measure as after hypophysation (Figure 1A,B). The interaction was statistically insignificant also for traits characterising the quality of eggs (Table 4). However, considering the value of the interaction for the percentage of fertilization and percentage of live embryos, the highest value was determined for fish of line 6 (i.e. the females that yielded the lowest weight of eggs) and the lowest for fish of line W (Figure 1C,D) that gave a high yield of eggs both after the Ovopel treatment and after hypophysation (Figure 1A,B).

### Interaction between the year and the ovulation stimulator

The interaction was statistically significant ( $P \leq 0.01$ ) for the two traits determining the weight of obtained eggs (Table 4). Figures 2A,B present the values of the above interaction for these two traits, showing that the highest value of the least-squares mean was found in 1999 for fish treated with Ovopel and the lowest one in 1998 in the

case of the same stimulator. In the two years of the investigation the stimulation conducted with carp pituitary resulted in very similar values of the least-squares means both for the weight of eggs in grams and for the percentage of female body weight (Figure 2A, B). The investigated interaction was insignificant either for the percentage of fertilization or for the percentage of live embryos (Table 4). In the case of two traits describing the quality of eggs the least-squares means for this interaction showed that eggs of the poorest quality (both after 12- and 24-hour incubation) were obtained from females treated with Ovopel in 1999 (Figure 2C,D). This finding concerned the females that yielded the highest weight of eggs. In 1998 both after 12- and 24-hour incubation the highest quality characterised eggs obtained from females treated with carp pituitary (Figure 2C,D).

#### Interaction between the year and the provenance of females

This interaction was statistically significant ( $P \leq 0.05$ ) for the two traits associated with the weight of obtained eggs (Table 4). The values of the interaction both for the weight of eggs in grams and in percentage of female body weight showed that the highest weight of eggs was obtained from females of line 6 which spawned in 1999. The lowest weight of eggs was recorded in the case of females of the same line in the previous spawning season (Figure 3A,B). The least-squares means estimated for these traits show that the value of the interaction for females of line W was almost identical in the two

years of the investigation (Figure 3A,B). No statistical significance of the investigated interaction for the traits representing the quality of eggs was ascertained (Table 4). The least-squares means, however, calculated for these traits distinctly show that the best quality of eggs was obtained from females of line 6 in 1998, i.e. from the fish yielding the lowest weight of eggs (Figure 3A,B,C,D).

#### Ovulation time and the weight and quality of eggs

The values of the least-squares means for three different times of egg yielding after Ovopel stimulation in the second year of the investigation show that the highest weight of eggs (both expressed in grams and in percentage of female body weight) was obtained from the latest ovulation (the respective values being 1 766.71 g and 15.69%) (Table 3B). It occurred within 14 h of the second injection of this stimulator. The weight of eggs obtained within 12 h after the first Ovopel treatment approximated the weight of eggs obtained 11 h after the second injection (the respective values in grams were 1 613.94 and 1 585.98 and in percentages of female body weight 13.76 and 13.99). The analysis of variance and  $F$ -test did not show any significant effect of the ovulation time on the two traits characterising the weight of eggs. This classification factor had no statistically significant effect on the investigated traits characterising the quality of eggs. However, the values of the least-squares means for the fertilization percentage and the percentage of live embryos suggest that the

Table 5. Correlation between the traits characterizing the female body weight and the results of propagation conducted in 1998 (above diagonal) and in 1999 (under diagonal)

Variables	Weight of females (kg)	Weight of eggs (g)	Weight of eggs (% of female body weight)	Percentage of fertilized eggs after 12 h incubation	Percentage of live embryos after 24 h incubation
	1	2	3	4	5
1		0.58*	-0.01	-0.06	0.07
2	-0.05		0.79*	0.02	0.13
3	-0.43	0.92*		0.06	0.12
4	0.31	0.06	-0.04		0.85*
5	0.34	-0.03	-0.19	0.88*	

\*correlation significant at  $P \leq 0.05$

latest eggs showed the best quality (the respective values reaching 95.63% and 93.05%). The poorest quality was found in the case of eggs yielded within 11 h of the second injection of Ovopel (the respective values 90.93% and 89.71%). The quality of eggs yielded after the first injection was good both after 12- and 24-hour incubation, amounting to 93.87% fertilization and 92.86% of live embryos (Table 3B).

### Relations between the investigated traits

The correlation between the investigated traits, calculated separately for the two investigated years, shows that in 1999 as many as a half of the coefficients took a negative symbol. For the year 1998 the coefficient of correlation between the female body weight and the weight of eggs in grams was +0.58, being statistically significant ( $P \leq 0.05$ ). For 1999 the correlation between the two traits mentioned above was insignificant and the coefficient had the value of -0.05. The statistically significant ( $P \leq 0.05$ ) correlation for the two years investigated was found between the percentage of fertilized eggs and that of live embryos while the values of coefficients were +0.85 for the year 1998 and +0.88 for the year 1999 (Table 5).

### DISCUSSION

The obtained results showed that in the two years Ovopel applied to fish of line W and line 6 induced the spawning in a higher percentage of females than that recorded after hypophysation. It should be stressed here that in the first year after the application of Ovopel all the females treated with this stimulator spawned. In relation to the percentage of fish spawning after hypophysation the Ovopel treatment affected a higher percentage of spawning females in the carp of Israeli line Dor-70 and in the crossbreed of this line with Hungarian line 8 (Brzuska and Grzywaczewski, 1999). The same was found in fish of Israeli line Dor-70 (Brzuska, 2001b), in French line F, and in the crossbreed of this line with Hungarian line W (Brzuska and Białowąs, 2002). In various fish species it was also found that the application of Ovopel as an ovulation stimulator induced the spawning in a higher percentage of females than the hypophysation. The data concerning these results were reported for

tench *Tinca tinca* L. (Kłodzińska and Okoniewski, 1988), bream *Abramis brama* L. (Kucharczyk *et al.*, 1998), European catfish *Silurus glanis* L. (Brzuska, 2001c), and African catfish *Clarias gariepinus* Burchell 1822 (Brzuska, 2002).

The results obtained in the present study distinctly show that the year when the controlled reproduction occurred, considerably affected three traits characterizing its effects. These traits are: percentage of ovulating females in relation to all the stimulated fish and the weight of eggs expressed in grams and in percentage of female body weight. The statistically significant effect of the reproduction year on the traits characterising the weight of eggs was also found in earlier investigations on 13 breeding lines of the carp whose ovulation was stimulated with various doses of carp pituitary (Brzuska, 1991). In the first year of the present investigation the percentage of spawning females was higher than in the second year, however the weight of eggs (both in grams and in percentage of female body weight) was much higher in the second year. It is known that heavier females yield eggs of higher weight than the lighter ones. In 1999 the average weight of females was higher, yet the regression on body weight used in the linear model unified the investigated material from the two years with respect to this trait.

The interpretation of the results associated with reproduction year is facilitated by data published by Augustyn (1998, 1999, 2000). The latter author characterized hydrological and meteorological conditions in Gołysz, the greatest attention being paid to water temperature owing to its decisive influence on the life processes of fish. The temperature of water is the chief environmental factor affecting the sexual maturity of fish and regulating the cycle of the development of gonads in adult individuals. For the results of reproduction the temperature in the pond where the fish are kept in the breeding season preceding the spawning, in the winter preceding the spawning, or the distribution of temperatures in spring before the spawners are taken to the hatchery cannot be indifferent. The data given in Figure 5 document the pattern of average temperatures of the whole water layer of Gołysz ponds in the period preceding the controlled fish propagation conducted in 1998 and 1999. In 1998 the last days of April, i.e. the period directly before the spawning, were characterised by fairly high (for that season of the year) water temperature (above 14°C) (Augustyn, personal communication). This

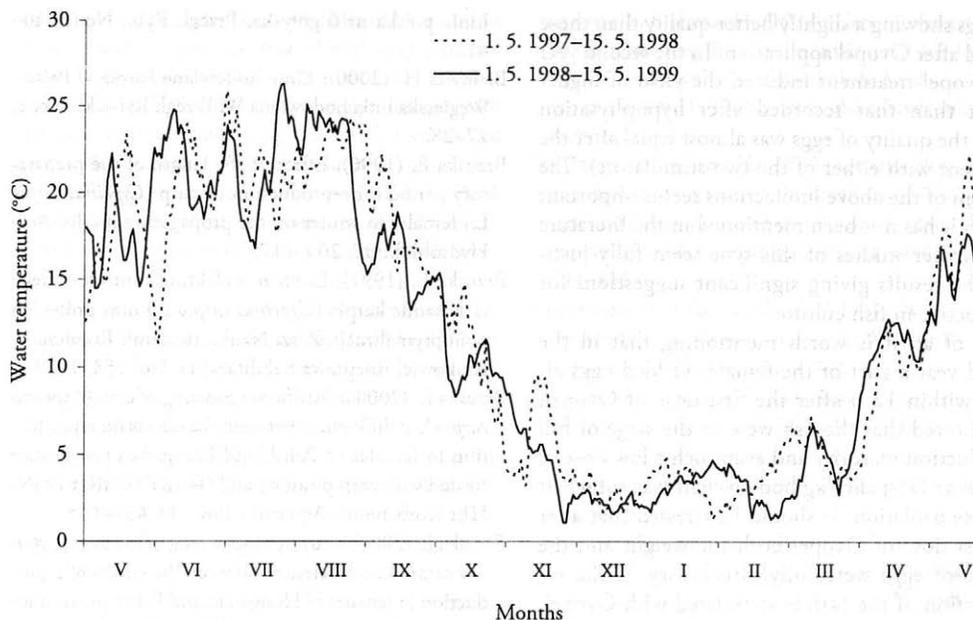


Figure 5. Mean daily pattern of water temperature in the Gołysz ponds (according to Augustyn)

can explain why such a high percentage of females were spawning in that year. In 1999 lower water temperatures characterised this period and the percentage of females yielding eggs was also lower. These observations are in agreement with earlier records by Brzuska (unpublished data).

In the second year of the experiment the high yield of eggs could most probably be attributed to a long period of high temperatures exceeding 19°C in June, July, and in the early part of the last 10 days of August 1998 (Augustyn, 1999). In the period of late spring and in summer there occurs vitellogenesis of this generation of eggs which are yielded by females in the breeding season of the next year. Since in the breeding season of 1997 the temperature of water in ponds decreased below the optimum level for the carp (on the turn of May even below the effective value) (Augustyn, 1998), the weight of produced eggs obtained from females in 1998 was low.

The more detailed data concerning the results of spawning conducted in controlled conditions in fish of different origin stimulated with either of the two stimulators could be obtained on the basis of the interaction between the year and the origin of females and between the year and the ovulation stimulator. The results concern the spawning carried

out in the two-year experiment. With respect to the interaction between the year and the origin of fish the obtained results give more profound information about this interesting dependence. The data show that the weight of eggs yielded by the females of Hungarian origin was similar in the two years of the investigation. The quality of eggs from the fish of line W did not differ in the two years either. On the other hand, the weight of eggs in grams and in percentage of female body weight considerably differed in the case of the females of Polish line 6. In 1998 the weight of eggs obtained from the fish of line 6 was much lower while the quality of eggs was much better than in the second year of the investigation. These records show that the effect of year on the traits characterizing the results of reproduction was stronger with females of Polish origin. The above data suggest a more general statement that the effect of year on the reproduction results is not the same in fish of different origin, the obvious condition being that the fish were kept in uniform environmental conditions.

An additional interesting piece of information was supplied by the results of investigation on the interaction between the year and the applied stimulator. In the first year a higher weight of eggs was obtained after pituitary treatment of the fish,

the eggs showing a slightly better quality than those yielded after Ovopel application. In the second year the Ovopel treatment induced the yield of higher weight than that recorded after hypophysation (while the quality of eggs was almost equal after the treatment with either of the two stimulators). The problem of the above interactions seems important though it has not been mentioned in the literature yet. Further studies of this type seem fully justified, the results giving significant suggestions for the practice in fish culture.

Last of all it is worth mentioning that in the second year a part of the females yielded eggs already within 12 h after the first dose of Ovopel. This showed that the fish were in the stage of full reproduction maturity and even such a low dose of Ovopel as 1/5 pellet/kg body weight was sufficient to cause ovulation. It should be stressed that after the first dose of Ovopel both the weight and the quality of eggs were fully satisfactory. If the reproduction of the carp is stimulated with Ovopel, the possibility of egg yielding by females after the first dose of this stimulator should be taken into consideration as it had already been reported by Kłodzińska and Okoniewski (1998).

### Acknowledgements

The author thanks Mr J. Adamek and Mrs U. Czudek working in the Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences in Gołysz, for technical assistance in the experiments, and Mrs U. Karosek for careful technical preparation of the manuscript. I also thank Dr. D. Augustyn for making available the data concerning the pattern of water temperatures in Gołysz ponds.

