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## Contents

### Part I

BAK B., WILDE J., SIUDA M., KOBYLINSKA M. Comparison of two methods of monitoring honeybee infestation with *Varroa destructor* mite 33

BORSUK G., BUŚ-KICMAN M., JEŻEWSKA-WITKOWSKA G., PALEOLOG J. The use of RAPD-PCR analysis in genetic diversity estimation in *Apis mellifera* honeybee 39

CHUDA-MICKIEWICZ B., PRABUCKI J., SAMBORSKI J., ROSTECKI P.

The response of *Apis Mellifera* L. workers to low air temperature 45

FLISZKIEWICZ M., WILKANIEC Z. Potential possibilities of insemination of mason bee (*Osmia rufa* L.) females by a single male in laboratory conditions 51

GĄBKA J., MADRAS-MAJEWSKA B., KAMIŃSKI Z., OCHNIO M., HOŃKO S. The influence of open brood in rearing colonies on eggs acceptance in different egg age 59


HOŃKO S., MADRAS-MAJEWSKA B., GĄBKA J. Comparison four races of bees in Finnish conditions 63

- KANIA A., JASIŃSKI Z., MADRAS-MAJEWSKA B. The influence of conditions of keeping the drones *A. mellifera* on quality of their semen 71
- MADRAS-MAJEWSKA B. The comparison of bee workers damages in their own colonies without queen 81
- MADRAS-MAJEWSKA B., JASIŃSKI Z., GĄBKĄ J., HOŃKO S., KANIA A. Effect of early supplemental feeding honeybee colonies with a substitute of bee bread made of pollen and honey on colony strength 87
- OLSZEWSKI K. Laboratory assessment of the production value of the honeybees (*Apis mellifera*) with various genotypes 93
- PALEOLOG J. Genetic-environment interactions resulting from different levels of competitiveness of workers in various *Apis mellifera* breeds 101
- SIUDA M., WILDE J., BAŁ B., KOBYLINSKA M. The impact of the sequence of particular drone semen administration on number of their own progeny after instrumental insemination 109
- SOKÓŁ R., MICHALCZYK M., RAŚ-NORYŃSKA M. Evaluation of winter hive debris in a commercial apiary 115
- SPODNIĘWSKA A., SOKÓŁ R. HCH and DDT concentration in bees, honey and bee bread in apiaries from Warmia and Mazury region with a variety of different nectar 121
- TOPOLSKA G., KRZYŻAŃSKA K., HARTWIG A., GAJDA A. The investigation of bee virus infections in Poland 125
- ZAJDEL B., JASIŃSKI Z., SZAFARSKA G. Injuries of honey bee workers and drones caged in native and foreign colonies 135
- Part II**
- DEAS A., GRUSZCZYŃSKA J., ŚWIDEREK W.P. Simulation analysis of correlation between the occurrence of the defined alleles of *Ovar-DRB1* gene in genotype and the health status of the sheep udders 143
- DROBIK W., MARTYNIUK E. Analysis of inbreeding in highly prolific Olkuska sheep flock 153
- FISZDON K., KOWALCZYK I. Litter size, puppy weight at birth and growth rates in different breeds of dogs 161
- KALETA T., GŁOWIENKA E. An analysis of incidents caused by captive cats in the world during 1990–2006 period on the basis of web database 169
- ŁUKASIEWICZ-ŚMIETAŃSKA D., WIRTH-DZIĘCIOŁOWSKA E., GAJEWSKA M. Identification of quantitative trait loci for body weight and body weight gain in two growth-differentiated mouse lines 177
- CHUDZICKA-POPEK M., KALINOWSKA A., MAJDECKA T. The conditions of Polish primitive horses' maintenance in some stables 185
- KLIMASZEWSKI K., JAROSIŃSKA P., ROMAŃSK B. The habitat preferences of amphibians in the catchment area of the Zagożdżonka River 191
- KLIMASZEWSKI K., TURLIK I., POPCZYK B. Characteristic of population and working of earthdogs in Poland in 2001–2007 197
- KUCHARSKA K., KUCHARSKI D., PEZOWICZ E. Entomopathogenic ne-

- matodes in agriculture – potential threat to protected beetle species 205
- STRUŻYŃSKI W. Restocking of the narrow-clawed crayfish (*Astacus leptodactylus* Esch.) in central-eastern Poland 211
- STRUŻYŃSKI W. The occurrence of the narrow-mouthed whorl snail (*Vertigo angustior*) in planned Natura 2000 sites in Masovian Province 217
- ŁOZICKI A., NIŻNIKOWSKI R., GŁOWACZ K., POPIELARCZYK D., KLIMASZEWSKI K., STRZELEC E. Evaluation of feeding value and yield of extensive pastures used in beef cattle grazing 221
- RUPIEWICZ M., NIEMIEC T., LEPIANKA A., BALCERAK M. Effects of excretions/secretions produced by larvae of *Lucilia sericata* on growth of poultry isolates *Salmonella* Enteritidis *in vitro* 229
- STUDNICKA A., SAWOSZ E., GRODZIK M., CHWALIBOG A., BALCERAK M. Influence of nanoparticles of silver/palladium alloy on chicken embryos' development 237
- WIĘSIK M., DYMNICKA M., ARKUSZEWSKA E., ŁOZICKI A. Effect of milk diet on the selected indicators of immunity of rats 243
- ZIELIŃSKA A.K., SAWOSZ E., GRODZIK M., CHWALIBOG A., KAMASZEWSKI M. Influence of nanoparticles of gold on chicken embryos' development 249
- GOLONKA M. Analysis of chosen behavioral forms of Konik Polski horses from the Popielno Reserve 255
- ŁOJEK J., KACZMAREK K., ŁOJEK A. The effect of a Thoroughbred filly participating in the Derby and its influence on her 3-year-old racing career 261
- ŁOJEK J., BURZYŃSKA K., LASKOWSKI M. Activity of herds of Konik Polski horses free ranging in Biebrza National Park 269
- ŁOJEK J., GĘBSKA M., ROMANOWSKI M., ŁOJEK A. Economic effects of racehorse stables management in Warsaw race track 281
- KUCZYŃSKA B., NAŁĘCZ-TARWACKA T. Relationship between content of phospholipids in milk and stability of milk fat globule membrane and the effect of some factors on the frequency of their's destabilization 289
- LITYŃSKI R., NIŻNIKOWSKI R., POPIELARCZYK D., STRZELEC E., GŁOWACZ K. PRNP gene polymorphisms in Polish population of East-Friesian milk sheep 295
- NIŻNIKOWSKI R., POPIELARCZYK D., STRZELEC E., GŁOWACZ K. Characterization of body constitution of old type Polish Merino ewes bred in mazowieckie voivodeship 301
- ŁUKASIEWICZ M., MICHALCZUK M., PIETRZAK D., ADAMCZAK L. Chemical composition and physico-chemical properties of meat of Ayam cemani hens and cocks 307
- ŁUKASIEWICZ M., MICHALCZUK M., GAJEWSKA J., WILCZYŃSKA-CZYŻ K., NIEMIEC J. Wheat distilled dried grains with solubles (DDGS) as a replacer of extraction soil meal in nutrition of broiler 315

- MICHALCZUK M., PIETRZAK D., MROCZEK J., NIEMIEC J., ŁUKASIEWICZ M. Results of rearing and technological traits of meat of slaughter chickens depending of their slaughter age 323
- CIEŚLA M., BŁASZCZAK B., LIRSKI A. Traditional carp farming in Poland as an example of sustainable animal production 329
- KAMASZEWSKI M., OSTASZEWSKA T. Development of digestive tract and swim bladder in larvae and juvenile stages of the Central American *Cichlidae* family on the example of T-bar cichlid (*Cryptoheros sajica*, Bussing 1974) 339
- WOJTYRA B. Regional differentiation of costs and profitability of milk production in Poland 349

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# PART I



## Comparison of two methods of monitoring honeybee infestation with *Varroa destructor* mite

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**Abstract:** *Comparison of two methods of monitoring honeybee infestation with Varroa destructor mite.* Investigations were carried out at the Apicultural Division of Warmia and Mazury University in Olsztyn (Poland) in 2009. The aim of this study was to compare the accuracy of the two different methods of diagnosing the level of infestation with *Varroa destructor* in bee colonies. Sixteen bee colonies were randomly chosen. Two samples of the adult bees were taken from each of them, from the same open brood comb (ca. 250 individuals). The first of these two samples was examined by sugar shake method in the apiary (3 minutes shaking), and the second, by flotation method in laboratory (5 minutes shaking). After the powdered sugar test, bees of the first sample were additionally checked by flotation method (2 minutes shaking). The average infestation in the samples examined by shaking with the powdered sugar amounted 10.43 (mites per 100 worker bees) and was only negligibly lower than the average value of the flotation method where this index amounted 11.09. No statistical differences were found. None of the methods gave always lower or higher results.

**Key words:** *Varroa destructor*, infestation level, flotation method, sugar shake method.

### INTRODUCTION

Monitoring of the level of infestation of bees with *Varroa destructor* is one of the techniques which allows one to determine

the invasion magnitude of the parasite in bee colonies, which in turn facilitates the choosing of a proper treatment method for bees infected with this mite. It also allows one to verify the effectiveness of *Varroa* control (Lodesani et al. 1995) as well as to check on the reinvasion (Sakofski 1989, Sakofski and Koeniger 1988).

There are four principal methods of examining bee colonies for the presence of *V. destructor*. The first method is inspection of dead bees, larvae or pupae. It's very labourconsuming and ineffective (Ritter and Ruttner 1980). The second method is observation of the natural mite drop (Hendrickson 2009). This way requires a period of time. The third method is floatation. In this method bees are shaken into a solution which will dislodge the mites. Several types of the solution were used: hot water (Grobov 1977), detergent solutions (De Jong et al. 1982), hexane or gasoline (Ritter and Ruttner 1980), ether (Macedo et al. 2002) or 96% ethanol. From evidence detergent solutions are very much recommended, because they are comparably effective with ethanol or ether, but are very cheap and non-toxic (De Jong et al. 1982). The

World Organization for Animal Health (OIE) also recommends flotation method for bee sample examination.

The last method is dust. To monitor the level of invasion of *Varroa* in bee colonies glucose, pollen, talcum powder, corn starch, baking soda and wheat flour were already tested (Shah and Shah 1988, Ramirez 1989, Loglio and Pinessi 1993, Macedo et al. 2002), and also the powdered sugar (Fakihimzadeh 2000, Macedo et al. 2002, Aliano and Ellis 2005, Caron et al. 2009). These substances have the same action method. The dusty particles adhere to the parasite body, in particular its ambulacrum making it difficult for it to remain on a bee (Ramirez 1989). The powdered bees clean themselves intensively, shaking off adherence devoid parasites. Ramirez and Malavasi (1991) and Ramirez (1994) found that parasites which had fallen down died from starvation. So the powdered sugar is a good tool for detecting and assessing the degree of infestation (Macedo et al. 2002).

The aim of the present investigation was to compare the accuracy of the two methods of determining the level of bee infestation with *V. destructor*: i.e. the powdered sugar shake method and flotation method. We have chosen the both methods because the one (the powdered sugar shake) is very friendly for bees and the while the other (flotation) is recommended by OIE.

## MATERIAL AND METHODS

Investigations were performed at the Apicultural Division of the Warmia and Mazury University in Olsztyn (Poland) in 2009. Sixteen bee colonies were randomly chosen for examinations. The

honeybee colonies were sampled at the end of August before the start of any *Varroa* control. Two samples of adult worker bees were taken from each of them, from the same open brood comb. The first of the samples was examined by the powdered sugar shake method in the apiary (method I) and the second by flotation method in laboratory conditions (method II).

### **The powdered sugar shake method (method I) (Caron et al. 2009)**

The sample of live bees (ca. 250 individuals) were transferred to the glass jar of 0.9 l capacity covered with 3 mm × 3 mm mesh screen which retains the bees and but allows for mites to drop out during shaking. Two tablespoons of powder sugar were next pushed through screening mesh. The jar with powdered bees was shaken for 2 minutes. The jar was next inverted over a white pan and the mites were shaken down until no more mites came out. The parasites were then counted. One tablespoon powder sugar was again poured into jar and bees were re-shaken for 1 minute. After that time the dropped parasites were also shaken down into pan where they were counted. Parasite numbers from both operations were summarized.

The effectiveness of the powdered sugar shaking method was additionally verified by the flotation method. For this aim, the bees of the powdered sugar shaken sample were killed by freezing them in -20°C temperature. Further, procedure was the same as those which will be described by flotation method except that these samples were shaken for only 2 min.



### Flotation method (method II) (modified method De Jong and al. 1982)

A sample of 250 frozen worker bees were placed into glass jar of 0.9 l capacity and flooded with 200-300 ml 1% detergent solution (this detergent was dish-washing liquid) and next the jar was closed tightly. One should remember, that too much detergent induces intensive foaming, which interferes with searching for mites.

The jars with the prepared samples were shaken on a shaking apparatus for five minutes. After that time the sample was poured down onto a two mesh screen set. The upper screen of 3 mm × 3 mm mesh retained bees corpses and below it was situated 0.5 mm × 0.5 mm mesh screen which did not allow sifted mites to pass. The sample was then rinsed with streaming warm water for 30 seconds and after which the *V. destructor* parasites retained on a bottom screen were counted.

### Calculation of infestation level of bees with *V. destructor*

The number of bees in each sample was counted and the infestation level calculated with the following formula:

$$IL = VN/BN \cdot 100$$

where:

IL – infestation level of bees with *V. destructor*

VN – *Varroa* parasites number found in a sample

BN – bees number in a sample.

Significance of differences had been verified by  $\chi^2$  test for observed values in relation to expected values.

### RESULTS

Average level of bee infestation with *V. destructor* in the samples tested by the powdered sugar shake method amounted to 10.54 (i.e. 10.54 *V. destructor* per 100 adult bee workers) and it was only slightly lower, than average index for samples examined by flotation method where it amounted 11.17. No statistical differences were found between both these mean values ( $\chi^2 = 9$ , df = 16 – 1 = 15, p = 0,87737), (tab).

Differences between infestation levels found by I and II methods, oscillated from 1.4% to 71%. It was also found, that samples originating from bee colonies which had infestation levels compiled as the both methods mean result, higher than 6% differed in this regard at most by 13.6% (colony No 14). Whereas greater differences existed within samples from colonies less invaded with *V. destructor* (Fig. 1). It was not found if one of the two methods always gave lower or higher results – the difference in infestation levels for particular colony samples, examined by the both methods, was in six cases a positive number (more mites were found after the powdered sugar shaking) while in ten cases it was a negative number (more mites were found by flotation method) (Fig. 1).

The effectiveness of the method I was additionally checked by flotation method. In one half of samples no more mites were found during the control examination, while in four of them the percentage of additionally found parasites constituted from 10.2% to 15.8% of total parasite number found in a given sample (Fig. 2).

On 412 mites found in all samples from Group I, 31 were detected by con-

TABLE. Mean *Varroa* infestation level of worker bees in the colonies detected by all methods (n = 16)

infestation level	Mean		
	method I (the powdered sugar shaken)	method II (flotation)	method I + subsequently method II
	10.54	11.17	11.21

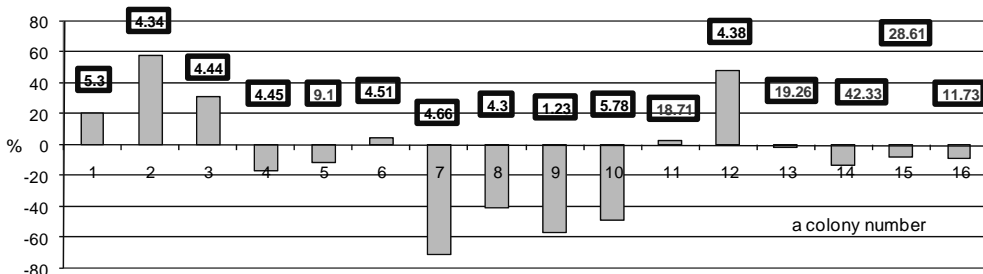


FIGURE 1. Differences between levels of *Varroa* infestation on bees obtained by the method I (the powdered sugar shake) and the method II (flotation) (expressed in % of the greater value) and arithmetical mean indexes values (shown in framings). Red colour shows infestation level >6. 0 level. This means the average infestation of the colony obtained by method I

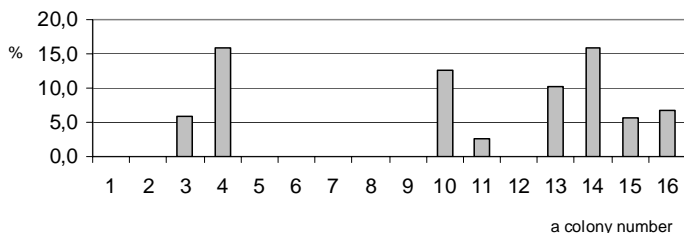


FIGURE 2. Percentage of parasites additionally found by control flotation method

trol flotation method. So, 93% of the mites were detected by the powdered sugar shake method.

The summarized numbers of mites found in bee samples after the powdered sugar shaking plus control flotation test changes the final infestation indexes for investigated honeybee colonies and a mean index for the colonies increases to 11.21 It is still not statistically different from that obtained by flotation method

(11.17) ( $\chi^2 = 8.17$ ,  $df = 16 - 1 = 15$ ,  $p = 0,91684$ ) (tab.).

No statistical differences were also found between the mean result obtained by sugar shake method (10.54) and the mean infestation level (11.21) calculated from examining the same samples by combined method I and next control flotation method ( $\chi^2 = 1.65$ ,  $df = 16 - 1 = 15$ ,  $p = 0,99999$ ) (tab.).

## DISCUSSION

The experiment has shown, that investigation of bee samples for *Varroa* by the powdered sugar shake method in field conditions can be used with the same result as the flotation method. Macedo et al. (2002) came to the same conclusions arrived but in the flotation method they used ether as opposed to detergent solution. They was conformed that the powdered sugar did not differ from ether in assessing low (1–5 mites per sample) infestation levels, but on a higher degree of infestation the powdered sugar is a more accurate method. In our experiment a similar dependence was not found. However, it was noticed, that results of research obtained by both methods in given bee colonies differed from each other more in cases of lower level infestation (< 6). Our experiment has shown, that the powdered sugar shake gave 93% mites on the samples of bees. Our data are similar to the results given by Fakimzadeh (2001) – 91% and much better to results given by Alliano and Ellis (2005) – 76.7%.

The powder sugar dose applied in the present experiment was 3 tablespoons, about 60 g. Fakimzadeh (2001) compared the effectiveness of dusting samples of bees (average 78 bees) with a dose 0.5 g and 5 g. His results indicate, that the first dose is less effective (62% mite fall), that the second one (91% mite fall).

Fakihimzadeh (2001) proved, that:

a) the powdered sugar did not penetrate and accumulate in the tracheal ducts. He believed that this fact is a result of the evolutionary adaptation of this insect to live in a dusty environment (pollen). He indicated, that bee mortality through dusting with the powdered sugar was 8%, so is not large in comparison with the

flotation method, where the bees must be killed regardless of the kind of solution used. Therefore, for ethically the powdered sugar shake method should be used.

De Jong et al. (1982) have shaken bees in a 1% detergent solution for 1 minute and got about 96% females *V. destructor*, which were on the bees, and 100% mite-fall from worker bees after 30 minutes shaking. In our experiment the samples were shaken for 5 minutes by flotation method. This method received comparable results with samples, which was examined by the powdered sugar method plus subsequent flotation method in a total time of 5 minutes. We did not check the effectiveness of flotation method by additional shaking for 25 minutes.

## CONCLUSIONS

1. Both the powdered sugar shaking and flotation methods are equally accurate and can be used interchangeably for monitoring of bees' infestation with *V. destructor*.
2. The powdered sugar shake method is more ethical since does not involve the necessity of killing the examined bees.
3. It is unnecessary to verify the powdered sugar shake method with aid of flotation technique.

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**Streszczenie:** Porównanie dwóch metod badania porażenia pszczoł przez *Varroa destructor*. Badanie przeprowadzono w Katedrze Pszczelnictwa UWM Olsztyn w 2009 roku. Celem doświadczenia było porównanie czułości dwóch metod badania stopnia porażenia pszczoł przez *Varroa destructor*. Do badań wyznaczono losowo 16 rodzin pszczoł z każdego plastra z czerwiami otwartym. Pierwszą próbkę przebadano metodą wytrząsania z cukrem pudrem na pasieczysku (wytrząsano 3 min), a drugą metodą flotacji w warunkach laboratoryjnych (wytrząsano 5 min.). Pszczoły z pierwszej próbki po wytrząsaniu z cukrem pudrem sprawdzono dodatkowo metodą flotacji (wytrząsano 2 min.) w celu weryfikacji skuteczności metody wytrząsania z cukrem pudrem. Średnia intensywność porażenia pszczoł przez *V. destructor* w próbach badanych metodą wytrząsania z cukrem pudrem wyniosła 10,54 i była tylko nieznacznie niższa niż średnia z prób badanych metodą flotacji, gdzie wyniosła 11,17. Różnica ta nie była istotna statystycznie. Wykazano, że obie metody badania porażenia pszczoł przez *V. destructor* są równie dokładne i można je stosować zamiennie. Metoda wytrząsania z cukrem pudrem jest bardziej etyczna, gdyż nie powoduje konieczności uśmiercania badanych pszczoł. Dodatkowe kontrolowanie metody wytrząsania pszczoł z cukrem pudrem za pomocą metody flotacji nie jest konieczne.

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## The use of RAPD-PCR analysis in genetic diversity estimation in *Apis mellifera* honeybee

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**Abstract:** *The use of RAPD-PCR analysis in genetic diversity estimation in Apis mellifera honeybee.* The study was designed to determine the utility of five RAPD-PCR arbitrary primers 539, 514, 694, 691 and 652 in estimation of genetic diversity in three honeybee breeds: *A. m. caucasica*, *A. m. carnica* and Buckfast. Arbitrary primer 514 proved most useful, since it generated the biggest number of RAPD-PCR products and bands characteristic for two honeybee breeds. The examined bee breeds displayed a large genetic similarity ranging from 0,9356 to 0,9818.

**Key words:** RAPD-PCR, genetic distances, genetic similarities, *Apis mellifera*.

### INTRODUCTION

The Caucasian bee (*A. m. caucasica*) and its hybrids are one of the foreign breeds that have been bred in Poland for the longest time (Woźnica 1968, Bornus et al. 1974). Still, beekeepers more frequently search for bees that would produce high yield with little input of human work (Bruder 1983). Therefore, foreign honeybee breeds are imported (Buckfast) in order to improve apiary farming and to gain more financial benefits. Due to the import of new breeds and uncontrolled natural selection and polyandry in bees,

the population of bees in our country is considerably hybridized (Woźnica 1968, Bornus et al. 1974, Gromisz and Skowronek 1982). In order to determine the purebreed population of bees bred in Poland, the usefulness of RAPD-PCR arbitrary primers for identification of genetic diversity should be assessed. This method had previously been used in estimation of genetic diversity of, among others, furbearing animals (Ślaska and Jeżewska 2007). Genetic investigations may facilitate discovery of purebreed, the least interrelated bees which, subsequently, could be regarded as initial breeding material or would be conserved, as in the case of mid-European bee (*A. m. mellifera*). The aim of the research was to check the utility of five RAPD-PCR arbitrary primers in estimation of genetic diversity of three bee breeds: *A. m. caucasica*, *A. m. carnica* and Buckfast.

### MATERIAL AND METHODS

The study was conducted on 12 larvae in each four purebreed groups of bees: Caucasian (*A. m. caucasica*), Carniolan (*A. m. carnica*) and Buckfast. DNA was

isolated with the use of isolation QIAamp DNA Mini Kit (Qiagen). Using five arbitrary primers (539, 514, 694, 691 and 652) we amplified DNA fragments by the RAPD-PCR method with the help of Taq PCR Core Kit (Qiagen). The reaction mixture for one sample contained from 12,7 to 21,6 ng DNA; 3 µl 10xPCR buffer, 4,2µl Q buffer, 2,5 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 0,3 µM arbitrary primer and 1,5 U polymerase. The final volume of the sample was 30 µl. The amplification of RAPD-PCR products was carried out in the thermocycler MJ Research PTC-225 Tetrad in accordance with the following thermal-temporal profile: preliminary denaturation at 94°C for 5 min; subsequently, a programme of 40 repetitive cycles was employed – denaturation at 94°C for 1 min, attaching the primers at 36°C for 2 and annealing of primers at 72°C for 2 min. The final annealing of primers was conducted at 72°C for 10 min. The amplification products were fractionated in 3% agarose gel with the addition of ethidium bromide. A 240 min electrophoresis was done at 70 V. The picture was viewed and analyzed with the use of ScionImage programme. Band length was assessed by GeneRuler™50bpDNA Ladder and pBR322 DNA BsuRI Marker, 5. Nei's genetic distances and similarities were calculated by the PopGene v 32 programme.

## RESULTS

Table 1 presents the results of the amplification of RAPD-PCR products obtained using the five arbitrary primers. When arbitrary primer 694 was used, the amplicons were observed in the smallest number of bees – 69.4 %, while in the case

of two arbitrary primers – 691 and 652, RAPD-PCR products were reported in all the bee larvae examined. The number of bands ranged from two – in the case of arbitrary primer 694 – to nine for 514. The lengths of the analyzed amplicons were in the range of 150 bp (bands of this length were observed when arbitrary primer 652 was used), to 1100 bp, in the case of arbitrary primer 514.

Table 2 presents the lengths of bands which were noted in all the individuals studied as well as band lengths present only in a particular bee breed. On using two arbitrary primers – 514 and 652 – there were four bands of 150 bp to 1000 bp observed in each individual. In the case of arbitrary primer 539, no common bands were observed, and RAPD-PCR products of 1150 bp were observed only in *A. m. carnica* bees. This length bands were noted in 25% of this bee breed. In the case of arbitrary primer 514, bands of 200 bp and 750 bp were visible only in 50% and 8.3% of individuals, respectively, in the Buckfast breed. This arbitrary primer also generated bands of 550 bp, typical only of *A. m. carnica* bees, in 75% of individuals. After the use of arbitrary primer 691, 480 bp bands were observed in 8.3% of *A. m. caucasica* bees. Furthermore, the use of arbitrary primer 652 allowed recognition of 1050 bp bands, which were noted in 58.3% and 33.3% of *A. m. caucasica* and Buckfast bees, respectively. No bands typically related to particular breeds were observed when arbitrary primer 694 was used.

In all the groups the total of 18 loci were observed with 55.56% of polymorphic loci noted in *A. m. caucasica* bees, 38.89% reported in *A. m. carnica* bees, and 11,11% of polymorphic loci in the

TABLE 1. Percentage of individuals with amplification products and the number and length of bands obtained after the use of particular arbitrary primers

Arbitrary primer	% of amplification	Number of bands	Length of bands (bp)
539	97,2	7	400–1150
514	97,2	9	200–1100
694	69,4	2	900–1000
691	100	6	400–1050
652	100	7	150–1050

TABLE 2. Arbitrary primers generating common bands and characteristic bands for particular breeds

Arbitrary primer	COMMON BANDS	CHARACTERISTIC BANDS					
		<i>A. m. caucasica</i>		<i>A. m. carnica</i>		Buckfast	
	bp	bp	% of individuals	bp	% of individuals	bp	% of individuals
539	–	–	–	1150	25	–	–
514	450,600,	200	50	550	75	–	–
	700,1000	750	8,3	–	–	–	–
694	1000	–	–	–	–	–	–
691	400,800,1050	480	8,3	–	–	–	–
652	150,300,550,650	1050	58,3	–	–	1050	33,3

TABLE 3. Genetic distances and similarities between the bee breeds in question (after Nei 1972)

GENETIC DISTANCE		GENETIC SIMILARITY		
		<i>A. m. caucasica</i>	<i>A. m. carnica</i>	<i>Buckfast</i>
		<i>A. m. caucasica</i>	*****	0,9818
<i>A. m. carnica</i>	0,0184	*****	0,9445	
Buckfast	0,0666	0,0571	*****	

group of Buckfast bees. Table 3 presents Nei's genetic distances and similarities.

## DISCUSSION

The largest genetic distances were noted between Buckfast breed and *A. m. caucasica* (0.0666) as well as between Buckfast breed and *A. m. carnica* (0.0571). Such genetic diversity implies that *A. m. caucasica* and *A. m. carnica* bees were not a direct cross components in pro-

duction of the synthetic Buckfast bee breed. However, the similarity between Buckfast and *A. m. caucasica* as well as *A. m. carnica* bees amounts to 0.9356 and 0.9445, which proves their close relatedness. This may be accounted for by the fact that in the production of the synthetic Buckfast breed, the Macedonian bee (Bruder 1983), which belongs to Carniolan (*A. m. carnica*) subspecies was used (Ruttner 1992). The smallest genetic distance (0.0184) was observed

between *A. m. caucasica* and *A. m. carnica*, which indicates notable similarity to Carniolan bees (*A. m. carnica*) that was acquired by Caucasian bee breed (*A. m. caucasica*). This is confirmed by the largest genetic similarity (0.9818) and may have resulted from the fact that the Caucasian breed was imported to Poland in the sixties (Bornus et al 1974, 1968). Preserving breed purity in these bees for approximately 50 years was difficult and might have pose problems related to inbreeding depression (Laidlaw 1986). Possibly, in order to avoid inbreeding issues, the Caucasian bee (*A. m. caucasica*) was recrossed with *A. m. carnica*, which is confirmed by a small genetic distance and a large genetic similarity between the investigated breeds.

## CONCLUSIONS

1. Buckfast is a newly imported bee in our country, thus, it displays the largest genetic distance, larger than that between Carniolan and Caucasian bees, which are already being bred in Poland.

2. Due to the inbreeding depression in purebreed bees, *A. m. caucasica* has been recrossed with *A. m. carnica*, therefore genetic similarities between these breeds proved the largest.

3. Arbitrary primer 514 turned out to be most useful in the research conducted on the honeybee, since it generated the biggest number of RAPD-PCR products and bands characteristic for two breeds, namely, *A. m. caucasica* and *A. m. carnica*.

4. Arbitrary primer 694 was apparently the least useful, because it only generated two bands; no characteristic amplicons were observed in any of the investigated groups, either.

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- Streszczenie:** Wykorzystanie analizy RAPD-PCR do różnicowania genetycznego pszczoły miodnej *Apis mellifera*. Pszczoła kaukaska i jej mieszańce są jedną z najdłużej użytkowanych obcych ras pszczół w Polsce. Jednak pszczelarze coraz częściej poszukują pszczoły, która będzie wymagała małych nakładów pracy przy jednoczesnej wysokiej produkcji. Dlatego coraz częściej prowadzone są obce rasy pszczół (Buckfast) w celu usprawnienia gospodarki pasiecznej, zwiększenia wydajności pasiek, a tym samym zwiększenia korzyści finansowych. Ze względu na import nowych ras pszczół oraz występujący u pszczół niekontrolowany naturalny dobór i poliandrię pogłowie pszczół w naszym kraju jest silnie zmieszane. W celu ustalenia czystorasowego pogłowia pszczół użytkowanych w Polsce należało określić przydatność pięciu starterów reakcji RAPD-PCR 539, 514, 694, 691,652 do oceny



zróznicowana genetycznego trzech ras pszczoł *A. m. caucasica*, *A. m. carnica* oraz Buckfast. Najbardziej przydatnym okazał się starter 514, gdyż generował największą liczbę produktów RAPD-PCR oraz prążki charakterystyczne dla dwóch ras pszczoł (*A. m. caucasica* i *A. m. carnica*). Badane rasy pszczoł wykazywały duże podobieństwo genetyczne mieszczące się w zakresie 0,9356–0,9818 (wg Nei).

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## The response of *Apis Mellifera L.* worker bees to low air temperature

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**Abstract:** *The response of Apis Mellifera L. worker bees to low air temperature.* The survivability and behaviour of worker bees of the Middle European, Carnolian and Caucasian breed at low air temperature was examined. Worker bees were individually placed in plastic cages and exposed to low temperatures – 5, 7 and 9°C – in a cooling incubator for a period of 60 and 120 min. After the fixed time elapsed, the number of moving worker bees as well as of chill-coma ones was recorded at a room temperature. The time after which worker bees recovered their vital activity, manifesting in abdomen, legs or antennae motion and assumption of full locomotor efficiency, was determined. All worker bees staying at 5, 7 and 9°C for a period of 60 and 120 min fell into the chill-coma state, irrespective of their breed and age (5, 10, 15 and 20 days). However, the time when bees remained motionless after removal from incubator differed. Worker bees kept at 5°C for, respectively, 60 and 120 min were motionless for the longest time, i.e.  $163 \pm 71$ – $236 \pm 84$  s. On the other hand, worker bees exposed to 9°C for 60 min as well as for 120 min were in the chill-coma for the shortest time, i.e.  $111 \pm 35$  and  $123 \pm 42$  s, respectively. The youngest, 5 day old, bees were the most susceptible to low temperature, both to 5, 7 and 9°C. The time of staying motionless in the worker bees examined as well as of returning to full locomotor efficiency was determined by their breed. The Middle European worker bees were being distinguished by the shortest time, whereas the Carnolian ones by the longest.