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Received: 02–05–09

Accepted after corrections: 03–04–06

## ABSTRAKT

Umělý výtěr kapra (*Cyprinus carpio*): výsledky dvouleté reprodukce jikernaček maďarské linie W a polské linie 6 po stimulaci ovulace kapří hypofýzou a přípravkem obsahujícím mGnRH-a a dopaminergní inhibitor

Byla zkoumána reprodukce jikernaček maďarské linie W a polské linie 6 ve dvou po sobě následujících letech. Jikernačky obojího původu byly k ovulaci stimulovány jednak kapří hypofýzou (I. dávka 0,3 mg/kg a za 12 h II. dávka 2,7 mg/kg), jednak přípravkem Ovopel – mGnRH-a a metoclopramid (I. dávka 1/5 pelety/kg a za 12 h II. dávka 1 peleta/kg). Bez ohledu na způsob stimulace ovulace bylo u maďarské i polské linie dosaženo vyšší procento ovulujících jikernaček v prvním roce sledování. V obou letech bylo získáno více jiker u jikernaček obojího původu při použití přípravku Ovopel, než při použití hypofýzy. Vliv roku byl na hmotnost vytřených jiker statisticky průkazný při hodnocení jak v gramech, tak v procentech hmotnosti vztažené k hmotnosti jikernaček ( $P \leq 0,01$  a  $P \leq 0,05$ ), při neprůkazném vlivu na kvalitu jiker. Stimulátor ovulace, původ jikernaček a interakce mezi stimulatory a původem, neměly statisticky průkazný vliv na hmotnost a kvalitu jiker. Interakce mezi rokem a stimulatory ovulace a mezi rokem a původem jikernaček měly statisticky průkazný vliv ( $P \leq 0,01$  a  $P \leq 0,05$ ) jen na hmotnost vytřených jiker.

**Klíčová slova:** umělý výtěr; kapr; hypofýza; Ovopel (mGnRH-a a metoclopramid); původ jikernaček; vliv roku

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# Artificial propagation of European catfish (*Silurus glanis*): application of a single dose of pellets containing D-Ala<sup>6</sup>, Pro<sup>9</sup>NEt-mGnRH and dopamine inhibitor metoclopramide to stimulate ovulation in females of different body weight

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**ABSTRACT:** Effects of reproduction of European catfish females of different body weight (4.60–11.00 kg) were investigated in two experiments after ovulation stimulation with two doses of carp pituitary (0.4 + 3.6 mg/kg body weight) or a single dose of Ovopel – a preparation containing mammalian GnRH analogue D-Ala<sup>6</sup>, Pro<sup>9</sup>NEt-mGnRH and dopamine receptor antagonist, metoclopramide (1 pellet/kg body weight). It was found in both experiments that all the lighter females spawned after Ovopel treatment while the percentage of spawning females of higher body weight treated with this ovulation stimulator was lower. No statistically significant effect of the experiment was observed with respect to traits characterising the weight of eggs or their quality. The effect of applied ovulation stimulator was statistically significant ( $P \leq 0.01$ ) for the percentage of live embryos after 56-h incubation, however the eggs obtained after Ovopel treatment were characterised by higher quality. The body weight of the females significantly ( $P \leq 0.05$ ) affected the weight of obtained eggs, though expressed in grams only, and the percentage of live embryos after 56-h incubation ( $P \leq 0.01$ ). The interaction between the ovulation stimulator and the body weight of females did not affect the traits determining the weight of eggs or the percentage of live embryos after 24- and 48-h incubation. However, it was statistically significant ( $P \leq 0.01$ ) for the percentage of live embryos after 56-h incubation.

**Keywords:** European catfish; artificial propagation; carp pituitary; single dose of Ovopel

In the course of long-term studies on the effects of controlled reproduction of females of various fish species, conducted in the Gołysz Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences (Brzuska and Adamek, 1997; Brzuska *et al.*, 1998; Brzuska, 1999a,b; Brzuska and Grzywaczewski, 1999; Brzuska, 2000a,b; 2001a,b, 2002) experiments are also carried out with European catfish (Brzuska and Adamek, 1999; Brzuska, 2001c). The results reported by different authors (Epler *et al.*, 1986; Kouřil *et al.*, 1987, 1996; Epler and Bieniarz, 1989; Linhart *et al.*, 1997) and the results of tests conducted in Gołysz (Brzuska and Adamek, 1999; Brzuska,

2001c) show that like in the case of numerous fish species of economic importance ovulation in the females of European catfish can also be stimulated using synthetic stimulators and give interesting results. Quite a small number of data in the literature concerning stimulated reproduction of this valuable fish species justifies further studies within the scope of this problem.

Ovopel (Horváth and Szabó, 1996; Horváth *et al.*, 1997) was found effective in the stimulation of ovulation in European catfish not only in the natural season of spawning (Kłodzińska and Okoniewski, 1998; Brzuska, 2001c) but also outside it (Ulikowski, 2001). Good effects

on reproduction of numerous fish species after Ovopel treatment (see review in Brzuska, 2001c) and numerous favourable traits of this stimulator (Kłodzińska and Okoniewski, 1998; Kucharczyk and Szabó, 1998) encourage further experiments with its use.

The application of a single dose of Ovopel gave interesting results in the reproduction of African catfish (*Clarias gariepinus*) (Brzuska *et al.*, 1998, 2000; Brzuska, 2002), instigating new studies on the ovulation stimulation in European catfish without a priming dose of this preparation. In the case of controlled spawning the treatment with one dose of this stimulator limits the necessary manipulations that are a source of stress to fish. The high costs connected with the reproduction of thermophilous fish in the conditions of hatcheries could also be reduced. These important aspects were taken into consideration in the project of the present investigation.

The aim of the presented investigation was to determine the possibility of obtaining eggs from European catfish treated only with one dose of Ovopel and whether the effects of reproduction after Ovopel application differed from those observed after traditional double hypophysation. The results of previous investigation conducted on this species showed that the spawning stimulated with carp pituitary or Ovopel applied in two doses (as recommended by Horváth *et al.*, 1997) was significantly affected by the weight of females used for reproduction (Brzuska, 2001c). Therefore in the present study it was attempted to determine the dependence between the body weight of females used for reproduction and the effects of spawning

after the stimulation of ovulation with a single dose of Ovopel or carp pituitary.

## MATERIAL AND METHODS

The data constituting the material of the study were derived from two experiments carried out in the season of natural spawning of this species, i.e. in June. The pooled data from the experiments conducted in the same conditions allowed to increase the number of investigated females. It was also attempted to determine whether the results obtained in one experiment significantly differed from those recorded in the other. In both experiments directly after the fish were taken from ponds, females were selected out of a larger population on the basis of external signs of maturity (Horoszewicz, 1971). They were transferred to a hatchery and divided into two groups. In both experiments a half of each group was composed of individuals of lower body weight and the other half of those of higher body weight (Table 1). The fish were placed in tanks (2.5 m<sup>3</sup> in volume) at the hatchery, two individuals in one tank (the one of lower and the other of higher body weight) in water at 22–23°C. After one day adaptation period ovulation stimulation began, the fish in group I being treated with carp pituitary homogenate and those in group II with Ovopel. The preparation Ovopel contains the mammalian GnRH analogue (D-Ala<sup>6</sup>, Pro<sup>9</sup>NET-mGnRH) and metoclopramide – a water-soluble blocker of dopamine receptors (Horváth and Szabó, 1996; Horváth *et al.*, 1997). The applied doses of the two stimulators of ovulation are given in Table 1. Both

Table 1. Numbers and average weight of females used in the investigation in group I and II of the experiments, the applied ovulation stimulators and their doses ( $\bar{x}$  = arithmetical mean; SD = standard deviation)

Experiment	Group	No. of females <i>n</i> = 24	Lighter females $\bar{x} \pm \text{SD}$	Heavier females $\bar{x} \pm \text{SD}$	Ovulation stimulator	Doses/kg body weight of females
1	I	6	5.30 ± 0.89	8.90 ± 1.11	carp pituitary	0.4 mg and 3.6 mg after 12 h
	II	8	6.05 ± 0.33	10.20 ± 0.93	Ovopel	1 pellet
2	I	4	6.30 ± 0.99	9.05 ± 0.35	carp pituitary	0.4 mg and 3.6 mg after 12 h
	II	6	6.47 ± 0.76	10.07 ± 0.83	Ovopel	1 pellet

the pituitary and Ovopel were applied to fish in the form of intraperitoneal injections.

A slight manual pressure on the abdomen of females (Littak and Okoniewski, 1975; Brzuska and Adamek, 1999; Brzuska, 2001c) was carried out to check ovulation. In both experiments the control

was started within 10 hrs after the second dose of pituitary and 10 hrs after Ovopel treatment. The checking was continued every hour during the successive six hours. Eggs were taken from each female separately, weighed and fertilized with mixed milt of controlled quality, taken from macerated testes

Table 2. Statistical characteristics of the data

Variable	Descriptive statistics					
	<i>n</i>	$\bar{x}$	$\bar{s}$	min	max	SD
Weight of females (kg)						
group I lighter females	5	5.70	0.43	4.60	7.00	0.97
heavier females	5	8.96	0.36	7.80	10.00	0.80
group II lighter females	7	6.23	0.21	5.60	7.00	0.54
heavier females	7	10.14	0.31	9.30	11.00	0.82
Weight of eggs (g)						
group I lighter females	4	535.00	125.12	265.00	805.00	250.23
heavier females	5	941.25	37.33	875.00	1 020.00	74.65
group II lighter females	7	655.00	99.30	255.00	920.00	262.71
heavier females	5	790.00	151.92	350.00	1 250.00	339.71
Weight of eggs (% of female body weight)						
group I lighter females	4	8.74	2.39	2.92	13.60	4.77
heavier females	5	9.29	0.66	8.31	11.20	1.33
group II lighter females	7	10.35	1.62	4.25	16.12	4.29
heavier females	5	7.67	1.29	3.68	11.30	2.89
Live embryos after 24-h incubation						
group I lighter females	4	85.75	5.95	68.00	93.00	11.89
heavier females	5	89.50	1.94	84.00	93.00	3.87
group II lighter females	7	92.57	1.04	90.00	98.00	2.76
heavier females	5	90.80	1.16	88.00	94.00	2.59
Live embryos after 48-h incubation						
group I lighter females	4	81.25	5.86	65.00	93.00	11.73
heavier females	5	83.50	1.32	80.00	86.00	2.65
group II lighter females heavier females	7	84.85	1.20	82.00	91.00	3.18
heavier females	5	80.60	1.78	76.00	87.00	3.97
Live embryos after 56-h incubation						
group I lighter females	4	61.75	3.33	54.00	70.00	6.65
heavier females	5	62.00	3.67	56.00	72.00	7.35
group II lighter females	7	81.71	0.94	79.00	85.00	2.50
heavier females	5	62.20	4.10	50.00	70.00	9.18

$\bar{x}$  = arithmetical mean;  $\bar{s}$  = standard error of the mean; SD = standard deviation

of several killed males. The males used for reproduction had been hypophysectomized with carp pituitary homogenate applied intramuscularly at a dose of 3.6 mg/kg body weight.

The incubation of fertilized eggs obtained in the two experiments was conducted in a Weiss glass for each female separately in water at 22–23°C. After 24, 48, and 56 hrs of egg incubation the percentage of live embryos was calculated using the method given by Brzuska and Adamek (1999). After the hatching of larvae the percentage of deformed individuals was also calculated for each female separately.

The statistical characterisation of data was presented in Table 2. The data obtained from the two experiments were subjected to analysis of variance using the least-squares method (Harvey, 1960, 1987). The aim of analysis was to estimate the effect of the main classification factors (experiment, ovulation stimulator, and female body weight) on the investigated traits. The investigated traits were: weight of eggs expressed in grams and in percentage of female body weight and percentage of live embryos after 24-, 48-, and 56-hour incubation. Analysis of variance was carried out according to a linear model also including the interaction between the ovulation stimulator (group) and the female body weight.

Analysis of variance was conducted according to the following linear model:

$$Y_{ijkl} = \mu + a_i + g_j + c_k + (gc)_{ij} + e_{ijkl}$$

where:  $\mu$  = overall mean

$a_i$  = effect of an experiment ( $i = 1 \dots 2$ )

$g_j$  = effect of an ovulation stimulator ( $j = 1 \dots 2$ )

$c_k$  = effect of the size of the female ( $k = 1 \dots 2$ )

$(gc)_{ij}$  = interaction between the ovulation stimulator and the size of the female

$e_{ijkl}$  = random error connected with observation  $l$

The significance of the effect of an experiment, group connected with an ovulation stimulator, body weight of the females, and the interaction between the ovulation stimulator and size of the female on the investigated traits was checked using the  $F$ -test. Analysis of the data allowed to estimate constants and least-squares means illustrating the values of the individual traits investigated within the main effects determined. The constants and

least-squares means are given in Table 3. The values of the least-squares means for the investigated interaction are presented in graphic form in Figure 1.

## RESULTS

### Percentage of females ovulating after hormonal stimulation

In experiment 1, 66.67% of lighter and 100% of heavier fish spawned after the pituitary treatment. After the application of Ovopel all the fish of lower body weight and 75% of heavier females spawned (Figure 1).

In experiment 2, eggs were obtained from all the fish, both of lower and higher body weight, treated with pituitary and from 100% of lighter fish treated with Ovopel. After Ovopel application eggs were obtained from 66.67% of the females of higher body weight (Figure 2).

### Ovulation time

The time between the second hypophysectomy and ovulation was 11 h for fish from group I in the first and second experiment. After Ovopel application all the females that passed ovulation in the two experiments yielded eggs within 15 h after the stimulator was injected.

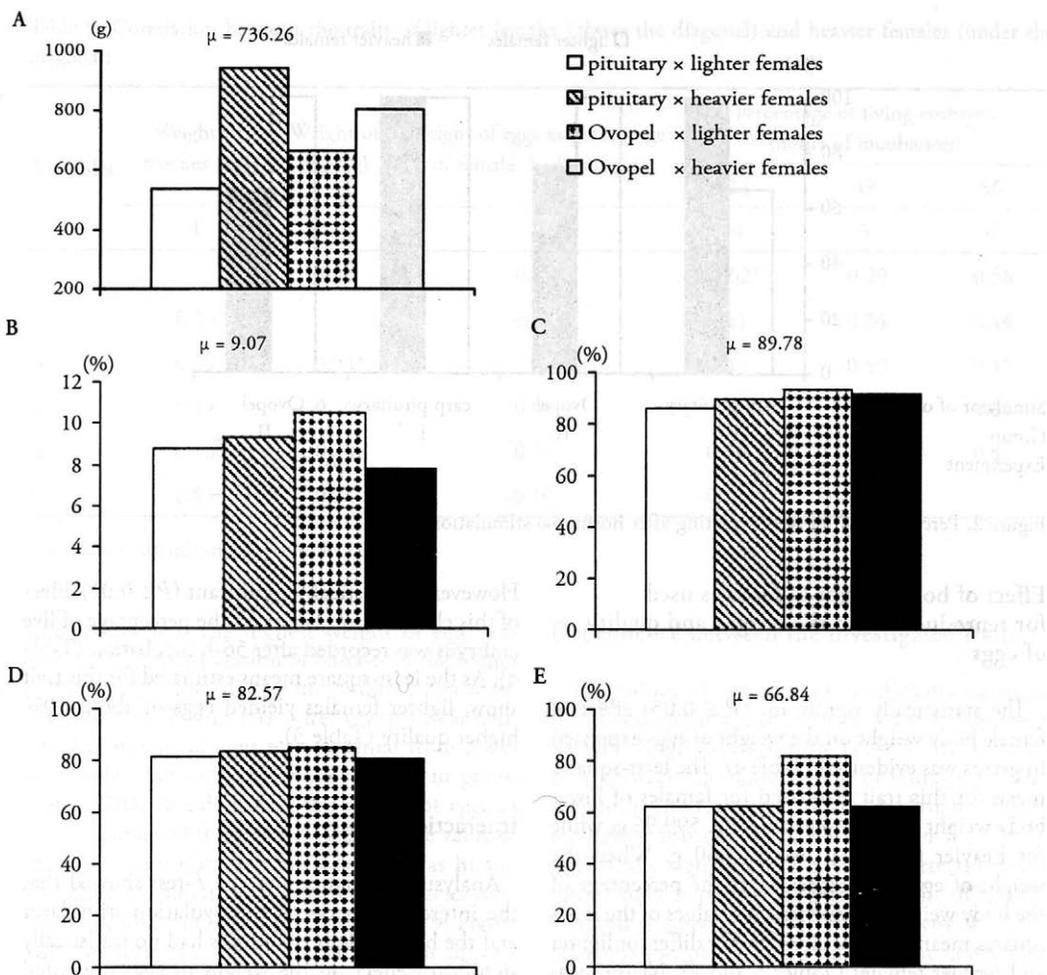
### Effect of experiment on the weight and quality of eggs

The results of analysis of variance and the  $F$ -test did not show any significant effect of the experiment on the traits determining the weight or quality of obtained eggs (Table 4). It should be mentioned, however, that with respect to the least-squares means determining the weight of eggs (both in grams and in percentage of female body weight) the obtained values show a higher weight of eggs recorded in experiment 2. In this experiment the constant of the least squares for the weight of eggs in grams manifested a deviation of +69.44 from the overall mean. For the weight of eggs expressed as percentage of female body weight the value of the deviation was only +0.58 (Table 3). As the values of the least-squares means estimated for the percent-

Table 3. Constants (LSC) and least-square means (LSM) estimated for investigated traits

Classification factor	Weight of eggs (g) $\mu = 736.26$			Weight of eggs (% of female body weight) $\mu = 9.07$			Percentage of living embryos								
							24-h incubation $\mu = 89.78$			48-h incubation $\mu = 82.57$			56-h incubation $\mu = 66.84$		
	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE
Experiment															
1	-69.44	666.82	79.09	-0.58	8.48	1.16	-1.44	88.34	1.78	-0.18	82.39	1.88	0.92	67.76	2.03
2	69.44	805.70	86.03	0.58	9.65	1.26	1.44	91.22	1.94	0.18	82.74	2.05	-0.92	65.92	2.21
Ovulation stimulator															
carp pituitary (group I)	1.86	738.13	90.62	-0.05	9.02	1.32	-2.15	87.63	2.04	-0.19	82.38	2.15	-4.96	61.88	2.33
Ovopel (group II)	-1.86	734.40	75.69	0.05	9.11	1.11	2.15	91.93	1.71	0.19	82.76	1.80	4.96	71.80	1.95
Body weight of fish															
lighter females	-136.30	599.96	80.43	0.52	9.59	1.18	-0.52	89.26	1.81	0.50	83.07	1.91	4.83	71.67	2.07
heavier females	136.30	872.60	86.17	-0.52	8.54	1.26	0.52	90.29	1.94	-0.50	82.07	2.05	-4.83	62.01	2.22

SE = standard error of least-squares means;  $\mu$  = overall mean



A – weight of eggs in grams; B – weight of eggs as percentage of female body weight; C – percentage of live embryos within 24-h of incubation; D – percentage of live embryos within 48-h of incubation; E – percentage of live embryos within 56-h of incubation

Figure 1. Least-squares means for the interaction of ovulation stimulator and body weight of females ( $\mu$  is overall mean)

age of live embryos after 24-, 48-, and 56-hour incubation show, the quality of eggs was very similar in the two experiments (Table 3).

#### Effect of ovulation stimulator on the weight and quality of eggs

No statistically significant effect of the ovulation stimulator was observed with respect to any of the

two traits determining the weight of obtained eggs or percentage of egg fertilization after 24- and 48-hour incubation. The effect of the ovulation stimulator was only significant ( $P \leq 0.01$ ) with respect to egg quality after 56-hour incubation (Table 4). As the values of the least-square means for this trait show, eggs obtained after Ovopel treatment were of much higher quality than those obtained after hypophysation (the respective values being 71.8 and 61.9; Table 3).

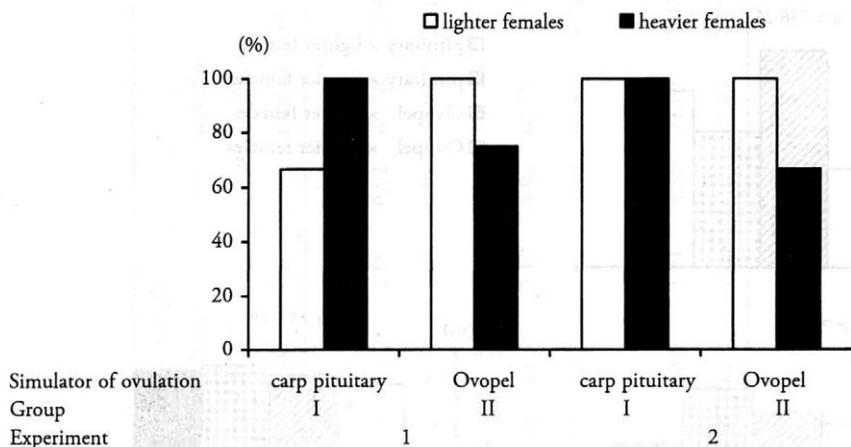


Figure 2. Percentage of females ovulating after hormonal stimulation

### Effect of body weight of females used for reproduction on the weight and quality of eggs

The statistically significant ( $P \leq 0.05$ ) effect of female body weight on the weight of eggs expressed in grams was evidenced (Table 4). The least-squares mean for this trait estimated for females of lower body weight had a lower value, i.e. 599.96 g, while for heavier females it was 872.60 g. When the weight of eggs was expressed as the percentage of the body weight of spawners, the values of the least-squares means did not significantly differ for lighter and heavier females (Tables 3 and 4). Neither was the effect of the body weight of spawners statistically significant with respect to the percentage of live embryos after 24- and 48-h incubation.

However, a statistically significant ( $P \leq 0.01$ ) effect of this classification factor on the percentage of live embryos was recorded after 56-h incubation (Table 4). As the least-square means estimated for this trait show, lighter females yielded eggs of about 10% higher quality (Table 3).

### Interaction

Analysis of variance and the  $F$ -test showed that the interaction between the ovulation stimulator and the body weight of females had no statistically significant effect on the weight of eggs presented either in grams or in percentage of female body weight (Table 4). The values of the investigated interaction for the weight of eggs expressed in

Table 4. Results of the  $F$ -test

Classification factor	Weight of eggs (g)	Weight of eggs (% of female body weight)	Percentage of living embryos (hours of incubation)		
			24	48	56
Experiment	–	–	–	–	–
Ovulation stimulator	–	–	–	–	**
Body weight of females	*	–	–	–	**
Interaction between ovulation stimulator and female body weight	–	–	–	–	**

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$

Table 5. Correlation between the traits of lighter females (above the diagonal) and heavier females (under the diagonal)

Variables	Weight of females (kg)	Weight of eggs (g)	Weight of eggs as percentage of female body weight	Percentage of living embryos (hours of incubation)		
				24	48	56
	1	2	3	4	5	6
1		0.45	0.26	0.62*	0.29	0.58
2	0.25		0.95*	0.41	0.34	0.44
3	0.09	0.93*		0.47	0.50	0.45
4	0.14	-0.16	0.04		0.85*	0.66*
5	0.06	0.24	0.26	0.46		0.52
6	0.53	0.01	-0.16	-0.22	0.31	

\*correlation significant at  $P \leq 0.05$

grams show that the highest weight of eggs was found after hypophysation of females of the higher weight and the lowest after the pituitary treatment of lighter fish (Figure 1A). After Ovopel treatment a higher weight of eggs was obtained from heavier females, but only if it was expressed in grams (Figure 1A). In calculating the weight of eggs as the percentage of female body weight, the value of the interaction obtained for this trait was higher for lighter females in comparison with heavier ones (the respective values being 10.44 and 7.79; Figure 1B). The least-squares means for this trait showed that for lighter females treated with Ovopel the interaction attained the highest value exceeding 10% (Figure 1B). No statistically significant effect of the investigated interaction on the percentage of live embryos after 24- or 48-h incubation of eggs was recorded (Table 4). The data presented in Figure 1C, however, distinctly show that already after 24-h incubation the best quality characterised eggs obtained from lighter females treated with Ovopel. The statistically significant ( $P \leq 0.01$ ) effect of the interaction was found for the percentage of live embryos after 56-h incubation (Table 4). The presented values of the least-squares means estimated for this trait show the highest quality of eggs (81.6% of live embryos after 56-h incubation) obtained from females of lower body weight stimulated with Ovopel in comparison with the remaining values investigated, which varied about 62% (Figure 1E).

#### Dependence between the investigated traits

The values of correlation coefficients between the investigated traits estimated for lighter and heavier females are given in Table 5. The correlation between the body weight of females and the remaining five traits investigated had higher values for lighter fish compared with heavier females. A statistically significant ( $P \leq 0.05$ ) correlation was found only between the body weight of lighter fish and the percentage of live embryos after 24-h incubation of eggs, the value of the coefficient being 0.62. In lighter fish a statistically significant ( $P \leq 0.05$ ) correlation was found between the percentage of live embryos after 24-h incubation and the percentage of live embryos after 48-h incubation and also between the percentage of live embryos after 24-h incubation and the percentage of live embryos after 56 h incubation of eggs (the respective values being 0.85 and 0.66). All the correlation coefficients estimated for lighter fish were characterised by positive values. In heavier females negative correlation values were recorded between the weight of eggs in grams and the percentage of live embryos after 24-h incubation, between the weight of eggs expressed as percentage of body weight of females and the percentage of live embryos after 56-h incubation, and between the percentage of live embryos after 24-h incubation and the percentage of live embryos after 56-h incubation.

## Occurrence of deformed larvae

The occurrence of deformed larvae not exceeding four individuals per 100 ones was observed after the application of the two ovulation stimulators. The body deformations were observed only among the larvae hatched from eggs obtained from heavier females.

## DISCUSSION

From the aspect of hatchery practice a very important result was obtained in the present investigation showing that in both experiments all the females spawning after Ovopel treatment yielded eggs at the same time, i.e. 15 h after injection of one dose of this stimulator in the amount of 1 pellet/kg. Synchronized ovulation in all the females after stimulation with one dose of Ovopel (1 pellet/kg) was also found in the investigation conducted on African catfish (*Clarias gariepinus*) (Brzuska *et al.*, 1998, 2000; Brzuska, 2001b). The same time of egg yielding by all the females of European catfish treated with one dose of Ovopel is important because after the application of two doses of Ovopel (1/5 + 1 pellet/kg female body weight) the spawning time was not the same for all the investigated females of this species (Brzuska, 2001c). It should be stressed that the above experiment was carried out in conditions corresponding with those of the present study. After the treatment with two doses most females yielded eggs within 11 h after the second Ovopel dose and the remaining ones two hours later (Brzuska, 2001c).