**Key words:** *Apis mellifera*, worker bee, temperature, chill-coma, locomotor activity.

## INTRODUCTION

Honey bee (*Apis mellifera L.*) colonies are being characterised by highly developed thermoregulation mechanism which enables them to inhabit also northern areas of Canada and Scandinavia (Ruttner 1992). However, a single bee dies of cold at –2 to –6°C after less than one hour (Free and Spencer-Booth 1960). According to Himmner (1926, after Free and Spencer-Booth 1958), a bee becomes rigid at 6–7°C, whereas it loses mobility at 11–12°C. A worker bee falls into chill-coma at a lower temperature, within a range of 9–12°C, while a drone in a temperature higher by 2–3°C. Most bees die in chill-coma after 50 hours (Free and Spencer-Booth 1960, Goller and Esch 1990). Return to vital activity may take place providing that ambient temperature increases to 16–20°C (Free and Spencer-Booth 1960, Heinrich 1980ab, 1987).

The cited authors refer their results to the species of honey bee, whereas our study relates to three breeds of honey bee, i.e. the response of their organisms to low temperatures.

## MATERIAL AND METHODS

Examinations were carried out in 2005 and 2006. They were made on worker bees of the Carnolian breed (Car), Caucasian breed (Cau) and Middle European breed (M) of different age: 5, 10, 15 and 20 days. In order to obtain worker bees of a specific age, queen bees were isolated on honeycombs for a period of twenty four hours under isolator made of queen excluder. After laying the eggs, a queen bee was set free, while a brood honeycomb was left under isolator until brood cells were sealed. One day prior to the worker bees emerging, honeycombs were placed in isolators made of 2x2 mm wire mesh. Young worker bees after reaching the age of 5, 10, 15 and 20 days, 16 in number in each age group, were placed individually in plastic cages and exposed to low temperature – 5, 7 and 9°C – in a cooling incubator, 8 worker bees for a period of 60 min and 8 ones for a period of 120 min. After the fixed time elapsed, the number of moving worker bees as well as of motionless ones (being in chill-coma) was recorded at a room temperature (20–22°C). The time after which worker bees recovered their vital activity, manifesting in abdomen, legs or antennae motion and assumption of the standing (marching) position, was determined in seconds. Observations were carried out at the turn of July–August, in two replications. Calculations and statistical analysis of findings were made with Statistica 7.0 computer software package, with significance being evaluated with ANOVA (Tukey test was applied) (Sokal and Rohlf 1981).

## RESULTS

All worker bees exposed to low temperature (5, 7 and 9°C) for a period of 60 and 120 min fell into chill-coma. However, the average time during which they remained motionless after removal from incubator differed significantly. Worker bees kept at 5°C for 120 min ( $236\pm 84$  s) remained motionless for the longest time, while those kept at 9°C for 60 and 120 min were motionless for the significantly short time, i.e.  $111\pm 71$  s and  $160\pm 63$  s, respectively (Table 1). The time when worker bees remained motionless was determined by their age since bees 5 days old kept at 5°C for 60 and 120 min as well those kept at 7 and 9°C for 120 min were motionless significantly longer than 10 day - and 15 day old worker bees. Worker bees aged 20 days were in chill-coma for a significantly shorter time than those aged 5, 10 and 15 days old kept at 7°C for 60 min and at 9°C for 120 min.

Table 2 presents the time of restoring full locomotor efficiency by worker bees, which consisted of chill-coma time (Table 1) and that of assuming the marching position. Together with a decrease in temperature, the time of recovering full vital activity by worker bees significantly extended. Worker bees kept at 5°C for 120 min recovered it on average after  $566\pm 173$  s, while those kept at 9°C for 60 min on average after  $315\pm 101$  s. Worker bees staying at 5 and 7°C for 120 min were reaching locomotor efficiency significantly longer than those kept at these temperatures for 60 min. On the other hand, worker bees staying at 9°C for 60

TABLE 1. Chill-coma mean time (in s) workers exposed to 5, 7 and 9°C for the periods of 60 and 120 min.

Age (days)	Breed	60 min			120 min		
		5°	7°	9°	5°	7°	9°
		$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$
5*	Car	119 ± 39	106 ± 64	82 ± 14	219 ± 101	136 ± 83	83 ± 37
	Cau	234 ± 90	122 ± 36	112 ± 41	292 ± 103	220 ± 77	130 ± 37
	M	228 ± 123	128 ± 42	121 ± 49	350 ± 155	242 ± 77	143 ± 46
$\bar{x}$	194 ± 103abc A	119 ± 49 ad A	105 ± 40 be A	287 ± 134 aef A	199 ± 90deg A	119 ± 49 cfg A	
10	Car	130 ± 26	135 ± 45	92 ± 32	204 ± 57	189 ± 62	106 ± 31
	Cau	115 ± 37	94 ± 25	88 ± 34	178 ± 73	124 ± 44	95 ± 35
	M	119 ± 60	96 ± 16	91 ± 28	192 ± 78	118 ± 35	97 ± 42
$\bar{x}$	121 ± 42 ac AB	108 ± 36 b B	90 ± 29 a B	191 ± 75 abc AB	144 ± 59abc AB	99 ± 34 c AB	
15	Car	144 ± 30	112 ± 22	82 ± 29	208 ± 55	131 ± 57	102 ± 37
	Cau	167 ± 78	120 ± 25	96 ± 54	186 ± 57	121 ± 30	136 ± 41
	M	140 ± 85	120 ± 65	78 ± 29	150 ± 53	113 ± 59	130 ± 34
$\bar{x}$	150 ± 64 ab AB	117 ± 38 ab C	85 ± 27 ab A	181 ± 54 ab AB	122 ± 44 a AB	123 ± 38 b B	
20	Car	167 ± 30	140 ± 24	189 ± 45	285 ± 84	148 ± 27	160 ± 45
	Cau	175 ± 30	131 ± 23	163 ± 40	279 ± 57	164 ± 36	155 ± 37
	M	218 ± 101	122 ± 25	133 ± 28	300 ± 91	215 ± 99	144 ± 44
$\bar{x}$	187 ± 66 ab B	131 ± 31 ab ABC	162 ± 44 ab AB	288 ± 77 ab B	176 ± 67a B	153 ± 40 b AB	
Total		163 ± 71 a	118 ± 39 b	111 ± 35 a	236 ± 84 ab	160 ± 63 bc	123 ± 42ac

\*n = 32 for each age group, breed, times and temperatures

Figures followed by the same big letter in columns and small in row are significantly different at p < 0.05.

and 120 min recovered their locomotor efficiency in a similar, not significantly different time, i.e. after 315±101 and 332±111 s on average.

When analysing the average time when worker bees recovered their locomotor efficiency in age groups, it appeared that it was determined by their breed. The 5 day- and 10 day old M worker bees staying at low temperatures for 60 min returned to their full locomotor efficiency significantly faster than the Car ones. Also the M worker bees aged 15 days

recovered significantly faster their locomotor efficiency than the Cau ones in the same age (Table 2). After staying at low temperatures for 120 min, significant difference between worker bees in recovering the locomotor activity was found between the M, Cau and Car ones aged 10 days as well as between the Cau and Car ones aged 20 days (Table 2). The average time from coming out of the chill-coma state to assuming the marching position between the M and the Car worker bees staying at 7°C for 60 min and at 9°C for

TABLE 2. Mean time of recovering full locomotor efficiency (marching position) by workers exposed to 5, 7 and 9°C for the periods of 60 and 120 min

Age (days)	Breed	60 min				120 min			
		5°		7°		9°		x̄ ± sd	
		x̄ ± sd	x̄ ± sd	x̄ ± sd	x̄ ± sd	x̄ ± sd	x̄ ± sd		
5*	Car	493 ± 99	436 ± 99	355 ± 67	428 ± 82 A	484 ± 91	400 ± 76	393 ± 74	426 ± 80 C
	Cau	491 ± 98	429 ± 79	330 ± 92	417 ± 86B	602 ± 138	485 ± 113	283 ± 124	457 ± 126 B
	M	505 ± 161	340 ± 64	221 ± 70	355 ± 91AB	564 ± 197	448 ± 150	239 ± 81	417 ± 147 A
	x̄	496 ± 20ab	402 ± 92ab	302 ± 96a	400 ± 87	550 ± 154a,b	444 ± 120a,b	305 ± 114b	433 ± 119
10	Car	484 ± 131	465 ± 139	328 ± 86	426 ± 119AB	593 ± 117	541 ± 150	349 ± 40	494 ± 108 A
	Cau	317 ± 142	306 ± 109	324 ± 97	316 ± 127A	499 ± 177	446 ± 143	306 ± 153	417 ± 161 A
	M	345 ± 90	338 ± 80	238 ± 79	307 ± 84B	400 ± 215	386 ± 136	266 ± 116	351 ± 169 A
	x̄	382 ± 141a	370 ± 172a	297 ± 95a	349 ± 107	497 ± 151a	458 ± 154a	307 ± 116a	421 ± 142
15	Car	378 ± 34	362 ± 88	269 ± 112	336 ± 79A	559 ± 123	464 ± 167	349 ± 160	457 ± 151 A
	Cau	424 ± 45	430 ± 112	305 ± 56	386 ± 7AB	701 ± 267	450 ± 82	318 ± 99	490 ± 137C
	M	354 ± 65	338 ± 65	282 ± 143	325 ± 84B	667 ± 270	434 ± 124	275 ± 68	459 ± 167B
	x̄	385 ± 57a	377 ± 96 b	285 ± 109a,b	349 ± 77	642 ± 234a,b	449 ± 127a,b	314 ± 117a,b	469 ± 149
20	Car	370 ± 73	407 ± 135	378 ± 105	385 ± 102 B	615 ± 131	461 ± 147	448 ± 126	508 ± 137 A
	Cau	495 ± 95	382 ± 96	312 ± 49	396 ± 81 B	506 ± 67	431 ± 117	379 ± 62	439 ± 79 A
	M	387 ± 53	322 ± 59	433 ± 153	381 ± 73 A	604 ± 234	454 ± 143	383 ± 80	480 ± 151B
	x̄	417 ± 93ab	370 ± 106a	374 ± 119b	387 ± 89	575 ± 164a,b	449 ± 134a,b	403 ± 97a,b	476 ± 133
Total	413 ± 112ab	380 ± 109 a,b	315 ± 101 b		566 ± 173ab	450 ± 131 a,b	332 ± 111a		

\*n = 32 for each age group, breed, times and temperatures

Figures followed by the same big letter in columns in the age group and small in row are significantly different at p < 0.05.

TABLE 3. Mean time (in s) of recovering the marching position by workers that stayed at 5, 7 and 9°C for the periods of 60 and 120 min. after coming out of chill-coma

Breed	5°		7°		9°		x̄
	60 min	120 min	60 min	120 min	60 min	120 min	
	x̄ ± sd	x̄ ± sd	x̄ ± sd	x̄ ± sd	x̄ ± sd	x̄ ± sd	
Car**	285±166 A	396±197 A	270±173 A	309±194 C	222±137 A	249±162a	289 ± 181*
Cau	303±165 C	406±228 B	253±158 B	306±175 B	217±120 B	226±131 B	285 ± 178
M	287±156 B	403±241 C	225±140 A	301±173 A	200±140 A	210±113a	271 ± 180*
x̄	347 ± 192a		277 ± 167a		221 ± 129a		292 ± 180

\*\*n = 128 for each breed, temperatures and times

Figures followed the same big letter in columns and small in row are significantly different at p < 0.05, in columns followed the star are significantly different at p < 0.0 6.

60 and 120 min was similarly different, with the M ones recovering it faster. On the other hand, locomotor efficiency recovering time in the Cau worker bees did not differ from that in the Car and M ones (Table 3). It can also be seen in Table 3 that the longer the bees stayed at low temperatures, the significantly longer was the time of coming out of the chill-coma to reaching the locomotor efficiency.

## DISCUSSION

All Car, Cau and M worker bees were motionless and fell into chill-coma after keeping them at 5, 7 and 9°C for 60 and 120 min. Free and Spencer-Booth (1958) reported that 6.2% of bees collected directly from colonies and kept at 9°C were moving after 60 min, while the other ones were in chill-coma. In our study, the youngest bees (5 days old) were the most susceptible to low temperature which is in conformity with the results of Free and Spencer-Booth (1960) who observed falling into chill-coma in bees in age groups 1–7, 8–14, 15–21 and 22–28 days at 9 to 14°C for one hour. The longer time of staying in chill-coma in young bees was a result of limited ability to raise body temperature by young bees at low temperature (Allen 1959). In our study, the average time of recovering full locomotor efficiency (marching position) by worker bees was 315–332 s for bees kept at 9°C for 60 and 120 min to 420–566 s for those kept at 5°C for 60–120 min. According to Free and Spencer-Booth (1960), bees kept at 5°C for 1h came out from chill-coma after 143.3 and 323.9 s, while those kept for 5 h after 325.2–487.8 s, thus in a similar time like in our study.

## CONCLUSIONS

1. Single worker bees of the Carnolian, Caucasian and Middle European breed staying at 5, 7, and 9°C for 60 and 120 min fall into the chill-coma.
2. The youngest bees remain motionless for the longest time after being affected by low temperature.
3. The most resistant to low temperature are worker bees of the Middle European breed. They have the shortest time of returning to the marching position.

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**Streszczenie:** *Reakcja robotnic Apis mellifera na niską temperaturę powietrza.* Badano przeżywalność i zachowanie się pszczoł robotnic rasy: środkowoeuropejskiej kraińskiej i kaukaskiej w niskiej temperaturze powietrza. Robotnice indywidualnie umieszczano w klateczkach plastikowych i poddawano działaniu temperatury 5, 7 i 9°C, w inkubatorze z chłodzeniem, przez okres 60 i 120 min. Po upływie wyznaczonego czasu, w temperaturze pokojowej, odnotowywano liczbę pszczoł poruszających się i będących w stanie odrętwienia. Określano czas, po którym pszczoły powracały do aktywności życiowej, przejawiającej się ruchami odwłoka, odnoży czy też czułków oraz odzyskania pełnej sprawności ruchowej.

Robotnice przebywające w temperaturze 5, 7 i 9°C przez okres 60 i 120 min bez względu na przynależność rasową i wiek (5, 10, 15, 20 dni) wszystkie zapadały w stan odrętwienia. Zróżnicowany był natomiast czas pozostawania ich w bezruchu po wyjęciu z inkubatora. Najdłużej w bezruchu  $163 \pm 71$ – $236 \pm 84$  s pozostawały robotnice przetrzymywane w temperaturze 5°C od-

powiednio przez 60 i 120 min. Najkrócej w stanie odrętwienia pozostawały robotnice poddane działaniu temperatury 9°C przez 60 min –  $111 \pm 35$  s i 120 min –  $123 \pm 42$  s. Najbardziej wrażliwe na działanie niskiej temperatury zarówno 5, 7 i 9°C były najmłodsze, 5dniowe, pszczoły. Pozostawanie pszczoł w bezruchu i dochodzenia do pełnej sprawności ruchowej było uwarunkowane ich przynależnością rasową. Najkrótszym okresem w wyróżniały się pszczoły rasy środkowoeuropejskiej, najdłuższym zaś rasy kraińskiej.

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## Potential possibilities of insemination of mason bee (*Osmia rufa* L.) females by a single male in laboratory conditions

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**Abstract:** *Potential possibilities of insemination of mason bee (*Osmia rufa* L.) females by a single male in laboratory conditions.* The mason bee, which belongs to the *Megachilidae* family is one of the wild-living bees which has been used for many years to pollinate early-spring orchard cultivations and isolated seed plantations. The aim of this study was to determine the number of females which a single mason bee male is capable to inseminate in laboratory conditions. The experimental material comprised males (30 individuals) and females (165 individuals) of mason bee. The trial included 10 experimental groups differing with regard to the number of females (1 to 10) at the same number of males – 1 for each group. Each group had three repetitions. The insects were kept in individual boxes to allow them to copulate. Next, the analysis of female prepared seminal receptacles was performed during which their size and the number of sperm cells found inside them were determined. Chi test – square, Duncan test and one-way analysis of variance were employed in the performed statistical calculations. Different numbers of inseminated females were observed in each experimental group. The performed experiment revealed that the best insemination results of females were recorded in the experimental group in which there was one male for three mason bee females and the maximum number of inseminated females by a single male was five. The mean volume of seminal receptacles in the inseminated females was slightly bigger than in non-inseminated females, although this difference was statistically non-significant. The mean number of sperm cells in the seminal receptacles of the inseminated females ranged from 1400 to 5683. The performed analysis of variance showed that there were no statistically significant differences between the

number of sperm cells in seminal receptacles and the number of inseminated females in individual combinations.

*Key words:* mason bee, insemination, seminal receptacle, number of sperm cells.

### INTRODUCTION

Mason bee, which belongs to the *Megachilidae* family, has been successfully used to pollinate cultivations intended to obtain high yields of seeds or fruits of plants pollinated by them (Wilkaniec, Fliszkiewicz, Giejdasz 2003). In Poland, investigations aiming to increase the population size of these insects by their artificial reproduction have been conducted for many years now (Wójtowski, Wilkaniec 1969). The mason bee is one of the most effective wild-living pollinators both of field cultivations as well as plants cultivated under cover (greenhouses, plastic tunnels, isolated boxes) (Giejdasz, Wilkaniec 2000). Most frequently, the above-mentioned bee is used to pollinate orchard plants (wild strawberries, black and red currants, sweet cherries, strawberries, sour cherries, raspberries and plums) (Zientak-Varga 2006).

Recognizing the advantages of the employment of the mason bee as a plant

pollinator, investigations are being carried out continuously with the aim to learn more about this species. Experiments involve not only the pollination effectiveness of various plant species but also its bionomy, including reproduction, etiology and improvement of breeding methods (Wilkaniec, Giejdasz 2003). In Poland, the breeding of the bee is carried out on an increasingly wider scale not only by research centres but also by private persons.

The objective of this research work was to determine the number of females which a single mason bee male is capable of inseminating in laboratory conditions.

## MATERIAL AND METHODS

The presented investigations were carried out at the Section of Useful Insect Breeding of the Institute of Zoology, University of Life Sciences in Poznań. They were conducted in April and May, that is to say, in the period of natural copulation of these insects. The experimental material included males (30 individuals) and females (165 individuals) of mason bee which were obtained from breeding carried out in a field station of Swadzim Station near Poznań. The insects in their cocoons, after taking them out of their nests, were stored in a refrigerator at the temperature of +4°C. In April, cocoon shells were taken out from the refrigerator and segregated according to the sex of insects found inside which was possible after cutting the top of the cocoon. The cocoon shells were put onto Petri dishes, separately males and females, and placed in a thermostat at the temperature of +35°C and air humidity of about 70% to allow the insects to break the cocoon

open. The control of cocoon shells was conducted daily until all insects emerged. Males emerged from the cocoon shells placed on Petri dishes after two days, while females – after three days.

Freshly emerged insects were transferred into plastic boxes (isolation chamber) measuring 26 × 17 × 16 cm. The trial comprised ten experimental groups differing with respect to the number of females (from 1 to 10) with the identical number (1) of males in each group. Each experimental group included three replications. Isolation chambers were placed in the laboratory near the window making sure that they received the same amount of daylight. Moreover, the isolation chambers were additionally illuminated using artificial light – infrared radiator. A 12-hour lighting cycle was applied controlled by an electronic controller. Two additional rectangular isolation chambers made of acrylic board, measuring 22 × 22 × 40 cm, were settled separately with males and females. Reserve insects were kept in them.

Insects kept in isolation chamber were supplied with nourishment on Petri dishes 3 cm in diameter which contained sugar-water syrup (1:1) and flower pollen in the form of ground pollen loads obtained from honey bee. Comb foundation disks were placed on the surface of sugar-water syrup to prevent insects from drowning. The nourishment was replaced every second day controlling the number of mason bee females replacing the dead ones with new individuals. When a male died, the entire experimental group was created again.

After the termination of the copulation period which lasted two weeks, female seminal receptacles were prepared and

measurements of their diameters were carried out using for this purpose a camera and the Axio Vision Rel. 4.0 program to analyse the microscopic images. The seminal receptacle is spherical in shape therefore its volume was calculated substituting the measurement results into the formula for the calculation of the sphere  $v = 4/3\pi r^3$ .

Next seminal receptacles were individually transferred into 200  $\mu$ l Hyes liquid, broken and their contents were vigorously mixed with the liquid. After placing a drop of solution in a Fusch-Rosenthal chamber, using a microscope with a phase contrast at  $20 \times 10$  magnification, sperm cells were counted in 10 large squares, i.e. in 2  $\mu$ l, which made it possible to determine the number of sperm cells in the seminal receptacle. In this way, the total of 165 mason bee females was analysed. In order to estimate how many females can one male of mason bee inseminate in various combinations, the chi-square test was applied, whereas Duncan test was employed to perform a detailed comparison of the significance of differences. Single-factorial

analysis of variance was used to compare the results of measurements of seminal receptacles of inseminated and non-inseminated mason bee females.

## RESULTS

As shown in Table 1, different numbers of fertilised females were observed in each experimental group.

Both in experimental groups as well as in replications, the number of inseminated females varied significantly.

The highest number of inseminated females was recorded in the experimental group comprising 10 females where the sum of fertilised females in three replications amounted to 9. The total number of 8 inseminated females was found in group VIII where 8 females fell on one male in individual replications. The total number of 6 inseminated females was determined in groups IX, V and III in which respectively: 9, 5 and 3 females fell per one male. In the case of the remaining experimental groups, the total number of fertilised females amounted to: 4, 2, 1 and 0.

TABLE 1. Number and percent of fertilised females in individual experimental groups

Experimental groups		Replication			Total
No	male : female	1	2	3	Number (%)
I	1:1	0	0	0	0 (0)
II	1:2	1	0	0	1 (16.6)
III	1:3	2	2	2	6 (66.7)
IV	1:4	1	1	2	4 (33.3)
V	1:5	3	1	2	6 (40.0)
VI	1:6	1	0	1	2 (11.1)
VII	1:7	1	1	2	4 (19.0)
VIII	1:8	2	5	1	8 (33.3)
IX	1:9	1	2	3	6 (22.2)
X	1:10	5	3	1	9 (30.0)
				Total	46 (27.9)

In order to find out whether the number of females inseminated by one male depended on the number of females in individual experimental groups, statistical calculations were performed using the chi-square test which yielded the value of  $\chi^2_{emp} = 15.85$  at the  $\chi^2_{tab} = 16.92$  value for the number of degrees of freedom  $df = 9$  and significance level  $\alpha = 0.05$ , in other words:

$$\chi^2_{emp} < \chi^2_{tab}$$

which confirmed that the number of inseminated females did not depend on the number of females falling on one male.

The experiment comprised the total number of 165 mason bee females of which 46 females were inseminated which constituted 27.9% (Tab. 1). The numbers of inseminated females in individual experimental groups and replications within group are presented in Figure 1.

The obtained results confirm considerable variations in numbers of inseminated females in individual replications within groups. This refers to all experimental groups with the exception of group I where the male to female ratio was 1:1 (the number of inseminated females in all replications was 0) and group III in which three females fell on one male (the number of inseminated females in all replications was 2). The results collated in Figure 1 show that one male can inseminate the maximum of 5 females (experimental groups VIII and X). On the other hand, in experimental group I, no inseminated females were found in any of the replications.

Table 2 presents minimal, maximal and mean volume values of seminal receptacles in inseminated and non-inseminated females.

It is evident from Table 2 that both in inseminated as well as in non-insemi-

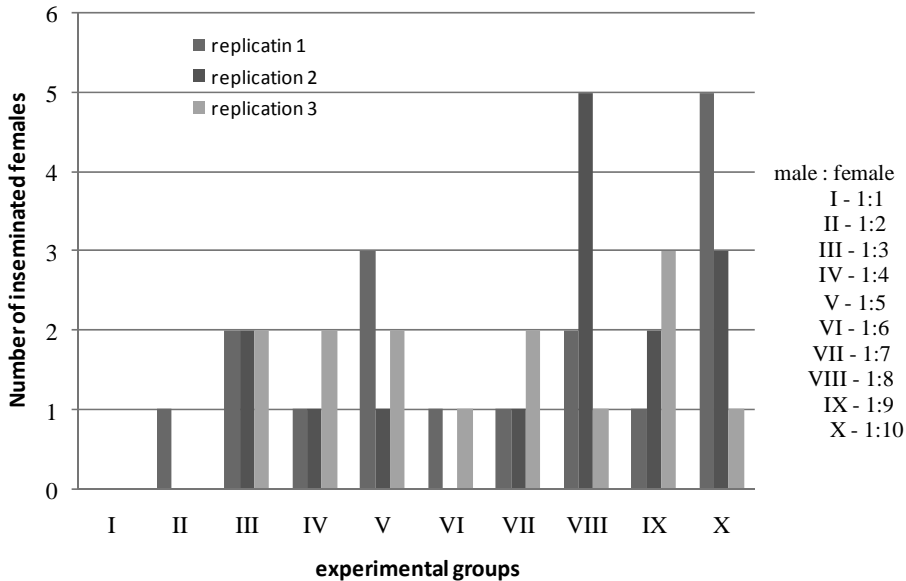


FIGURE 1. Numbers of inseminated females in individual experimental groups

nated females minimum and maximum volume values of seminal receptacles were identical, while mean values differed slightly. Absence of significant differences is confirmed by the range test at the level of significance  $\alpha = 0.05$ . In addition, the performed single factorial analysis of variance revealed that F value was smaller than the F value read from tables for the significance level of  $\alpha = 0.05$ .

$$F_{(\alpha = 0.05)} = 3.19 < F_{tab(\alpha = 0.05)} = 3.84$$

hence, there were no statistically significant differences between the volume of seminal receptacles in inseminated females and those of non-inseminated ones. This means that the size of the seminal receptacle is an individual feature of a given female and does not undergo changes following insemination.

Mason bee females in individual experimental groups differed with regard to the filling of seminal receptacles with sperm cells. These values are presented in Table 3.

The mean number of sperm cells in the seminal receptacle of mason bee inseminated females in individual experimental groups ranged from 1400 to 5683, whereas the mean from all experimental groups was 4035. In order to check whether there was a correlation between the number of sperm cells in the seminal receptacle and the number of females falling on one male in individual experimental groups statistical calculations were performed employing Duncan test. The obtained F value = 1.29 for the level of significance of  $\alpha = 0.05$ .

$$F_{(\alpha = 0.05)} = 1.29 < F_{tab(\alpha = 0.05)} = 2.35$$

TABLE 2. Volume of the seminal receptacle in mason bee females [ $\mu$ l]

Inseminated females	Non-inseminated females
min = 0.00038	min = 0.00038
max = 0.00418	max = 0.00418
mean = 0.0015	mean = 0.0013

TABLE 3. Mean number of sperm cells in a given experimental group

Experimental groups		Mean number of sperm cells
No	male:female	
I	1:1	0
II	1:2	1600
III	1:3	5450
IV	1:4	4200
V	1:5	5683
VI	1:6	1400
VII	1:7	4000
VIII	1:8	4050
IX	1:9	4466
X	1:10	2488
<b>Mean</b>		4035

The obtained F value was smaller than the value of F read from tables, therefore it can be said that no correlation was observed between the number of sperm cells in seminal receptacles of inseminated females and the number of females falling on one male in individual experimental groups.

## DISCUSSION

Since mason bee females take active part in plant pollination, therefore for economical reasons, it is more favourable for breeders of these insects to 'manufacture' populations in which females are in majority (Giejdasz, Wilkaniec 2000). In natural conditions, there is a numerical equilibrium between males and females in populations of *Osmia rufa* (Kristjansson 1992). In breeding conditions, gender structure can, to some extent, be regulated by supplying females nesting tubes of specific diameter (Wilkaniec et al. 2000). Therefore, it is important to know to what level it would be possible to decrease the number of males in the populations without a negative impact on female insemination. In natural conditions, the male waiting for females near the nest or flowering plants is capable to inseminate, on average, 4 females (Seidelmann 1999). Our experiments which were conducted in laboratory conditions showed that one male can inseminate up to 5 females. However, the ratio of 1 male for 3 females turned out to be most favourable because in all three replications two females were inseminated which constituted 66.65% of all females in this experimental group. The results of insemination obtained in laboratory conditions were relatively low and hetero-

geneous. They could have been caused by worse conditions than in natural environment or could be attributed to the individual feature of a given male used in experiments.

In the described trial, out of 165 mason bee females, 115 females failed to become inseminated and in one case maceration of the seminal receptacle was recorded. Lack of similar investigations carried out on mason bee makes comparison of the obtained results impossible. The obtained results can only be referred to other hymenoptera, e.g. bumble bees. In his experiments, Schousboa (1994), who investigated the filling of seminal receptacles in bumble-bee queens, failed to find any of them empty. The mean size of this organ in bumblebee mothers in different experimental groups ranged from 0.007 to 0.0072  $\mu\text{l}$ , whereas the size of this organ in the examined mason bee females on average fluctuated from 0.0013 to 0.0015  $\mu\text{l}$ . The performed statistical calculations revealed that the filling of the seminal receptacle with sperm cells did not affect its size.

The mean number of sperm cells in the seminal receptacles of analysed bumblebee mothers fluctuated at the level from 5800 to 36200 sperm cells (Tasei, Aupinel 1994). Even higher numbers of sperm cells of over 52000 were reported by Röseler (1973) who examined bumblebee queens caught both in spring and in autumn. Investigations carried out by Fliszkiewicz (2002) revealed that the number of sperm cells in naturally overwintered queens of *Bombus terrestris* amounted, on average, to 67080, whereas in queens that fell into winter diapause – to 54140. Bearing in mind the fact that *Bombus terrestris* families may consist of

up to 1000 members, the reserve of sperm cells in the seminal receptacle of the female is very large. A similar situation occurs in the examined mason bee. In natural conditions, a mason bee female can build and supply 20-30 hatching cells in flower pollen (Teper 2004), hence lay the same number of eggs. According to our investigations, the mean number of sperm cells in seminal receptacles of females from individual experimental groups amounted from 1400 to 5683. Seidelmann (1999) reported that a mason bee female copulates only with one male. In his earlier studies, the same researcher reported that the filling of the female seminal receptacle 8 hours after copulation was of the order of  $10^3$  (Seidelmann 1995), which was also confirmed by the results of the above-described investigations.

## CONCLUSIONS

1. The performed investigations revealed that one mason bee (*Osmia rufa* L.) male can inseminate maximum of 5 females and the number of inseminated females does not depend on the number of females falling on one male.

2. The mean volume of semen receptacles of inseminated and non-inseminated mason bee females does not exhibit statistically significant differences.

3. The number of semen cells in the seminal receptacle of inseminated mason bee females varied and, on average, amounted to over 4000.

4. The volume of the seminal receptacle which was not filled with sperm cells did not show significant differences in comparison with the volume of the seminal receptacle which was filled with sperm cells.

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**Streszczenie:** *Potencjalne możliwości inseminacji murarki ogrodowych (*Osmia rufa*) przez pojedynczego samca.* Murarka ogrodowa należąca do rodziny Megachilidae jest jednym z gatunków pszczoł dziko żyjących wykorzystywanym od wielu już lat do zapylania wczesnowiosennych upraw sadowniczych oraz izolowanych upraw nasiennych. Celem pracy było określenie liczby samic, jakie zdolny jest unasiennić jeden samiec murarki ogrodowej w warunkach laboratoryjnych. Materiał doświadczalny stanowiły samce (30 osobników) i samice (165 osobników) murarki ogrodowej. Doświadczenie obejmowało 10 grup doświadczalnych, różniących się liczbą samic (od 1 do 10) przy stałej liczbie samców – 1 w każdej grupie. Każda grupa posiadała trzy powtórzenia. Owady przetrzymywano w izolatorach przez 2 tygodnie w celu odbycia przez nie kopulacji. Następnie przeprowadzono analizę wy-preparowanych zbiorniczków nasiennych samic. Określano ich wielkość oraz liczbę znajdujących się w nich plemników. Do obliczeń statystycznych zastosowano test chi – kwadrat, test Duncan oraz jednoczynnikową analizę wariancji. W każdej grupie doświadczalnej zaobserwowano różną liczbę unasiennionych samic. Wydaje się, iż najkorzystniejszym stosunkiem liczbowym

jest 1:3 (samiec : samica), ponieważ w każdym z 3 powtórzeń samiec unasiennił po 2 samice, co stanowi 66,65% wszystkich samic w tej grupie. Najmniej efektywnym stosunkiem był 1:1, ponieważ w żadnym powtórzeniu nie odnotowano unasiennionych samic. Maksymalnie samiec unasiennił 5 samic. Średnia objętość zbiorniczków nasiennych u samic unasiennionych była nieco wyższa aniżeli u samic nieunasiennionych. Jednakże przeprowadzona analiza wariancji na poziomie istotności  $\alpha = 0,05$  wykazała, iż różnice te są statystycznie nieistotne. Średnia liczba plemników w zbiorniczkach nasiennych przeanalizowanych samic wahała się między 1400 a 5683 i jak wykazała przeprowadzona analiza wariancji nie ma statystycznie istotnych różnic pomiędzy liczbą plemników w zbiorniczkach nasiennych, a liczbą unasiennionych samic w poszczególnych grupach doświadczalnych. Doświadczenie wykazało, iż najlepsze wyniki unasiennienia samic uzyskano w grupie doświadczalnej, gdzie na jednego samca przypadają trzy samice murarki ogrodowej, natomiast maksymalna liczba unasiennionych samic przez jednego samca w doświadczeniu wynosiła pięć.