The varied time of egg yielding, disorganising the work of the hatchery, was also recorded by Brzuska and Adamek (1999) in European catfish females treated with des-Gly<sup>10</sup>, [D-Ala<sup>6</sup>] LHRH-Ethylamide + pimozone or with Ovaprim [(D-Arg<sup>6</sup>, Pro<sup>9</sup>-NEt) sGnRH + domperidone; Peter *et al.*, 1993]. In the case of the former stimulator the latency time ranged from 26 to 30 h while after Ovaprim treatment from 24 to 30 h. Neither did Kouřil *et al.* (1996) obtain the synchronised time of ovulation in all fish of the investigated groups treated with des-Gly<sup>10</sup>(D-Ala<sup>6</sup>) GnRH-Ethylamide (at the dose twice higher than that applied by Brzuska and Adamek, 1999) without the blocker of dopamine receptors or with Isofloxythepin, a dopamine inhibitor. The latency time for this stimulator, as reported by Kouřil *et al.* (1996), was also very long, ranging about 30 hours.

The latency time naturally depends on the temperature of water in which fish are kept (Drori *et al.*, 1994) although in all the experiments described above the temperature showed optimum values for the reproduction of European catfish, i.e. about 23°C. It should be mentioned as well that the synchronization of egg yielding by all the females stimulated with a given preparation is possible only if the degree of maturity of the females is similar at the time of treatment.

From the practical standpoint it seems worth stressing that in European catfish the ovulation stimulation with Ovopel at one or two doses makes the occurrence of ovulation probable after a much shorter time than after GnRH-a treatment with dopamine receptor blocker or without it. It was suggested by the results obtained by the following authors: Epler *et al.* (1986), Epler and Bieniarz (1989), Kouřil *et al.* (1987, 1996), Kłodzinska and Okoniewski (1998), Brzuska and Adamek (1999), Brzuska (2001c) and present data; Ulikowski (2001).

The results concerning the percentage of spawning fish in relation to all the individuals treated with stimulators show that the application of one Ovopel dose to lighter females led to ovulation in all of them in both experiments discussed in this paper. Heavier fish responded to the synthetic stimulator to a lower degree as expressed by the lower percentage of spawning females. The above observation corroborates the results of an earlier study carried out on the same fish species with two doses of Ovopel (1/5 + 1 pellet/kg) (Brzuska, 2001c). The comparison of results concerning the percentage of spawning females after one or two doses of Ovopel (Brzuska, 2001c) distinctly shows that a decrease in the amount of the stimulator by omitting the priming dose did not negatively affect this important trait determining the reproduction efficiency of spawners.

The data obtained from the present investigation clearly show that the results of reproduction depend on whether the ovulation stimulation was carried out on heavier or lighter females. The values of interaction between the ovulation stimulator and the weight of evaluated fish for the investigated traits illustrate the effects of reproduction separately for heavier and lighter females.

Analysis of the least-squares means for the investigated interaction shows that the weight of eggs expressed in grams did not depend on the ovulation stimulator applied but on the body weight of spawning females. Heavier females stimulated

either with pituitary or with Ovopel yielded eggs of higher weight in comparison with lighter fish. However, in the case of one-dose Ovopel treatment of lighter females the weight of eggs expressed as a percentage of female body weight was much higher than that obtained from heavier females.

The values of interaction between the ovulation stimulator and weight of females for the percentage of live embryos after 24-hour incubation were higher in fish treated with Ovopel irrespective of their higher or lower weight. After 56-hour incubation of eggs, however, these obtained from heavier fish were characterised by about 20% poorer quality than the eggs yielded by lighter females. It should be stressed that after a single-dose Ovopel treatment lighter fish yielded eggs of the best quality out of the four combinations investigated. The same observation was made when the ovulation stimulation was carried out with two doses of Ovopel (Brzuska, 2001c). In the investigation on African catfish (*Clarias gariepinus*) the application of a single dose of Ovopel also resulted in the yield of eggs of better quality in comparison with those obtained from heavier females (Brzuska, 2001b).

Another interesting observation also made on the basis of the present work shows that after 56-hour incubation the quality of eggs yielded by lighter or heavier hypophysectomized females did not differ. This information agrees with the results of studies conducted on African catfish (*Clarias gariepinus*) though the percentage of live embryos of this species was calculated only after 24-hour incubation (Brzuska, 2002).

In the discussed investigation containing the results of two separate experiments an important finding was that no eggs of very poor quality were yielded either by lighter or by heavier fish treated with Ovopel. This could be determined already after 24-hour incubation. The above observation is in agreement with data obtained after applying this ovulation stimulator though at a higher amount and at two doses (Brzuska, 2001c). In the case of Ovaprim application to females of this species 20% of them yielded eggs, which showed 0–20% of live embryos after 24-hour incubation (Brzuska and Adamek, 1999).

The problem of reproduction effects observed in lighter and heavier females is important from the aspect of practice. As the presented data show after ovulation stimulation with a single dose of Ovopel (greatly reducing the stress to fish and the labour-consuming operations necessary in controlled re-

production) better results were recorded in lighter females than in the heavier ones. Higher percentage of lighter females spawned and the obtained eggs were of better quality. It seems important that no deformed larvae hatched from eggs obtained from lighter fish. In analysing the obtained results there arises a question whether it is worthwhile to rear and reproduce spawners of body weight exceeding 7 kg. The results of earlier studies conducted on various fish species (Brzuska, 1987, 1991, 2001a; Brzuska and Adamek, 1997; Kłodzińska and Okoniewski, 1998; Brzuska *et al.*, 1998, 2000) stressed the problem, highly important in hatchery practice, of minimising doses of the applied ovulation stimulators. The results of former experiments and those obtained in the present work show that the investigations concerning reduced amounts of applied stimulators are fully justified. They should be continued and aim at the polyoptimization of reproduction effects (Brzuska, 1991). The attempts at reducing the high costs connected with controlled reproduction of European catfish (both by reducing the amounts of applied stimulators and selecting females of body weight not exceeding 7 kg) cannot be regarded as of no avail.

## Acknowledgements

The author thanks Mr J. Adamek and Mrs U. Czudek working in the Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences in Gołysz, for technical assistance in the experiments, and Mrs U. Karosek for careful technical preparation of the manuscript.

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Received: 02–07–01

Accepted after corrections: 03–04–06

## ABSTRAKT

### Umělý výtěr sumce velkého (*Silurus glanis*): jednorázové podání přípravku obsahujícího analog GnRH a dopaminergní inhibitor pro stimulaci ovulace jikernaček různé hmotnosti

Byly provedeny dva experimenty s dosažením ovulace jikernaček sumce velkého (o hmotnosti v rozpětí 4,60 až 11,00 kg) pomocí stimulace dvěma dávkami kapří hypofýzy (0,4 + 3,6 mg/kg hmotnosti jikernaček) a jednorázové podanou dávkou přípravku Ovopel (1 peleta/kg hmotnosti těla). V obou experimentech bylo zjištěno nižší procento ovulovaných jikernaček o vyšší hmotnosti těla při použití přípravku Ovopel. Nebyl zjištěn statisticky signifikantní vliv hmotnosti jikernaček na hmotnost jiker v gramech a jejich kvalitu. Byl zjištěn statisticky průkazný účinek ( $P \leq 0,01$ ) použitého přípravku k dosažení ovulace na procento živých embryí po 56 hodinách inkubace; jikry získané při použití přípravku Ovopel měly lepší kvalitu. Hmotnost jikernaček měla statisticky signifikantní vliv ( $P \leq 0,05$ ) nejen na hmotnost získaných jiker vyjádřenou v gramech, ale rovněž na procento živých embryí po 56 hodinách inkubace ( $P \leq 0,01$ ). Nebyl nalezen vliv stimulatorů ovulace a hmotnosti jikernaček na hmotnost vyřetěných jiker a procento živých embryí za 24 a 48 h inkubace. Byl ale zjištěn statisticky signifikantní ( $P \leq 0,01$ ) vliv na procento živých embryí po 56 h inkubace.

**Klíčová slova:** sumec velký; umělý výtěr; kapří hypofýza, jednorázová dávka Ovopelu

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# Analysis of reproduction and production parameters in dam populations of pigs and genealogical sire populations by means of M BLUP-AM method in Slovakia

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**ABSTRACT:** This paper evaluates whole population averages of dam pig population concerning both reproduction and production parameters in Slovakia in the years 1996–2002. The prediction of breeding value of the best sire populations of Large White (LW), White Meaty (WM), Landrace (L) in the years 2000–2002 was estimated by means of M BLUP-AM method. Based on the achieved total population averages, the best breeds in reproduction performance were LW and WM, in 2002 the number of all born piglets Large White 11.23, White Meaty 11.33 and Landrace 10.70; live-born piglets LW 10.77, WM 10.65, L 10.42, reared piglets LW 9.82, WM 9.63 and L 9.82. On the basis of total breeding value (TBV), the best genealogical populations of dam breeds were evaluated and included in the “top ladder”. In Slovakia the best genealogical sire populations of LW breed were as follows: Pintos with total breeding value (TBV) 806.2, Sunar with TBV 784.1, Faber with TBV 771.8. In White Meaty breed the best results in genealogical sire populations were achieved in Don with total breeding value (TBV) 701.7, Rank with TBV 636.7 and Norik with TBV 614.5. In Landrace breed the best populations were as follows: Floppy with total breeding value (TBV) 730.6, Damborek with TBV 634.3 and Don with TBV 723.4. The M BLUP-AM method enables to make genetic values of individual animals and breeds more precise, and subsequently to provide for more objective controlled selection and further improvement of pig breeds.

**Keywords:** pigs; animal model; fattening and carcass value

In recent years the main question of breeding and hybridisation in Slovakia was how to improve meat efficiency at reduced feed consumption as well as how to maintain reproduction properties on a certain level. Further improvement in this area is impossible without optimisation of breeding value prediction methods.

The problem of reproduction parameters has been dealt with by many authors – Anderson and Karras (1994). Selection indices and prediction of reproduction breeding values were dealt with by Bobček *et al.* (1991), Flak *et al.* (1997), Wolfová and Wolf (1997), Peškovičová *et al.* (1997), Bobček and Řeháček (1999), Matoušek *et al.* (1999) and others.

The more precise the breeding value, the higher the breeding advancement can be expected. Therefore breeders are looking for a method of precise prediction of values in pigs included in selection. Such precise prediction is provided by BLUP method (Best Linear Unbiased Prediction). At present it is the most progressive method of breeding value prediction. The most optimum programme using M BLUP-AM method is Pest – Groeneveld and Peškovičová (1999). This programme enables to calculate breeding values for the individual traits as well as multidimensional models and effects with more traits while respecting additive – genetical affinity.

Predictions by means of M BLUP-AM method include parameters of field test, fattening and carcass values from testing stations as well as reproduction parameters. The evaluation of dam populations of pigs emphasises mainly the reproduction parameters since they have a significant impact on breeding effectiveness and become an important part of breeding programmes.

## MATERIAL AND METHODS

The results of progeny testing of degree I in Slovakia as well as data provided by the State Centre of Information Technology in Žilina helped us to evaluate total reproduction and production averages of breeding population in Slovakia in the years 1996–2002. Genealogical sire populations in the individual mother breeds Large White (LW), White Meaty (WM) and Landrace (L) were evaluated by means of M BLUP-AM method. This method has been used widely in Slovakia since January 2000. Recently, two-year results of efficiency of individual hybridisation breeding have been evaluated.

In 2002 there were 65 nucleus herds in Slovakia. The representation of individual breeds was as follows: dam pig population LW – 45 nucleus herds (5 144 sows), WM – 14 nucleus herds (1 317 sows) and L – 6 nucleus herds (574 sows), which is 65 nucleus herds in total (7 035 dam populations of pigs).

In addition, the M BLUP-AM method was used for the evaluation of 675 genealogical sire populations of LW breed, 243 genealogical sire populations of WM breed, and 309 genealogical sire populations of L breed, carried out from 2000 to 2002.

Methodology of evaluation respected valid standards STN 46 6164 and 46 6150 used for the progeny testing of individual breeds. The calculations of genetic and environmental trends were based on the multidimensional animal system M BLUP by Groeneveld and Peškovičová (1999).

## RESULTS

The evaluation of reproduction parameters in dam populations of pigs for the years 1996–2002 is given in Table 1. As far as the individual reproduction trends are concerned, the achieved results were as follows: LW and WM trend to increase slightly

all live born piglets, namely in LW the number rose from 10.7 (1996) to 11.2 piglets (2002), in WM from 10.9 (1996) to 11.3 piglets (2002). The same tendency was seen in the number of new born alive piglets and reared piglets. Mortality of LW was 0.8–0.9 piglets and of WM 0.8–1.1 piglets. Landrace breed showed a decreased reproduction performance in all parameters. The evaluation of dam populations of pigs carried out at progeny testing stations based on whole population averages in the years 1996–2002 is shown in Table 2.

Landrace breed received the best evaluation as far as the production parameters of dam populations of pigs are concerned. It also achieved the whole population average from 30 to 100 kg, namely 840.7 g,  $s = 94.3$  with difference min/max  $-23.4$  g/+31.3 g at consumption 37.0 ME/MJ,  $s = 0.32$ , and feed consumption/1 kg gain  $-2.93$  kg,  $s = 0.39$ .

The second best results were achieved by LW pigs whose daily gain was 811.9 g,  $s = 110.4$ , with difference min/max  $-25.5$  g/+29.1 g at consumption 37.19 ME/MJ,  $s = 0.29$ , and feed consumption 2.95 kg,  $s = 0.40$ .

Landrace pigs also achieved the highest carcass value parameters, namely: backfat thickness  $-17.9$  mm,  $s = 0.39$ , difference min/max  $-1.4$  g/+1.7 mm, MLLT 478.6 mm<sup>2</sup>,  $s = 6.86$ , and carcass loin value 52.08%,  $s = 2.69$ , and difference min/max  $-1.66$ %/+1.62%.

LW breed had the lowest values of all production parameters in the years 1996–2001 with respect to whole population averages. The evaluation of the best genealogical sire populations based on dam populations of pigs is shown in Table 3.

The evaluation of individual genealogical populations in Slovakia in 2000–2002 was based on the estimation of total breeding value. In LW breed the best values were achieved by Pintos 1 857/4 015 ranging min–max from 554.3 to 806.2 (TBV) total breeding value from Agro Insemas in Ratka. Further best values were achieved in the following populations: Sunar 1 342/3 001 from 570.2 to 784.1 AC Sof Faber 1 398/4 003 ranging from 633.5 to 771.8 (TBV) total breeding value from ISK Kružno, Amulet 1 818/4 016 ranging from 618.2 to 708.5 (TBV) total breeding value from AC Agrosopol in Bolkovce.

In WM breed the best genealogical sire populations ranked as follows: Don 884/4 103 ranging min–max from 550.6 to 701.7 (TBV) total breeding value from Agricultural Co-operative (AC) Hontianske Moravce, Rank 884/4 114 ranging

Table 1. Results of reproduction performance of dam populations of pigs in Slovakia in 1996–2002

Breed	Year	Number of sows	Piglets/litter						
			born piglets in total		live-born piglets		reared piglets		mortality
			$\bar{x}$	di	$\bar{x}$	di	$\bar{x}$	di	
LW	1996	4 602	10.75	-0.32	10.36	-0.22	9.53	-0.19	0.8
	1997	5 428	10.93	-0.14	10.44	-0.14	9.62	-0.10	0.8
	1998	5 477	11.04	-0.03	10.56	-0.02	9.63	-0.09	0.9
	1999	5 743	11.05	-0.02	10.58	0.00	9.66	-0.06	0.9
	2000	5 106	11.26	+0.19	10.63	+0.05	9.87	+0.15	0.8
	2001	5 002	11.28	+0.21	10.75	+0.17	9.96	+0.24	0.8
	2002	5 144	11.23	+0.16	10.77	+0.19	9.82	+0.10	0.8
WPA 96/02			11.07	-0.32/+0.21	10.58	-0.22/+0.19	9.72	-0.19/+0.24	0.82
WM	1996	1 348	10.95	-0.22	10.52	-0.15	9.71	-0.02	0.8
	1997	1 542	11.07	-0.10	10.54	-0.17	9.58	-0.15	1.0
	1998	1 580	11.11	-0.09	10.66	-0.01	9.66	-0.07	1.0
	1999	1 508	11.09	-0.08	10.75	+0.08	9.82	+0.09	1.1
	2000	1 338	11.32	+0.15	10.82	+0.15	9.88	+0.15	1.0
	2001	1 337	11.34	+0.17	10.78	+0.11	9.88	+0.15	0.9
	2002	1 317	11.33	+0.16	10.65	-0.02	9.63	-0.10	0.8
WPA 96/02			11.17	-0.22/+0.17	10.67	-0.17/+0.15	9.73	-15/+15	0.94
L	1996	361	10.91	+0.16	10.81	+0.31	10.02	+0.23	0.8
	1997	520	10.68	-0.07	10.57	+0.07	9.78	-0.01	0.8
	1998	538	10.83	+0.08	10.59	+0.09	9.92	+0.13	0.6
	1999	653	10.85	+0.10	10.58	+0.08	9.78	-0.01	0.8
	2000	535	10.69	-0.06	10.39	-0.11	9.57	-0.22	0.8
	2001	558	10.59	-0.16	10.18	-0.32	9.68	-0.11	0.5
	2002	574	10.70	-0.05	10.42	-0.08	9.82	+0.03	0.9
WPA 96/02			10.75	-0.16/+0.16	10.50	-0.32/+0.31	9.79	-0.22/+0.23	0.74

Legend: LW = Large White, WM = White Meaty, L = Landrace, WPA = whole population averages in 1996–2002

from 570.1 to 636.7 (TBV) total breeding value from the AC in Krakovany-Stráže, Norik 1 479/4 005 ranging from 576.9 to 614.5 (TBV) total breeding value from the AC in Senica.

In L breed the best Floppy 1 917/4 015 ranging min–max from 300.9 to 730.6 (TBV) total breeding value from the AC in Kovaľov, Damborek 1 470/4 012 ranging from 612.7 to 634.3 (TBV) from the AC Krakovany-Stráže, Don 884/4 038 ranging from 563.6 to 723.4 (TBV) from ISK Hurbanovo. The distribution of breeding values of traits according to M BLUP-

AM based on extreme limits for sire populations and total breeding value in the “top ladder” are shown in Table 4.

The breeding values for each trait express a predicted genetic deviation of the animal in question with respect to the average value of a particular mother breed expressed in total breeding value and/or in Sk.

The highest total breeding values in the “top ladder” by December 2002 were achieved in LW – total breeding value 741.4 (top 01). The same value was achieved by 6 sire populations.

Table 2. Results of whole population averages of dam populations of pigs concerning fattening performance and carcass parameters in 1996–2002

Breed		Daily weight gain (g)	Consumption (ME/MJ)	Feed mixture consumption per kg	Backfat thickness (mm)	MLLT (mm <sup>2</sup> )	Carcass loin (%)
LW {n = 5 629}	$\bar{x}$	811.9	37.19	2.95	19.2	455.1	52.13
	s	110.4	0.29	0.40	4.6	5.83	3.12
	di	-25.5/+29.1	-1.07/+1.07	-0.13/+0.14	-2.3/+1.7	-20.6/+16.1	-1.64/+1.22
WM (n = 1 980)	$\bar{x}$	801.6	38.46	3.09	19.6	449.0	51.80
	s	105.4	0.31	0.42	3.9	5.04	2.73
	di	-15.6/+30.5	-3.31/+1.57	-0.27/+0.14	-3.2/+1.9	-27.7/+16.1	-2.55/+1.29
L (n = 795)	$\bar{x}$	840.7	37.0	2.93	17.9	478.6	52.08
	s	94.3	0.32	0.39	3.9	6.86	2.69
	di	-23.4/+31.3	-0.83/+1.35	-0.09/+0.12	-1.4/+1.7	-13.8/+14.7	-1.66/+1.62

Legend: di = difference interval of whole population averages of dam populations of pigs in 1996–2002

Table 3. Evaluation of the best sires of Large White, White Meaty and Landrace genealogical population based on total breeding values in top ladder in 2000–2002

Number	Sire population	Registration number	TBV Range min–max	Breeder
Large White				
1	Pintos	1 857–4 015	554.3–806.2	Agro Insemas, s.r.o, Ratka
2	Sunar	1 342–3 001	570.2–784.1	AC Sol'
3	Faber	1 398–4 003	633.5–771.8	ISK Kružno
4	Amulet	1 818–4 016	618.2–708.5	AC Agropol, Bolkovce
5	Tyrsk	1 951–4 005	521.6–660.5	ISK Hurbanovo
White Meaty				
1	Don	884–4 103	550.6–701.7	AC Hontianske Moravce
2	Rank	884–4 114	570.0–636.7	AC Krakovany-Stráže
3	Norik	1 479–4 005	576.9–614.5	AC Senica
4	Calesh	1 430–1 024	498.8–617.6	AC Mojmirovce
5	Ossi	1 433–4 014	480.4–538.9	AC Uhrovec
Landrace				
1	Floppy	1 917–4 015	300.9–730.6	AC Kovalov
2	Damborek	1 470–4 012	612.7–634.3	AC Krakovany-Stráže
3	Don	884–4 038	563.6–723.4	ISK Hurbanovo
4	Deniso	1 837–2 001	513.9–514.9	Agrofarma, s.r.o., Kysuca
5	Filan	1 466–4 003	274.0–508.0	ISK Rybníčky-Dolná Krupá

Table 4. Economic factors for prediction of breeding values M BLUP–AM based on extreme limits according to the individual sires and dam populations

Breed	Top ladder	Number of sires	Field test			Station test		Reproduction		TBV (Sk)
			average daily gain (g)	average back-fat thickness (cm)	average daily gain (g)	valuable carcass loin (%)	average back-fat thickness (cm)	live born piglets 1	live born piglets 2	
LW	01	6	52.2	−0.26	88.4	3.59	−0.58	1.07	0.99	741.4
LW	05	30	44.0	−0.18	71.2	2.68	−0.41	0.80	0.78	528.8
LW	10	59	37.3	−0.14	57.6	2.26	−0.35	0.70	0.64	470.9
LW	20	118	30.1	−0.10	45.8	1.82	−0.25	0.54	0.49	382.3
LW	50	296	18.1	−0.02	19.8	0.89	−0.12	0.23	0.23	252.2
WM	01	2	54.9	−0.25	101.2	3.67	−0.47	1.30	1.03	660.7
WM	05	9	43.3	−0.23	69.2	3.03	−0.40	1.02	0.79	545.4
WM	10	18	34.8	−0.18	59.6	2.65	−0.34	0.92	0.73	483.2
WM	20	35	29.1	−0.14	51.6	2.19	−0.30	0.70	0.56	408.0
WM	50	89	15.6	−0.04	24.8	1.08	−0.14	0.31	0.20	241.4
L	01	3	58.6	−0.23	105.2	3.90	−0.51	1.04	0.79	654.4
L	05	13	45.0	−0.17	80.4	3.04	−0.36	0.75	0.58	520.6
L	10	53	28.2	−0.09	49.4	1.65	−0.23	0.38	0.29	324.6
L	20	53	28.2	−0.19	49.4	1.65	−0.23	0.38	0.29	324.6

Legend: TBV = total breeding value, Sk = Slovak crown

The second best values were achieved in two sire populations of WM with total breeding value (TBV) 660.7 (top 01). The lowest values were found in three sire populations of L with TBV 654.4 (top 01). The low values recorded in reproduction parameters resulted in the markedly low values of total breeding values.

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Received: 02–09–05

Accepted after corrections: 03–04–09

## ABSTRAKT

**Analýza reprodukčných a produkčných vlastností materských populácií prasíat a genealogických líníi otcovských populácií pomocou metódy M BLUP-AM na Slovensku**

V predloženej práci sme sa zamerali na vyhodnotenie celopopulačných materských plemien v reprodukčných a produkčných ukazovateľoch za roky 1996 až 2002 na Slovensku. Odhad plemennej hodnoty sme vyhodnotili metódou M BLUP-AM u najlepších líníi kancov plemien biele ušľachtilé (BU), biele mäsové (BM) a landrase (L). Na základe dosiahnutých celopopulačných priemerov, ako najlepšie plemená v reprodukčnej úžitkovosti boli plemená BU a BM. V roku 2002 dosiahli počet všetkých prasíat BU 11,23, BM 11,33 a L 10,70, v počte živonarodených prasíat BU 10,77, BM 10,65 a L 10,42, v počte odchovaných prasíat BU 9,82, BM 9,63 a L 9,82. Pri hodnotení celkovej plemennej hodnoty (CPH) metódou M BLUP-AM sme vyhodnotili najlepšie genealogické línie materských plemien a zoradili v rámci „top rebríčka“. Na Slovensku medzi najlepšie genealogické línie kancov plemena BU patria línie Pintos s maximálnou hodnotou CPH 806,2, línia Sunar s hodnotou 784,1, línia Faber s hodnotou CPH 771,8. Plemeno BM najlepšie genealogické línie malo Don s hodnotou CPH 701,7, Rank 636,7 CPH, Norik 614,5 CPH. Plemeno L najlepšie línie mal Floppy s hodnotou CPH 730,6, línia Damborek 634,3 CPH a línia Don 723,4 CPH. Metóda M BLUP-AM nám určuje presnejšiu genetickú hodnotu genealogických líníi a plemien, čím nám dáva objektivnejšie možnosti na usmernenú selekciu šľachtenia ošípaných.