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## The influence of open brood in rearing colonies on eggs acceptance in different egg age

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**Abstract:** *The influence of open brood in rearing colonies on eggs acceptance in different egg age.* Quality of bee queens depends primarily on the age of the brood used to queen rearing. The best queens are obtained by rearing from the eggs. The aim of the study was to investigate whether the unsealed brood presence in the rearing colonies affects eggs acceptance.

To colonies rearing with the unsealed brood there were introduced eggs at the age of 0–18 hours, 24–42 hours and 48–66 hours. After three days the number of built queen cells was verified. When in the colonies was no longer open broods, eggs were introduced to those colonies again and after three days the number of built queen cells was checked. Rearing colonies with unsealed brood accepted 42% of eggs at the age of 0–18 hours, 40% of eggs at the age of 24–42 hours and 51% of eggs at the age of 48–66 hours. Colonies without an open brood accepted 31% of eggs at the age of 0–18 hours, 61% of eggs at the age of 24–42 hours and 78% of eggs at the age of 48–66 hours. It was found that the best accepted eggs were the oldest ones in colonies without unsealed brood.

*Key words:* honey bee, bee eggs, bee queens rearing, rearing colonies.

### INTRODUCTION

Age of the brood used to rearing queens have a major impact on queens quality. Queen laying yield depends on the

number of ovarian tubes and the volume of the spermatheca (Chuda-Mickiewicz 1998). Larvae used for rearing queens should be as young as possible. Queens with the highest weight, the highest number of ovarian tubes and the volume of the spermatheca are obtained by rearing from eggs (Jordan 1960, Woyke 1971). Öroösi Pal (1964) created a method of rearing queens from eggs by moving them with the cut bottom boards of cell. In the Jenter method it is possible to rear queens from eggs or from larvae without moving them (Jenter 1983, Sieger 1983). Some authors (Winston 1987, Chuda-Mickiewicz et al. 1993, Pidek 1999) indicate that the age of the eggs can be specified by the angle of inclination to the cell bottoms. The more mature eggs are, the more skewed. However, Woyke (1987) showed that the change of eggs position is not related to their age. Eggs are skewed due to the heating of the honey bee heads. When it is hot egg stands and when is cold then egg lies. Weiss (1960) investigated the survival of eggs outside the hive in order to determine the most appropriate egg age to transfer as breeding material. He stated that the best way to store eggs is age from 1.5 to 2.5 days.

He also stated that some of the larvae hatched from stored eggs are abnormal formed. According to Ostrowska (1974) eggs used for rearing queens should be 2.5–3 days old, and according to Pidek (1999) not older than 2 days. Most of the authors (Ostrowska 1974, Pidek 1999, Chuda-Mickiewicz 1998) believes that in rearing colonies should be present unsealed brood, but by Skubida and Skowronek (1988) the presence of open brood negative affects the abundance of feeding larvae in queen cells and the best are accepted larvae in colonies without brood. In the Tworek's experiments (1986), which used Jenter frame, bees accepted 69% of eggs at the age of three days and 83% of larvae. Pidek (1987) reported that queen rearing colonies usually accept no more than 50% of the eggs.

The aim of the study was to investigate whether the unsealed brood presence in the rearing colonies affects eggs acceptance.

## MATERIAL AND METHODS

Material for this study were 432 bee eggs introduced in two series to six rearing colonies. In the experiment the Jenter methodology was applied, allowing to rear queens from the eggs. Jenter frame has removable bottom of the cells that with the eggs or larvae are placed in special tubes which make up the queen cell cup to be introduced to rearing colonies. To obtain eggs of a certain age queen was isolated on the three Jenter frames for three consecutive days from 18.00 to 10.00 the next day. In this way after three days on the frames was obtained eggs at the age of 0–18 hours, 24–42 hours and 48–66 hours. Eggs were placed in bree-

ding frames and were introduced to the six rearing colonies from which about an hour before queens had been taken away. To each colony were introduced 36 eggs – every 12 from each age group. After three days the number of built queen cells was checked and breeding frames were removed. When in rearing colonies there was no unsealed brood, wild queen cells were cut off. Queen was isolated again for 3 days at Jenter frames to obtain eggs in different age. To rearing colonies eggs were introduced as before and after three days the number of built queen cells was verified. In the whole experiment a total of 216 eggs to colonies with open brood and 216 eggs to colonies without open brood has been introduced.

## RESULTS AND DISCUSSION

Rearing colonies with unsealed brood accepted 42% of eggs at the age of 0–18 hours, 40% of eggs at the age of 24–42 hours and 51% of eggs at the age of 48–66 hours. Colonies rearing without an open brood accepted 31% of eggs at the age of 0–18 hours, 61% of eggs at the age of 24–42 hours and 78% of eggs at the age of 48–66 hours.

There was no significant effect of unsealed brood presence in rearing colonies on the eggs 0–18 hours old (Table 1) and eggs 24–42 hours old (Table 2) acceptance. Between colonies with open broods and without there were found statistically significant differences in the acceptance of eggs at the age of 48 hours and older (Table 3). The largest number of queen cells was obtained in colonies with no open brood which confirms the results of research of Skubida and Skowronek (1988).

TABLE 1. Number of eggs 0–18 hours old approved in the rearing colonies

	Number of colonies	Number of eggs in each colony	Min-Max	Mean $\pm$ se	Median
Colonies with open brood	6	12	0 – 8	$5.0 \pm 1.21$	5.5
Colonies without open brood	6	12	0 – 6	$3.67 \pm 0.96$	4

TABLE 2. Number of eggs 24–42 hours old approved in the rearing colonies

	Number of colonies	Number of eggs in each colony	Min-Max	Mean $\pm$ se	Median
Colonies with open brood	6	12	0 – 7	$4.83 \pm 1.14$	6
Colonies without open brood	6	12	3 – 11	$7.33 \pm 1.38$	7.5

TABLE 3. Number of eggs 48–66 hours old approved in the rearing colonies

	Number of colonies	Number of eggs in each colony	Min-Max	Mean $\pm$ se	Median
Colonies with open brood	6	12	3 – 8	$6.17 \pm 0.79$	6.5
Colonies without open brood	6	12	6 – 12	$9.33 \pm 0.88$	9.5

In the colonies rearing with open brood there was no significant effect of eggs age on their acceptance to colonies. Statistically significant differences were found in colonies without unsealed brood. Eggs 48–66 hours old were accepted by colonies without unsealed brood at the highest percentage (78). Similar results were obtained by Tworek (1986). In his study bees accepted 69% of the eggs at the age of three days and 83% of the larvae, but the eggs in different age was not introduced. Those results are consistent with the observations by Ostrowska (1974), that the eggs used for rearing queens should be at the age of 2.5–3 days. Pidek (1999) notes that the eggs should not be older than 2 days, but in the experiment carried out it can be stated that eggs older than 2 days were accepted the best.

## CONCLUSIONS

1. Unsealed brood cells presence in rearing colonies do not effect significantly the eggs at the age of 0–42 hours acceptance.
2. Colonies rearing without open brood accepted eggs at the age of 48 hours and older significantly more than colonies with open brood.
3. The highest percentage of eggs acceptance are eggs at the age of two days and older in colonies without the unsealed brood.

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**Streszczenie:** *Wpływ czerwiu otwartego w rodzinach wychowujących na przyjmowanie jaj w różnym wieku.* Jakość matek pszczelich zależy przede wszystkim od wieku czerwiu użytego do ich wychowu. Najlepsze matki uzyskuje się przy wychowie z jaj. Celem doświadczenia było zbadanie czy czerw otwarty w rodzinach wychowujących wpływa na przyjmowanie poddawanych jaj. Do rodzin wychowujących z czerwem otwartym poddano jaja w wieku 0–18 godzin, 24–42 godziny i 48–66 godzin. Po trzech dniach sprawdzono liczbę odciąganych mateczników. Gdy w rodzinach nie było już czerwiu otwartego ponownie poddano do nich jaja i po trzech dniach sprawdzono liczbę odciąganych mateczników. Rodziny wychowujące z czerwem otwartym przyjęły 42% jaj w wieku 0–18 godzin, 40% jaj w wieku 24–42 godziny i 51% jaj w wieku 48–66 godzin. Rodziny wychowujące bez czerwiu otwartego przyjęły 31% jaj w wieku 0–18 godzin, 61% jaj w wieku 24–42 godziny i 78% jaj w wieku 48–66 godzin. Stwierdzono, że najlepiej przyjmowane są jaja najstarsze w rodzinach bez czerwiu otwartego.

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## Comparison four races of bees in Finnish conditions

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**Abstract:** *Comparison four races of bees in Finnish conditions.* The aim of work was to investigate influence amounts of brood on production of honey at four the bees' races used in Finland. It was compared also wintering and spring development during three years. There was not found the influence of races on winter fall and nosema paralysis. The winter food consumption was dependent on race: Central European race – 1.04 kg, Carniolan race – 1.06 kg, Italian – 1.26 kg and Buckfast – 1.34 kg. The spring development, measured the quantity of sealed brood was the lowest in Central European race (33.7 dm<sup>2</sup>, the highest in Buckfast (43.5 dm<sup>2</sup>). Central European race had the lowest honey production (33 kg). Races: Italian and Buckfast had similar honey production (38 kg), Carniolan race – 42 kg. The ratio of honey production to quantity of sealed brood was in Carniolan and Central European races identical (1.08 kg/dm<sup>2</sup>) and was higher than in Buckfast and Italian races (0.99 and 0.97 kg/dm<sup>2</sup>). There were not found statistically significant differences.

*Key words:* bees, brood, bee races, fall of winter, honey production.

### INTRODUCTION

The subject of work was to investigate which of the prevalent races in Finland bees guarantee the best production results in the southern parts of the country in the region that is the biggest quantity of bees. During winter time was examined wintering period which identified bees Nosema paralysis and size of families and their productivity within three apiarian seasons.

In Norway and Finland till 1750 year there were no honey bee (Ruttner 1992). First mention of the bees in Finland come from the year 1761 (Huotari 1994). This Central-European bees were imported from Sweden. According to Alpatow (1948), Central-European bees populated in Scandinavia have a strong tendency to swarming. The Italian bee was brought to Finland for the first time in 1867 (Huotari 1994). Its massive inbreeding started in the 60s of last century (Vesterinen 1967). Bees imported from Italy, according to Brat Adam (1967), have little in common with the old, dark lines of Italian bees. According Ruttner (1988) some selected lines with appropriate methods of wintering give the best results also in Finland.

In the 70s – the twentieth century has also started to import Buckfast bees which, according to Brat Adam (1971) are: diligent, nonswarmly, economically managing supplies and fairly resistant to disease.

Size of winter fall was studied by Borisenko (1973), who argues that the main part winter fall which constitute bees with a sudden cooling and shrinkage of the cluster remained outside its boundaries. Root (1959) believes that in a healthy family during the winter may die up to 15% of the bees. Studies of

Madras (1999) conducted on Caucasian, Carniolan and Italian bees, the highest winter fall were found in Italian breed. Zmarlicki (1978) examining wintering in Langstroth hives stated that food consumption in insulated hives was 0.5 kilograms per comb and 0.68 kilograms per comb in no insulated hives. During wintering Nosema paralysis depends from climate and weather according Kostecki (1976). Gromisz and Bobrzecki (1984) obtained results: 64.3% in Carniolan bees and 37% in Central-European. The experience of Gromisz (1986) a leading position in terms of speed in spring development was taken by the Carniolan bees. Woyke (1981) stated dependence on the honey production to the length of the life bees. The average of length of bees depends from effort and body consumption during feeding larvae (Wille and Gerig 1976, Woyke 1980). Intensity of flights with foragers is genetically determined (Bratkowski and Wilde 1999). In studies of Timosinowa (1973) the most honey were obtained from Caucasians race, second place took Carniolan bees, the third – Italian and the last – local bees. Research of Levcenko and others (1972) points to the earlier acquisition and capabilities to collect nectar by Carniolan bees. This breed is highly evaluated by Vesely (1974) and Singer (1971). The last one is recommending her for breeding in different regions of the world.

#### MATERIAL AND METHODOLOGY

Research conducted in the breeding station – apiary Finnish Union of Beekeepers, located in south-western Finland. Apiary is located in many places distant from each other by a few to several kilo-

metres. Number of hives in one place did not exceed 10. Among bee plants occurring this area most important are: rape, clovers, willows, bilberry, raspberry and willowherb. Studies were conducted in the years 1997–1999. Compared the following breed bees: Italian (*Apis mellifera ligustica* Spin.) Buckfast, Carniolan (*Apis mellifera carnica* Poll.) and Central-European (*Apis mellifera mellifera* L.). Italian bees came from a line maintained for many years in Finland, Buckfast bees came from the Finnish breeder and lines received from Canada, the Carniolan bees were represented by the lines of “Willy” and line “Singer” – company from Austria, and Central-European bees were from three Finnish apiaries. Examinations were conducted in 1997 with 36 families, in the next year to 42 families and the third year for 40 families. Apiary consisted of multiple-storey Langstroth hives, with insulated walls. They were spending the winter depending on the size, on one or two 9-frame corps. On apiaries well protected from the wind hives spent the winter without additional shielding. On worse shielded places hives were being wrapped up with paper silo, who was being removed during the first spring inspection.

Spring precipitating of families development wasn't practiced. Contraction of brood nest wasn't being practiced. Preventing the coming into swarming impulse consisted in enlarging the capacity of hives with corps with foundation. The swarming impulse was being eliminated through shaking bees off to corps with foundation, and then corps with the brood were moved above queen excluder which was removed after few days. Honey have been taken away from

the hives once after the termination from main flow it means half of August. Replenishing winter supplies started in the second-half of August and they were finishing in middle of September. Winter food consumption was determined by weighing hives two times: in the last days of October and the second half of April at the first overview of the Spring. Measurement sealed brood was made for the first time in full blooming apple trees, which accounted for experiences during the 23 week of the year. Second measurement was made at 3 weeks after first.

Honey production was adopted as the sum of the quantities of the centrifuged honey and leftover honey in the hive. The ratio of the production of honey for brood quantities expressed in kilograms of honey per 1 dm<sup>3</sup> sealed brood. Because of unequal size of families both: winter fall and winter food consumption was converted to relative values, i.e. in relation to number of combs. Nosema disease paralysis test results are given in the average quantity of spores to 1 bee.

Values of investigated properties were characterized by giving the arithmetic mean, standard deviation and standard error. There was conducted a analysis of variance. Statistically significant differences between the means was identified by a test of Student – Newman – Keuls with probability 95%.

## RESULTS

### Wintering

**Average of winter fall** per one comb was from 197 bees of the breed Carniolan to 269 for a group of Buckfast bees. However, there was no statistically significant

difference between races. The average size of winter fall for the whole apiary was not big between years (from 202 per comb during the winter of 1997/98 to 261 per comb during the winter 1996/97 and also the differences were not significant.

**Nosema disease paralysis** expressed by the mean number of spores per 1 bee was from 0.98 million for the Central-European race to 6.66 million for the race Carniolan. Extent of paralysis was comparative varied inside groups. Between the races there were no significant differences.

**Winter food consumption** ranged from 1.04 kilograms per comb in the group of families of the Central-European up to 1.34 kg per comb in the group of families Buckfast. There was found statistically significant difference between these groups. Also the consumption of food among the families bees Central-European was significantly lower than in the group of families Italian. Carniolan bees consumed significantly less food than Buckfast bees. Between the Italian and Buckfast bees was no significant differences. Also between Central – European and Carniolan race there wasn't statistically significant differences. Numerical values of parameters characteristic for wintering are summarized in Table 1.

### Families spring development

The smallest number of brood within three years of comparisons, were Central-European bees (an average of 33.7 dm<sup>2</sup>), and the largest Buckfast bees (43.4 dm<sup>2</sup>).

Difference between these two races was statistically important. It was significantly more brood from Italian bees than Central-European. The Carniolan

TABLE 1. Wintering parameters characterizing the results, that is winter fall of 1 comb, food consumption per 1 comb, the number of spores per 1 bees and percent of colonies infestation, (Races: BU – Buckfast, IT – Italian, KR – Carniolan, PM – Central-European)

		Winter fall (bees/comb)		Consumption of food (kg/comb)		Nosema (number of spore/ /bee x 1 000 000)		% Affected families
Winter	Race	Limit values	Average	Limit values	Average	Limit values	Average	
1996/97	BU	20-1475	337	0.92-3.0	1.46	0-18.8	2.61	36.4
	IT	24-378	166	0.83-1.86	1.24	0-15.37	3.31	50
	KR	43-472	164	0.88-1.5	1.15	0-6.4	2.17	80
	PM	24-944	519	0.8-1.75	1.38	0.75-5.6	2.46	100
<b>Average</b>		---	<b>261</b>	---	<b>1.32</b>	---	<b>2.79</b>	<b>54.8</b>
1997/98	BU	83-545	199	0.81-2.0	1.09	0-0.125	0.043	45.5
	IT	79-944	259	0.89-2.4	1.24	0-71.6	7.94	63.6
	KR	59-581	179	0.63-1.23	0.98	0-43.6	8.72	20
	PM	67-511	164	0.72-1.5	0.98	0-0.138	0.034	25
<b>Average</b>		---	<b>202</b>	---	<b>1.08</b>	---	<b>4.24</b>	<b>45.2</b>
1998/99	BU	126-730	273	0.87-2.45	1.47	0-40.4	9.5	80
	IT	103-654	252	0.79-1.54	1.3	0.037-2.8	6.56	100
	KR	66-617	229	0.75-2.11	1.09	0-52	8.01	70
	PM	79-515	223	0.75-1.36	1.0	0-64	0.91	40
<b>Average</b>		---	<b>244</b>	---	<b>1.22</b>	---	<b>6.2</b>	<b>72.5</b>
Average of the three years	BU	20-1475	269	0.81-3.0	1.34	0-40.4	3.88	53.1
	IT	24-944	223	0.79-2.4	1.26	0-71.6	5.84	69.7
	KR	43-617	197	0.63-2.11	1.06	0-52	6.66	60
	BU	24-944	237	0.72-1.75	1.04	0-6.4	0.98	47.1
Standard deviation			215.3		0.39		1.13E7	49.5
Average mean			20.15		0.04		1.12E6	4.90

bees had significantly more brood than Central-European.

Average number of brood for all groups was little differed between years of research, despite the different weather. In 1997 was an average of 39.24 dm sq brood, and in 1998 was 41.05 dm sq and in 1999 – 39.44 dm sq brood. Statistically was no significant differences between years.

### Honey production

Between 1997 and 1999 years examined four races bees in terms of honey produc-

tion did not differ significantly. In 1998 years the lowest honey production was a group of Central-European families breed.

Statistically more honey had race Carniolan and Buckfast. Low production of honey by bees of the Central-European race, in 1998 was the result of the swarming impulse. The arrangement of the weather in this season was particularly favourable for it.

In the group of families of the Central-European race you could see the apparent lack of selection for swarming, these families during the main honey flow, per-

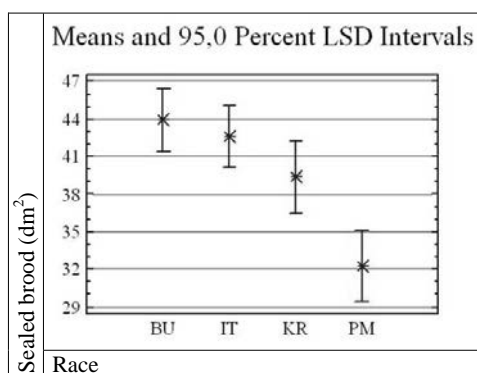


manently were in the swarming impulse, while the families of other races, was able to relatively easily eliminated.

In the period of 3 years of families breeds: Buckfast and Italian had an almost identical average honey production (respectively 37.9 and 38.1 kg), the Carniolan Bees produced an average of 42 kg, and the family of race Central-European – 33.2 kg. There was statistically significant differences between the group of families of the Central-European race which has the lowest production of honey and a group of race Carniolan which, in each seasons was characterized the largest production. There was no statistically difference in honey production between Carniolan races and races: Buckfast and Italian.

### Relation of honey production to the amount of brood

There was calculated the ratio of the production of honey to the average amount of brood during the development of families, and thus the number of bees



BU – race Buckfast, IT – Italian, KR – Carniolan, PM – Central-European

FIGURE 1. Comparing the average brood areas during the spring development of families (in dm sq.)

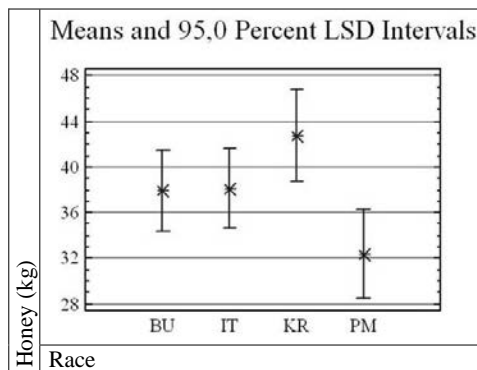


FIGURE 2. Average size of honey production in four breeds studied bees

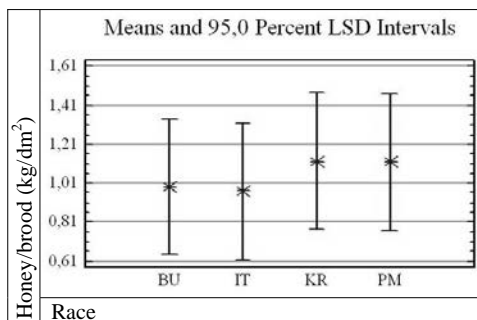


FIGURE 3. Average size compared to the amount of honey production to the brood in four test races

during the main honey flow. Results are illustrated in Figure 3.

The lowest ratio of honey production to the amount of brood was found in brood Buckfast bees (0.97 kg/dm<sup>2</sup> brood), the highest – with bees breed Carniolan and Central-European (1.08 kg/dm<sup>2</sup> brood). However, there was no statistically significant differences between races.

### CONCLUSIONS

1. Breed had no significant effect on the size of wintering.

2. There were significant influence of race on the winter food consumption. Carniolan and Central-European bees consumed less food than Italian and Buckfast bees.

3. Paralysis Nosema disease wintering bees wasn't dependent on the breed.

4. Central-European bees had spring development lower than the other races. There were found significant differences between Central-European and Italian and Buckfast race.

5. The smallest brood had Central-European race what caused that they had the lowest production of honey. Bee races: Buckfast and Italian were very close to each production of honey and brood. Carniolan bees obtain in each year of the highest of all groups honey production.

6. Carniolan and Central-European bees had an identical ratio of honey production to brood. It was higher than Buckfast and Italian bees.

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**Streszczenie:** *Porównanie czterech ras pszczoł w warunkach fińskich.* Celem pracy było zbadanie wpływu ilości czerwiu na produkcję miodu u czterech ras pszczoł użytkowanych w Finlandii. Porównano także zimowlę i rozwój wiosenny w ciągu trzech lat. Nie stwierdzono wpływu rasy, zarówno na wielkość osypu zimowego, jak i porażenie nosemozą zimujących pszczoł. Zimowe zużycie pokarmu było natomiast uzależnione od rasy i wynosiło, w przeliczeniu na obsiadany plaster: dla rasy środkowoeuropejskiej – 1,04 kg, dla kraińskiej – 1,06 kg, dla włoskiej – 1,26 kg i dla

Buckfast – 1,34 kg. Tempo rozwoju wiosennego, mierzone ilością czerwiu krytego, było najniższe u pszczoł rasy środkowoeuropejskiej (33,7 dm<sup>2</sup>), najwyższe zaś, u pszczoł buckfast (43,5 dm<sup>2</sup>). Rasa środkowoeuropejska miała również najniższą produkcję miodu (ok. 33 kg). Rasy: włoska i Buckfast, miały zbliżoną do siebie produkcję miodu (ok. 38 kg), rasa kraińska uzyskała średnio 42 kg. Stosunek produkcji miodu do ilości czerwiu krytego był u ras: kraińskiej i środkowoeuropejskiej identyczny (1,08 kg/dm<sup>2</sup>) i był wyższy, niż u rasy buckfast i włoskiej (odpowiednio: 0,99 i 0,97 kg/dm<sup>2</sup>). Nie stwierdzono tutaj statystycznie istotnych różnic.

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## The influence of conditions of keeping the drones *A. mellifera* on quality of their semen

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**Abstract:** *The influence of conditions of keeping the drones A. mellifera on quality of their semen.* The investigation was conducted in the apiary of Bee Division of Warsaw Agricultural University in 2003-2004. The aim of this experiment was to study the influence of the temperatures on survival and changes of body weight of drones and quality of their semen. The quality of semen was evaluated organoleptically by comparison of semen characteristic of the drones from control group and the experimental one. For the investigation unknown origin drones were used as well as collected from colonies.

Basing on investigations the conclusions have been drawn, that increased temperature in which the drones are kept may have negative influence on survival of drones and causes disorders in drone's activities.

It has also affected on the loss or the increase of body weight of drones. In many cases it induces death of drones.

The body weight of drones does not influence on the volume of collected semen, and the difference of body weight after keeping drones under evaluated conditions influences on the volume of collected semen ( $r = -0,12$ ,  $N = 323$ ,  $\alpha = 0,04$ ). Some conditions (temperatures over  $42^{\circ}\text{C}$ ) of keeping the drones can make it impossible to collect the semen, and to cause the decrease of its volume as well as increase the frequency of collecting semen with changes in its consistency. Collecting such semen is more difficult and we can take smaller volume of semen. These influences the increased number of drones to collect the necessary dose of semen as well as difficulty in collecting semen samples. The age of drones does not influence on the volume as well as the consistency of collected semen. Statistically confirmed the influence of age on colour of collected

semen ( $r = 0,44$ ,  $N = 231$ ,  $\alpha = 0,0001$ ). There was found, that semen of drones, which were kept in temperatures  $42-50^{\circ}\text{C}$  is more acid then semen of drones of control group and pH semen from different colonies was significantly different. Semen of individual drones in colonies can differ in colour, consistency, volume and pH.

*Key words:* the survival of drones, semen of drones, semen colour, semen consistency, semen pH, volume of semen of drones.

### INTRODUCTION

The quality the drones needed for insemination depends on their vitality, possibility of eversion the copulatory apparatus, execution of semen on surface of copulatory apparatus, the volume of taken semen as well as its quality. The quality of drones semen plays significant role during insemination queens.

Transportation drones from breeders to points of insemination in inappropriate conditions (high temperature) can cause, that part of drones will get lost and living drones could have thickened semen. The good inseminator pays special attention on appearance, the consistency as well as the volume of semen. Semen not fulfilling parameters of standard semen, that is i.e. of unsuitable consistency should be thrown aside, because it can cause clogging of the queen's oviducts. This can effect with infections and even falls

down of queens. Queen's losses bring to prolongation pauses in eggs laying and they cause delay in the development of bees' colony.

The aim of experiences during years 2003-2004 in apiary of Bee Division SGGW in Warsaw was to examine the influence of different conditions of keeping the drones on quality of their semen.

Premises for undertaking the investigations were founded by drones semen with consistency of solid body after transportation in unfavourable conditions (Jasiński 1993), the inseminated queens with semen coming from older drones has more often the bunged oviducts than the queens inseminated with the drones' semen in optimum aged 14-21 days (Woyke and Jasiński 1978). Investigations were to show whether semen is in fact subjected to the thickening under influence of increased temperatures, or this is connected with drones' age.

Moreover the experiments were to show if:

- high temperature influences parameters of taken semen, i.e.: consistency, colour, volume and pH,
- hickening of semen is caused by the decrease of body weight of drones,
- with the drones' age flow changes the colour, consistency, volume and pH of semen,
- change of semen's colour is connected with change of semen's pH.

The observations conducted during survey can be the first stage of investigations of influence of the drones' age and the high temperatures of keeping on survivability of sperms in semen. The results of investigations can also serve to the optimization of conditions of transport and keeping of drones.

## MATERIAL AND METHODS

The investigations were conducted in years 2003 and 2004 in apiary of Bee Division SGGW. The investigative material were drones of unknown age and the origin as well as the drones brought up in the bee's families of apiary of Bee Division (2003 year). In year 2004 drones were both control and experimental group taken from families brought from queens excluder. The control group was established by drones not kept in incubator and not subjected under influence of increased temperatures. These drones after bringing from the queens excluders of families were weighed, was taken from them the semen as well as evaluated his quality.

Totality in draught of two seasons were used 2024 drones. In season 2003 from 17 June to 22 August were taken 17 times from several to tens drones, together 918 in which 153 drones made up control group and 765 experimental group. These 765 drones were kept in temperatures from 40 to 43°C. 135 drones were kept without bees and 530 drones with increasing bees' number (15, 50, 100, 150 bees). Majority of drones used in these investigations were caught from outlets and their age was unknown (they made about 70% experience - 639 drones), and remaining drones came from insulators and their age were known i.e. 13, 19, 23, 28 and 30 days. 179 drones were kept in box with drinking water, and 586 drones didn't have water. Incubator didn't have ventilator.

In season 2004 from 27 May to 24 August were taken 25 times with tens, the most often 45 drones, together 1106 drones. Drones were 14 to 85 days old (20

groups of old aged drones). Drones came from 6 families, several races among others.: Caucasian, Italian and Carniolan (probably hybrids). Control group made of 371 drones (33,5%), and experimental group 735 drones (66,5%). The drones of experimental group with constant number 50 bees were kept in cases in incubator in temperature 42, 44, 46, 48 and 50°C. In season 2004 incubator were stocked in ventilator. The part of cases were stocked in syringe with water.

## METHODS

The drones of experimental group were kept in cases (boxes) with bees in incubator in temperature from 40-50°C by about maximally 20 hours. The part of cases (44 szt.) was stocked in water.

In year 2003 to earlier prepared cases of dimensions  $11,3 \times 17,3 \times 6$  cm stocked in cake honey-sugar, piece comb as well as water in syringe (or without water) were put bees and then marked, previously weighed drones, it were had put in to incubator with definite temperature. Temperature moisture were defined mobile – hygrometer and had elevate from 25-79%. In cases relation drones to bees carried out 15:0, 15:15, 15:50, 15:100, 15:150. Before inserting drones to cases permit oneself them “to fly round”. In tract observation specimens which die were removed. It were weighed, to qualify their weight.

Toward beginning of experience was tried to establish the time of life the drones in definite conditions and the temperature, to estimate the length of period of keeping their in incubator. The time of keeping the drones in the same temperature considerably differed. It put in the

way, that the foreseen time of keeping the drones was shorter and then observed larger number of falls. Decided so, that final and the time most proper moment of investigation of influence definite conditions keeping on quality of semen will time fall about half drones.

After extraction, alive drones from incubator were weighed, eversion their copulatory apparatus, estimating the possibility of taken of semen. Subsequently from copulatory apparatus by help of needle was taken semen defining his consistency, later volume as well as pH.

In year 2004 drones were brought to laboratory, they were assured to flight. They were marked, weighed and it had let in to cases in which were already 50 bees. The supply of cases were similar how it in year 2003. Cases were put to incubator possessing the sensor of temperature and the moisture as well as the possibility of extorting through ventilator the circulation of air. The investigation of quality of semen were made after extraction cases with drones from incubator.

The drones of control group did not put in to incubator and after extraction they from queens excluder of family were weighed as well as taken from them semen for organoleptic evaluation.

In table no 1 presented conditions in whatwere kept the drones of experimental group in season 2004.

Evaluation of parameters of drones semen in both groups in season 2004 depended on qualification:

1. Consistency of semen in track taking the semen to needle;
2. Volume of semen (in glass tube);
3. Colour of semen by use the Colour book of Pantone Process Color System Guide, Edition 1996;

TABLE 1. Conditions of keeping the drones in season 2004

Temp. in °C	Number and % drones		Ventilator		With water	Without water
			on	off		
			number of drones in given conditions			
42	195	26,5	150	45	150	45
44	180	24,5	30	150	90	90
46	195	26,5	75	120	135	60
48	105	14,3	30	75	75	30
50	60	8,1	30	30	30	30
Total	735	100	315	420	255	480

#### 4. pH of semen by use indicatory paper Duotest (pH 5,0-8,0).

The evaluation of colour of semen was made after dropping of semen on transparent foil and the one put on little window of colour book. The colour of semen was compared with colours of colour book to which attributed defined code. Occur cases, that the semen dried quickly and colour changed on darker, before the observer on the time to attribute him the definite code of colour. Semen from foil were transferred on piece of indicatory paper Duotest (pH 5,0-8,0) in aim of qualification his pH.

Results evaluation parameters of semen control group were compared with results for parameters semen the drones of experimental group. Analysis of results were executed in programme SPSS in support if crossed table, the analysis of correlation, analysis the variance (the tests of significance differences) and the linear regression.

## RESULTS

### Survivability the drones

Drones in incubator in temperature 40-43°C were kept even by over 20 h, and in temperature 42-50°C maximally by 7,44 h. In temperature 48 and 50°C

drones were kept maximally by about 4h. There was found, that in conditions of increased temperature the keeping the drones the access to water in case as well as the presence concurrent bees, they contributed to prolongation of their life. The drones without water of live more briefly. Survivability the drones depends on their origin, temperature of keeping: 42, 44, 46, 48 and 50°C, presence concurrent bees', access to water in cases, the time of keeping in given temperature.

### The body weight the drones

There was found also, that drones unknown age and origin in season 2004 had the most the differential body weight. The differences between minimum and maximum body weight they carried out over 200 mg, that is the average weight one drone. This results probably from this, that part drones of low weight came from hunchbacked brood, that drones is mannered in honeybee comb. The average body weight of 1106 drones to experience in season 2004 carried out 208,17 mg (min = 134, max = 327). There was found differences between body weight the drones in different known age.

Drones in time of keeping in increased temperatures in season 2003 lost or gained on body weight. The averages the



differences of body weight the drones kept in different temperatures were statistically significant. Therefore keeping the drones in increased temperatures influenced on change of body weight of drones.

In season 2003 and 2004 was found, that differences in average the body weight the drones kept in cases with water and without water were statistically significant. In 2003 year the larger differences in body weights the drones were during keeping drones with bees at access to water than without access to water. There was found, that drones during keeping in increased temperatures increase on average weight 18,7 (7,4) mg (max = 116), there was found also, that they lost on weight (min = -44(-46 mg)). There was found, that the drones from the same colony and in the same conditions change the body weight in both directions losing or gaining.

### **Possibility of taking the semen from drones kept in increased temperatures**

The keeping the drones in increased temperatures contributed to decrease of number the drones from which were taken semen. The smaller number the drones from which were taken semen were caused falls the drones. From drones kept in temperatures above 42°C harder were taken semen, because they were weak. It happened, that drones had dried body or softened muscles and membrane between abdomen segments what made difficult or made impossible taken from them semen. Observed also, that the high temperature of keeping causes in short time bees' inability to finding and taking water despite possibility access to her.

### **The consistency of drones' semen**

The suitable consistency of semen facilitates separating semen from mucus, it decides about possibility his taken, influences also on easy emptying oviducts by inseminated queen bees.

According to Woyke and Jasiński (1978) semen of drones 10-14 days is thin and easily can be taken to insemination needle. Semen drones older than 4 weeks is more thick and more difficult taken to insemination needle. Thick semen wrongly separates from mucus. Jasiński (1993) was found, that often the cause of thickening semen in drone organism is the overheating in track of inappropriate transportation the drones and sometimes at overheated drones the semen stops being liquid and becomes solid body.

In experimental group was found more drones from which taken semen with changes in consistency. However statistically does not found significant influence increased temperatures on consistency of taken semen. The lacks of semen in experimental group were larger, this were results among other falls drones kept in unfavourable for them conditions.

In control group light thick semen was found both by drones of young (in age 16 days) how and by older (33, 40 and 67 days). The does not found statistically significant influence of drones' ages on consistency of taken semen.

Condensed and thick semen were found by drones which had small losses in body weight and even large shrines. It was can affirm, that the thickening the semen (the decrease of plasma) is not connected with loss of body weight. Thin semen were taken from drones with

of decreases how and the increases of body weight.

### **The volume of drones' semen**

The volume of semen taken from drones plays big role in insemination queen bees. They larger volume semen taken from individual drone reduce number drones needed to collect the required to insemination dose of semen, what significantly accelerates insemination.

Woyke (1960) taken average  $1,7 \text{ mm}^3$  of semen with deviation  $1,5-1,75 \text{ mm}^3$ . Bobrzecki (1964) give in, that average from drone was taken  $0,58 \text{ mm}^3$  of semen during when the minimum volume of sperm carried out  $0,1 \text{ mm}^3$ , meanwhile maximum  $1,8 \text{ mm}^3$ . Author was found, that needle Mackensen's take average only 45% total volume of semen ejaculated by drones. About half of semen undergoes destruction mixed with mucus, or burst during ejaculation (Bobrzecki 1966).

In season 2003 average volume of taken semen in control group carried  $0,73 \text{ mm}^3$  and experimental group  $0,81 \text{ mm}^3$ . Don't found significant difference between average volume of semen taken from drones of control and experimental group. Volume drones' semen in control and experimental group in season 2004 was statistically significant differed. Both in season 2003 and 2004 in experimental group observed larger variation in volume semen taken from drones than from drones control group.

In season 2004 from drones control group were taken average significant and high significant larger semen then from drones experimental group. In control group most larger semen taken from drones were in range volume  $0,61-0,81$

$\text{mm}^3$ . There was found, that seldom taken semen below  $0,2 \text{ mm}^3$  and above  $1,41 \text{ mm}^3$ . High significant (lower) differences between average volume of semen was found in drones kept in temperatures  $46-50^\circ\text{C}$ , and significant in temperature  $44^\circ\text{C}$  compared to volume of drones' semen of control group.

From drones kept in temperature  $42^\circ\text{C}$  were taken significantly and high significantly larger volume of semen than from drones kept in higher temperatures. Also from drones kept in temperature  $44^\circ\text{C}$  and  $48^\circ\text{C}$  were taken high significantly more semen than from kept in temperature  $50^\circ\text{C}$ . In season 2004 was found, that together with increasing the temperatures of keeping the drones decrease volume semen taken from them.

In season 2003 was found correlation ( $r = -0,32$ ,  $N = 172$ ,  $\alpha = 0,000$ ) between differences of body weight and the volume of taken semen. In experimental group was found differences between of body weight and the volume of taken semen ( $df = 170$ ,  $\alpha = 0,000$ ). In season 2004 showed significantly correlation between difference of body weight the formed during keeping drones in increased temperatures and the semen volume ( $r = -0,1$ ,  $N = 323$ ,  $\alpha = 0,04$ ). The analysis of regression showed, that exists dependence between these variables.

### **The colour of drones' semen**

The colour of drones semen makes possible the recognition and the distinction the semen from mucus. Observed, that the colour of semen is very diverse and enclose over 30 colours from pale yellow to ochraceous beige definite on basis of colour book. In investigation was found, that the colours of semen were compati-

ble with codes of colours of colour book and contains in range from pale karamel orange (E 22-9), by pale yellowly beige (E 18-9), darkly beige orange (E 18-8), beige orange (E 22-8), beige (E 23-8), pale beige (E 6-7), pale beigly yellow (E 19-8), orange beige (E 22-7), beige orange (E18-7, E 18-6), pale peach, beige brown orange, peach orange and peach (E 32-7, E 32-6, E 22-6, E 22-5, E 32-5), beige orange (E 18-6), darkly beige orange (E 18-5), beige brown, orange beige (E 33-7, E 19-7, E 19-6), brown beige, beige brown (E 23-6, E 33-6, E 33-5), orange brown (E 19-5), beige brown (E 23-5, E 23-4), orange olive and olive (E 29-5, E 29-6) to ochraceous beige (E 46-5, E 46-6, E 34-4) (total 33 codes). The descriptive onomastics colours not always it's not able exactly and correctly to reflect (show) evaluated colour. Using with basic colours helps mainly orientate in what range occur evaluated colour.

According to Blum et other (1962) sperm is cream colour. According to Woyke and Jasiński (1978) semen drones 10-14 days has yellowly-cream colour, and drones older than 4 weeks is creamly-brown. Jasiński (1978) claims also, that good semen has beigly-orange colour.

In control group was found high significantly correlation ( $r = 0,44$ ,  $N = 231$ ,  $\alpha = 0,000$ ) between drones age and the semen colour. Statistically proved, that together with age drones semen their darkens. Analysis variance showed significantly influence age of drones on semen colour was taken from them ( $df = 19$ ,  $\alpha = 0,000$ ).

In experimental group was found high significant regression between temperatures of keeping the drones and colour of their semen ( $df = 321$ ,  $\alpha = 0,00001$ ). It

was found high significantly negative correlation ( $r = -0,21$ ,  $N = 317$ ,  $\alpha = 0,001$ ) between colour of semen and the temperature of keeping as well as the high significantly influence of temperatures on semen colour ( $df = 4$  (316),  $\alpha = 0,02$ ). The most often semen with specific colour in control group was taken also from drones in experimental group.

Drones in the same age came from one same colony can have the different semen' colour.

In control group was found high significantly correlation between semen' colour and his pH ( $r = 0,2$ ,  $N = 229$ ,  $\alpha = 0,03$ ). In experimental group correlation-carried out ( $r = 0,3$ ,  $N = 312$ ,  $\alpha = 0,001$ ). There was found, that the darkest semen taken from drones of control group has pH 7,7.

### **The pH of drones' semen**

The optimum pH of drones semen influences on correctly running metabolism of sperms, it affects on vitality as well as the motility of sperms in different conditions. The suitable pH of semen can have influence on transfer sperms to spermatheca after insemination as well as it can influence on long-lasting storage sperms in spermatheca of queens. According to Smirnov (1953) in majority of cases pH of drone's semen is close neutral (pH = 6,8-7,1) and only in two, three cases is lightly acid (pH = 6,0).

In control group the drones semen had pH in range from 6,2-8,0. In season 2003 was found high significantly correlation ( $r = 0,36$ ,  $N = 162$ ,  $\alpha = 0,000$ ) between temperatures the keeping the drones and pH of taken semen as well as the high significantly influence of temperatures of keeping on pH of semen.

Statistically analysis showed, that drones kept in increased temperatures 42-50°C had semen of pH significantly or high significantly lower than drones of control group. In draught 12 days of investigations the averages the pH of drones semen of control group were significantly or high significantly higher then average pH of drones semen in experimental group. There was found also statistically significantly differences in value the pH of semen taken from drones kept in temperature 42°C, and the pH of semen the drones kept in remaining temperatures.

In season 2004 in experimental group was found the significantly positive correlation between the pH and the volume of semen ( $r = 0,3$ ,  $N = 313$ ,  $\alpha = 0,000$ ) as well as the significant linear dependence (regression) between pH of taken semen and his volume.

## CONCLUSIONS

1. There was found, that body weight the drones is very differential and the differences can come even to 200 mg, that is to achieve the body weight of average weight of drone.

2. Keeping of drones in increased temperatures result in enlargement their mortality.

3. In time keeping is present the considerable changes weight of drones. There was found, that the change of weight of body the drones significantly influence on possibility the taken from them the semen.

4. Semen of drones from the same bee's family differs both under in relation to colour, volume how and pH.

5. Initial of body weight the drones both in control and experimental group

has not influence on volume of taken semen.

6. Age of the drones has not significantly influence on semen volume and consistency.

7. High temperatures of keeping the drones influence on decrease volume of taken semen (significantly difference for season 2004) as well as high significantly influence decrease on pH of semen.

8. Value pH of semen the drones from control and experimental group is include in range 6,2-8,0 and had the most often value 7,1-7,7 (reaction lightly alkaline).

9. Young how and old drones have the most often semen of pH 7,1-7,7. Age of drones is high correlated with pH of semen ( $r = 0,22$ ,  $N = 230$ ,  $\alpha = 0,001$ ). Analysis variance showed significantly influence drones' age on pH their semen.

10. High temperatures of drones keeping do not influence on thickening the drones semen, but contribute to growth of number drones from which collect of semen with changes consistency.

11. There was found significantly correlation between drones' age and colour of their semen ( $r = 0,44$ ,  $N = 231$ ,  $\alpha = 0,0001$ ) as well as the significantly influence of drones' age on colour their semen (the analysis of variance).

12. In experimental group with growth of volume of taken semen increased his pH ( $r = 0,3$ ,  $N = 313$ ,  $\alpha = 0,000$ ), but the most often in range pH not larger then 7,7.

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**Streszczenie:** Wpływ warunków utrzymania trutni (*A. mellifera*) i ma jakość ich nasienia. Celem pracy było zbadanie wpływu podwyższonych temperatur przechowywania na przeżywalność trutni, zmianę masy ich ciała oraz jakość nasienia. Jakość nasienia trutni oceniano głównie organoleptycznie przez porównanie cech nasienia trutni grupy kontrolnej pobranych z rodzin wychowujących, nie przechowywanych w podwyższonych temperaturach z cechami nasienia trutni grupy doświadczalnej przechowywanych w podwyższonych temperaturach. Do badań wykorzystano trutnie nieznanego pochodzenia i wieku oraz trut-

nie w znanym wieku pochodzące z rodzin wychowujących.

Stwierdzono, że przechowywanie trutni w podwyższonych temperaturach przez określony czas powodowało zmniejszenie ich przeżywalności oraz wpływało na stratę lub przyrost masy ciała trutni. Stwierdzono ponadto, że masa ciała trutni nie była skorelowana z objętością pobieranego nasienia, a różnica masy ciała trutni będąca wynikiem przechowywania w podwyższonych temperaturach korelowała ( $r = -0,12$ ,  $N = 323$ ,  $\alpha = 0,04$ ) z objętością pobieranego nasienia. Dowiedziono, iż przechowywanie trutni w wysokiej temperaturze ( $42-50^{\circ}\text{C}$ ) może uniemożliwiać pobranie nasienia, powodować zmniejszenie jego objętości oraz zwiększać częstotliwość pobierania nasienia ze zmianami w konsystencji. Wpływa to m.in. na zwiększenie liczby trutni potrzebnych do pozyskania określonej dawki nasienia oraz na trudności w jego pobraniu. Nie stwierdzono wpływu wieku trutni na objętość oraz konsystencję pobieranego nasienia. Statystycznie stwierdzono wpływ wieku na barwę nasienia trutni ( $r = 0,44$ ,  $N = 231$ ,  $\alpha = 0,0001$ ). Stwierdzono, że nasienie trutni przechowywanych w temperaturach  $42-50^{\circ}\text{C}$  ma istotnie niższe pH niż pH nasienia trutni grupy kontrolnej, a pH nasienia trutni w różnym wieku różni się wysoko istotnie. Nasienie poszczególnych trutni pochodzących z tej samej rodziny różniło się zarówno pod względem barwy, konsystencji, objętości jak i pH.

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## The comparison of bee workers damages in their own colonies without queen

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**Abstract:** *The comparison of bee workers damages in their own colonies without queen.* The investigations were performed at Bee Division, Faculty of Animal Science, Agricultural University of Warsaw, during the summer 2007. The aim of the study was to evaluate degree of damages and damages intensities bee workers kept on the bee's own colonies without queen. Those studies can explain causes of queen damage kept in the queens banks.

In the investigation were used 912 bees, which were observed on body damages during the time of keeping them in colonies in transport plastic cages with the crevices in the upper wall of cage. Damages were evaluating twice, after three and ten days, in 912 bees kept in cages. There were inspected: 13824 legs, 4216 wings and 4608 antennas.

*Key words:* bee worker, damage, mortality, survivability.

### INTRODUCTION

The phenomenon of damaging queens by the colony occurs in case of place more than one queen in dequeen colonies (Woyke 1956), (Jasiński 1984, 1986, 1987, 1995b). In the base of results obtained by Zamarlicki i Mors (1964) one of the reason of this phenomenon may be pheromones which decide of queen attractiveness to bees. Some role in this problem can play scent glands situated at the base of queen legs arolium. Those

glands produce the substance, which is left on the glass in the place where arolium contacts glass surface (Lensky i Slabesky 1981). Those authors also claimed that this substance effect the bee workers by hamper their desire to set up queen cells.

Jasiński (1986, 1987, 1995a) described damages and classified into four group:

1. changes in arolium pigmentation,
2. lac of leg segment or whole legs,
3. arolium deformations as well as partial and total arolium and claws losses,
4. other damages like frayed wings membrane, come off antennas segments or whole antenna.

Jasiński (1995a) prevail that damages from group 2, 3, 4 causes handicap of queens normal movement ability. Author observed ability to movements of queens with different damages. Aroliums are soft and are used by queens and other bees to walk on smooth surfaces.

Jasiński (1995a) stated that after damages pigmentation of aroliums is changed from brightly grey to yellowish brown and black. Jasiński (1995a) ascertained also that aroliums become hard so queens movement on the glass is obstructed. Losses of leg segments or even whole legs hamper queens walking on the comb.

Damages from group 4 like wings damages hamper queens flying and the losses of whole antennas limit the sense ability and causes usually changing the queen. According to Jasiński (1995a) damages like this disqualify queens like a breeding material. It was observed that queens damages are held in the first two days of keeping (Jasiński 1987, 1995b, Jasiński, Fliszkiewicz 1992). Jasiński, Fliszkiewicz (1996) affirmed subtly less aggressiveness of young bees to queens kept with them. Jasiński (1988, 1995a, 1998) continuing his researches on queens damages noticed, that keeping at least two queens in one colony resulted in damages, but keeping more than two queens in one colony during the period of two weeks resulted in situation where more than 50 percent of queens failed from damages and were useless in breeding. Very important notice made by Jasiński (1990, 1995a, 1998) was a fact that it was an influence of sort of cage where the queen were kept on damage intensity. On the base of his studies can be affirmed that in the wooden cages with one net wall with meshes  $2.5 \times 2.5$  mm, bees less harmed queens than in plastic transport cages with crevices  $2.6 \times 2.6$  mm. It was turned out, that keeping queens in dequeens colonies in cages together with bees, queens were harmed by bees from colony which they were kept (Jasiński, Fliszkiewicz 1995). It is an evidence that bees from colony where the queens are kept are aggressive to queens and assisted bees. There were also tested drones and bees closed in cages in colony and damages were observed. Many authors noticed that warroza has conductive influence on damaging queens (Jasiński, Trzybiński 1991, Jasiński, Kawecki 1992,

Woyke et al. 1994, Jasiński, Fliszkiewicz 1995). Bees irritated by this parasite harm more heavily than bees from the same colony but cured from warroza

## MATERIAL AND METHODS

The investigation were performed at Bee Division, Faculty of Animal Science Agricultural University in Warsaw in Ursynów during the summer 2007. For the study were used 912 worker bees, which were observed on the body damages during the time of keeping them in colonies in plastic transport cages with crevices in the upper wall. There were evaluate body damages in 912 bees twice after three and ten days of keeping. There were inspected: 13824 legs, 4216 wings, 4608 antennas.

To evaluate damages of bee workers kept in their own colonies without queen, there were closed 6 bees in one plastic cage with 27 crevices in one wall. Crevices sized  $2.5 \text{ mm} \times 11 \text{ mm}$ . Cages with bees placed in special frames, which were put into the colonies. In the feed compartment of cage were placed honey and sugar candy as a food for bees. In every 3 repetition, bees were placed in 3 colonies. Damage control were after 3 and 10 days after the placement frames with cages. During the investigation there were evaluated number of living and dead bees – so were estimated survivability and mortality of bees. There were also observed under the microscopy bee damages, especially on legs, wings and antennas. It was noted number and severity of damages and also it was quoted number of bees damaged in strange colony. On that base it was definite the degree of damage number and the percentage of



damaged bees. It was also evaluated damage intensity in a percent Student-Newman-Keula's test was used for statistical analysis.

## RESULTS AND DISCUSSION

On the Figure 1 it is shown that 24 bees kept in the cages during 3 days in colony where they were origin from were damaged, that is about 3% from all in colony. After 3 days of keeping bees in cages in its own colonies died 88 bees,

that is 11% of all. It was ascertained that bee workers had damaged mainly legs and less damaged antennas and wings. It can be concluded that survivability in bees own colonies were 86,5% respectively.

On the Figure 2 is shown that 56 bees kept in cages during the 10 days in its own colony were damaged (7% of all bees). After 10 days died 200 bees kept in cages in its own colonies (25% of all). It was ascertained that bee workers had damaged mainly legs and less damaged antennas and wings.

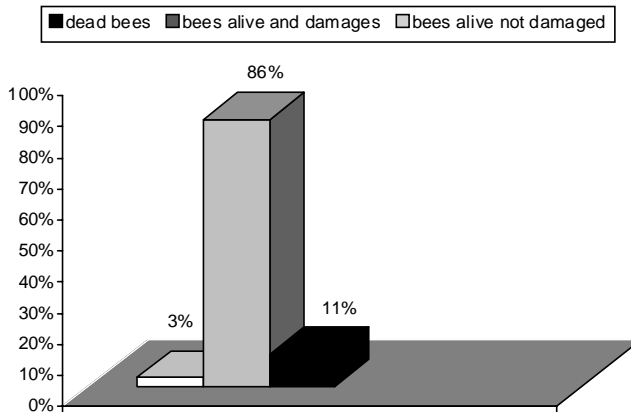


FIGURE 1. The comparison of bee keeping results in its own colonies without queen after 3 days

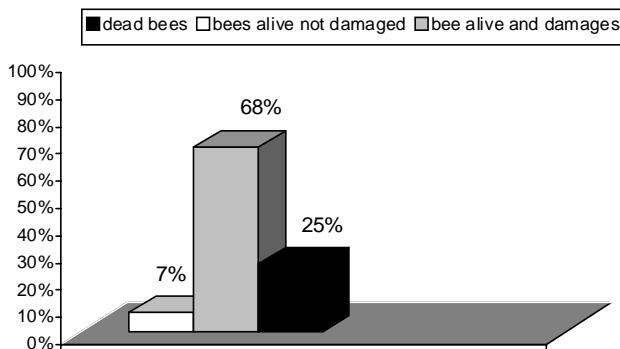


FIGURE 2. The comparison of results of bees kept results in its own colonies and in the strange colonies after the 10 days

After 10 days of keeping it was affirmed that bees in its own colonies without queen were not damaged and alive intensely than after 3 days of keeping. The bees in its own colonies without queen after 3 days has not significantly then the bees in its own colonies without queen after 10 days.

Standard error for own colonies alive not damages after 3 days is  $24 \pm 9.74$ . Standard error for own colonies alive not damages after 10 days is  $56 \pm 30.17$

After 3 days of keeping it was affirmed that bees in its own colonies without queen were damaged more intensely than after 10 days of keeping. The bees in its own colonies without queen after 3 days has not significantly damaged then the bees in its own colonies without queen after 10 days. Standard error for own colonies damages after 3 days is  $688 \pm 149.8$ . Standard error for own colonies damages after 10 days is  $544 \pm 12.8$ .

After 10 days of keeping it was affirmed that bees in its own colonies without queen were died more intensely than after 3 days of keeping. The bees in its own colonies without queen after 10 days has significantly more dead then the bees in its own colonies without queen after 3 days. Standard error for own colonies dead after 3 days – honey is  $88 \pm 1.34$ .

TABLE 1. The percent and number of alive bees not damaged after 3 and 10 days kept in its own colonies without queen queen

Number of days of keeping	Own colonies	
	Percent of alive not damaged bees	No of alive not damaged bees
After 3 days	3%	24
After 10 days	7%	56

TABLE 2. The intensity of damages in percent and number of alive bees damaged after 3 and 10 days kept in its own colonies without

Number of days of keeping	Own colonies	
	Percent of damaged intensity	No of damaged bees
After 3 days	86%	688
After 10 days	68%	544

TABLE 3. The intensity of dead in percent and number of bees after 3 and 10 days kept in its own colonies without queen

Number of days of keeping	Own colonies	
	Percent of dead intensity	No of dead bees
After 3 days	11%	88
After 10 days	25%	200

Standard error for colonies dead after 10 days – sugar is  $200 \pm 1.72$

## CONCLUSIONS

1. Bees kept in colonies in transport cages with crevices in one wall were damaged by bees.

2. The most often bees had lesion on legs, less often on wings and antennas.

3. Bees were damaged during the period of keeping from the first to 10<sup>th</sup> day.

4. Mortality of bees kept in cages rose from 3<sup>th</sup> day to 10<sup>th</sup> day.

5. Bees damage intensity kept in its own colonies during the period of 3 days and 10 days was 3% and 7% respectively.

6. The bees in its own colonies without queen after 10 days has significantly more dead then the bees in its own colonies without queen after 3 days.

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**Streszczenie:** Porównanie uszkodzeń robotnic w osieroconych rodzinach pszczelich. Doświadczenie wykonano w pasiece Pracowni Hodowli Owadów Użytkowych SGGW latem 2007 roku. Celem doświadczenia było zbadanie uszkodzeń robotnic przetrzymywanych w osieroconych rodzinach własnych. W doświadczeniu wzięło udział 912 pszczoł, które umieszczono w plastikowych klateczkach transportowych. Uszkodzenia sprawdzano dwukrotnie po trzech i po dziesięciu dniach przechowywania ich w osieroconych rodzinach pszczelich. Stwierdzono następujące uszkodzenia robotnic: 13824 nóg, 4216 skrzydeł i 4608 czółek.

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## **Effect of early supplemental feeding honeybee colonies with a substitute of bee bread made of pollen and honey on colony strength**

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**Abstract:** *Effect of early supplemental feeding honeybee colonies with a substitute of bee bread made of pollen and honey on colony strength.* The experiment was run in a stationary apiary, at Otrębusy, Pruszków region in the spring of 2005. The trial involved nineteen honeybee colonies divided into two groups: group I – 10 honeybee colonies were fed candy mix made of pollen and honey; group II – 10 colonies fed honey-and-sugar candy. The development of colonies was assessed based on brood area measurements. The experiments furnished evidence that early spring feeding of honeybee colonies with a mix of powder sugar and honey raised brood number in the colonies but generally when the bees were unable to forage for pollen. Later in the season, i.e. in April feeding with pollen – honey candy not failed so intensive to increase colony strength.

*Key words:* colony strength, pollen, colony development.

### **INTRODUCTION**

Colony strength before the nectar flow is a critical factor behind honey yield. Of particular importance is to bring the colony up to a high strength early in the spring which allows an effective use of early flows nowadays prevailing in this country. A frequently used method to hasten colony development is to feed the

colonies with bee bread (Maurizio 1950, Standifer 1967, Campana and Moeller 1977), candy or with other substances. Hayes (1984) and Loper and Berder (1980) hold the opinion that pollen is an indispensable food to be given to honeybee colonies in the spring. According to them, with the absence of pollen honeybee colonies can be fed substitutes which stimulate egg-laying and maintain brood rearing under less-than-optimum conditions. Hayes (1984) recommends soybean flour and yeast added to candy as the food to be given to colonies in the spring. Cook and Wilkinson (1986) believe that pollen substitute diets produce inconsistent results and not always stimulate egg-laying. Konopacka (1986) was also sceptical about the use of substitutes. She found that feeding soybean meal and yeast to honeybees failed to have a clear positive effect on colony development and performance. Likewise, Zmarzlicki and Marcinkowski (1980) failed to find a clear effect of early spring feeding a hydrocarbon diet on the number of reared brood. Baryczko and Szymaś (2001) found a higher average degree of pharyngeal gland development in bees that were fed bee bread. Like-

wise, they found fresh body weight to be higher by 8% in bees that were fed pollen from pollen loads. The rate of ingestion of food given to the bees increases with decreasing external temperature and with increasing temperature inside the hive (Łagowska, Szymaś 2001). Rogala and Szymaś (2001) determined the effect of non-protein diet on the content of amino acids in honeybee bodies. After two weeks on such a diet they found a lower content of essential amino acids in bee bodies as compared to that in one day-old bees. The effect of early-spring cold spells on bee activity was found by Dustmann and Ohe (1988). Pollen and honey mix was used in that study.

The objective of the study was to investigate the effect of early supplemental feeding of honeybee colonies with pollen – honey candy on colony strength expressed in terms of brood area.

## MATERIAL AND METHODS

The study was conducted in the spring of 2005 in a stationary apiary at Otrębusy, Pruszków region. The Carniolan queens used in the experiment were siblings; they were inseminated naturally by drones of unknown origin.

The experiment area was dominated by medium early nectar flows mainly from different willow species as well as from bass, acacia, maple, dandelion and sycamore.

The experiment was carried out in Wielkopolski type hives. Two colony groups of similar strength were formed:

Group I – 10 honeybee colonies were fed candy made of pollen and honey;

Group II – 10 colonies fed honey-and-sugar candy.

The candy was made of tree ingredients: pollen (kept for over half a year in a drying), sugar powder and honey in a weight ratio of 1:1:0,5. The candy was placed on the escape board and was replenished as the colonies consumed it. A single candy portion weighed 0.5 kg. It was in the form of a pat 1.5 cm thick wrapped in thin plastic sheet and placed on the escape board. Honey and sugar candy was served in a similar fashion. The candy was replenished as the bees used it up. The rate of consumption per colony was 1.5 kg of candy.

Three weeks after the supplemental feeding was started brood area in each colony was measured. The first measurement was done on March 13, 2005. The next two measurements were done at 21 day intervals, on April 06, 2005 and on April 27, 2005. Brood area was measured using a modified ellipse area formula and expressed as square decimeters:

$$P = 0.7854 \times D \times d$$

where: P = ellipse area, D = the longer diameter, d = the shorter diameter.

Student-Newman-Keula's test was used for statistical analysis.

## RESULTS OF RESEARCHES

Following the first period of the diet, the colonies fed the cake enriched with sugar powder showed an increase of brood area by 6.49 dm<sup>2</sup> as compared to the colonies that were fed pollen-honey candy (Fig. 1).

However, three weeks later, during the second measurement, the brood area in colonies fed pollen – honey candy was found to be smaller as 19 dm<sup>2</sup> than that of the control group being 24.12 dm<sup>2</sup>.

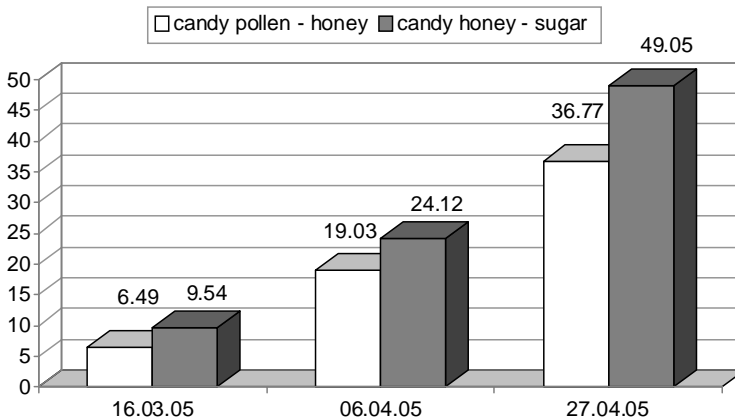


FIGURE 1. Average brood area in colonies

However, three weeks later, during the second measurement, the brood area in colonies fed pollen – honey candy was found to be smaller as 36.77 dm<sup>2</sup> than that of the control group being 49.05 dm<sup>2</sup> (Fig. 1).

The measurement data allowed the assessment of brood area growth rate.

In the first study period i.e. from 22.02.05 to 16.03.05 a greater brood area growth rate was found in colonies fed suger powder – honey candy.

Similarly, during the next measurements made in April and in May a greater brood growth rate was found in colonies fed suger powder – honey candy.

The data revealed that early supplemental feeding of colonies with candy enriched with pollen protein brought brood area. The brood area in colonies fed suger powder – honey candy was, on average, greater by 42% than that in pollen-honey colonies.

Another three weeks passed, the colonies fed suger powder – honey candy showed brood area greater by 19% than

that of supplementally colonies fed power powder – honey candy.

In the last study period i.e. from April 06, 2005 to April 27, 2005 a greater brood area growth rate was found in colonies fed suger powder – honey candy. This colonies showing 9% more brood than the colonies fed honey – sugar candy.

Colonies fed candy honey-sugar has significantly more brood then colonies fed candy pollen-honey from 22.02.05 to 16.03.05. Standard error for colonies fed candy pollen – honey is 6,49 ±1.28. Standard error for colonies fed candy honey – sugar is 9–54 ±1,61.

Colonies fed candy honey-sugar has significantly more brood then colonies fed candy pollen-honey from 16.03.05 to 06.04.05. Standard error for colonies fed candy pollen – honey is 19.03 ±1.86. Standard error for colonies fed candy honey – sugar is 24.12 ±3.11.

Colonies fed candy honey-sugar has significantly more brood then colonies fed candy pollen-honey from 06.04.05 to 27.04.05. Standard error for colonies

fed candy pollen – honey is  $36.77 \pm 2.05$ . Standard error for colonies fed candy honey – sugar is  $49.05 \pm 4.37$ .

## CONCLUSIONS

1. Early spring supplemental feeding of honeybee colonies with candy made of suger powder and honey become a promoter of egg-laying by the queens and an enhancer of the amount of brood in the colonies.

2. Later in the season, i.e. in April feeding of pollen – honey candy does not enhance so intensive colony strength.

3. Feeding bee bread substitutes in April is not warranted.

4. Colonies fed candy honey-sugar has significantly more brood then colonies fed candy pollen-honey.

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**Streszczenie:** Wpływ wczesnego żywienia uzupełniającego kolonii pszczół miodnych substytutem ciasta pszczelego składającego się z pyłku i miodu na siłę kolonii. Eksperyment został przeprowadzony w pasiece Pracowni Hodowli Owadów Użytkowych, Szkoły Głównej Gospodarstwas Wiejskiego, latem 2004 roku. Badanie odbyły się w dwudziestu rodzinach pszczelich podzielonych na 2 grupy: grupa I – 10 rodzin pszczelich podkarmianych ciastem miodowo-pyłkowym; group II – 10 rodzin pszczelich podkarmianych ciastem miodowo-cukrowym. Podkarmianie rodzin pszczelich wczesną wiosną ciastem zrobio-



nym z cukru pudru i miodu spowodowało przyspieszenie czerwienia matek pszczelich. Podobnie w kwietniu rodziny podkarmiane ciastem miodowo-pyłkowym nie przyspieszyły rozwoju tak intensywnie jak rodziny z drugiej grupy doświadczalnej karmione ciastem miodowo-cukrowym. W pozostałym okresie badawczym sytuacja nie uległa zmianie.