**Kľúčové slová:** prasa; animal model; hodnota výkrmnosti; jatočná hodnota

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# The effect of iodine supplementation on its output and thyroid gland status in dairy cows on a diet containing rapeseed meal

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**ABSTRACT:** Effects of iodine supplement ethylenediamine dihydroiodide and rapeseed meal (RM) on iodine output in milk and urine and on the thyroid gland status were studied in an experiment involving eight dairy cows. The experiment was established as a  $4 \times 4$  Latin square; two groups of dairy cows received a feed mixture containing RM (group RM) and two groups were without RM (group SM). In both variants one group received an iodine supplement (RM<sub>1</sub>; SM<sub>1</sub>) while the other did not (RM<sub>0</sub>; SM<sub>0</sub>). The iodine supplement increased iodine concentration in milk significantly ( $P < 0.01$ ). In iodine supplemented groups, RM reduced iodine concentration in milk (SM<sub>1</sub> vs. RM<sub>1</sub>,  $P < 0.01$ ). In groups without iodine supplement, the effect of RM was manifested only as a tendency to a reduced iodine concentration in milk (SM<sub>0</sub> vs. RM<sub>0</sub>;  $P > 0.05$ ). Iodine supplementation resulted in a significant increase in urinary iodine concentration ( $P < 0.01$ ). The evaluation of the thyroid gland status in cows of the groups with iodine supplements showed an insignificant trend to increased concentrations of T<sub>3</sub>, decreased concentrations of total and free T<sub>4</sub>. Iodine supplements showed a tendency to increase the T<sub>3</sub>/T<sub>4</sub> ratio ( $P > 0.05$ ). RM did not influence the concentrations of T<sub>3</sub>, total T<sub>4</sub>, and free T<sub>4</sub> significantly; the T<sub>3</sub>/T<sub>4</sub> ratio remained unchanged as well.

**Keywords:** blood; plasma; thyroxine; triiodothyronine; milk; urine

The concentration of iodine in milk of dairy cows is dependent on many factors. Changes in its concentration in milk can be influenced by the variability of its dietary supply and by the application of iodoform sanitation solutions used in the primary milk production (Pennington, 1990). Iodine can be supplied to animals in drinking water, forage, concentrates, feeding supplements, mineral salts and veterinary drugs.

However, the amount of iodine intake is primarily determined by the composition of the diet offered to dairy cows. As far as the type of diet for dairy

cows is concerned, feeding rations for ruminants based on roughage are not generally sufficient to assure an adequate supply of iodine. Besides roughage, a low content of iodine can also be found in concentrates, soybean and rapeseed meals and feed mixtures without mineral supplements (Groppel *et al.*, 1989). Low concentrations of iodine in milk and diseases resulting from iodine deficiency can reflect a very low content of this element in roughage, its problematic supply from other sources and/or an unqualified approach to the extensification of animal production (Kursa *et al.*, 1996). The low

efficiency of compensation of iodine requirements by means of roughage feeding in regions with the endemic occurrence of struma was mentioned by Wemheuer (1993). Studies presenting results of comparative estimations of iodine concentration in milk of dairy cows originating from various herds often indicate a really alarming inadequate supply of this element to animals (Kursa *et al.*, 1996). This author found very low concentrations of iodine in milk not only in cows with new-born calves showing symptoms of struma but also of those cows that bore calves without symptoms of the enlarged thyroid gland.

The composition of the diet can have an indirect effect on those physiological processes associated with iodine metabolism that determine its reserves in the body of dairy cows. This concerns above all those numerous chemical substances that commonly occur in some plants and can induce a secondary iodine deficiency. Rape (*Brassica napus*, L.) and feeds made of this crop belong to this group. The anti-quality components present in rapeseed involve fibre, phytin, tannin, sinapin, phenolic compounds and, especially, glucosinolates (Zukalová and Vašák 2001a,b).

Based on their physiological effects, glucosinolates can be classified into three groups. The first involves compounds with anti-microbial, anti-fungal, anti-bacterial and thyroidal effects (isothiocyanates) that are produced by hydrolysis taking place in a neutral or alkaline medium. They can selectively bind iodine and prevent its intake by the thyroid gland; these negative effects can be compensated by a supplement of iodine salts (Zukalová and Vašák, 2001a). The second group comprises substances produced by transformation of specific isothiocyanates showing strumigenous effects and inhibiting the synthesis of thyroidal hormones; their effects cannot be eliminated by an increased intake of iodine. The third group consists of thiocyanates produced by hydrolysis. Neither antinutritive effects nor toxicity of these compounds have been described until now. Physiological effects of glucosinolates and their metabolites can also be affected by the level of iodine supply and it is to expect that the strumigenous effects of isothiocyanates are dependent on the extent of potential iodine deficiency. Possible negative effects of rapeseed feeds are therefore determined not only by their type, variety and proportion in the diet but also by the spectrum of glucosinolates and their metabolites. Under normal conditions of the management

of high-producing dairy cows it is not possible to eliminate competitive inhibitors of iodine intake that involve nitrates and thiocyanates (Wemheuer, 1993).

Metabolic products of glucosinolates are partly transferred into blood and milk of dairy cows. Zech *et al.* (1995) mentioned a significant increase in the concentration of thiocyanates in milk and blood serum of dairy cows receiving a mixture containing rapeseed meal of the “double low” type. A similar increase in the concentration of thiocyanates in milk of dairy cows during a long-term experiment based on a diet containing rapeseed meal was reported by Emanuelson *et al.* (1993). Strumigenous effects of glucosinolates and their metabolites consist in the inhibition of either iodination or iodisation of thyroid gland (Zech, 1993). Such an inhibition of thyroid gland can be manifested by a change in concentrations of thyroidal hormones in blood serum. Zech (1993), however, did not observe any significant changes in concentrations of thyroidal hormones even in animals with increased levels of thiocyanates in blood serum and milk. Similarly, Ehlers *et al.* (1994) found in a long-term experiment with dairy cows on a diet containing rapeseed meal that a reduced intake of iodine resulted in an only insignificant decrease in  $T_4$  concentration in blood serum. Trávníček *et al.* (2001) also studied the effects of an adequate iodine supply on concentrations of thyroid hormones in sheep on a diet containing glucosinolates (i. e. rapeseed meal); the concentration of  $T_4$  significantly increased in their experiments.

In several other experiments the effects of iodine supplements and of glucosinolates on the metabolism of iodine and on the thyroid gland status were studied separately (Zech *et al.*, 1995; Emanuelson *et al.*, 1993; Berg *et al.*, 1988; Swanson *et al.*, 1990; Kaufmann *et al.*, 1998; Herzig *et al.*, 1999).

This study was aimed at a periodic experiment that was conducted to estimate the effects of a combination of surplus organic iodine and glucosinolates originating from rapeseed meal on the output of iodine in milk and urine as well as on the status of thyroid gland.

## MATERIAL AND METHODS

Eight dairy cows of the Czech Red Pied breed (mean BW  $626 \pm 50$  kg) yielding  $23 \pm 3$  l of milk per day were used in an experiment conducted from

February to April 2000 in an experimental stall in the Research Institute of Animal Nutrition, Ltd., Pohořelice. The cows were kept individually and received a basal diet (maize silage, 33% DM, and lucerne hay) plus concentrate feed mixture twice daily (at 5:00 a.m. and 3:00 p.m.) (Table 1). The basal diet was calculated both for the maintenance and production of 10 l milk and concentrates were offered at the amount of 0.45 kg per each litre of milk produced above the basic milk production. The amounts of offered and refused feed were

weighed daily. The experiment was designed to involve 4 periods of 21 days; the first 14 days were used for adaptation of animals to the diets. Samples of milk, urine and blood were collected during the last week of the periods. Treatments involved four groups of animals given four experimental concentrate feed mixtures (i. e. a 4 × 4 Latin Square experiment). Two mixtures contained 270 g/kg of rapeseed meal, either with (RM<sub>1</sub>) or without (RM<sub>0</sub>) mineral premix containing organic iodine (supplied in the form of ethylenediamine dihydroiodide,

Table 1. Ingredient and nutrient composition of feed mixtures

Ingredient	Concentrate feed mixture			
	RM <sub>1</sub> <sup>1</sup>	RM <sub>0</sub> <sup>2</sup>	SM <sub>1</sub> <sup>3</sup>	SM <sub>0</sub> <sup>4</sup>
Barley (g/kg)	100	100	180	180
Maize (g/kg)	350	350	275	275
Flax (g/kg)	30	30	30	30
Oat (g/kg)	80	80	125	125
Wheat (g/kg)	138	140	98	100
Soybean meal (46%CP) (g/kg)	–	–	180	180
Rapeseed meal (g/kg)	270	270	–	–
Dried sugar beet pulp (g/kg)	–	–	80	80
Feed salt (g/kg)	5	5	5	5
Mineral and vitamin supplement* (g/kg)	25	25	25	25
Iodine premix (EDDI, wheat meal) (g/kg)	2	–	2	–
Nutrient	RM <sub>1</sub>	RM <sub>0</sub>	SM <sub>1</sub>	SM <sub>0</sub>
DM** (g/kg)	879	879	882	882
CP** (g/kg)	180	180	167	167
NEL (MJ)	7.09	7.10	7.02	7.03
PDIN (g/kg)	117	117	117	117
PDIE (g/kg)	108	108	115	115
Ca** (g/kg)	5.97	5.98	6.07	6.08
P** (g/kg)	6.38	6.39	5.17	5.18
I** (mg/kg)	6.63	0.410	6.59	0.450

<sup>1</sup>RM<sub>1</sub> = feed mixture with rapeseed meal and iodine supplement

<sup>2</sup>RM<sub>0</sub> = feed mixture with rapeseed meal

<sup>3</sup>SM<sub>1</sub> = feed mixture with soybean meal and iodine supplement

<sup>4</sup>SM<sub>0</sub> = feed mixture with soybean meal

\*Mineral and vitamin supplement: calcium 150 g/kg; phosphorus 50 g/kg; sodium 90 g/kg; magnesium 80 g/kg; iron 2 000 mg/kg; zinc 7 000 mg/kg; manganese 7 000 mg/kg; copper 1 500 mg/kg; iodine 0 mg/kg; selenium 20 mg/kg; cobalt 20 mg/kg; vitamin A 1 000 000 i.u.; vitamin D3 100 000 i.u.; vitamin E 1 000 mg/kg; niacin 4 000 mg/kg

\*\*Marked values were obtained from analyses

C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>·2HI, EDDI). The other two mixtures were without rapeseed meal (it was replaced by soybean meal at amounts respecting the isonitrogenous levels of mixtures), either with (SM<sub>1</sub>) or without (SM<sub>0</sub>) iodine supplement. The content of iodine in RM<sub>1</sub> and SM<sub>1</sub> mixtures was calculated as a surplus amount and corresponded to 250% of the recommended daily iodine requirement for dairy cows (Šimek *et al.*, 1994). Ingredients and nutritional composition of experimental feed mixtures are presented in Table 1. The average daily dietary iodine intakes were 3.00 mg and 42.4 mg in treatments without (SM<sub>0</sub> and RM<sub>0</sub>) and with iodine supplementation (RM<sub>1</sub> and SM<sub>1</sub>), respectively. The dietary iodine intakes are presented in Table 2.

Samples of forage, feed mixtures and refused feed were collected in the 3rd week of each experimental period and analysed for crude protein by Kjeldahl method (Kjel-Foss Automatic 16210), crude fibre by Henneberg-Stohmann method (Fibretec, Tecator), calcium by atomic absorption (Perkin-Elmer 4000) and phosphorus by a spectrophotometric method. Feed mixtures were also analysed for the content of glucosinolates (GLS; gluconapin, glucobrassicinapin, progoitrin) using the gas chromatography method (Zukalová and Vašák, 1978). The values of glucosinolate content in feed mixtures were converted to total daily intake of glucosinolates: 35.4 mmol and 35.8 mmol per animal in RM<sub>1</sub> and RM<sub>0</sub> treatments, respectively. The values of GLS intake are also presented in Table 2.

Milk yield was recorded daily. Milk samples were collected during morning milking on Days 16, 17 and 18 in each of the experimental periods. Blood and urine were sampled after morning milking on D 19 of each period.

Milk, urine and feed samples were analysed for iodine concentrations by means of Sandell-Kolthoff spectrophotometric method after alkaline digestion (Bednář *et al.*, 1964).

Milk samples were also analysed for milk fat by Gerber acidobutyrometric method, total protein (Pro-Milk Mk-II, Foss Electric), whey protein (Pro-Milk Mk-II, Foss Electric, after casein agglomeration), casein (Pro-Milk Mk II: Instruction manual. A/S N., Foss Electric), lactose by a polarimetric method (according to ČSN 57 0530), urea by a spectrophotometric method (Gajdůšek *et al.*, 1996) and citric acid (Milkoscan 4000, Foss Electric). Solids-non-fat content was calculated from solids content and milk fat content.