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## Laboratory assessment of the production value of the honeybees (*Apis mellifera*) with various genotypes

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**Abstract:** *Laboratory assessment of the production value of the honeybees (*Apis mellifera*) with various genotypes.* The object of the study was the assessment of the usefulness of laboratory cage tests in the estimation of honey yield and the parameters characterising the springtime colony development and the colony strength. Each experiment comprised 10 colonies representing three groups of honeybees: pure-bred Buckfast (Bcf), Caucasian queen × Carniolan drone hybrids (Cau × Car) and Norwegian queen × Caucasian drone hybrids (Nor × Cau). In the course of the experiments, laboratory cage tests were carried out with the aim of checking the foraging efficiency and life span of bees. The results of those tests were compared with the honey yield and the parameters characterizing springtime colony development.

In the Bcf group no significant correlations were found between the average quantity of the foraged food per one cage, per one day of the test, and the honey yield. Such correlations were present in the Cau × Car group, and those traits were negatively correlated in the Nor × Car group. Only in the Bcf group were significant correlations found between the average quantity of the foraged food per one bee per one day of the test and the honey yield. The correlation coefficient was especially high in May, which may have resulted from the fact that it was in May that the bees were acquired for use in the experiments. Only negative correlations between food foraging and the honey yield were found in the Nor × Cau group under laboratory conditions. Negative correlation between the life span and the honey yield was found in the Nor × Cau group. Similar result was achieved in the Bcf group. In the Cau × Car group those traits were positively correlated. In all groups under assessment, the correlation coefficient between the life

span and the brood area was positive at the second measurement and negative at the third. Positive values of the correlation coefficients at the third measurement of the brood area as opposed to their negative values at the second measurement may have resulted from the fact that the date of the third measurement was close to the date of collecting the bees for the experiments. Positive correlation between the colony strength at the last autumn inspection and the life span was found in all the groups. Unexpected negative correlation between the life span and the colony strength occurred in the Nor × Cau group in March. In the remaining groups, correlation coefficients were positive and significant.

The conformity between the results of the laboratory tests, the real honey yield and the parameters characterizing the springtime colony development and the colony strength may be considerably influenced by the genotype/breed of the bees as well as by the date of collecting the bees for the laboratory test. Detailed explanation of that issue, however, requires further studies.

*Key words:* honey bee, laboratory tests, foraging, honey yield.

## INTRODUCTION

Honey yield is a complex trait with predominant impact on the results that Polish apiaries achieve in terms of economy. Honey yield is mostly influenced by the foraging and hoarding efficiency and it also depends on the proportion of

the stored supplies that is used to meet the colony's subsistence needs (Paleolog 1996). These processes are conditioned by both environmental and hereditary factors (Woyke 1998; Woyke 1984). The assessment of complex traits, which are to a large extent dependent on the environment, is difficult to carry out in a real apiary. Hence, some researchers used laboratory cage tests in order to concentrate on genetic background and to minimise the influence of the environment (Olszewski, Paleolog 2005, Paleolog, Olszewski 2005, Milne 1985b, Kulinčević, Rothenbuhler 1982). Such tests are also cost-efficient and they facilitate conducting the experiments under similar conditions in different research centers. A strong relationship between the results obtained by means of laboratory tests and the real honey yield of a bee colony would allow the assessment of the honey production capabilities of that colony long before the nectar flow. Nevertheless, there are different opinions on whether the foraging efficiency during cage tests and the honey yield can, in fact, be correlated (Paleolog, Flis 1999; Milne 1985; Milne 1977).

In recent years information of the increasing number of cases of colony collapse disorder (CCD) has been coming from all over the world. CCD seems to affect beekeeping also in Poland. In response to that fact, there is an increasing interest in the production of nucleus colonies among beekeepers, as a means of compensating the losses. If such tendencies continue in the future, and if foreign markets open for Polish nucleus colonies and package bees, such traits as springtime colony development and inclination to intensive brood rearing may turn out to be of equal importance as honey

yield. Because of that, in addition to the relations between the foraging efficiency under laboratory conditions and the real honey yield, the author decided to examine whether there is any relation between the life span of bees assessed under laboratory conditions, the springtime colony development and the colony strength.

## MATERIAL AND METHODS

The study comprised 10 colonies representing three groups of genetically different honeybees. They were pure-bred Buckfast (Bcf), Caucasian queen × Carniolan drone hybrids (Cau × Car) and Norwegian queen (Middle European bee of the Norwegian line) × Caucasian drone hybrids (Nor × Cau). Laboratory cage tests were carried out with the aim of checking the foraging efficiency and life span of the bees. The results of those tests were compared with the honey yield evaluated in the apiary throughout the entire season and with the parameters characterizing springtime colony development as well as the colony strength. The experiments were repeated in three subsequent seasons.

In May, one-day-old bees were collected from each colony in each group. The bees from each colony were introduced into 3 wooden cages, (125 × 125 × 48 mm), 50 bees per cage. The cages with bees were kept in the dark room at constant temperature and humidity (temp. = 28°C; H = 65%). Each cage was equipped with a feeder made of a plastic test tube that was cut so that the bees could forage the candy being a mixture of powdered sugar and honey. Candy was replenished every day and several drops of water was injected through

ventilation openings. The feeders were weighed every day, in order to measure the weight of the foraged food, and dead bees were counted to measure their life span. Food foraging was expressed as the average quantity of food foraged by all bees in one cage from a given group on one day of the test and as the average quantity of food foraged by an individual bee from a given group. Life span was expressed as the day until which the average of 70% (D70%), 50% (D50%) and 30% (D30%) bees survived in one cage in a given group.

In order to assess the springtime colony development, brood area was measured three times in each colony. The first measurement was carried out on the day of the spring cleansing flight. The interval between subsequent measurements was 21 days. Brood area was used for computing the daily increase of brood cells at each measurement. Brood increase was computed from the difference of brood cell number between subsequent (2<sup>nd</sup> and 1<sup>st</sup>; 3<sup>rd</sup> and 2<sup>nd</sup>) measurements. The colony strength was expressed as the number

of combs fully populated by bees. It was evaluated at the brood area measurements and at the last autumn inspection.

Honey yield was evaluated on the basis of the weight of the extracted honey. It was computed by means of weighing the combs, for each colony separately, before and after extracting the honey. Three honey harvests were carried out in each season, one in May, June and July. In the first two harvests the harvested honey came from the spring nectar flow and it was referred to as the spring honey. At the third harvest, in July, honey was obtained from the summer nectar flow.

The results were analysed statistically (Pearson correlation) using a packet SAS (2000).

## RESULTS

In the Bcf group no significant correlations were found between the average quantity of the foraged food per one cage and the honey yield (Tab. 1). Such correlation occurred in Cau × Car and it concerned spring honey, extracted in

TABLE 1. Coefficients of correlation between the honey field and the average weight of foraged food per one cage and one bee on one day of the test

Group	Food foraging	Honey yield			
		Spring		Summer (July)	Sum season
		May	June		
Bcf	per one cage	-.165	.236	.178	.124
	per one bee	<b>.622**</b>	<b>-.586**</b>	<b>.365*</b>	<b>.378*</b>
Cau × Car	per one cage	-.165	<b>.594**</b>	<b>.562**</b>	<b>.491*</b>
	per one bee	.326	-.001	-.020	.212
Nor × Cau	per one cage	-.597	-.455	<b>-.678*</b>	<b>-.694*</b>
	per one bee	-.487	-.060	-.238	-.334

**Bcf** – Buckfast; **Cau × Car** – Caucasian × Carniolan; **Nor × Cau** – Norwegian × Caucasian; **Spring** – spring honey; **May** – honey extracted in May; **June** – honey extracted in June; **Summer** – summer honey; **Sum season** – honey extracted in the entire season

\* - correlations significant at  $P \leq 0.05$ ; \*\* – correlations significant at  $P \leq 0.01$ .

June, summer honey and the total mass of honey extracted in the entire season. In the Nor × Car group those traits were negatively correlated. Only in the Bcf group were significant correlations found between the average quantity of the foraged food per one bee and the honey yield. The correlation coefficient was especially high in May, which may have resulted from the fact that it was in May that the bees were acquired for use in the experiments. In June, on the other hand, honey yield was negatively correlated with the average quantity of food foraged by one bee. Only negative correlations between food foraging and the honey yield were found in the Nor × Cau group under laboratory conditions.

Negative correlation between the life span and the honey yield was found in the Nor × Cau group (Tab. 2). Similar result was obtained in the Bcf group. In the Cau × Car group, however, those traits were positively correlated. Only in that group were significant correlations found.

As far as the relations between the life span, the parameters describing the springtime colony development and the colony strength are concerned, only those of them were included in Table 3 for which significant correlations were found in at least one experimental group. The largest number of such correlations was found in the Bcf group. In Cau × Car and Nor × Cau groups only isolated cases of significant correlations occurred. In all bee groups under assessment, the correlation coefficient between the life span and the brood area had a positive value at the second measurement and negative at the third. Nor × Cau group was an exception at D70%. Positive values of the correlation coefficients at the third measurement of the brood area as opposed to their negative values at the second may have resulted from the fact that the date of the third measurement was close to the date of acquiring the bees for the cage tests. Similar tendencies were observed with regard to the brood

TABLE 2. Coefficients of correlation between the life span of the bees in the laboratory test – day until which 70% (D 70%), 50% (D 50%) and 30% (D 30%) survived, respectively, and the honey yield of the colonies

Group	Honey yield	Life span of bees		
		D 70%	D 50%	D 30%
Bcf	Spring	-,196	-,121	-,033
	Summer	-,244	-,059	,068
	Sum season	-,266	-,097	,035
Cau × Car	Spring	,073	,155	,277
	Summer	<b>,469*</b>	,398	<b>,513*</b>
	Sum season	,292	,356	<b>,491*</b>
Nor × Cau	Spring	-,209	-,175	-,186
	Summer	-,471	-,241	-,167
	Sum season	-,384	-,230	-,193

**Bcf** – Buckfast; **Cau × Car** – Caucasian × Carniolan; **Nor × Cau** – Norwegian × Caucasian; **Spring** – spring honey; **Summer** – summer honey; **Sum season** – honey extracted in the entire season

\* - correlations significant at  $P \leq 0.05$ .

TABLE 3. Coefficients of correlation between the life span of the bees in the laboratory test – day until which 70% (D 70%), 50% (D 50%) and 30% (D 30%) survived, respectively, and the brood area, the increase of the brood and the colony strength

Parameters		Life span of bees		
		D 70%	D 50%	D 30%
Bcf	Brood area at the first measurement [dm <sup>2</sup> ]	<b>-.494**</b>	<b>-.417*</b>	-.294
	Brood area at the third measurement [dm <sup>2</sup> ]	.300	<b>.466**</b>	<b>.504**</b>
	Brood increase (number of cells between 2 <sup>nd</sup> and 1 <sup>st</sup> measurement)	<b>-.400*</b>	-.315	-.242
	Brood increase (number of cells between 3 <sup>rd</sup> and 2 <sup>nd</sup> measurement)	<b>.546**</b>	<b>.638**</b>	<b>.596**</b>
	Colony strength at the last autumn inspection (number of combs)	<b>.526**</b>	.316	.262
	Colony strength in March (number of combs)	<b>.582**</b>	<b>.500**</b>	<b>.462**</b>
Cau × Car	Brood area at the first measurement [dm <sup>2</sup> ]	-.047	-.055	-.024
	Brood area at the third measurement [dm <sup>2</sup> ]	.306	.410	.455
	Brood increase (number of cells between 2 <sup>nd</sup> and 1 <sup>st</sup> measurement)	-.177	-.096	-.085
	Brood increase (number of cells between 3 <sup>rd</sup> and 2 <sup>nd</sup> measurement)	.249	.331	.350
	Colony strength at the last autumn inspection (number of combs)	.350	.162	.258
	Colony strength in March (number of combs)	.350	<b>.553*</b>	<b>.657**</b>
Nor × Cau	Brood area at the first measurement [dm <sup>2</sup> ]	-.400	-.289	-.215
	Brood area at the third measurement [dm <sup>2</sup> ]	-.011	.231	.265
	Brood increase (number of cells between 2 <sup>nd</sup> and 1 <sup>st</sup> measurement)	-.298	-.153	-.089
	Brood increase (number of cells between 3 <sup>rd</sup> and 2 <sup>nd</sup> measurement)	.387	<b>.612**</b>	<b>.586**</b>
	Colony strength at the last autumn inspection (number of combs)	.499	<b>.574*</b>	<b>.577*</b>
	Colony strength in March (number of combs)	-.367	-.094	-.024

**Bcf** – Buckfast; **Cau × Car** – Caucasian × Carniolan; **Nor × Cau** – Norwegian × Caucasian;

\* - correlations significant at  $P \leq 0.05$ ; \*\* - correlations significant at  $P \leq 0.01$ .

increase. Positive correlation between the colony strength at the last autumn inspection and the life span was found in all groups. Significant correlations were found in Bcf and Nor × Cau. Unexpected negative correlation between the life span and the colony strength occurred in the Nor × Cau group in March. In the remaining groups, correlation coefficients were positive and significant.

## DISCUSSION

Some authors (Kepeňa 1985; Milne 1985a; Kulinčević, Rothenbuhler 1982) claim that the honey field of a bee colony may be predicted on the basis of its ability to forage food under laboratory conditions. Not everybody, however, shares that view (Collins et al. 1984; Milne 1980). Also in the experiments conducted by

the author, the results obtained in the laboratory did not correlate with the honey production in the apiary. However, the basis for the lack of such correlation was complex. Taking into consideration the production of honey throughout the season, in Bcf and Cau  $\times$  Car positive correlations were found between the honey yield and food foraging under laboratory conditions. In the Nor  $\times$  Car group those correlations were negative. Therefore, the conformity of the laboratory tests with the actual results obtained in the field, may be genotype/breed dependent. That could explain the lack of authors' unanimity regarding the usefulness of laboratory tests in the evaluation of the productivity of bee colonies (Collins et al. 1984; Milne 1980). The acquisition date of the bees to be used in laboratory tests also seems to be significant.

Weak negative correlation between the honey yield of the colonies and the life span of worker bees was proved by Woyke (1984). The results obtained by the author conform with that, but only in the case of Bcf and Nor  $\times$  Cau. In the Cau  $\times$  Car those traits were positively correlated. The lack of relation between the effectiveness of the foragers' work and their life span was proved by Rinderer and Silvester (1978) and Milne (1980). In the author's studies, the highest values of the correlation coefficient between the honey yield and the life span were found in Cau  $\times$  Car, perhaps because Caucasian bees are considered to have the longest longevity. The longevity of Middle European bees is also considered to be long and in the author's studies, the component of that breed contained hybrids of the Norwegian bee (Konopacka 1999; Ruttner 1992). In that case, however,

negative correlation was found. That fact seems to confirm the view that genotype/breed of the bees may also, to a considerable extent, influence the results of laboratory tests.

Also with regard to the correlations between the longevity and the parameters that characterise the springtime colony development and strength, the influence of the genotype/breed was clearly visible. The values of the correlation coefficients were usually the highest in the Bcf group. The largest number of positive correlations which were statistically significant were also found in that group. Apparently, it is the result of the predisposition of the Buckfast bee to the dynamic springtime colony development, rearing large amounts of brood and maintaining higher than average colony strength throughout the season (Maul et al. 1999, Büchler 1998a, Büchler 1998b) and, as the studies point out, longer life span than that of Caucasian and Norwegian bee hybrids (Paleolog, Olszewski 2005).

## CONCLUSION

The conformity between the results of the laboratory tests, the real honey yield in the apiary and parameters characterizing springtime colony development and colony strength may be considerably influenced by the genotype/breed of the bees as well as by the date of the acquisition of the bees for laboratory test.

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**Streszczenie:** *Laboratoryjna ocena wartości produkcji pszczoł miodnych o różnych genotypach.* Celem pracy była ocena przydatności laboratoryjnych testów klatkowych do szacowania wydajności miodowej oraz parametrów charakteryzujących rozwój wiosenny i siłę rodzin. Do badań wykorzystano po 10 rodzin z trzech grup pszczoł: czystorasowe pszczoły Buckfast (Bcf), mieszańce po matkach kaukaskich inseminowanych nasieniem trutni krańskich (Cau × Car) oraz mieszańce po matkach linii norweska inseminowanych nasieniem trutni kaukaskich (Nor × Cau). Przeprowadzono laboratoryjne testy klatkowe, w których sprawdzano efektywność pobierania pokarmu i długowieczność pszczoł. Wyniki tych testów porównano z wydajnością miodową i parametrami charakteryzującymi rozwój wiosenny i siłę rodzin.

W grupie Bcf nie stwierdzono istotnych korelacji między ilością pobranego pokarmu przypadającego średnio na jedną klatkę w jednym dniu testu a wydajnością miodową. Takie korelacje wystąpiły u Cau × Car, a Nor × Car, cechy te były skorelowane ujemnie. Tylko u Bcf stwierdzono istotne korelacje między ilością pobranego pokarmu przypadającą średnio na jedną pszczołę w jednym dniu testu a wydajnością miodową. Współczynnik korelacji przyjął szczególnie wysoką wartość w maju, być może dlatego, że pszczoły do doświadczenia pozyskano właśnie w maju. U Nor

× Cau stwierdzono wyłącznie ujemne zależności między pobieraniem pokarmu w warunkach laboratoryjnych a wydajnością miodową. W grupie Nor × Cau stwierdzono ujemną korelację między długowiecznością pszczół a wydajnością miodową. Podobnie było w przypadku Bcf. U Cau × Car cechy te były skorelowane dodatnio. Dla wszystkich ocenianych grup współczynniki korelacji między długowiecznością a powierzchnią czerwii przy drugim pomiarze przyjmowały wartości ujemne, a przy trzecim pomiarze dodatnie. Dodatnie współczynniki korelacji przy trzecim pomiarze czerwii w odróżnieniu do ujemnych przy drugim mogą wynikać z tego, że termin trzeciego pomiaru był zbliżony do terminu pozyskania pszczół do testów laboratoryjnych. We wszystkich grupach stwierdzono dodatnią zależność między siłą rodzin w czasie ostatniego przeglądu jesiennego a długowiecznością. W grupie Nor × Cau zaskoczyły ujemne wartości współczynników korelacji między długowiecznością pszczół

a siłą rodzin w marcu. W pozostałych grupach współczynniki korelacji przyjmowały wartości dodatnie i były istotne.

Zgodność między wynikami testów laboratoryjnych a rzeczywistą wydajnością miodową oraz parametrami charakteryzującymi rozwój wiosenny i siłę rodzin może zależeć od genotypu/rasy pszczół a także terminu pozyskania pszczół do doświadczenia laboratoryjnego. Szczegółowe wyjaśnienie tej kwestii wymaga jednak dalszych badań.

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## Genetic-environment interactions resulting from different levels of competitiveness of workers in various *Apis mellifera* breeds

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**Abstract:** *Genetic-environment interactions resulting from different levels of competitiveness of workers in various *Apis mellifera* breeds.* The influence of the inter-breed interactions between the bees of the Caucasian (CAU), Carnelian (CAR) and Buckfast (BC) genotypes on the results of their hoarding activity was studied. For the experiments three environments with the artificial nectar flow were created. The intensity of bee competition was different in each environment. The CAU were poor competitors, i.e. they were efficient only in the environment without competition ( $p < x$ ;  $x 0.0001 \div 0.004$ ) whereas the CAR were good with that respect, i.e. they were better in the environments with competition ( $p < x$ ;  $x 0.01 \div 0.02$ ). The competitiveness of the BC was medium or more similar to the CAR (differences between BC and CAU, as well as BC and CAR were statistically not significant;  $p > 0.05$ ), i.e. they were insensitive to either competition or no competition, since their hoarding efficiency was relatively similar in each of the three experimental environments ( $p < x$ ;  $x 0.07 \div 0.33$ ). The occurrence of the huge genotype-environment interactions ( $F_{001} = 5.09$ ;  $p < 0.0013$  and  $F_{001} = 6.01$ ;  $p < 0.0004$ ) and significant influences of the experimental environments ( $F_{001} = 14.7$ ;  $p < 0.0001$  and  $F_{001} = 18.1$   $p < 0.0001$ ) have been revealed. The genotype ranking was distinctly different in each of the environments due to the bee competition. Effects of genotypes (independently on environment) were insignificant ( $p > 0.23$  and  $p > 0.98$ ). Consequently, bees of some genotypes are more sensitive to bee competition whereas bees of other genotypes are less sensitive. The worker-bee competitive abilities should be considered by bee breeders, especially in the regions densely populated by various honeybee breeds (sub-species).

*Key words:* *Apis mellifera*, competition, flying cage, hoarding, interactions.

### INTRODUCTION

The results of the studies that were conducted in the regions where the *Apis mellifera* is native and also the regions where it has been introduced revealed its significant impact of on indigenous species (Roubik P.W. 1978, Bernhardt and Weston 1996) because *Apis mellifera* utilize over 80% of the floral resources produced (Paton 1996). Therefore, many studies concerned the competition between Honeybees, and other indigenous *Apoidea* (Steffan-Dewenter and Tschardt 2000, Roubik and Wolda 2001, Goulson and Sparrow 2009, Paini 2009). Such studies have also been performed in Poland (Banaszak 2000, Pawlikowski and Celary 2006). It is amazing, however, that almost no researchers paid attention to the competition between the breeds (ecotypes, subspecies) within the *Apis mellifera* species, even though, beekeepers are spreading those subspecies throughout the world intensively. There is evidence that in Poland the competition between different honey bee subspecies/breeds influenced the Honeybee breeding programs (Gromisz 1997, Paleolog 2002). Our former studies

also showed that interactions between European bee breeds could be an important environmental factor (Paleolog *et al.* 1999). Moreover, Pham-Deleque *et al.* (1987) suggested that crossbreds were better producers than purebreds because of their better competing abilities. Consequently, there is a need for the research concerning the inter-breed (inter-subspecies) competition (interactions) in *Apis mellifera*.

The aim of this study was to estimate the influence of the inter-breed interactions resulting from different levels of competitiveness of workers in Caucasian, Carnelian and Buckfast bees on their hoarding efficiency under the artificial nectar flow conditions.

## MATERIAL AND METHODS

Nine source colonies were maintained during two consecutive years and were stimulated to the colony build up. Three of them were Buckfast, three Caucasian and three Carniolan. They were headed by sister purebred queens. Brood combs were transferred between the hives within each of the breeds. The source colonies were also supplied with the emerging brood before overwintering.

Eighteen Langstroth hives, each of them containing four previously weighted empty combs (435 x 230 mm), were prepared. Worker-bees from each of the nine source colonies were smoked and shaken to an empty wooden box in the evening, when the flight activity was terminated. The colony age structure was maintained. Then, the workers were sprayed with water : mint : ethanol : honey solution and the bees belonging to each of the three breeds were polled together in three se-

parate boxes and smoked/sprayed again. After that, each of the three half-dozen of the Langstroths were populated respectively by bees of the Buckfast (**BC**), the Caucasian (**CAU**) and the Carniolan (**CAR**) genotype – one liter of workers *per* hive. One of the four hive combs was supplied with a caged queen and a comb cutting (10 x 10 cm) containing 1–2 day old larvae/eggs. The queens remained caged during the whole test period. Each hive was supplied by 200g of honey : sugar candy which contained 100g of pollen. After being kept in the cellar for 12 hours the experimental colonies were transported to the test region. Six of them (environment with no concurrence; **NoC**) were placed separately in six flight cages (plastic black mesh; 5 m long, 2 m wide and 2.2 m high) with artificial feeding stations (water : sugar syrup – 1:1), the next six (environment with concurrence; **C**) were placed together into one flight cage (4.5 m long, 3.5 m wide and 2.2 m high + two artificial feeding stations) and the remaining six were kept in the field, without flight cages in the vicinity of a bee yard (environment with concurrence but without flying cages; **C+**). These bees had access to two artificial feeding stations and to natural nectar resources. All the bees were supplied with sugar syrup incessantly. So, the intensity of bee competition and density of bee population, were different in **NoC**, **C**, and **C+**, whereas remaining environment factors were similar in these environments. Each half-dozen of hives placed in each of the three environments consisted of two **BC**, two **CAU** and two **CAR** colonies. The procedure described above was repeated twice (May, July) in each of the two consecutive years, which

means that four test repetitions were performed.

All combs in all hives were weighed individually in 3, 9, 14 and 19 days after the beginning of the test in order to evaluate weight of the hoarded supplies. Two-way ANOVA with an interaction was performed separately for data obtained from measurements performed at the 9<sup>th</sup> and 19<sup>th</sup> day. The following model was applied:  $x_{ijk} = \mu + B_i + C_j + (B \times C)_{(i \times j)} + e_{ijk}$  ( $B_i$  is the breed effect,  $C_j$  is the environment effect). Data from all repetitions were pooled but before that, in order to eliminate the influence of the year/repetition, they were standardized (Falconer, 1981) by expressing all colony values from a given repetition as a decimal fraction of the average of all the colonies examined in that repetition. Results presented in Tables 3 and 4 and also at Figure 2 were obtained using the standardized data and results presented in table 1 and 2 and at figure 1 were obtained using original data.

## RESULTS

Bees of the **CAU** genotype (Fig. 1 and Tab. 1) hoarded the highest amount of supplies whereas the **CAR** bees were the worst ( $p < 0.01$ ) under **NoC** (no concurrence) conditions. The **BC** bees achieved medium results in this environment. Under **C** and **C+** (concurrence) conditions, however, bees of the **CAU** genotype hoarded the lowest amount of supplies ( $p < 0.01$ ). The **BC** bees were similar to the **CAR** ( $p > 0.05$ ) and better than the **CAU**. On the other hand, when the standardized data were used, the hoarding efficiency of the **CAR** (Fig. 2) was the best, the **BC**'s medium and the **CAU**'s was the worst

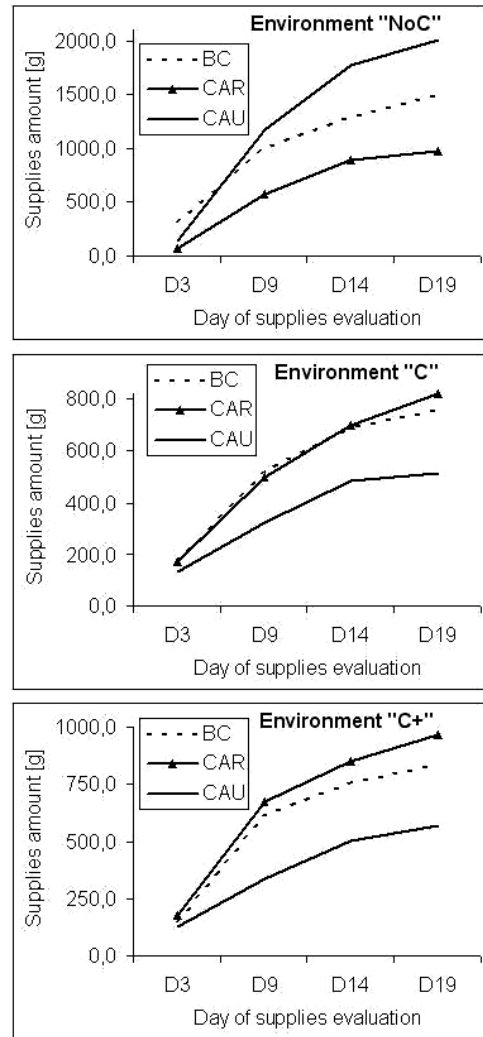


FIGURE 1. The average amount of sugar supply evaluated in bees of each the genotypes within each of the environments for each of the colonies (Y axes)

**D3, D9, D14** and **D19** stand for the 3<sup>rd</sup>, the 9<sup>th</sup>, the 14<sup>th</sup> and the 19<sup>th</sup> day of the test, respectively (X axes). **NoC**: – environment under flying cages without bee competition. **C**: – environment under flying cages with bee competition. **C+**: – environment under no flying cages with bee competition. **BC, CAR** and **CAU** stand for the bees of Buckfast, Carniolan and Caucasian genotypes.

TABLE 1. The average amount of sugar supply (4 repetition were pooled) gathered by bees of the Buckfast (**BC**), the Caucasian (**CAU**) and the Carniolan (**CAR**) genotype in three environments during 19 days of the test

Environment	Genotype of bees			Overall mean
	CAU	CAR	BC	
NoC	2006.0 <sup>A</sup>	976.7 <sup>B</sup>	1490.5 <sup>C</sup>	1491.1 <sup>A</sup>
C	515.9 <sup>B</sup>	819.8 <sup>Aa</sup>	759.1 <sup>Aa</sup>	698.3 <sup>Bb</sup>
C+	571.5 <sup>a</sup>	975.1 <sup>b</sup>	834.2 <sup>b</sup>	793.6 <sup>Bb</sup>
<b>Overall mean</b>	1031.1 <sup>a</sup>	923.9 <sup>a</sup>	1027.9 <sup>a</sup>	

NoC – no competition. C – competition with flying cages. C+ – Competition with no flying cages. At the shadowed part the statistical comparison were made in rows (between the breeds). At the no shadowed part the statistical comparison were made in the column (between the environments). Different capital letters – means differ significantly ( $p < 0.01$ ). Different lowercase letters – means differ significantly ( $p < 0.05$ ).

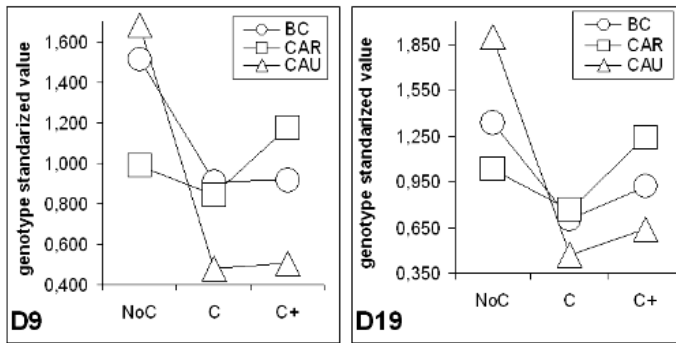


FIGURE 2. The interaction graph. The genotype standardized values obtained by expressing all colony values from a given repetition as a decimal fraction of the average of all the colonies examined in that repetition have been plotted against the environments. Data from four repetitions were pooled

**BC**, **CAR** and **CAU** stand for the average hoarding results estimated for bees of Buckfast, Carniolan and Caucasian colonies/genotypes. **D9** and **D19**: – the 9<sup>th</sup> and the 19<sup>th</sup> day of the test (X axes). **NoC**: – environment under flying cages without bee competition. **C**: – environment under flying cages with bee competition. **C+**: – environment under no flying cages with bee competition.

( $p < 0.05$ ) in the **C+** environment ( $p < 0.05$ ). It may, therefore, be concluded that the **CAU** were bad and the **CAR** were good competitors whereas the competitiveness of the **BC** was medium or good (respectively in **C+** or **C**).

Better weather (Tab. 2) conditions resulted in higher storage of supplies in the repetitions 1 and 2. An influence of the poor weather was particularly visible

in the repetition 4 ( $p < 0.01$ ). The standardized data, however, were free of the repetition (i.e. weather) influences (i.e. residual error), and therefore, they were more reliable for statistical comparisons.

The total amount of storage (Tab. 3 – standardized) was significantly lower in the **C** and the **C+**, i.e. in the environments with bee competition ( $p < 0.01$ ) than in the **NoC**, whereas it was similar ( $p > 0.2$ )

TABLE 2. Numbers of days of good, moderate and poor weather conditions within the 19 days of the field test (Part 1) and amount of sugar supply (Part 2) evaluated at 9<sup>th</sup> (D9) and at 19<sup>th</sup> (D19) day of the test expressed in original and standardized values

	Repetition			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
<b>Weather – Part 1</b>				
Good	11	14	6	4
Moderate	6	5	9	7
Poor	2	0	4	8
<b>Supply – Part 2</b>				
D9 – standardized	1.000	1.000	1.000	1.000
D19 – standardized	1.000	1.000	1.000	1.000
D9 – original [g]	803.4 <sup>Ad</sup>	1064.0 <sup>Ae</sup>	485.6 <sup>B</sup>	185.3 <sup>C</sup>
D19 – original [g]	1445.7 <sup>Ad</sup>	1703.6 <sup>Ae</sup>	722.5 <sup>B</sup>	105.5 <sup>C</sup>

Different capital letters – means differ significantly ( $p < 0.01$ ). Different lowercase letters – means differ significantly ( $p < 0.05$ ). Comparisons were made only in rows.

TABLE 3. Least Squares Means (LSM)\* calculated separately for each of the genotypes (pooled environments) and for each of the environments (pooled genotypes). The standardized data were used

(D9) LSM $\pm$ se		(D9) LSM $\pm$ se	
<b>BC</b>	1.1128 $\pm$ 0.0924 <sup>A</sup>	<b>C</b>	0.7414 $\pm$ 0.0924 <sup>A</sup>
<b>CAR</b>	1.0001 $\pm$ 0.0924 <sup>AB</sup>	<b>C+</b>	0.8649 $\pm$ 0.0924 <sup>A</sup>
<b>CAU</b>	0.8863 $\pm$ 0.0924 <sup>B</sup>	<b>NoC</b>	1.3938 $\pm$ 0.0924 <sup>C</sup>
(D19) LSM $\pm$ se		(D19) LSM $\pm$ se	
<b>BC</b>	0.9848 $\pm$ 0.0922 <sup>A</sup>	<b>C</b>	0.6458 $\pm$ 0.0922 <sup>A</sup>
<b>CAR</b>	1.0130 $\pm$ 0.0922 <sup>A</sup>	<b>C+</b>	0.9321 $\pm$ 0.0922 <sup>B</sup>
<b>CAU</b>	1.0023 $\pm$ 0.0922 <sup>A</sup>	<b>NoC</b>	1.4221 $\pm$ 0.0922 <sup>C</sup>

**BC**, **CAR** and **CAU** stand for bees of Buckfast, Carniolan and Caucasian genotypes. **D9** and **D19** – the 9<sup>th</sup> and the 19<sup>th</sup> day of the test. **NoC**: – environment under flying cages without bee competition. **C**: – environment under flying cages with bee competition. **C+**: – environment under no flying cages with bee competition.  $\text{Pr} > |t|$  for each LSM was  $< .0001$ . \* – SAS System, GLM procedure. Different capital letters – means differ significantly ( $p < 0.01$ ).

in colonies of the three tested genotypes (**CAU**, **CAR** and **BC**). This finding was confirmed by results presented at the table 1. So, the environment influenced but the genotype did not influenced the total amount of storage hoarded by bees.

The two-way ANOVA (Tab. 4) shown (standardized data) that the *environment* (i.e. competition) was the highest source of variability because the mean-square

of this source surpass the mean-square of the *error* over 13-time for D9, and over 18-time for D19. The *environment* impact was statistically significant. When the original data were used (for D19 only), the *environment* still was the highest and statistically significant source of variability, but in this case, *environment/error* mean-squares' ratio amounted only 6.6. The mean-square of the *genotype*-

TABLE 4. The two-way ANOVA\* results. Genotype, environment and their interaction were considered

Source of variability	Standardized data						Original data [g]		
	At the 9 <sup>th</sup> day of the test			At the 19 <sup>th</sup> day of the test			At the 19 <sup>th</sup> day of the test		
	Mean square	F	Pr > F	Mean square	F	Pr > F	Mean square	F	Pr > F
Genotype	0.30779615	01.50	0.2305	0.00485629	00.02	0.9765	89318,5	0,132	0,876
Environment	2.88247941	14.07	<.0001	3.69864621	18.11	<.0001	4496273,5	6,666	0,002
Genot * Env	1.04308575	05.09	0.0013	1.22831236	06.01	0.0004	1286021,8	1,907	0,120
Error	0.20491458			0.20423188			674508,2		

At the 9<sup>th</sup> day of the test: – R square = 0.449777, F = 6.44 and p > 0.0001. At the 19<sup>th</sup> day of the test: – R square = 0.489154, F = 7,54 and p > 0.0001. The model is accurate enough. \* – SAS System, GLM procedure.

*environment-interaction* (standardized data) was also statistically significant and surpass the mean-square of the *error* over four-time for D9, and over six-time for D19. However, when the original data were used, the *genotype-environment-interaction* impact was statistically insignificant and *genotype-environment-interaction/error* mean-squares' ratio was 1.6. On the other hand, the genotype ranking (Figure 2 – standardized data) was distinctly different in **NoC versus C** and **NoC versus C+** environments, and therefore, the occurrence the big *genotype-environment-interaction* has been confirmed. An influence of the *genotype* was not proved because *genotype : error* mean square ratios amounted only 1.5 for D9, 0.05 for D19 and 0.13 for the original data. The influence of this source of variability was statistically insignificant (“p” ranged from 0.23 to 0.97).

Concluding, the highest, huge influence of worker competition on the average amount of the sugar supply gathered by bees was confirmed. No genotype itself but only the genotype-environment interaction (i.e. genotype-competition) influenced the supplies.

## DISCUSSION

An intensity of bee competition was the main factor that differentiated the NoC, C and C+ environments in my studies. Therefore, genotype-environment interaction and a huge, significant effects of the experimental environments suggest that inter-breed (subspecies) competition within the Honeybee is an essential factor that could strongly influence of honey yield, a phenomenon similar to inter-species competition between other *Apoidea/Apidae* and *Apis mellifera* (Roubik 1978, Eggelston 1985, Banaszak 2000, Pawlikowski and Celary 2006, Goulson and Sparrow 2009, Paine 2009). Previous studies on the Caucasian and European Black bee in Poland (Gromisz 1997, Paleolog *at al.* 1999, 2002) and on the Italian bee crossbreeds (Pham-Deleque *at al.* 1987) suggest, in the context of my present research, that the impact of inter-breed competitive abilities in bees is underestimated. My results also revealed that, under some circumstances, the competition between the Honeybee breeds would be even as intensive as that between the Honeybee and the other bee



species (Steffan-Dewenter, Tschartke 2000).

Bees that are good achievers under the weak but are poor under the strong competition conditions could be defined as of “expansive”, whereas those that do not change their efficiency when they are placed under either weak or strong competition conditions could be defined as bees of “resistant” genotypes (Mather and Calligari 1983, Egelston 1985, Paleolog 2000). My present studies confirmed that one could find such types of bees also among the bee breeds that are kept in Poland.

## CONCLUSIONS

1. Bees of some genotypes (subspecies) could be more sensitive whereas bees of another genotypes (subspecies) could be less sensitive to bee competition, therefore the level of the competitiveness of bee breeds kept at a given area is an important environmental factor.

2. The worker-bee competitive abilities should be considered by bee breeders, especially in the regions densely populated by various honeybee breeds (sub-species), because no genotype itself but rather the genotype-environment interaction may influence the honey yield.

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**Streszczenie:** *Interakcje genetyczno-środowiskowe spowodowane różną zdolnością do konkurencji robotnic pochodzących z różnych ras Apis Mellifera.* Ważnym, potocznie niedocenianym czynnikiem środowiska naturalnego, jest konkurencja ze strony innych jego mieszkańców. U pszczoł badana jest raczej konkurencja pomiędzy zbieraczkami różnych rodzin/rodzajów *Apoidea/Apidae* niż pomiędzy zbieraczkami różnych ras (podgatunków) *Apis mellifera*. By zbadać te ostatnią stworzono po 6 rodziniek doświadczanych (4 plastry Langstroth) pszczoł o genotypach rasy Buckfast (BC), krainka (CAR) i kaukaska (CAU), co dało razem 18 rodziniek. Z pośród nich wybrano po dwie rodziniki BC, CAR i CAU i każdą umieszczono pod oddzielnym izolatorom (6 izolatorom) tworząc środowisko bez konkurencji. Następne 6 rodziniek, także po dwie BC, CAR i CAU, umieszczono razem pod jednym izolatorom, tworząc środowisko z konkurencją. Ostatnie 6, także po dwie każdej rasy, umieszczono na pasieczysku tworząc środowisko z konkurencją ale bez izolatora. Pszczoły miały dostęp do podkarmiaczek zewnętrznych z syropem 1:1 podawanym nieprzerwanie. Natężenie konkurencji było więc różne w każdym z tych środowisk. CAU okazały się słabymi (wydajne

przy braku konkurencji;  $p < x$ ;  $x \ 0,0001 \div 0,004$ ) a CAR dobrymi (wydajniejsze w warunkach konkurencji;  $p < x$ ;  $x \ 0,01 \div 0,02$ ) w konkurowaniu o zasoby pożytkowe. BC Były jedynie nieco słabsze od CAR ( $p > 0,05$ ) i gromadziły podobne ilości zapasom w każdym ze środowisk ( $p < x$ ;  $x \ 0,07 \div 0,33$ ) będąc tym samym mało wrażliwe na brak/występowanie konkurencji. Uszeregowanie genotypom było różne w każdym ze środowisk, a wpływ środowiska, czyli konkurencji ( $F_{001} = 14,7$ ;  $p < 0,0001$  i  $F_{001} = 18,1$   $p < 0,0001$ ) oraz interakcji genetyczno-środowiskowej ( $F_{001} = 5,09$ ;  $p < 0,0013$  i  $F = 6,01$ ;  $p < 0,0004$ ) były największymi i istotnymi źródłami zmienności. Natomiast wpływ genotypu (rasy), badany niezależnie od środowiska, okazał się nieistotny ( $p > 0,23$  i  $p > 0,98$ ). Potwierdzono, że konkurencja pomiędzy zbieraczkami w znaczny sposób wpływa na wydajność jednych, a w nieznaczny, na wydajność innych ras pszczoł. Dlatego wydajność danej rasy należy oceniać także biorąc pod uwagę jej zdolności do konkurowania o źródła wziętku. Hodowcy winni to brać pod uwagę.

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## The impact of the sequence of particular drone semen administration on number of their own progeny after instrumental insemination

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**Abstract:** *The impact of the sequence of particular drone semen administration on number of their own progeny after instrumental insemination.* During the mating flight, a queen copulates with several, sometimes more than ten, drones. It is known that a queen preserves semen from all the drones with which she had copulated. However, it is not clear if the particular drone position in the mating sequences influences the number of this male's progeny. Investigations were performed in the apiary of Apiculture Department of Warmia and Mazury University in Olsztyn (Poland) from 1 July till 30 Sept. 2009. The experiment was carried out on 36 Carniolan queens (*Apis mellifera carnica*). Each of them was instrumentally inseminated with semen collected from nine drones. The drones represented two bee races – *Apis mellifera ligustica* and *A.m.carnica* – that have different body colouring. It was found that each of the drones which copulated with a queen had a chance for acquiring progeny. Although the findings indicated that the progeny of drones' semen which was the last to be injected into queen's oviduct had the smallest range.

*Key words:* drones, queens, insemination, bee workers, colour inheritance, number of progeny.

### INTRODUCTION

Within a healthy bee colony drones are produced only during the reproduction

period – May to August. Their main function is to mate and inseminate the young queens with semen. Queen and drone copulations occur in the air during mating flights (Soczek 1958). A queen is successively mated with several drones (Woyke 1960) during nuptial flights. Except for the mating flight periods, drones do not display interest in young queens whether they are in nest or its vicinity (Zmarlicki and Morse 1963). Virgin queens fly to drone congregation areas to get mated with mature drones.

Drones aggregate in the chosen areas which can be used even for several years (Ruttner 1966, Loper 1985). Drones mark congregation areas and flight tracks with a specific pheromone (Lensky et al. 1985). Such a mechanism probably facilitates the young queens to find the drone congregation area. Drones congregate around a flying queen lured by her secreted pheromones (Butler 1976). Koeniger et al. (1979) managed to film the copulation process of the honey bees. After copulation, the drone dies from a total tectonic contraction of abdominal muscles, that is necessary for copulation

apparatus extrusion and semen injection into oviducts of queen (Woyke 1958).

The biology of queens insemination makes extreme demands on the drone. To convey its genes, a drone must overcome the great concurrence of the other drones. Koeniger's et al. (1979) film showed that the drones are not waiting passively on their chance but they are fighting to get the best position all the time. In order to do this, they hit their heads against the competing drones, not only those nearby but even at the moment of with a queen (Koeniger et al. 1979).

After her mating flight, the queen comes back to the hive and pushes the collected semen inside her body by contracting the oviduct's muscles. All the time a small amount of semen (ca. 6%) penetrates into spermatheca of queen (Woyke 1960). The queen preserves semen from all the drones with which she has mated (Jasiński 1994). This happens as the semen from each drone is mixed in queen oviducts as a consequence of injection force and contractions of oviduct's muscles (Jasiński 1994, Woyke 2008b). In spite of this mixing process, the quantity of semen that enters into queen's spermatheca from particular drones may be differentiated. So, this raises the question if a place of a particular drone in mating sequence influences quantity of its progeny.

The aim of this study was to determine the possible impact of the order of a particular drone's semen injection during instrumental insemination on the amount of its own progeny.

## MATERIAL AND METHODS

The study was carried out in apiary of Apiculture Department of Warmia and

Mazury University in Olsztyn between 1 July and 30 September 2009. Thirty six Carniolan queens (*Apis mellifera carnica*) were used. The queens were instrumentally inseminated with semen from nine successive drones according to the following schedule:

- Group I (12 queens) – the first drone of Italian race (*A.m. ligustica*) and next eight Carniolan drones (*A.m. carnica*);
- Group II (12 queens) – first four Carniolan drones followed by one Italian drone and again four Carniolan drones;
- Group III (12 queens) – first eight Carniolan drones followed by one Italian drone.

During instrumental insemination, semen collected from one drone was singly injected into queen's oviduct and the insemination needle was later thoroughly rinsed with physiological salt solution. After insemination queens were introduced into a new settled nucleus. Combs of sealed brood with the progeny of investigated queens were isolated in an incubator before the young bees emergence. Emerging worker bees were counted and recorded as light (after Italian drone) and dark (after Carniolan drones) coloured separately.

In order to verify the hypothesis that a queen's insemination by a given sequence of drones does not influence percentage of light and dark coloured workers, a chi-square independence test was used. Statistical calculation was performed for three independent trials.

## RESULTS

The average distribution of light-coloured bees in Groups I and II were similar to each other and amounted to 28.4

and 27.9 individuals that was respectively 22% and 23% of the emerging workers population. In comparison with groups I and II, light-coloured worker population distribution in the third group was lower with an average of 19.7 individuals. It constituted 20% of all worker population emerging in that group (Tab. 1). A statistical (chi-square) independence test for 3 independent trials to verify hypotheses showed that the sequence of semen injections during instrumental insemination did not influence a percentage of light- and dark-coloured workers. The Chi-square test value amounted 3.28. Critical values for  $(2-1)(3-1) = 2$  freedom degree and reliance range  $p = 0.05$  and  $p = 0.01$  are 5.5991 and 9.210 respectively. The performed test showed no influence on light- and dark-coloured workers percentage in their progeny by semen injection sequence during instrumental insemination of queens.

A wide span has been found between extreme results within the progeny of particular queens. The greatest span appeared in the second group which amounted 41.1 (Fig. 1) having both the least (8%) and the greatest (49.1)% contribution of

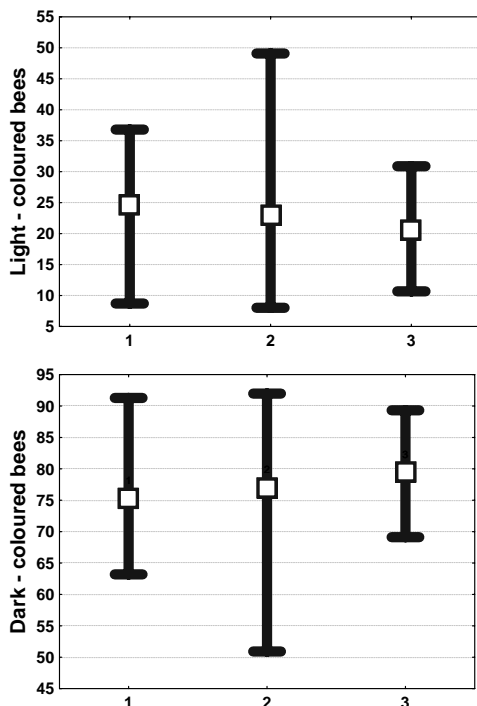


FIGURE 1. Mean value as well as minimal and maximum values of light – and dark-coloured bees proportions (%)

Explanations: 1 – the first drone of Italian race (*A.m. ligustica*) and next eight Carniolan drones (*A.m. carnica*), 2 – first four Carniolan drones followed by one Italian drone and again four Carniolan drones, 3 – first eight Carniolan drones followed by one Italian drone.

TABLE 1. Total and average number and percentage of the light – and the dark-coloured bees

Order of semen injection	n	Light-coloured bees		Dark-coloured bees	
		individuals	%	individuals	%
I+8C	12	28.4 n = 341	23	96.1 n=1153	77
4C+II+4C	12	27.9 n = 335	22	97.3 n=1168	78
8C+II	12	19.7 n = 236	20	78.5 n = 942	80

Explanations: I + 8C – the first drone (of the light colour) of Italian race (*A.m. ligustica*) and next eight Carniolan drones (*A.m. carnica*), 4C + II + 4C – first four Carniolan drones followed by one Italian drone and again four Carniolan drones, 8C + II – first eight Carniolan drones followed by one Italian drone.

light-coloured bees in progeny of particular queens. The least differentiated bees were found in the third group where the extreme results values differed by 20.2. Within this group, the least proportion of light-coloured bees amounted by 10.7% and the highest by 30.9% of particular queen progeny.

## DISCUSSION

Drones were often not appreciated due to their haploid nature and their lack of direct engagement in bee colony productivity. Only in recent years has more interest has been shown for the drones' presence for intensive apiculture production (Woyke 2008a). Drones, originating from unfertilized eggs, were neglected in bee genetics they in favour of the genetics of their mothers (Rinderer 1986, Paleolog 1996 a and b).

Bees' body colour depends on one pair of the main genes and seven modulator genes (Woyke 1977). As a result of their interaction ten classes of bee colouring exists with various proportions of yellow and black colours. The Carniolan bees used in our studies were generally dark-coloured although they can possess some more or less yellowish tones. In this study, all bees which did not have an entirely dark body colour were classified as the progeny of Italian drones which characterize themselves with light, yellow body colour. That is why a theoretical 11% proportion of light-coloured workers was found only within progeny of two queens. Jasiński (1994) found the same proportion of mutant worker bees after inseminating queens with mutant drones' semen administered in various sequences. In this experiment considerable seasonal

variation was observed in the number of bees of dark and yellow colour.

## CONCLUSIONS

Our study has shown that every drone whose semen was inseminated to a queen has a chance to produce own progeny.

The findings indicated that the progeny of drones' semen which was injected into queen's oviduct as the latest, had the smallest range.

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- wpływ na ilość powstałego po nich potomstwa. Celem pracy było określenie wpływu kolejności wprowadzania nasienia od poszczególnych trutni podczas sztucznego unasieniania na ilość ich potomstwa. Doświadczenie wykonano w paśmie Katedry Pszczelnictwa UWM w Olsztynie w okresie od 1.07.2009 do 30.09.2009 roku. W doświadczeniu użyto 36 matek rasy kraińskiej (*Apis mellifera carnica*). Matki te unasieniono sztucznie nasieniem pobieranym od 9 trutni dwóch ras *Apis mellifera ligustica* i *A. m. carnica* różniących się ubarwieniem. Stwierdzono, że każdy truteń unasieniający matkę ma szansę na pozostawienie potomstwa. Potomstwo po trutniach, których nasienie wprowadzane jest do jajowodów matki jako ostatnie mają, charakteryzowało się najmniejszym rozstępem.

**Streszczenie:** *Wpływ kolejności wprowadzania nasienia trutni podczas sztucznego unasieniania na ilość potomstwa własnego. Matka pszczeła podczas lotu godowego kopuluje z kilkoma lub nawet kilkunastoma trutniami. Wiadomo, że matka pszczeła przechowuje nasienie od wszystkich trutni z którymi kopulowała, natomiast nie jest jasne czy kolejność kopulujących trutni ma*

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## Evaluation of winter hive debris in a commercial apiary

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**Abstract:** *Evaluation of winter hive debris in a commercial apiary.* In the spring of 2009, 82 winter hive debris samples were collected from 17 bee yards in a commercial apiary. The weight of winter hive debris ranged from 57.1 g to 122.8 g, and it comprised from 610 to 1298 bees with average individual weight of 82.3–91.5 mg. Wax debris had a varied share of the total weight of hive debris. The lowest weight of wax debris was observed in colonies infested with both pathogens – *Nosema apis* and *Varroa destructor* (10.7%), and the highest – in colonies affected only by varroosis (21.2%). The number of *V. destructor* females observed in hive debris reached 16.6–16.9, and the number of pollen grains in the intestine observed in a microscopic field of view (400x) ranged from 8.1 (colonies infested with *Nosema apis*) to 11.1 (colonies infested with *Nosema apis* and *V. destructor*). Parasites were not found in 26 (31.7%) of the studied hive debris samples, *V. destructor* females were noted in 9 (11%) samples, *N. apis* spores were observed in 28 (43.1%) samples, and both parasites were detected in 19 samples (23.2%).

*Key words:* bees, hive debris.

### INTRODUCTION

Winter hive debris consists of dead bees that have dropped to the floorboard, and wax debris that fall as bees chew through cappings. Healthy bee colonies which are well prepared for wintering and are settled in a peaceful location produce small quantities of debris. Anxious bees which

are infested with, for example, *Varroa destructor*, *Nosema apis*, rodents or birds, are less adequately prepared for wintering. Some diseased and irritated bees fall to the floorboard and die. Low, sub-zero temperatures and low humidity levels in the hive support wintering. High, above-zero temperatures, temperature fluctuations and high humidity levels in the hive increase the food demand of bees, thus overfilling the rectum and deteriorating the bees' overall health condition. Other factors that significantly contribute to the wintering of bee colonies and the volume of winter hive debris include the age of wintering bees, the method of preparation for the winter, feed type, winter feeding dates and stored pollen reserves (Hartwig, Topolska 1995).

In view of the encountered instances of poor wintering and winter bee losses, the objective of this study was to determine the factors affecting the volume and the composition of hive debris in apiaries with similar hives, similar late summer pastures and similar type of preparation for wintering.

### MATERIALS AND METHODS

The study was carried out in the spring of 2009, and it covered 17 out of 50 bee

yards in a commercial apiary. Full debris samples were randomly collected from the floorboards of 82 hives. In a laboratory, the samples were weighed and sifted through a 3 mm mesh sieve. Bees and wax debris were weighed. The percentage content of wax debris was calculated as the difference between full debris weight and wax debris weight. Sifted bees were counted, and 20 randomly selected individuals were weighed. The weight of an individual bee was determined based on the computed values. The same bees were used to evaluate the rate of infestation with *Nosema apis* spores and to determine the number of pollen grains in the intestine. The number of *Varroa destructor* females was determined in wax debris. Bee colonies were fed syrup in the winter in the amount of 15 kg sugar per colony. Varroosis was controlled in late August/early September by fumigating the bees with 3–4 treatments of Apiwarol AS. In the second half of the summer season, bee flight was observed mainly in buckwheat and weed pastures.

## RESULTS

In the group of 82 analyzed debris samples, parasites were not found in 26 (31.7%) samples, *V. destructor* females were observed in 9 (11%) samples, *N. apis* spores were reported in 28 (34.5%) samples, and both parasites were detected in 19 (23.2%) samples. The weight of winter hive debris ranged from 57.1 g to 122.8 g, and it comprised from 610 to 1298 bees with average individual weight of 82.3–91.5 mg. Wax debris that has fallen to the floorboard as a result of bee chewing through cappings had a varied share of the total weight of hive debris.

TABLE 1. Results of winter hive debris evaluations in a commercial apiary

Hive debris from colonies where:	No. of studied hive debris samples	Hive debris weight (g)	% share of wax debris in total hive debris	No. of bees in hive debris	Bee weight (mg)	No. of <i>V. destructor</i> females in debris	% debris samples containing <i>N. apis</i> spores with intensity level		No. of pollen grains in microscopic field of view (400x)
							++	+++	
<i>V. destructor</i> and <i>N. apis</i> were not determined	26	62.7	14.8	610	84.4	0	0	0	10.3
Only <i>V. destructor</i> females were determined	9	57.1	21.2	556	91.5	16.6	0	0	8.9
Only <i>N. apis</i> spores were determined	28	122.8	11.5	1231	90.5	0	41.7	58.3	8.1
<i>V. destructor</i> females and <i>N. apis</i> spores were determined	19	111.1	10.7	1298	82.3	19.6	15.8	84.2	11.1

The lowest percentage share of wax debris was reported in colonies infested with both parasites (10.7%), and the highest – in colonies affected only by varroosis (21.2%). The number of *V. destructor* females observed in hive debris reached 16.6–16.9. *N. apis* spores were found in more than 57% debris samples. The highest rate of *Nosema apis* invasion was reported in colonies additionally infected with varroosis (84.2%). The number of pollen grains from the intestine ranged from 8.1 (colonies infested with *N. apis* only) to 11.1 (colonies invaded by both parasites) (Table 1).

## DISCUSSION

There is a limited number of published studies discussing various factors that affect the volume of winter hive debris. The existing publications focus mostly on the number of bees, the physical condition of debris (damp, mould), the rate of infestation with *N. apis* spores and *Varroa destructor* mites. In a study of wintering bees, Kasperek et al. (2009) observed that while some bees dropped to the floorboard, other bees left the hive at an average rate of 15 bees per day. The above authors also noted that the weight of bees leaving the hive in the winter was higher than the weight of bees which collapsed to the floorboard. Pohorecka (2008), who monitored the health condition of Polish bees based on hive debris samples from 353 colonies submitted by beekeepers, did not find *V. destructor* mites in 36.5% samples, and observed a low rate of infestation in 30.9% samples. The lowest rate of varroosis infestation was observed in bees from medium-sized apiaries of 31–80 colonies. 36.5% of the

studied samples were free of nosemosis, 28.9% showed low infestation rates, and 8.2% – high infestation rates. No samples containing more than 400 million *N. apis* spores per bee were determined in hive debris from apiaries with up to 10 colonies and from 51 to 80 colonies. The highest rate of infestation with *N. apis* spores was noted in apiaries with 31 to 50 colonies.

In addition to the above indicators, this study also analyzed hive debris samples, dead bees, the weight of wax debris and the number of pollen grains in the intestine. The latter factors seem to adequately reflect the bees' wintering behavior. Researchers find it difficult to explain why the volume of hive debris, the weight of bees and the number of pollen grains in the intestine vary in colonies which are similarly prepared for wintering, use similar pastures in the second half of the summer season and have queens of the same race and age. Romaniuk et al. (2008) observed that in colonies affected by varroosis in 11.6–14.8% the weight of hive debris reached 51.5 g, whereas it was 1.7 times lower in colonies infested with the investigated parasite in 4.4–4.7%. Similar results were reported in respect of the number of bees in debris samples. The lowest individual weight was noted in debris samples collected from colonies affected by varroosis at the rate of 4.4–4.7%. In the remaining colonies, the weight of the studied bees ranged from 63.5 mg to 75.3 mg. Pollen grain counts were low in the examined abdomen samples. The lowest number of pollen grains in the abdomen was observed in colonies affected by varroosis in 15.5–25%, and the highest (more than 15) – in bees infected with the disease at the rate of

11.6–14.8%. In this study, hive debris from the colonies infected with varroosis had a much higher content of wax debris than the colonies affected by nosemosis. The share of wax debris in colonies infested only with *V. destructor* mites and in colonies free of that parasite produced interesting results. Colonies affected by varroosis produced nearly twice the volume of wax debris in comparison with non-infested colonies, suggesting that the parasite produced an irritating effect on bees, thus contributing to a higher level of winter stock consumption in the winter. The highest quantity of pollen grain was found in bees from colonies affected by both parasites. The above indicates that in addition to carbohydrates, bees also consumed bee bread. The low weight of bees in colonies infested with *N. apis* and *V. destructor* is difficult to explain. It seems that bees emerging from cells infected with varroosis were characterized by low body weight which increased their susceptibility to *N. apis* infections. *V. destructor* females were not found in 30% of the studied hive debris samples, which suggests that varroosis can be effectively controlled through regular preventive treatment.

Bee wintering and hive debris volume are affected by a number of factors. The key determinants are difficult to identify as even minor changes in pasture conditions, disease infestation and, above all, winter temperatures and humidity levels inside the hive affect wintering behavior and the quantity of hive debris. Anxious and infected bees consume more feed to maintain the right temperature within a cluster which leads to higher production of wax debris, overfilled intestinal tract and higher hive debris volume.

## CONCLUSIONS

1. The volume of hive debris is determined by the health condition of a bee colony prepared for wintering and the type of parasite infestation.
2. Regular varroosis prevention in an apiary significantly minimizes or eliminates parasite invasions.
3. The occurrence of nosemosis and combined nosemosis and varroosis infestations affect hive debris volume and composition.

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**Streszczenie:** Ocena osypu zimowego w pasiekach wielkotowarowych. Wiosną 2009 r. z 17 pasiek w wielkotowarowej pasiece pobrano 82 osypy zimowe. Masa osypu zimowego wahała się od 57,1 g do 122,8 g, a w nim znajdowało się od 610 do 1298 pszczół o średniej masie każdego owada 82,3-91,5 mg. Masa zgryzów wyliczona w procentach w stosunku do pełnego osypu, była zróżnicowana. Najniższą stwierdzono w rodzinach zarażonych obydwoma pasożytami – *Nosema apis* i *Varroa destructor* (10,7%), a najwyższą w dotkniętych tylko warrozą (21,2%). Liczba samic *V. destructor* wykryta w osypach wynosiła 16,6-19,6, a ziaren pyłku z jelita w polu widzenia mikroskopu (pow. 400x) wahała się od 8,1 – rodziny z nosemozą do 11,1 – rodziny zarażone dodatkowo *V. destructor*.

Wśród zbadanych osypów w 26 (31,7%) nie wykryto żadnych pasożytów, w 9 (11%) występowały tylko samice *V. destructor*, w 28 (43,1%) spory *N. apis*, a w 19 (23,2%) obydwaj pasożyty.

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## HCH and DDT concentration in bees, honey and bee bread in apiaries from Warmia and Mazury region with a variety of different nectar

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**Abstract:** HCH and DDT concentration in bees, honey and bee bread in apiaries from Warmia and Mazury region with a variety of different nectar. Bees, bee bread and honey from 11 apiaries from Warmia and Mazury region were examined. In apiaries from Olsztyn the average concentration of HCH in bees was 0.0220 µg/g of lipid matter (l.m.), while from the apiaries situated on the fields of the former State Farms and individual farmers was more than twice and from Piska Forest 1.4 time higher than in Olsztyn. In bee bread from Olsztyn apiaries HCH content was 0.0012 µg/g l.m. whereas in those situated in other locations (fields of the former State Farms, individual farmers and Piska Forest) was from 3.4 to 7 time higher. HCH content in honey from Olsztyn was the least (0.0003 µg/g l.m.) and the greatest (0.0024 µg/g l.m.) from fields of former State Farms. Residues of DDT were very high (0.1200–0.1862 µg/g l.m.) in bees from all apiaries but low in honey and ranged from 0.0011 µg/g l.m. (Olsztyn and Piska Forest) to 0.0014 µg/g l.m. (the remaining apiaries). Concentration of total DDT in bee bread was the least in Olsztyn (0.0001 µg/g l.m.) and very high in the others. Particularly high level of this pesticide (0.0927 µg/g l.m.) was noted in bee bread from Piska Forest.

**Key words:** bees, bee bread, honey, HCH, DDT.

### INTRODUCTION

Recent investigations conducted on bees and bee products as well as entomophilous plants, have showed that residues of

chlorinated hydrocarbons in biological material depend on apiary location and even on the race of bees. Usually, level of these pesticides in soil and plants on areas with intensive cultivation is greater in comparison to the regions with extensive agriculture. Similarly, in apiaries located in the vicinity of forests sprayed previously with pesticides against black arches (*Lymantria monacha*) invasion, the residues of HCH and DDT in bees and bee products are high (Romaniuk et al. 2003 a,b; 2004; Witkiewicz et al. 2000).

The previous research concerning pesticides degradation in the environment has shown that they are resistant to bacterial decomposition. Therefore they remain in the soil for a long time and afterwards with plants penetrate into animals' organism. The main product of bacterial decomposition of DDT, due to DDT-dehydrochlorinase activity, is DDE; the second one – created by aquatic microorganisms – is DDD (Rózański 1992).

In Poland, chlorinated hydrocarbons – mainly DDT, were recalled from production and application in agriculture and veterinary medicine in the late seventies, but in many European countries they

were used until the end of eighties. Preparations containing chlorinated hydrocarbons are still in use in some countries of Asia, Africa, South and Middle America and with air drift they spread even to Europe. Therefore in Poland, despite the discontinuance the application of chlorinated hydrocarbons in agriculture, these pesticides are still found in the soil, animal tissues and products of animal origin (e.g. milk, eggs) (Januskiewicz and Niewiadowska 1984; Niewiadowska at al. 1995, Smoczyński at al. 1996).

The aim of the studies were to estimate the HCH and DDT residues in bees, honey and bee bread from apiaries located in places with a variety of different nectars and type of agriculture.

## MATERIAL AND METHODS

Studies were carried out in randomly selected apiaries from Warmia and Mazury region in the second half of July 2008.

Bees, bee bread and honey from 11 apiaries were examined. Two of them were located on the outskirts of Olsztyn, four situated on the fields of former State Farms, four on the fields and waste land belonged to individual farmers and one the edge Piska Forest.

Samples of biological material were examined to detect HCH and DDT. Analyses were performed by the Chair of Commodity Science and Food Analysis UWM in Olsztyn, using Amarowicz and Smoczyński (1986) method.