Blood samples were analysed for concentrations of triiodothyronine (T<sub>3</sub>), total thyroxine (T<sub>4</sub>) and free thyroxine (FT<sub>4</sub>) by means of a radioimmuno-logical method (commercial kits, manufacturer Immunotech, Prague). The T<sub>3</sub>/T<sub>4</sub> ratio was estimated on the basis of concentrations of triiodothyronine and total thyroxine in plasma. Plasma concentrations of total protein, albumin, globulin, glucose, creatine kinase, AST and GMT were determined using an automated analyser (Cobas Mira S, Roche) and according to the methods described in

Table 2. Dietary intake of iodine and glucosinolates

	<i>n</i>	Group RM <sub>1</sub>		Group RM <sub>0</sub>		Group SM <sub>1</sub>		Group SM <sub>0</sub>		
		mean	SD	mean	SD	mean	SD	mean	SD	
Iodine intake										
Feed mixtures (mg/d)	2	42.4 <sup>A</sup>	2.70	2.3 <sup>B</sup>	0.11	40.6 <sup>A</sup>	6.86	2.5 <sup>B</sup>	0.34	
Total dietary intake (mg/d)	2	42.8 <sup>A</sup>	8.31	2.9 <sup>B</sup>	0.46	42.0 <sup>A</sup>	9.76	3.1 <sup>B</sup>	0.52	
Total dietary intake (mg/kg DM)	2	2.2 <sup>A</sup>	0.34	0.1 <sup>B</sup>	0.02	2.2 <sup>A</sup>	0.43	0.2 <sup>B</sup>	0.02	
Glucosinolate intake										
Gluconapin (mmol/d)	2	7.9	2.20	8.0	2.47	–	–	–	–	
Glucobrassicinapin (mmol/d)	2	0.9	0.42	0.9	0.46	–	–	–	–	
Progoitrin (mmol/d)	2	26.6	7.48	26.9	7.98	–	–	–	–	
Total glucosinolates (mmol/d)	2	35.4	9.43	35.8	10.26	–	–	–	–	

Means with different superscripts in lines differ significantly (<sup>A, B, C</sup>*P* < 0.01)

the diagnostic kits. Glutathion peroxidase activity (GSH-Px) was measured by the method developed by Paglia and Valentine (1967), using the set supplied by Randox and automatic analyser Cobas Mira S (Roche).

The effects of rapeseed and supplemental iodine inclusion in diets were tested by the multifactorial analysis of variance (ANOVA), calculations were carried out using the statistical software Statgraphics Ver. 7.0.

## RESULTS

The mean intake of iodine in the diet of experimental dairy cows was calculated on the basis of data on the analyses and consumption of feeds

(Table 2). The intake of glucosinolates in feeding mixtures was calculated (calculation was based on glucosinolate analyses). The content of glucosinolates in mixtures SM<sub>1</sub> and SM<sub>0</sub> was under the sensitivity level of the analytical method of estimation. The average daily intake of glucosinolates in groups receiving feed mixtures with rapeseed meal (RM<sub>1</sub> and RM<sub>0</sub>) was as follows: gluconapin 7.95 mmol, glucobrassicinapin 0.9 mmol, progoitrin 26.75 mmol and glucosinolates in total 35.6 mmol (Table 2).

Milk performance was not significantly influenced by the experimental factors under study (Table 3). Neither did these factors cause any significant changes in the concentrations of milk constituents presented in Table 3. Similarly, none of the parameters of blood plasma was significantly affected by the experimental factors (Table 4).

Table 3. Mean milk yield and mean basic milk constituents

		n	Group RM <sub>1</sub>		Group RM <sub>0</sub>		Group SM <sub>1</sub>		Group SM <sub>0</sub>	
			mean	SD	mean	SD	mean	SD	mean	SD
Milk yield	(l/day)	2	21.7	2.93	21.0	3.24	21.1	2.48	21.5	3.24
Milk fat	(%)	2	3.99	0.461	4.11	0.622	4.05	0.588	4.06	0.643
Total protein	(%)	2	3.40	0.179	3.41	0.210	3.44	0.185	3.40	0.131
Whey protein	(%)	2	0.754	0.106	0.765	0.093	0.735	0.116	0.729	0.095
Casein	(%)	2	2.79	0.172	2.76	0.225	2.86	0.199	2.83	0.174
Lactose	(%)	2	5.08	0.111	5.05	0.113	5.04	0.136	5.06	0.106
Solids-non-fat	(%)	2	9.05	0.142	9.03	0.212	9.05	0.165	9.03	0.158
Urea	(%)	2	263	76.0	243	83.1	293	84.3	286	62.6
Citric acid	(%)	2	0.162	0.0300	0.170	0.0320	0.202	0.209	0.163	0.023

Table 4. Mean plasma parameters within the experiment

		n	Group RM <sub>1</sub>		Group RM <sub>0</sub>		Group SM <sub>1</sub>		Group SM <sub>0</sub>	
			mean	SD	mean	SD	mean	SD	mean	SD
Urea	(mmol/l)	2	4.76	1.00	4.45	0.621	4.89	0.746	5.19	0.994
Proteins	(g/l)	2	72.4	4.51	70.4	4.75	72.3	4.41	70.7	4.05
Albumins	(g/l)	2	37.6	2.63	37.2	1.06	38.0	1.66	37.6	2.56
Globulins	(g/l)	2	34.9	5.60	33.2	4.71	34.4	3.93	33.2	5.53
Glucose	(mmol/l)	2	3.45	0.435	3.54	0.376	3.53	0.261	3.55	0.425
Creatine kinase	( $\mu$ kat/l)	2	5.34	5.35	4.78	6.25	8.51	11.4	6.82	13.2
AST	( $\mu$ kat/l)	2	1.19	0.147	1.16	0.246	1.22	0.233	1.22	0.334
GMT	( $\mu$ kat/l)	2	0.295	0.0843	0.328	0.0800	0.300	0.110	0.326	0.0898
GSH-Px	( $\mu$ kat/l)	2	878	232	974	298	931	123	920	122

The effect of iodine supplement and rapeseed meal (RM) on the concentration of iodine in milk is illustrated in Table 5. The highest concentration of iodine in milk of a group of cows was measured in SM<sub>1</sub> (i.e. in one of the groups supplemented with iodine, 595 µg/l). As compared with the groups without iodine supplement (RM<sub>0</sub> and SM<sub>0</sub>), the experimental iodine supplementation of feeding mixtures significantly increased milk iodine concentrations in groups RM<sub>1</sub> and SM<sub>1</sub> (by 265 µg/l and 472 µg/l, resp.;  $P < 0.01$ , Table 5). In groups with iodine supplement, RM reduced the milk iodine concentration; as compared with group SM<sub>1</sub>, the iodine concentration in group RM<sub>1</sub> was lower by 253 µg/l ( $P < 0.01$ ). In the groups without iodine supplement the effect of RM was manifested only as a tendency to its reduced concentration in milk (as compared with SM<sub>0</sub>, the decrease in RM<sub>0</sub> group was 46 µg/l;  $P > 0.05$ ).

The diurnal iodine output in milk was calculated on the basis of data on milk production in 24 hours. The supplement of iodine increased the iodine output in milk by 5.87 mg/d and 10.03 mg/d in RM<sub>1</sub> and SM<sub>1</sub> groups, resp., as compared with groups RM<sub>0</sub> and SM<sub>0</sub>, respectively ( $P < 0.01$ ; Table 6). The supplement of RM, however, reduced the daily output of iodine in milk but significant differences

were found only in groups with iodine supplements (as compared with SM<sub>1</sub>, daily output of iodine in milk was reduced by 5.20 mg/d in RM<sub>1</sub>;  $P < 0.01$ ). The ratio of dietary iodine intake to iodine output in milk (I/O) was calculated as another parameter of iodine output. Iodine supplementation increased the I/O ratio by nearly 3.97 mg/d and 1.76 mg/d in groups SM<sub>1</sub> and RM<sub>1</sub>, resp., as compared with groups RM<sub>0</sub> and SM<sub>0</sub>, respectively ( $P < 0.01$ , Table 6). Feeding of RM also increased the I/O ratio; this increase was 2.59 in groups with iodine supplements (RM<sub>1</sub> and SM<sub>1</sub>) and it was statistically significant (as compared with SM<sub>0</sub> group, the I/O ratio in group RM<sub>0</sub> was lower by 0.38,  $P > 0.05$ ).

The highest urinary iodine concentration in a group of cows was found in RM<sub>1</sub> (1 005 µg/l, Table 5). As compared with groups without iodine supplementation (RM<sub>0</sub> and SM<sub>0</sub>), the supplement of iodine significantly increased its concentration in urine of dairy cows in groups RM<sub>1</sub> and SM<sub>1</sub> (by 767 µg/l and 619 µg/l, resp.;  $P < 0.01$ , Table 5). This result was similar to that of iodine concentration in milk. In the groups with iodine supplement, RM increased the concentration of iodine in urine of experimental cows. As compared with group SM<sub>1</sub>, the concentration of iodine was higher by 282 µg/l in group RM<sub>1</sub> ( $P > 0.05$ ). In the groups

Table 5. Concentration of iodine in milk and urine

	n	Group RM <sub>1</sub>		Group RM <sub>0</sub>		Group SM <sub>1</sub>		Group SM <sub>0</sub>	
		mean	SD	mean	SD	mean	SD	mean	SD
Iodine concentration									
Milk (µg/l)	2	342 <sup>A</sup>	64.7	77.0 <sup>B</sup>	31.6	595 <sup>C</sup>	178	123 <sup>B</sup>	76.3
Urine (µg/l)	2	1 005 <sup>A</sup>	686	238 <sup>B</sup>	170	723 <sup>A</sup>	373	104 <sup>B</sup>	73.0

Means with different superscripts in lines differ significantly (<sup>A, B, C</sup> $P < 0.01$ )

Table 6. Iodine output in milk (calculated values)

	n	Group RM <sub>1</sub>		Group RM <sub>0</sub>		Group SM <sub>1</sub>		Group SM <sub>0</sub>	
		mean	SD	mean	SD	mean	SD	mean	SD
Iodine output									
Milk (mg/day)	2	7.50 <sup>A</sup>	2.06	1.63 <sup>B</sup>	0.670	12.7 <sup>C</sup>	4.52	2.67 <sup>B</sup>	1.59
Dietary intake/output ratio									
Milk (–)	2	5.95 <sup>A</sup>	1.38	1.98 <sup>B</sup>	0.83	3.36 <sup>C</sup>	0.53	1.60 <sup>B</sup>	1.14

Means with different superscripts in lines differ significantly (<sup>A, B, C</sup> $P < 0.01$ )

Table 7. Plasma concentrations of thyroidal hormones

Thyroidal hormones	n	Group RM <sub>1</sub>		Group RM <sub>0</sub>		Group SM <sub>1</sub>		Group SM <sub>0</sub>	
		mean	SD	mean	SD	mean	SD	mean	SD
Triiodothyronine (T <sub>3</sub> ) (nmol/l)	2	2.25	0.293	2.19	0.290	2.19	0.242	1.98	0.212
Total thyroxine (T <sub>4</sub> ) (nmol/l)	2	55.0	7.88	63.1	11.0	59.3	10.5	60.3	8.33
Free thyroxine (FT <sub>4</sub> ) (nmol/l)	2	14.0	2.71	15.5	2.31	14.4	2.84	15.0	2.92
	2	0.0421	0.0106	0.0353	0.00591	0.0377	0.00708	0.0332	0.00498

without iodine supplements, the effect of RM was also manifested as a statistically insignificant increase in urinary iodine concentrations (+134 µg/l,  $P > 0.05$ ).

To define the status of thyroid gland, concentrations of hormones triiodothyronine (T<sub>3</sub>), free thyroxine (FT<sub>4</sub>) and total thyroxine (T<sub>4</sub>) in blood plasma were estimated. Using their concentrations, we were able to calculate the ratio of triiodothyronine to total thyroxine (T<sub>3</sub>/T<sub>4</sub>). The concentrations of thyroidal hormones are presented in Table 7.

In the experimental groups with iodine supplement there was a tendency to higher concentrations of T<sub>3</sub> (+0.06 nmol/l and +0.21 nmol/l in RM<sub>1</sub> and SM<sub>1</sub>, resp., as compared with RM<sub>0</sub> and SM<sub>0</sub>;  $P > 0.05$ ). In the groups with iodine supplement there was a tendency to decreasing concentrations of T<sub>4</sub> and FT<sub>4</sub> in blood plasma. As compared with RM<sub>0</sub> and SM<sub>0</sub>, the decrease in the level of total thyroxine was -8.10 nmol/l and -1.00 nmol/l in RM<sub>1</sub> and SM<sub>1</sub> groups, respectively. As compared with RM<sub>0</sub> and SM<sub>0</sub>, the concentrations of free thyroxine decreased by -1.50 nmol/l and -0.60 nmol/l in RM<sub>1</sub> and SM<sub>1</sub>, respectively. Iodine supplementation increased the T<sub>3</sub>/T<sub>4</sub> ratio by 0.0068 and 0.0045 in RM<sub>1</sub> and SM<sub>1</sub>, resp., as compared with RM<sub>0</sub> and SM<sub>0</sub>. Within the groups supplemented with iodine, the rapeseed meal factor showed a tendency to increased concentrations of T<sub>3</sub> (RM<sub>1</sub>+0.06 nmol/l vs. SM<sub>1</sub>,  $P > 0.05$ ). A similar trend was found for the rapeseed factor within the groups without iodine supplement (RM<sub>0</sub>+0.21 nmol/l vs. SM<sub>0</sub>,  $P > 0.05$ ). The concentrations of T<sub>4</sub> and FT<sub>4</sub> were not explicit in this case and the T<sub>3</sub>/T<sub>4</sub> showed a trend to an increased ratio (+0.0044 and +0.0021 in RM<sub>1</sub> and SM<sub>1</sub>, resp., as compared with RM<sub>0</sub> and SM<sub>0</sub>;  $P > 0.05$ ).