## RESULTS

The occurrence of chlorinated hydrocarbons was recorded in all examined samples. Residues of mentioned pesticides were depended on the type of examined material and apiary location. In apiar-

ies from Olsztyn the average concentration of HCH in bees was 0.0220 µg/g of lipid matter (l.m.), while from those situated on the fields of former State Farms as well as individual farmers was more than twice higher (0.0474 µg/g l.m. and 0.0525 µg/g l.m., respectively). Also in bees from Piska Forest HCH content was about 93 µg/g l.m. higher than in Olsztyn.

In bee bread from apiaries from Olsztyn the level of HCH was 0.0012 µg/g l.m., whereas from those located on the cultivations of former State Farms and individual farmers was about 3.7-4.4 µg/g l.m. times and from Piska Forest more than 7 times higher.

Residues of HCH in honey from Olsztyn were 0.0003 µg/g l.m. and they were about 15 times lower in comparison to apiaries of individual farmers fields, more than 5 times lower than in Piska Forest and 8 times lower than those located on the fields of former State Farms.

Residues of DDT in bees in all examined apiaries were very high (from 0.1200 µg/g l.m. – Olsztyn to 0.1862 µg/g l.m. – apiaries on the fields and waste land of private farmers), while in honey were low and fluctuated from 0.0011 µg/g l.m. (Olsztyn and Piska Forest) to 0.0014 µg/g l.m. (the remaining apiaries).

Concentration of DDT, including mainly DDE, in bee bread was low in apiaries from Olsztyn (0.0001 µg/g l.m.) and very high in the others. Particularly high level of this pesticide was found in bee bread from Piska Forest (0.0927 µg/g l.m.).

## DISCUSSION

Various chemical substances are brought into hive by bees, which collect nectar, pollen and water within their flight dis-



tance. The amount of these compounds is depended on the apiary location, type of nectar and its area. Our investigation conducted on bees and bee products from apiaries located in places with a variety of different nectars, indicated differences in amount of chlorinated hydrocarbons in these material. Similar investigation concerning utilization of entomophilous plants and bees to estimate the HCH and DDT were done by Witkiewicz and co-workers (2000). Authors demonstrated the presence of these compounds by evaluating of its residues in flowers of entomophilous plants, bees and bee products. Concentration of HCH and DDT in examined material was not equal. It depended on the apiary location, type of the plant, year of investigation and even the race of bees. Romaniuk and co-workers (2003a) researching the residues of chlorinated hydrocarbons in honey from 45 apiaries from Warmia and Mazury province, found the occurrence of HCH in honey from all districts, while DDT were detected only in 31. HCH content in honey was vestigial. For instance, in honey from 31 districts HCH concentration amounted to 0.0001–0.0020  $\mu\text{g/g}$  l.m.; in 9 it was 0.0022–0.0030  $\mu\text{g/g}$  l. m. and 0.0034–0.050  $\mu\text{g/g}$  l. m. in 5 districts. The greatest concentration of HCH was ascertained in honey from apiaries situated on the fields of former State Farms. DDT was not found in 14 districts and in the remaining its content ranged from 0.0001 to 0.0068  $\mu\text{g/g}$  l.m. Similarly to HCH, the greatest amount of DDT was also noted in honey from apiaries situated on the fields of former State Farms. Besides honey, pollen and bee bread, pesticides occurs also in other bee products. In Germany, Gayger and Dustman (1985) determined the concentration

of chlorinated hydrocarbons in beeswax. They found not only HCH and DDT, but also its isomers. They also demonstrated that old beeswax contains considerably greater amount of chlorinated hydrocarbons that the recent one. Spodniewska and Romaniuk (2006) estimating concentration of HCH and DDT in bees proved, that residues of these pesticides were not equal and depended not only on apiary location, but also on bee colony. According to the authors, it testifies to multidirectional bee flights and their custom to collect nectar from specified group of plants.

Taking into consideration bee race or period of nectar collection, the evaluation of pesticides residues in bees and bee products may be more complicated. This kind of dependence was ascertained by Romaniuk and co-workers (2001) in some races of bees working the same entomophilous plants in Piska Forest. It was occurred that the greatest amount of HCH and DDT accumulated Caucasian bees and the least Augustan ones.

Taking note of mentioned above, it should be supposed that amount of chlorinated hydrocarbons in bees and bee products depends on apiary location. Areas where growing entomophilus plants are frequently protected against pests with pesticides, contain considerably greater its amount than in regions without intensive agriculture.

## CONCLUSIONS

1. The level of chlorinated hydrocarbons in bees, bee bread and honey depends on the apiary location as well as type of examined material. The greatest amount is found in bee bread, and the least in honey.

2. Among the detected chlorinated hydrocarbons in the examined material, DDT and its metabolites predominated. It indicates its slow degradation in the environment.

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**Streszczenie:** Zawartość HCH i DDT w pszczołach, miodzie i pierdze z rejonu Warmii i Mazur. Zbadano pszczoły, pierzgę i miód z 11 pasiek województwa warmińsko-mazurskiego. W pasiekach z miasta Olsztyn, średnia zawartość HCH u pszczoł wynosiła 0,0220 µg/g substancji lipidowej (s.l.), natomiast z pasiek położonych na polach po byłych PGR i rolników indywidualnych była ponad dwa razy, a z pasieki z Puszczy Piskiej 1,4 razy wyższa niż w Olsztynie. W pierdze z pasiek Olsztyna zawartość HCH wynosiła 0,0012 µg/g s.l., natomiast w usytuowanych na uprawach po byłych PGR, rolników indywidualnych oraz Puszczy Piskiej o 3,4 do 7 razy wyższa. Zawartość HCH w miodzie była najniższa (0,0003 µg/g s.l.) u pszczoł z pasiek z Olsztyna, a najwyższa (0,0024 µg/g s.l.), z pasiek z terenów byłych PGR. Pozostałości DDT u pszczoł we wszystkich pasiekach były bardzo wysokie 0,1200-0,1862 µg/g s.l., natomiast w miodzie niskie i wahały się od 0,0011 µg/g s.l. (miasto i Puszcza Piska) do 0,0014 µg/g s.l. (pozostałe pasieki). Zawartość sumy DDT w pierdze była najniższa w pasiekach z Olsztyna (0,0001 µg/g s.l.), a bardzo wysoka w pozostałych. Szczególnie wysoki poziom tego pestycydu występował w pierdze z Puszczy Piskiej (0,0927 µg/g s.l.).

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## The investigation of bee virus infections in Poland

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**Abstract:** *The investigation of bee virus infections in Poland. In 1995–1996 adult dead bee samples were collected from April to September from apparently healthy colonies in nine apiaries located in different parts of Poland. The samples were tested by agar gel immunodiffusion (AGID) for black queen cell virus (BQCV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV) and sacbrood virus (SBV), also by microscopy for *Nosema* spores and by electron microscopy for filamentous virus (FV). *Nosema* was a very common infection in the investigated apiaries. It was detected in 80% of tested colonies in 1995 and in 79% of colonies in 1996. FV and BQCV were found in all the apiaries, respectively in 70% and 60% of investigated colonies. The percentage of samples infected with FV and BQCV was very high in the period April – June, with the peak in May. ABPV was found in six apiaries – located in the area where a marine climate predominates over a humid continental one – in 24% of investigated colonies, mainly in samples collected from July to September. SBV was detected in five apiaries (approximately in 10% of colonies), mainly in April and May. CBPV also occurred in five apiaries (15% of colonies), mainly in May – June. In three apiaries five viruses and in the other three – four viruses per apiary were found. In about 6% of colonies infection by at least three viruses was detected.*

**Key words:** honey bee, viruses, Poland, 1995–1996.

## INTRODUCTION

According to the latest discoveries of American scientists viruses can be one of the main causes of CCD – Colony Collapse Disorder which since 2006 has caused each year the disappearance of a considerable number of bee colonies in the USA. Johnson and co-authors (2009) report that “The reduced protein synthetic capabilities that would accompany ribosomal hijacking by multiple picorna-like viruses would leave bees unable to respond to additional stresses from pesticides, nutrition, or pathogens”. Fifty years ago only two virus infections attracted the attention of scientists – chronic bee paralysis virus and sacbrood virus (Bailey et al. 1963). 20 years later about 16 honey bee viruses were known and their importance increased when the mite *Varroa destructor* arrived in Europe and was found to be a vector of many viruses and an activator of virus infections. At the beginning of the 21<sup>st</sup> century molecular biology methods (RT-PCR) became the main tool in investigating the most important of these viruses (Benjeddou et al. 2001; Grabensteiner et al. 2001). Since then new reports have been

appearing periodically about detection of particular viruses in successive countries (Siede and Büchler 2004, Antúnez et al 2006, Berényi et al. 2006, Nielsen et al. 2008, Teixeira et al 2008, Sanpa and Chantawannakul 2009, Haddad et al. 2009,). Earlier, the detection of such infections was based mainly on the immunodiffusion test – AGID which requires diagnostic antisera or extremely laborious biological tests (Anderson 1984, Ball 1999). Because of this only a few laboratories in the world worked on bee viruses. The sensitivity of the AGID test is low. The test detects only heavy infections, when the amount of the virus is high enough to kill individual bees (Ball 1999), in contrast to RT-PCR which detects also extremely common virus infections with small amounts of virus. To distinguish slight from heavy infection expensive real time RT-PCR is necessary (Tentcheva et al. 2004, Gauthier 2007, Siede et al. 2008). In 1992 in bee samples from Poland using AGID and electron microscopy, for the first time seven bee viruses were identified (at Rothamsted Experimental Station) (Topolska et al.). These were acute bee paralysis virus (ABPV) chronic bee paralysis virus (CBPV), sacbrood virus (SBV), deformed wing virus (DWV), black queen cell virus (BQCV), bee virus X (BVX), bee virus Y (BVY) and filamentous virus (FV). The samples were collected in Warsaw. A few years later the chronic paralysis virus associate was detected (CPVA) (Allen and Ball 1996). In recent years RT-PCR was introduced as one of the methods used in our laboratory for identification of BQCV, DWV, SBV, ABPV, CBPV (Topolska 2008).

Owing to cooperation with scientists in other countries the genomes of Polish strains of ABPV, DWV and BQCV were sequenced (Bakonyi 2002, Berenyi 2007, Tapaszti 2009).

In 1995–1996 bee samples were collected in apiaries located in different parts of Poland. Some of the results of testing these samples by AGID for the presence of bee viruses were presented during research conferences. This work contains all the results. Differences in seasonal incidences of viruses, quite rarely found in the literature, are also reported.

## MATERIAL AND METHODS

In 1995–1996 adult dead bee samples were collected from April to September from apparently healthy colonies in nine apiaries located in different parts of Poland. Ten colonies in each apiary were sampled by placing a dead bee trap in front of each hive entrance. Bees were taken from traps monthly and kept at  $-18^{\circ}\text{C}$  until investigated. The samples were extracted by the method described by Ball and Allen (1988) and tested by agar gel immunodiffusion (AGID) against the following sera: anti-SBV, anti-ABPV, anti-CBPV, and anti-BQCV. Some extracts were tested at Rothamsted against slow bee paralysis virus (SPV), cloudy wing virus (CWV) and Kashmir bee virus (KBV) antisera. The initial clearing pellets were tested for *Nosema* spores. Electron microscopy examination of material for FV was also carried out.

The results were expressed as the presence or absence of *Nosema* spores and viruses in a sample. The Yates corrected  $\chi^2$  test (Statistica 8.0 software, StatSoft)

was used to compare occurrence of viruses in samples from different months of the two year survey (Stanisz 2006).

## RESULTS

A total of 564 samples were tested: 264 collected in 1995 and 300 collected in 1996 (Table 1). The reason why some samples were missing was usually the foraging activity of wasps which collected bees from traps. In 1996 we managed to collect samples from seven of nine apiaries, which were investigated in 1995. The owner of apiary II retired his bees from the studies for personal reasons and

colonies from apiary I collapsed at the end of winter; the cause was honeydew honey, left in colonies for winter time, in association with *Nosema* infection. All the investigated colonies were apparently healthy. However, as *Varroa destructor* was a well established parasite in Polish apiaries, all the colonies were infested with this mite, although beekeepers did not observe any mites on bees and no mites on collected dead bees were found. *Nosema* was a very common infection in the investigated apiaries. It was detected in 80% of tested colonies in 1995 and in 79% of colonies in 1996. FV and BQCV were found in all the apia-

TABLE 1. The number of samples collected in investigated apiaries during a two year study

No. of apiary	No. of examined samples											
	April		May		June		July		August		September	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
I	8	0	8	0	0	0	2	0	3	0	1	0
II	0	0	0	0	7	0	8	0	2	0	3	0
III	4	10	10	9	9	7	9	5	5	4	2	7
IV	5	1	10	8	4	10	1	8	2	1	4	8
V	6	9	5	8	4	5	2	3	2	6	2	9
VI	0	10	10	8	8	7	4	7	6	8	2	10
VII	0	6	10	6	0	9	0	10	3	6	5	7
VIII	0	0	10	10	8	9	5	10	10	10	9	10
IX	0	10	10	10	6	10	10	9	10	0	10	0
<b>Total</b>	<b>23</b>	<b>46</b>	<b>73</b>	<b>59</b>	<b>46</b>	<b>57</b>	<b>41</b>	<b>52</b>	<b>43</b>	<b>35</b>	<b>38</b>	<b>51</b>

TABLE 2. Comparison of occurrence of viruses in samples from different months of two year survey. Values followed by different letters in a row differ significantly (Yates corrected  $\chi^2$  test,  $p < 0.05$ )

Virus	Percentage of infected samples (1995+1996)					
	April n=69	May n=132	June n=103	July n=93	August n=78	September n=89
ABPV	0.0	3.7ac	1.0a	12.0b	9.0c	6.7c
CBPV	1.4a	3.8a	3.9a	0.0	0.0	2.5a
FV	62.2a	56.1a	36.9b	4.3c	5.1c	0.0
BQCV	24.6a	41.7b	30.1ab	7.5c	2.6c	0.0
SBV	5.8a	4.5a	0.0	0.0	0.0	1.1a

ries (respectively in 70% and 60% of investigated colonies) – in colonies with *Nosema* infection. The percentage of samples infected with BQC

high in the period April – June, with the peak in May (Table 2.) when the peak of *Nosema* infection occurred (Fig. 1). At that time, usually numerous FV particles

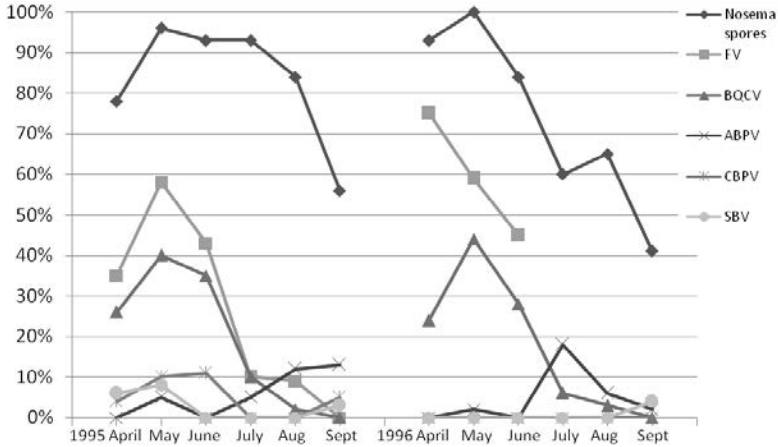


FIGURE 1. Percentage of samples per month in which FV, BQC, ABP, CBP or SBV were detected

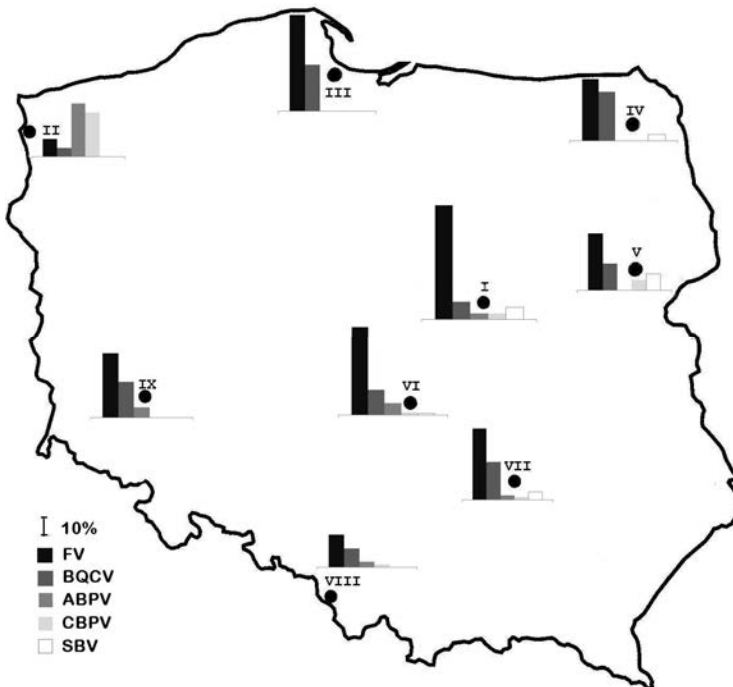


FIGURE 2. Map of Poland with location of investigated apiaries and percentage of samples positive for FV, BQC, ABP, CBP or SBV

were detected by electron microscopy. In the period July-August the incidence of viruses was low, even if *Nosema* incidence was still high (July-August 1995). ABPV was found in six apiaries (Fig. 2) – located in the area where a marine climate predominates over a humid continental one – in 24% of investigated colonies, mainly in samples collected from July to September. However, a few samples from May (originating from two apiaries) also contained the virus.

SBV was detected in five apiaries, approximately in 10% of colonies, in April and May (mainly in May – in statistical analysis the difference not significant), although in September some incidences of SBV were also noted. CBPV also occurred in five apiaries (15% of colonies), mainly in May – June, but in April and September as well (in statistical analysis the difference not significant).

In three apiaries five viruses and in the other three – four viruses per apiary were found. In about 6% of colonies the infection by at least three viruses was detected. SPV, CWV and KBV were not found.

## DISCUSSION

Surveys of bee viruses undertaken in various countries, using RT-PCR, revealed that DWV is the most prevalent virus in France, Austria and Hungary (Tentcheva et al. 2004, Berenyi et al. 2006, Forgach et al. 2008). In France and Austria it was found in most of the investigated samples. Although the virus can be transmitted by larval food, widespread *Varroa destructor* is a very effective vector of the virus and activator of its unapparent infections (Bowen-Walker 1999, Nord-

strom 2003, Schen et al. 2005). We did not investigate the samples collected in 1995 and 1995 for this virus, however, in samples investigated by AGID in 1992 the virus was almost ubiquitous.

The current surveys of bee viruses do not include FV. A brief analysis of the results presented in this paper suggests that in Polish apiaries FV is the most prevalent of the five tested viruses. Bailey with co-authors (1983) described the same situation in Great Britain. However, electron microscopy used to detect this virus is a very sensitive method and can reveal infections even at a very low level. The detection of ABPV, CBPV, BQCV and SBV using AGID, which is a method of low sensitivity, demonstrated that colonies were heavily infected, although the beekeepers did not observe any signs of infection. BQCV was also found frequently. FV and BQCV had been found to be associated with *Nosema apis* infection (Bailey et al. 1981) and, according to Bailey and coauthors (1983), they showed the annual cycle of incidence corresponding with that of microsporidian. This was also observed in Poland but contrary to the situation found by these authors the viruses occurred much less frequently in July, even if *Nosema* infection was still very common. Since BQCV is very common in Poland, it is not surprising that this virus was found to be the main cause of death of queen brood in queen rearing apiaries (Topolska 2008).

ABPV, was detected in a much lower percentage of samples, although in Germany it was determined as the main cause of death of bees in colonies infested with *Varroa* mites (Ball and Allen 1988). In Poland heavy virus infection was often found in dead bee samples originating

from colonies which collapsed, or almost collapsed, during the winter of 2007/2008, when in some provinces colony losses reached 30% (Topolska et al. 2008). ABPV is another virus associated with the *Varroa* mite (Ball 1989). The population of mites builds up during the season so it is no wonder that the virus was found mainly in summer and autumn. The detection of the virus in May came as rather a surprise, suggesting that despite the fact that beekeepers in these colonies did not observe *Varroa destructor* on bees, the infestation must have been quite severe. In the investigations performed in 1992 ABPV was also found in samples from May (Topolska et al. 1995). The detecting of heavy virus infection in areas where marine climate predominates over humid continental climate may be caused by the better conditions for *Varroa* reproduction in these regions than in the rest of Poland. During examination of clinical samples performed in recent years, ABPV has never been found as one of the main causes of colony death in these areas (unpublished own results).

SBV and CBPV are the viruses which do not need feeding activity of *Varroa* mites to cause overt disease in the colony. The development of disease from unapparent infection is usually caused by environmental factors. Bailey (1981) did not observe any regularity in outbreaks of chronic paralysis but in France the highest rates of bee mortality occurred in spring and summer (Ribière et al. 2002). In Poland CBPV was detected mainly in spring and early summer however it seems that this virus infection is not a serious problem in our country. This was confirmed by the studies done in 2007–2009 when CBPV was found as a cause

of bee mortality in 2,5% of samples received from colonies in which abnormal bee death was observed (unpublished own results).

SBV by AGID was found usually in dead bee brood (Bailey 1981) and many cases of overt disease were reported in Australia (Anderson 1983). Since in our findings the virus was found in adult bees, probably brood in these colonies was heavily infected, although beekeepers did not observe any brood mortality. This heavy SBV infection was detected mainly in April and May, which corresponds to some extent with the results obtained by real time RT-PCR in France (Gauthier 2007).

Detecting CBPV and SBV by AGID in September may be connected with the fact that these viruses can be transmitted by *Varroa destructor*, although this probably did not influence the incidents of outbreaks of chronic paralysis and sacbrood.

The presence of heavy infection with three of the investigated viruses at once in the same colony did not noticeably influence the condition of bee colonies. Only colonies from apiary I died during the winter of 1995/1996, however the recent investigation of historical samples revealed that bees in this apiary were heavily infected by *Nosema ceranae*, which is much more harmful to bees than *N. apis*.

The investigation did not reveal the presence of SPV, CWV and KBV in Polish apiaries but the examined samples were not numerous. KBV is rarely found in Europe and SBPV was mainly detected in United Kingdom. These viruses were rarely found in Europe, apart from United Kingdom where, in some surveys, SPV



and CWV were detected quite often. (Carreck 2005, Rebiere 2008).

## CONCLUSIONS

1. In 1995 and 1996 in Poland the heavy infection of bee colonies with FV and BQCV was very common.

2. The seasonal incidences of FV, BQCV, CBPV and SBV in Poland generally did not differ much from those described for Great Britain by Bailey.

3. Climatic factors can probably influence the development of ABPV infection in colonies infested by *Varroa destructor*.

4. The presence of heavy infection with three of the investigated viruses at once in the same colony does not noticeably influence the condition of bee colonies.

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**Streszczenie:** *Badanie dotyczące infekcji wirusowych pszczół w Polsce. Zainteresowanie wirusami pszczół w ostatnich latach jest coraz większe. Przypuszcza się, że odgrywają one bardzo istotną rolę w zespole masowego giniecia rodzin pszczelich (CCD) w USA. Odkąd na przełomie dwudziestego i dwudziestego pierwszego wieku*

wprowadzono technikę RT-PCR do identyfikacji wirusów pszczelich w publikacjach naukowych pojawiają się często doniesienia o stwierdzeniu za pomocą tej metody zakażeń poszczególnymi wirusami w kolejnych krajach. Wcześniej wykrywanie tego typu zakażeń opierało się głównie na stosowaniu immunodyszufacji w żelu agarowym (AGID), do której niezbędne były surowice diagnostyczne, lub niezwykle pracochłonnymi próbami biologicznymi. W 1992 r. w próbkach pszczół z polskich pasiek testem AGID i mikroskopią elektronową po raz pierwszy stwierdzono (w Rothamsted Experimental Station – w Wielkiej Brytanii) obecność 7 wirusów pszczelich. W latach 1995–1996 w 9 polskich pasiekach, w rodzinach rozwijających się normalnie i niewykazujących objawów zakażeń wirusowych zbierano od kwietnia do września próbki martwych pszczół. Testem AGID badano w próbkach obecność wirusa ostrego paraliżu pszczół (ABPV), wirusa chronicznego paraliżu pszczół (CBPV), wirusa choroby woreczkowej czerwiu (SBV), wirusa choroby czarnych mateczników (BQCV) oraz mikroskopią elektronową – wirusa włókienkowego (FV) (Ball i Allen, 1988). FV i BQCV wykryto we wszystkich badanych pasiekach (w 70% i w 60% badanych rodzin), w rodzinach ze stwierdzoną obecnością spor z rodzaju *Nosema* – głównie w okresie kwiecień-czerwiec. SBV stwierdzono w 5 pasiekach (ok. 10% rodzin), głównie w kwietniu i maju, CBPV w 5 pasiekach (15% rodzin) – głównie w maju i czerwcu. ABPV stwierdzono

w 6 pasiekach (w 24% badanych rodzin), głównie w okresie od lipca do września. W ok. 6% rodzin stwierdzono jednocześnie zakażenie 3 wirusami. Wykrycie ABPV, CBPV, BQCV i SBV mało czułą metodą, jaką jest AGID, świadczy o silnym zakażeniu rodzin, pomimo iż pszczelarze nie obserwowali objawów zakażenia. Natomiast późniejsze badanie próbek pszczół pochodzących z rodzin zmarłych zimą 2007/2008 wykazało, że w połączeniu z silną inwazją *Varroa destructor* ABPV był bardzo istotnym czynnikiem prowadzącym do wyginięcia wielu rodzin pszczelich. Zakażenie rodzin pszczelich wirusami BQCV i FV jest w Polsce bardzo powszechne. Obecność znacznego zakażenia rodziny pszczoły nawet 3 wirusami nie musi w widoczny sposób wpływać na stan rodziny pszczoły.

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## Injuries of honey bee workers and drones caged in native and foreign colonies

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**Abstract:** *Injuries of honey bee workers and drones caged in native and foreign colonies.* In June and July 2008 worker bees and drones were kept in plastic transport cages in native and foreign colonies. 4,224 honey bees were used in the investigation (1,920 workers and 2,304 drones). After 3 and 7 days of storage the bees' injuries were checked with a stereoscopic microscope. The results were used to determine the percentage of injuries and mortality of the bees. Both worker bees and drones stored in native and foreign colonies were subject to injuries. The injuries were limited mainly to legs and arolium, with occasional injuries to wings and antennae. The highest level of injuries was recorded for workers stored for 7 days in foreign colonies (almost 8%) and the lowest for drones stored for 3 days in native and foreign colonies (1.5%). The highest mortality was recorded for drones stored in native and foreign colonies for 7 days (around 71%), and the lowest for workers stored in native colonies for 3 days (almost 6%). It was observed that the location of cages influenced the number of injured and dead workers. The percentage of injured bees was found to be dependent on the duration of their storage in the colony. The longer the storage time of worker bees and drones, the higher the injury rate. The number of injured bees was also influenced by sex, as males were injured distinctly less often than females. The death rate of drones stored in cages was significantly higher than that of worker bees.

*Key words:* injuries, cage, worker bees, drones.

### INTRODUCTION

When several queens are stored in queenless colonies, they are often injured by

workers from these colonies (Woyke 1995, Jasiński 1984, 1986, 1987, 1995b). This phenomenon is often observed in the so called “queen banks”. The reasons for this phenomenon are not clear. It is probably related to the queens' pheromones (Zmarlicki and Morse 1964) and mandibular gland secretion (Lensky and Slabesky 1981).

Jasiński (1986, 1987, 1995a) detected 26 different types of injuries and categorized them in four groups:

1. Changes in the color of arolium.
2. Loss of leg segments or whole legs.
3. Deformations of arolium and partial or complete loss of arolium and claws.
4. Other injuries – frayed wings and loss of antennae or antennae segments.

The injuries negatively influence the queens' motor abilities, flying abilities and their sense of feeling. Injured queens are not eligible as breeding material (Jasiński 1995a). If several queens are stored in a colony, they are exposed to injuries from worker bees. It has been recorded that 30–40% of queens which are stored in a colony are injured after 2 days (Jasiński 1995a). The level of injuries is influenced by the type of the storage cage. More queens are injured in plastic transport cages with 2.6mm slots than in cages covered with wire gauze with apertures

of 2.5 mm (Jasiński 1990, 1995b). Age is also an important factor. Young workers are less aggressive towards queens than older bees (Jasiński and Fliszkiewicz 1996). Jasiński and Kawecki (1992) observed that workers assisting the queens stored in cages are also injured. Many observations show that caged drones are injured too (Madras-Majewska et al. 2008).

The purpose of the investigation was to compare the level of injuries and death rate of workers and drones stored in native and foreign colonies.

## MATERIAL AND METHODS

The investigation was carried out in June and July 2008 in the apiary of Warsaw Agricultural University. 4,224 bees (1,920 workers and 2,304 drones) were observed during storage in native and foreign colonies. The level of injuries and mortality were checked twice, after 3 and 7 days of storage. In total, 25,344 legs, 16,896 wings and 8,448 antennae were checked.

The bees were stored in plastic transport cages with 27 slots of 2.5 mm × 11 mm in one side. Each cage was provided with a “bee cake”. In each cage there were 6 workers or 6 drones. Cages with honey bees were put into holding frames located in a colony. Each holding frame contained 16 cages arranged in 4 lines. There were four frames in each colony – two with workers, from the native (WN) and foreign colonies (WF), and two with drones, from the native (DN) and foreign colonies (DF). The holding frames were separated with two combs. The colonies used in the investigation were of medium strength and had queens. The honey

bees from nurse colonies could contact the caged honey bees through slots. The level of injuries and the death rate were checked twice, after 3 and 7 days of storage. The injuries were checked using a stereoscopic microscope.

## RESULTS

The level of injuries is defined as the percentage of injured honey bees in relation to all caged honey bees. The level of injuries of workers stored in native colonies for 3 days was 2.5%, and after 7 days it increased to 6.17%. In the group of workers from foreign colonies, the level of injuries was 4.17% after 3 days and 7.83% after 7 days. The duration of storage had a significant impact on the level of injuries of workers from native and foreign colonies ( $P \leq 0.002$ ). The longer the time of storage, the more injuries of workers from native and foreign colonies were observed.

After 3 days of storage, the level of injuries for workers from native and foreign colonies varied significantly ( $P = 0.005$ ). There were, however, no statistically significant differences in the level of injuries between workers from native and foreign colonies after 7 days of storage.

In both groups of drones (from native and foreign colonies) stored for 3 days the level of injuries was equal (1.5%). After 7 days the level of injuries for the drones from native colonies increased to 2.83% and for those from foreign colonies to 1.83%.

The level of injuries in all groups was significantly lower for drones than for workers ( $P < 0.01$ ).

The death rate of workers stored for 3 days in native colonies was 5.83%, and

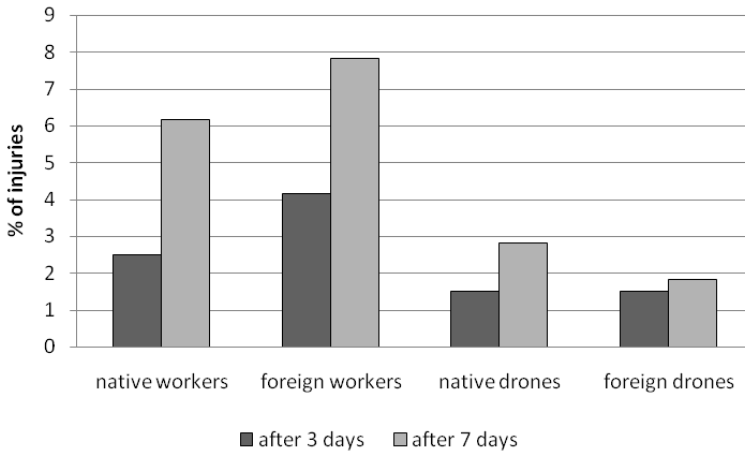


FIGURE 1. Level of injuries for workers and drones stored in cages in native and foreign colonies for 3 and 7 days

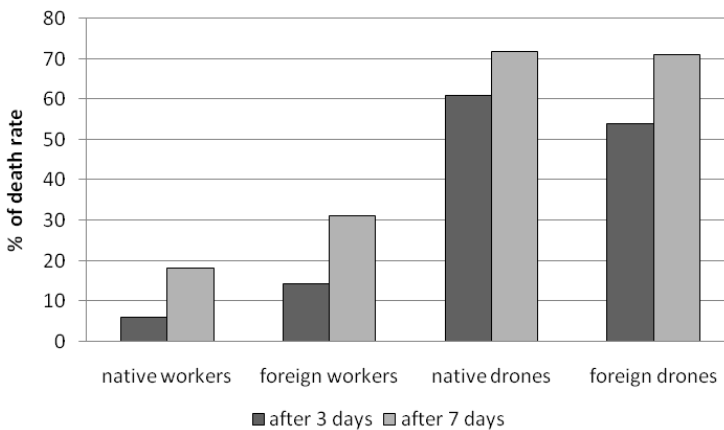


FIGURE 2. Death rate of workers and drones stored in cages in native and foreign colonies for 3 and 7 days

increased to 18% after 7 days. For workers stored in foreign colonies the death rate was 14.17% and 31% respectively. In both groups the mortality of workers after 3 and 7 days varied significantly ( $P < 0.001$ ). It was observed that the longer time of storage was, the more dead workers there were.