## DISCUSSION

### Milk

During the experiment, the concentrations of iodine in milk ranged from 77.0 µg/l (RM<sub>0</sub>) to 595 µg/l (SM<sub>1</sub>). Franke *et al.* (1983) estimated the concentrations of 166 µg/kg in milk of dairy cows without iodine supplements while in the experiments with supplements of 4 ppm of ethylenediamine dihydroiodide (EDDI) the estimated iodine levels were as high as 745 µg/kg. Swanson *et al.* (1990) supplemented feeding rations of dairy cows with 1, 2 and 4 mg/kg iodine in the form of potassium iodide and/or EDDI and the resulting levels of iodine concentrations in milk were 404; 477 and 757 ng/ml vs. 467; 535 and 869 ng/ml, respectively. In milk of controls, an average iodine concentration was 205 ng/ml. Kaufmann *et al.* (1998) used potassium iodide as a source of iodine at amounts of 0; 20; 60 and 150 mg/d and found milk concentrations ranging from 470 µg/l to 819 µg/l; in controls, the average concentration was 128 µg/l.

As expected, a higher amount of iodine supplied to feeding rations in the form of EDDI significantly increased the concentrations of iodine in milk of dairy cows (groups RM<sub>1</sub> and SM<sub>1</sub>, Table 5). Iodine concentrations in milk of dairy cows without its supplements were comparable with literary data about controls (77.0 µg/l and 123 µg/l in RM<sub>0</sub> and SM<sub>0</sub>, resp.). Our results are also comparable with literary data with respect to the intake of iodine in the groups of cows with iodine supplements (2.16 mg/kg vs. 2.0 mg/kg, in the study of Swanson *et al.*, 1990); their levels of iodine in milk of experimental animals (477 ng/ml) were similar to our results in group SM<sub>1</sub> (595 µg/l). Further comparable data were published for example by

Herzig *et al.* (1999), who mentioned a surprising increase in iodine concentrations in milk to 20 µg/l; 37 µg/l; 64 µg/l and 121 µg/l after increasing doses of EDDI (corresponding to 0; 33; 66 and 100% of recommended daily requirement of iodine).

In dairy cows with marked deficiency of iodine (as manifested through deliveries of calves with enlarged thyroid glands) the concentration of iodine in milk can drop below 30 µg/l (Kursa *et al.*, 1996). In our study the lowest iodine level was 77.0 µg/l in group RM<sub>0</sub>; as compared with data published by Herzig *et al.* (1999), this value was similar to that recorded in animals with 66% of recommended daily supply of iodine. As compared with Kursa *et al.* (1996), however, these values did not imply an excessive iodine deficiency.

Diurnal output of iodine in milk was calculated on the basis of its content in milk and produced milk; the values were significantly higher in groups with iodine supplements than in controls. Zech *et al.* (1995) mentioned that the output of iodine was higher with increasing dietary iodine intake.

In the groups of dairy cows receiving feeding mixtures with rapeseed meal there was a decrease in iodine concentration in milk (Table 5). This effect can probably be associated with strumigenous effects of glucosinolates and their metabolites originating from rapeseed meal (contents of glucosinolates in feed mixtures are presented in Table 2). There are different mechanisms of action of compounds with strumigenous effects and some of them prevent iodine retention in the thyroid gland (Pisaříková *et al.*, 1996). A similar effect was described also in sheep (Trávníček *et al.*, 2001). This author explained decreased concentrations of iodine in milk of ewes on a diet containing rapeseed meal and nitrates as an inhibition of active transport of iodine into the mammary gland due to competitive effects of nitrates and glucosinolates. Zech *et al.* (1995) reported an insignificant decrease in iodine levels in milk of dairy cows receiving a production feed mixture with 30% of rapeseed meal of the "double low" type (to 107 µg/l) as compared with controls (110 µg/l). In our experiments a significant decrease in iodine concentration in milk was observed only in variants with a parallel supply of iodine. The average daily intake of glucosinolates was 35.6 mmol (Table 2); as compared with data published by Zech *et al.* (1995) this value was much higher (18.6 mmol/d).

Data on the diurnal output of iodine in milk are presented in Table 6. A decreased output of iodine in milk due to rapeseed meal was observed also by Zech *et al.* (1995), who explained it on the basis of an increased renal clearance of iodine due to a higher concentration of thiocyanates in the blood of dairy cows. An increased excretion of iodine in urine results in its reduced concentration and output in milk. In our experiment the levels of thiocyanates were not estimated and for that reason their increased blood levels could not be documented. In the course of this experiment, however, there was a tendency to increased levels of iodine in urine of animals receiving feed mixtures with rapeseed meal (Table 5). The increased ratio of iodine intake to output (O/I) in milk observed in the groups receiving rapeseed meal can be interpreted in a similar way (Table 6).

## Urine

As compared with milk, there was a significant increase in iodine concentrations in the urine of dairy cows on a diet containing supplemental iodine. As compared with animals with the iodine supplement, dairy cows without it showed a decreased concentration of iodine in urine (Table 5). Herzig *et al.* (1996) estimated iodine concentrations in the urine of 672 dairy cows originating from 22 localities of the Czech Republic. In 27% and 31% of the examined animals iodine concentrations were lower than 21 µg/l and higher than 100 µg/l, respectively. The lowest average concentration of iodine in urine was found in group SM<sub>0</sub> (104 µg/l); this value corresponds with the above-mentioned ranges. In groups RM<sub>1</sub> and SM<sub>1</sub> iodine concentrations in urine were 1005 µg/l and 723 µg/l, respectively. To compare, Herzig *et al.* (1999) reported a concentration of 346 µg/l in the urine of dairy cows with complete (100%) coverage of prescribed iodine requirements by means of EDDI supplements. Regarding the intake of iodine in both experiments it can be concluded that these data are comparable because in our experiment the iodine supply corresponded to 250% of its prescribed requirements.

In groups receiving rapeseed meal there was also an insignificant tendency to an increased iodine concentration in the urine of dairy cows (Table 5); this effect was mentioned above in the discussion about iodine excretion in milk.

## Thyroidal hormones

Zech *et al.* (1995) studied effects of rapeseed meal on the status of thyroidal hormones and found no changes in thyroxine concentration in blood serum. This means that the functions of thyroid gland were not affected. Kaufmann *et al.* (1998) was not able to demonstrate the effect of increasing doses of potassium iodide on concentrations of  $T_3$  and  $T_4$ . Their average concentrations of  $T_3$  and  $T_4$  were 2.87 nmol/l and 65.3 nmol/l, resp., and these data are comparable with our results.

In this experiment no decrease in  $T_4$  concentration due to rapeseed meal was observed (this decrease is generally considered as a typical goitrogenous effect of glucosinolates). This means that  $T_4$  concentration could not be increased even in the groups with iodine supplements. Trávníček *et al.* (2001) found a decreased concentration of  $T_4$  and an increased concentration of  $T_3$  in sheep on a diet containing rapeseed meal. This fact was explained on the basis of a preference of  $T_3$  synthesis to that of  $T_4$  in the thyroid gland because  $T_3$  contains less iodine than  $T_4$ . Unchanged concentrations of thyroidal hormones observed in our study were probably affected by the length of experimental periods that were too short to enable the manifestation of the effects of glucosinolates present in rapeseed meal. In our study the intake of glucosinolates from rapeseed meal was higher than that mentioned by Zech *et al.* (1995); in spite of this, however, the level of glucosinolates was not high enough to influence the concentrations of thyroidal hormones within our relatively short three-week experimental periods.

## CONCLUSIONS

The aim of this experiment was to estimate the effect of a combined supplementation of surplus organic iodine and glucosinolates present in rapeseed meal on the output of iodine in milk and urine of dairy cows and on the status of their thyroid glands (expressed by a concentration of the thyroid gland hormones in blood). A periodic experimental model with three-week periods was chosen purposely. The factor of organic iodine surplus in the diet of dairy cows adequately increased its concentration in milk and its quantified output in milk. It was demonstrated that organic iodine, supplied in the form of ethylenediamine dihydroiodide, was a suit-

able source of iodine for supplementation of diets showing iodine deficiency.

The factor of rapeseed meal in diets (that was used as a source of glucosinolates) changed the iodine dietary intake to milk output ratio, expressed by a statistically significant decrease of iodine output in milk and by an increased urinary iodine concentration.

Otherwise as we presumed, the thyroid gland hormone concentrations were unaffected by rapeseed addition into the experimental feed mixtures.

It can therefore be concluded that, from the viewpoint of short-term effects, rapeseed meal supplied in feed mixtures at amounts of 27.5% affected the kinetics of iodine. The effects of rapeseed meal were not directly associated with changes in thyroidal hormone concentrations, we suppose that this fact resulted from a relatively low content of glucosinolates in rapeseed meal and by the duration of the experimental periods.

## Acknowledgements

The authors would like to thank J. Kříž, V. Hlaváček and especially D. Vrzalová for their assistance during the experimental work.

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Received: 01–12–13

Accepted after corrections: 03–03–31

## ABSTRAKT

### Vliv přídatku jodu na jeho výdej a činnost štítné žlázy u dojnic krměných dávkou s řepkovým extrahovaným šrotem

Na osmi dojnicích plemene české strakaté byl sledován vliv jodového doplňku (etylendiamin dihydrojodid) spolu s řepkovým extrahovaným šrotem (ŘEŠ) na výdej jodu mlékem a močí a na činnost štítné žlázy. Pokus byl založen jako 4 × 4 latinský čtverec, dvě skupiny dostávaly směsi s ŘEŠ, z nichž jedna byla dotována jodovým doplňkem (RM<sub>1</sub>). Směsi dalších dvou skupin byly bez ŘEŠ, jedna z nich byla taktéž dotována jodem (SM<sub>1</sub>). Přídavek jodu průkazně zvýšil obsah jodu v mléce, skupina RM<sub>1</sub> 342 µg/l, resp. SM<sub>1</sub> 595 µg/l ve srovnání s nedotovanými skupinami RM<sub>0</sub> 77,0 µg/l, resp. SM<sub>0</sub> 123 µg/l ( $P < 0,01$ ). V rámci skupin s přídatkem jodu došlo vlivem ŘEŠ ke snížení obsahu jodu v mléce, SM<sub>1</sub> 595 µg/l oproti RM<sub>1</sub> 342 µg/l ( $P < 0,01$ ). Ve skupinách nedotovaných jodem se vliv ŘEŠ projevil pouze tendencí k nižšímu obsahu jodu (SM<sub>0</sub> 123 µg/l ve srovnání s RM<sub>0</sub> 77,0 µg/l;  $P > 0,05$ ). Vlivem

přídavku jodu byl denní výdej jodu průkazně vyšší ( $P < 0,01$ ). ŘEŠ denní výdej jodu snížil, průkazně rozdíly se projevíly pouze u skupin současně dotovaných jodem ( $RM_1$  oproti  $SM_1$ ;  $P < 0,01$ ). Přídavkem jodu se průkazně zvýšil poměr jodu přijatého dietou k jodu vyloučenému mlékem ( $P/V$ ) ( $P < 0,01$ ). Vlivem ŘEŠ se podíl  $P/V$  zvýšil také, u skupiny současně dotované jodem ( $RM_1$  oproti  $SM_1$ ) bylo zvýšení statisticky průkazné. Po dodatku jodu se průkazně zvýšil obsah jodu v moči ( $RM_1$  1 005  $\mu\text{g/l}$ , resp.  $SM_1$  723  $\mu\text{g/l}$  ve srovnání s  $RM_0$  238  $\mu\text{g/l}$ , resp.  $SM_0$  104  $\mu\text{g/l}$ ;  $P < 0,01$ ). Vyhodnocení koncentrací hormonů štítné žlázy u skupin dotovaných jodem ukázalo na neprůkazný trend k vyšší koncentraci  $T_3$ , nižší koncentraci celkového  $T_4$  a nižší koncentraci volného  $T_4$ . Dále byla nalezena tendence rozšiřujícího se poměru  $T_3/T_4$  vlivem přídavku jodu ( $RM_1$  0,0421, resp.  $SM_1$  0,0377 ve srovnání s  $RM_0$  0,0353, resp.  $SM_0$  0,0332;  $P > 0,05$ ). Koncentrace  $T_3$ , celkového  $T_4$ , volného  $T_4$  a poměru  $T_3/T_4$  v rámci skupin s přídavkem jodu nebyla vlivem ŘEŠ ovlivněna. Ve skupinách nedotovaných jodem byl v případě hormonů štítné žlázy vliv ŘEŠ také neprůkazný.

**Klíčová slova:** krev; krevní plazma; tyroxin; trijodtyronin; mléko; moč

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**Czech Journal of Animal Science** (Živočišná výroba) • Published by Czech Academy of Agricultural Sciences  
– Institute of Agricultural and Food Information • Editor Office: Slezská 7, 120 56 Praha 2, Czech Republic, phone:  
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