The death rate of drones was very high. It was 61% for drones stored for 3 days in

native colonies and 53.8% in foreign colonies. After 7 days the death rate in both native and foreign colonies was equal (71%). The duration of storage had a significant influence on the death rate of drones ( $P \leq 0.001$ ). The mortality of drones was significantly higher than the mortality of workers in each group ( $P < 0.001$ ).

It has been observed that the location of cages in the holding frame influenced

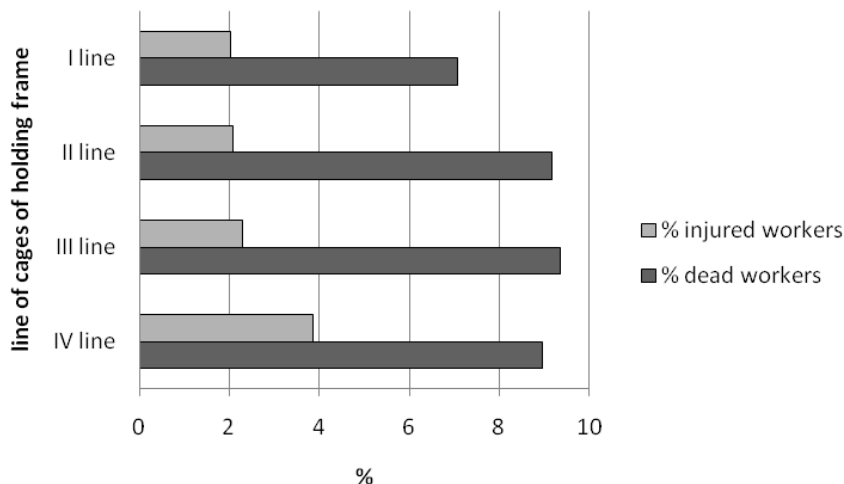


FIGURE 3. The percentage of injured and dead workers in each line of cages in relation to all injured and dead workers

the level of injuries and the death rate of worker bees. In cages located in the bottom line of the holding frame the level of injuries was almost 4%, in the other three lines it was about 2%.

The lowest death rate (7%) was observed in cages located in the first (top) line of the holding frame. The death rate in the other lines was about 9%.

## DISCUSSION

Up to now researchers have focused exclusively on the injuries of queen bees. The aim of this research was to determine the level of injuries and the death rate of the other honey bees: workers and drones stored in native and foreign colonies.

Jasiński and Kawecki (1992) observed that workers from colonies injured not only queens, but also the bees which were caged with the queens. Jasiński and Fliszkiewicz (1997) examined the injuries of bees assisting queens in cages.

They discovered that the level of injuries of caged workers was 35% and the death rate was almost 50% after 2 weeks of storage. In our investigation workers and drones were stored for 7 days. After this time the level of injuries for workers was 6.6% and the death rate was 24.6%. Comparison of the results shows that the presence of the queen in cages increases the level of injuries of workers. Each queen exudes a distinctive smell which is characteristic of the colony. Workers from cages had the same smell as the queens. Such accumulation of different smells can irritate workers from the nurse colony and cause increased aggressiveness towards the caged bees. In our investigation the workers were caged without queens, which may explain why the level of injuries was lower.

In our research workers and drones were divided in two groups: native – from the same colony – and foreign – from a different colony. Workers from colonies were more aggressive towards



workers from foreign colonies, while for drones the level in injuries and the death rate were higher in native colonies than in foreign colonies. Madras-Majewska et al. (2008) recorded similar results.

One of the reasons for injuring the queen is the mandibular gland secretion (Lensky and Slabesky 1981). Mandibular glands of workers and drones also secrete substances which are likely to influence the behavior of workers from colonies. Thus, the most frequently injured body parts were the legs and arolium.

In the years to come it is planned to compare the level of injuries between queens, workers and drones in native and foreign colonies.

## CONCLUSIONS

1. Workers and drones which are caged and stored in native and foreign colonies are injured by workers from the colonies. Most common are leg injuries – loss of segments, black arolium. Wings and antennae are rarely injured.

2. The level of injury is influenced by the duration of storage. The longer the time of storage, the more injuries of workers and drones from native and foreign colonies.

3. Sex of honey bees has an influence on the rate of injuries. Males were injured much less than females.

4. Both in native and foreign colonies, the death rate of drones was much higher than that of workers.

5. The location of cages in the holding frame influences the level of injuries and the death rate of worker bees. Most injuries were observed in the bottom lines of cages. The lowest death rate was observed in the top lines of cages.

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**Streszczenie:** *Uszkodzenia pszczoł robotnic i trutni przechowywanych w rodzinach własnych i obcych.* W czerwcu i lipcu 2008 roku przechowywano pszczoły robotnice i trutnie w plastikowych klateczkach transportowych w rodzinach własnych i obcych. W badaniach użyto 4224 pszczoł (w tym 1920 robotnic i 2304 trutni). W 3 i 7 dniu przechowywania dokonywano kontroli uszkodzeń używając mikroskopu stereoskopowego. Na podstawie wyników określono stopień uszkodzenia oraz śmiertelność osobników pszczelich. Zarówno pszczoły robotnice jak i trutnie podczas przechowywania w rodzinach własnych i obcych ulegały uszkodzeniom. Najczęściej uszkodzane były nogi i przyłgi, sporadycznie skrzydła i czułki. Najwyższy stopień uszkodzeń stwierdzono u robotnic przechowywanych w rodzinach ob-

cych (prawie 8%) po 7 dniach przechowywania, a najniższy u trutni w rodzinach własnych i obcych po 3 dniach przechowywania (1,5%). Najwięcej osobników martwych stwierdzono w grupach trutni przechowywanych w rodzinach własnych i obcych po 7 dniach przechowywania (ok. 71%), a najmniej w grupie robotnic przechowywanych w rodzinach własnych po 3 dniach przechowywania (niecałe 6%). Zaobserwowano pewną zależność, że położenie klateczek wpływało na liczbę uszkodzonych i padłych robotnic. Na stopień uszkodzenia pszczoł ma wpływ długość przechowywania ich w rodzinie. Im dłuższy jest okres przechowywania zarówno pszczoł jak i trutni w rodzinach własnych i obcych, tym uszkodzeń jest więcej. Na liczbę uszkodzonych osobników ma wpływ płeć, samce uszkodzane są znacznie rzadziej niż samice. U trutni przetrzymywanych w klateczkach śmiertelność jest znacznie wyższa niż u pszczoł robotnic.

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# PART II



## Simulation analysis of correlation between the occurrence of the defined alleles of *Ovar-DRB1* gene in genotype and the health status of the sheep udders

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**Abstract:** *Simulation analysis of correlation between the occurrence of the defined alleles of Ovar-DRB1 gene in genotype and the health status of the sheep udders.* In simulation analysis, the results of the molecular studies (RFLP of DRB1 gene) and the results of evaluation of the health status of udder (SCC in 1 ml of milk) being conducted on Polish Heath Sheep and Polish Lowland Sheep of Żelaźnieńska variety were utilized. Besides it, the data base for simulation analyses was created from the nucleotide sequences of exon 2 of *Ovar-DRB1* gene, and their corresponding amino acid sequences, published in GenBank. The simulation cutting of the collected sequences with restriction enzymes (*Bst*YI, *Bsu*RI, *Rsa*I) was performed and then, the comparison with the real results of restriction analysis and with the results of evaluation of health status of the udders of the examined sheep was carried out. In the female carriers of the allele 141 (*Bst*YI, *Bsu*RI, *Rsa*I) the statistically significantly higher ( $P \leq 0.05$ ) mean somatic cell count in milk was recorded; and in the female carriers of allele 112, the lower mean somatic cell count in milk was found, as compared to the female carriers of the remaining alleles. Due to the fact that the mentioned alleles in the examined ewes may have an antagonistic effect, the comparison of virtually created sequence of allele 141 and the sequence, corresponding to allele 112 was carried out. Twenty six nucleotide changes were found between the mentioned above sequences what corresponded to 8 changes in sequence of amino acids. The recorded correlation between the defined alleles of *Ovar-DRB1* and health status of the sheep udders (SCC) indicates that the developed procedure of simulation analyses may be utilized in breeding practice.

*Key words:* *Ovis aries*, *Ovar-DRB1*, MHC, SCC.

### INTRODUCTION

The inflammatory status of udders (*mastitis*) constitute a serious reason for economic losses in the sheep breeding. The mentioned disease affects negatively the quantity and the quality of the produced milk and also, the results of lamb rearing (Charon 1990, Fthenakis and Jones 1990). The main cause of *mastitis* includes pathogenic microorganisms, first of all *Staphylococci* and *Streptococci*. The udder diseases have a differentiated pathogenesis, clinical run and symptoms and they affect the functions of the discussed organ in a different way. Due to the run of the disease, we may distinguish acute inflammation (clinical), sub-clinical (without symptoms) and chronic. The most important features which characterize the health status of the mammary gland – apart from clinical changes – include somatic cell count (SCC) and the presence of pathogenic bacteria in milk (Krzyżanowski et al. 1983, Jones 1993, Świderek 1996, Bergonier et al. 2003). The basic treatment of *mastitis* consists in the application of the general and intra-

mammary injections of antibiotics. The effectiveness of the therapy is, however, dependent mainly on the stage of the disease and sensitivity of the pathogenic microorganisms to the administered antibiotics. Insufficient progress in therapy and prophylaxis of the udder diseases has caused the increase of the interest of the researchers in genetic background of the discussed disease (Shook 1989).

Resistance of the organism is a complex physiological process in which the major system of tissue compatibility (*Major Histocompatibility Complex* – MHC) plays the fundamental role. The mentioned system includes many genes, distinguished by a very large polymorphism and coding the surface structures of the body cells, being defined as MHC particles (Hediger et al. 1991). The discussed particles participate actively in process of genes' presentation to the specialized cells of immunological system; as a result of it, production of antibodies directed against a given antigen takes place.

In the so-far studies, it was revealed that some of alleles of MHC genes may be connected with the resistance of the sheep to leukemia (Nagaoka et al. 1999) or parasite invasions (Schwaiger et al. 1995, Paterson 1998, Charon et al. 2002, Sayers and Sweeney 2005). Also, the relationship of MHC with the yield and quality of the ewe milk and body weight gain of the lambs was also recorded (Barillet et al. 2005, Gruszczyńska et al. 2000, Geldermann et al. 2006). In the available scientific literature, there are only few reports concerning the relationship of *Ovar*-MHC gene polymorphism and *mastitis* in the sheep. The studies dedicated to the mentioned problems were implemented mainly on the dairy

cattle (Kelm et al. 1997, Sharif et al. 1998, Park et al. 2004).

In the present studies, the simulation analysis of the relationship occurring between the defined alleles of *Ovar*-DRB1 gene in the sheep genotype and health status of mammary gland, as expressed by somatic cell count in the milk, was carried out.

## MATERIAL AND METHODS

The research material consisted of 101 ewes of Polish Lowland Sheep of *Żeleźnińska* variety and 99 ewes of Polish Heath Sheep. The animals derived from Experimental Farm of Division of Sheep and Goat Breeding in *Żelazna*.

In the simulation analysis, the results of the evaluation of restriction fragment length polymorphism (RFLP) of *Ovar*-DRB1 gene (Gruszczyńska et al., 2005) and the results of the studies on the health status of the sheep udders were utilized. The genome DNA was isolated from whole blood of the sheep by the phenol-chloroform method. The selected fragment of *Ovar*-DRB1 gene with the length of 279 bp, consisting of part of intron 1 (9bp) and the whole exon 2 (270bp) was amplified by PCR method, using starter sequences, published in the paper of Ammer et al. (1992). The obtained product was digested successively, in separate test tubes, with three successive restriction enzymes *Bsu*RI, *Rsa*I and *Bst*YI. The restriction fragments were separated by electrophoresis method in native 10% polyacrylamide gel and then, stained with silver nitrate. The length of the restriction fragments was determined, using Programme ScanPack 3.0 (Biometra). The health status of the sheep udders was

evaluated on the ground of the somatic cell count (SCC) in 1 ml of milk, as being determined in flow cytometer Somacount 150 (Bentley).

The results of restriction polymorphism analysis of exon 2 of *Ovar-DRB1* gene served for creation of basis of the potential alleles, resulting from the simulation combination of restriction of *BsuRI*, *RsaI* and *BstYI* pattern. Then the relationship between the obtained alleles RFLP and the health status of the sheep udder, as expressed by somatic cell count in 1 ml of milk, was evaluated using ANOVA. In order to standardize the distribution of variable, somatic cell count in milk was transformed into natural logarithm (Ali and Shook, 1980, Person-Waller and Colditz, 1999). The analysed alleles were compared – in respect of nucleotide and amino acid sequences – with the analogical alleles, obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) as a result of appropriate computer simulations. To this end, the published nucleotide sequences were at first subjected to standardization (unification of starter site (agta) and length – 270bp). Then, virtual cutting of the mentioned sequences with the use of restriction enzymes (*BsuRI*, *RsaI* and *BstYI*) was carried out, using Webcutter programme (<http://rna.lundberg.gu.se/cutter2/>); it enabled creation of virtual base of alleles. Determination of differences in nucleotide sequences of the obtained alleles RFLP of exon 2 of *Ovar-DRB1* gene, resulting from point mutations and the evaluation of the effect of these mutations on changes in amino acid sequences was performed, using GeneDoc Programme (<http://www.nrbsc.org/gfx/genedoc/index.html>).

## RESULTS AND DISCUSSION

On the base of restriction analysis of exon2 of *Ovar-DRB1* gene, the list of the possible alleles, resulting from simulation combination of restriction patterns: *BsuRI*, *RsaI* and *BstYI*, was created. Thus, the base of 137 different alleles with a very much differentiated frequency was obtained (Polish Heath Sheep: 0.018–0.155; Żelazieńska variety: 0.0016–0.130). Then, the simulation analysis of the relationship between the incidence of the defined alleles of *Ovar-DRB1* gene in the genotype and the health status of the sheep udders, evaluated on the ground of somatic cell count (SCC) in 1 ml of milk, was carried out. The results of the conducted analysis are given in Table 1.

As was found on the base of presented data, the highest, statistically significant ( $P \leq 0.05$ ), somatic cell count ( $\chi = 443\ 634$ ) in the milk of the sheep was connected with the presence of allele 141 in genotype; the lowest one ( $\chi = 157\ 118$ ) – with the presence of allele 112. A similar relationship was also recorded between the remaining analysed alleles of *Ovar-DRB1* gene. The recorded differences supported statement that the potential carriers of alleles 141, 241, 218 and 213 may reveal a higher inclination to status of the udders inflammatory as compared to the carriers of alleles 112, 125 and 222.

To compare the construction of nucleotide sequences of the analysed alleles, the available gene bank resources were utilized. From among 147 sequences of *Ovar-DRB1* gene, published in GenBank (Aida 1998, Schwaiger et al. 1994, Griesinger 1997, Kostia et al. 1998, Gutierrez-Espleta 2001, Jugo and Vicario 2000, Jugo and Arietta-Agirre 2003, Nicolini et al.

2004, Gruszczyńska et al. 2005, Sayers et al. 2005), subjected to virtual digestion with restriction enzymes (Tab. 2), only 6 alleles possessed identical restriction pattern as the analysed alleles given in Table 1. Two sequences corresponded to patterns 112 and 241 each and one sequence corresponded to each of patterns 213 and 222.

Figures 1 and 2 contain the comparison of all selected six sequences (Tab. 2). As it results from the submitted figures, the sequences with the same pattern 112 differed in four substitutions of bases in nucleotide: 108, 231, 232 and 244. Nevertheless, the mentioned changes did not have any effect on the coded amino

acid sequences (Fig. 2). It should be mentioned that the both sequences derived from the sheep of different breeds and they were published by other authors. The sequence U00223 was identified in the Finnish sheep (Schwaiger et al., 1994) whereas the sequence Z92728 was found in the sheep of Red Masai breed (Griesinger 1997). Sequences AF126440 and AF036562 with the restriction pattern 241 occurred to be identical within the examined segment in respect of nucleotide as well as amino acid sequence; they were recorded in the sheep of Bighorn breed (Gutierrez-Espleta et al. 2001). From the comparison of the remaining sequences, as shown in Figure 1, it is followed that

TABLE 1. Evaluation of the effect of the selected alleles of *Ovar*-DRB1 gene on variability of somatic cell count in milk of the examined sheep

Allele RFLP	n	mean LSCC	mean SCC	p
112	29	5.057	157 118	0.16
125	36	5.137	170 204	0.22
141	18	6.095	443 634	0.05
213	15	5.760	317 348	0.39
218	16	5.745	312 623	0.41
222	37	5.113	166 168	0.18
241	14	5.943	381 076	0.20

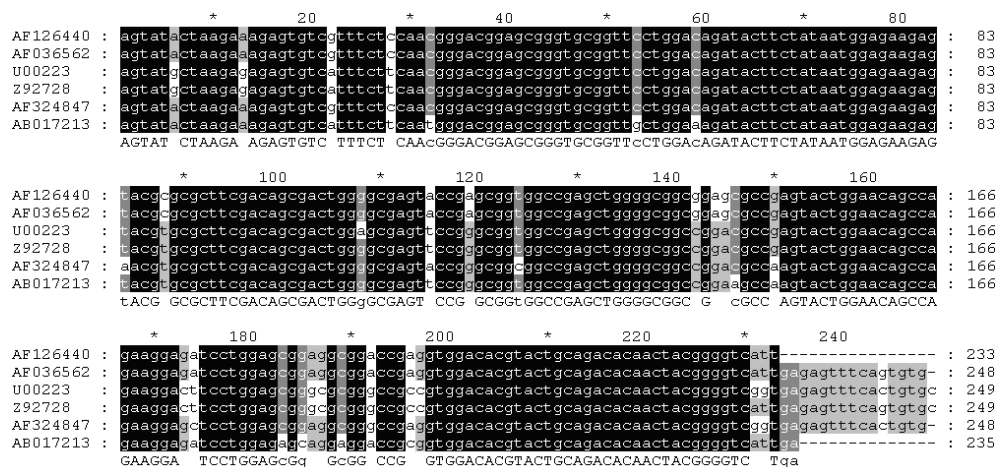
LSCC – somatic cell count expressed in natural logarithms

SCC – real somatic cell count, p – value

TABLE 2. The results of virtual digestion of the selected sequences of *Ovar*-DRB1 gene (source GenBank, different authors)

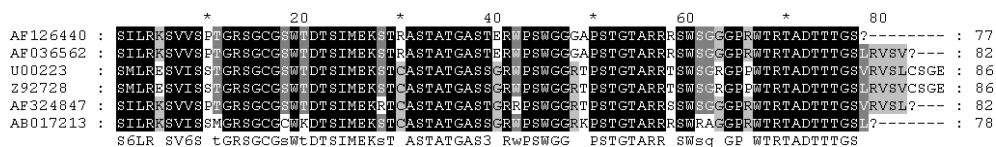
No of sequence (Genbank)	Pattern of digesting with enzymes			Restriction allele
	<i>Bst</i> YI	<i>Bsu</i> RI	<i>Rsa</i> I	
U00223	1	1	2	112
Z92728	1	1	2	112
AF324847	2	1	3	213
AB017213	2	2	2	222
AF126440	2	4	1	241
AF036562	2	4	1	241





Black colour: identical sites; white colour: difference; grey colour: dominating version

FIGURE 1. Comparison of nucleotide sequences of the analysed alleles of *Ovar-DRB1* gene



Black colour: identical sites; white colour: difference; grey colour: dominating version

FIGURE 2. Comparison of amino acid sequences of the analysed alleles of *Ovar-DRB1* gene

they differed in several nucleotide changes, which affected also the changes in amino acid sequences (Fig. 2). The greatest differences, i.e. 20 single nucleotide changes, causing 9 changes in amino acid sequence, were observed between the sequences of alleles 112 (Z92728) and 241 (AF126440). It should be stressed that the analysed alleles differed considerably in the results of the evaluation of the health status of the sheep (Tab. 1), as expressed by somatic cell count in the milk (allele 112 –  $x=157$  118, allele 241 –  $x=381$  076).

The analysis of sequence of alleles 112 and 141 brings some interesting facts. The sheep which possessed both

mentioned alleles were characterized by extreme results of the evaluation of the health status of the udders (the lowest and the highest mean SCC – Tab. 1). Due to the lack of sequence with pattern 141 in the GenBank, virtual nucleotide pattern of the mentioned allele was constructed on the ground of two most approximate sequences AF126434, with pattern 121 and AF 126433 with pattern 142, identified in the sheep of Latxa breed (Jugo and Vicario, 2000). From the comparison of virtually created sequence 141 and sequences (U00223, Z92728) corresponding to pattern 112 (Figs 3 and 4) it is followed that they differed in 24–26 single nucleotide changes, the consequence of

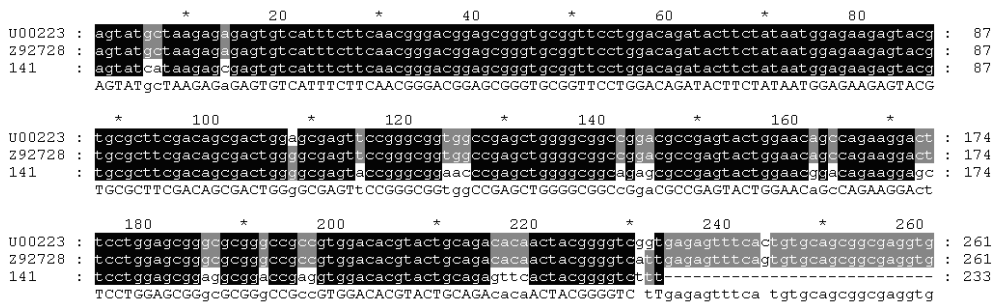
which, 8 changes in amino acid sequence took place.

In the so-far conducted studies, several hundreds of different alleles of Ovar-MHC genes were identified in the sheep (Scott et al. 1991, Blattman et al. 1993, Fabb et al. 1993, Escayg et al. 1996, Konnai et al. 2003). *Ovar-DRB1* gene (exon 2), coding  $\beta$ 1 domain of the particle MHC class II, playing a significant role in the correct recognition of antigen, is one of MHC genes, which focused the researchers interest. The conducted molecular studies revealed the existence of a large restriction polymorphism of the discussed gene. However, the relationship between the recorded genetic differentiation and resistance or susceptibility of the sheep to *mastitis* was analysed only in innumerous studies. More such studies were conducted in case of the dairy cattle.

Diets et al. (1997a) showed a significant relationship of allele BoLA-DRB3.2\*16 with subclinical forms of inflammations of mammary gland in cows. Similar dependence was recorded by other authors (Kelm et al. 1997, Diets et al. 1997b, Park et al. 2004). They stated the significant inclination to *mastitis* in carriers of alleles DRB3.2\*16, DRB3.2\*24 and DRB3\*0101. On the other hand, different results were obtained by Sharif et al. (1998) when revealing – in opposition to the cited above authors – lower susceptibility of the cows, carriers of DRB3.2\*16 allele, to the diseases of the udders.

CONCLUSIONS

The relationship of the analysed alleles RFLP of *Ovar-DRB1* gene and health status of the mammary gland in the sheep



Black colour: identical sites; white colour: difference; grey colour: dominating version

FIGURE 3. Comparison of nucleotide sequences with pattern 112 and simulated sequence with pattern 141



Black colour: identical sites; white colour: difference; grey colour: dominating version

FIGURE 4. Comparison of amino acid sequences with pattern 112 and simulated sequence with pattern 141

as well as the recorded big differences in construction of nucleotide sequence and coded amino acid sequence of certain alleles, as being revealed in the present studies, is the evidence of the possibility to identify MHC markers, connected with the resistance of animals to the diseases. Besides it, the obtained results of the studies indicate that the submitted procedure of simulation analyses may be utilized in scientific considerations as well as in breeding practice.

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**Streszczenie:** *Symulacyjna analiza korelacji pomiędzy występowaniem określonych alleli genu DRB1 w genotypie a stanem zdrowia owcy.* W analizie symulacyjnej wykorzystano wyniki badań molekularnych (RFLP genu DRB1) oraz wyniki oceny stanu zdrowotnego wymion (SCC w 1 ml mleka) przeprowadzonych na owcach rasy wrzosówka i owcach nizinnych odmiany żelaźnieńskiej. Ponadto z opublikowanych w GenBank sekwencji nukleotydowych eksonu 2 genu *Ovar-DRB1* oraz odpowiadających im sekwencji aminokwasowych, utworzono bazę danych do analiz symulacyjnych. Przeprowadzono symulacyjne cięcie zgromadzonych sekwencji

enzymami restrykcyjnymi (*Bst*YI, *Bsu*RI, *Rsa*I), a następnie porównano z rzeczywistymi wynikami analizy restrykcyjnej oraz z wynikami oceny stanu zdrowotnego wymion badanych owiec. U nosicieli allelu 141 (*Bst*YI, *Bsu*RI, *Rsa*I) odnotowano statystycznie istotnie wyższą ( $P \leq 0,05$ ) a u nosicieli allelu 112 niższą średnią liczbę komórek somatycznych w mleku w porównaniu z nosicielkami pozostałych alleli. Ze względu na fakt, iż wymienione allele mogą u badanych owiec mieć antagonistyczne działanie, dokonano porównania wirtualnie utworzonej sekwencji allelu 141 z sekwencją odpowiadającą allelowi 112. Stwierdzono 26 zmian nukleotydowych między w/w sekwencjami, co odpowiadało 8 zmianom w sekwencji aminokwasów. Odnotowane współzależność między określonymi allelami genu *Ovar-DRB1* a stanem zdrowotnym wymion owiec (liczba SCC) wskazują, że opracowana procedura analiz symulacyjnych, może być wykorzystana w praktyce hodowlanej.

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## Analysis of inbreeding in highly prolific Olkuska sheep flock

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**Abstract:** *Analysis of inbreeding in highly prolific Olkuska sheep flock.* The aim of this study was to analyze the dynamics of the inbreeding increase in the flock of Olkuska sheep, belonging to a private breeder, Mr Tomasz Korczyński. The experimental material included 65 ewes and 21 rams and their progeny, composed of 661 lambs that were born between years 1989-2009. The reproduction performance of the flock was very high. The mean litter size, in the analyzed period was  $3.15 \pm 0.63$ . The rate of inbreeding in the flock was high. The mean inbreeding level in lambs was growing continuously until 2009, when it reached the level of 13.3% for ram-lambs and 13.1% for ewe-lambs. No significant inbreeding depression affecting ewe prolificacy was found. In general, the results indicate that inbreeding became a serious problem in the Olkuska sheep population.

*Key words:* inbred, Olkuska sheep, litter size, reproduction performance, inbreeding depression.

### INTRODUCTION

Olkuska sheep is a native Polish breed belonging to the group of long-wool sheep, originating from the southern part of the country. The prolificacy of Olkuska sheep is the highest among Polish local breeds; litters of three and four lambs are frequent (Instytut Zootechniki, 2005). The breed has also very good maternal abilities and milk yield (Berska et al., 1997). High prolificacy of Olkuska sheep is determined by a single gene with the major effect on prolificacy,

which was shown by statistical methods; the gene was proposed to be named as *FecO* (Martyniuk, 2009).

While the population of Olkuska sheep in the 1960s were about 10.000 (Knothe, 1988), by the next thirty years its size decreased rapidly; in 1997, only 58 registered ewes were being kept in five flocks (Hodowla..., 1997). After the year 2000, the population of Olkuska sheep started to increase; this process continues until now. Over time, three flocks became the most important for development of Olkuska sheep population: the flock belonging to the Agricultural University of Cracow, Sheep and Goat Breeding Department (Bielany flock), a private flock of Mr. Korczyński's and a flock established at Warsaw University of Life Sciences, Department of Animal Genetics and Breeding (Żelazna flock). In 2008, the sheep genetic resources conservation programme covered 456 ewes kept in 29 flocks (Sikora, 2008). Most of genetic material in the current population of Olkuska sheep originated from the three leading flocks mentioned above.

In any small, closed population there is a danger of uncontrolled increase of inbreeding, what brings the risk of inbreeding depression (Boujenane and Chami, 1997). The majority of research indicates negative effect of sheep inbreeding on

several traits, as presented in the review by Lamberson and Thomas (1984). Ercanbrack and Knight (1991), Wiener et al. (1992a), Annala et al. (1998), Boujenane and Chami (1997), Norberg and Sørensen (2007) and Wyk et al. (2009), which found inbreeding depression for lamb body weight. Negative effects of inbreeding have been also found for prolificacy and fertility (Ercanbrack and Knight, 1991; Wiener et al., 1992b; Lamberson et al., 1982).

Taking into account a dramatic decrease of Olkuska sheep population size in the 1990s and the possible bottleneck effect, there is a high risk of inbreeding in the Olkuska sheep population. High levels of inbreeding in some individuals from the flock belonging to private breeder, Mr Korczyński were observed by Martyniuk (2007). The purpose of this study was to quantify the level of inbreeding in Mr. Korczyński's flock, examine its reproduction performance and analyze a possible impact of inbreeding level on litter size.

## MATERIAL AND METHODS

The experimental material included 65 ewes and 21 rams and their progeny composed of 661 lambs born between years 1989-2009. The number of animals in successive years is shown in the Table 1. All animals born in the flock or introduced into the flock in the period from 1989 to 2009 were included in the study. The pedigree database was based on all available breeding documentation provided directly by the breeder.

The inbreeding coefficient was calculated using SPIN software based on a tabular Quass-Henderson method developed

by Olech (2003). Due to exchange of breeding material between leading flocks, inbreeding coefficients were calculated using the pedigree database of Mr. Korczyński's flock alone, and subsequently with the addition of pedigree databases of Żelazna flock, Bielany flock and both. This approach allowed to substantially enhance the pedigree information.

The reproduction performance was analyzed using standard parameters routinely applied by Sheep breeders association, such as fertility, litter size, lamb rearing and fecundity.

The  $\chi^2$  test was applied to evaluate the relationship between the inbreeding level and the average and maximum litter size of the ewes.

## RESULTS

Mr. Korczyński's flock size was relatively small in the first years after its establishment, comprising less than 10 ewes. Since 2000, the flock increased in size, reaching 24 ewes in 2009. The results of the present study demonstrate a very high reproduction potential of the flock, with average litter size well over 3.50 in some years (Table 1).

Over time, nine ewes in the flock had an average litter size higher than 4.50; one of them, the ewe named Gwiazda, in 1998, had seven lambs, a litter size first recorded for this breed. Extremely high reproduction potential was also observed for the ewe 358-001-0091, which gave birth to 46 lambs in 10 litters. Distribution of litter size in Mr. Korczyński flock is shown on Figure 1.

The value of inbreeding coefficients differed substantially depending on the pedigree database used; in some indivi-



TABLE 1. Summary of reproductive performance of Mr. Korczyński's flock in 1989–2009

Year	Number of ewes	Number of rams	Number of lambs born	Litter size	Fertility %	Lamb rearing %	Fecundity %
1989	3	1	8	2.67	100	/	/
1990	5	1	10	2.50	80	/	/
1991	6	1	11	1.83	100	/	/
1992	4	/	11	2.75	100	/	/
1993	5	1	15	3.00	100	/	/
1994	8	1	23	2.88	100	100	213
1995	8	3	25	3.13	100	68	188
1996	9	4	35	3.89	100	68	189
1997	9	1	35	3.89	100	93	311
1998	6	1	29	4.83	100	90	433
1999	7	2	22	3.14	100	88	200
2000	5	1	12	2.40	100	75	180
2001	10	1	28	3.11	90	92	220
2002	10	1	27	2.70	100	78	210
2003	11	2	38	3.45	100	97	336
2004	10	1	35	3.50	100	72	260
2005	14	2	41	2.93	100	100	221
2006	14	3	48	3.43	100	100	293
2007	18	3	62	3.44	100	90	306
2008	25	3	70	3.33	84	80	224
2009	24	3	76	3.30	96	100	208
Mean	-	-	-	3.15	-	87	249

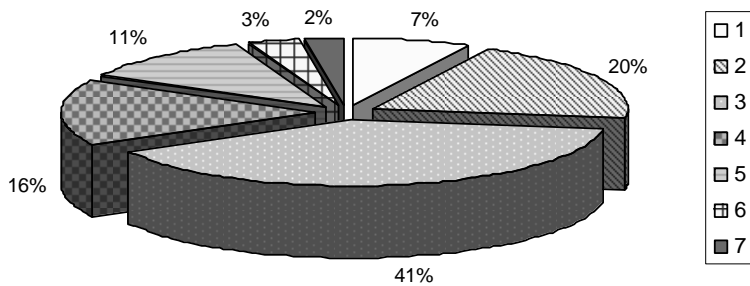


FIGURE 1. Distribution of litters consisted of 1 to 7 lambs born in the flock in years 1989–2009

duals the differences were very high. For instance, the ewe 100000611913 was non inbred if inbreeding coefficient was calculated from the database provided by the breeder. Her inbreeding coefficient increased to 6.25% for Korczyński

+ Bielany database and up to 9.58% for full database (Korczyński + Bielany + Żelazna). As an example the differences in the average inbreeding level of ram-lambs depending on the database used, is shown on Figure 2.

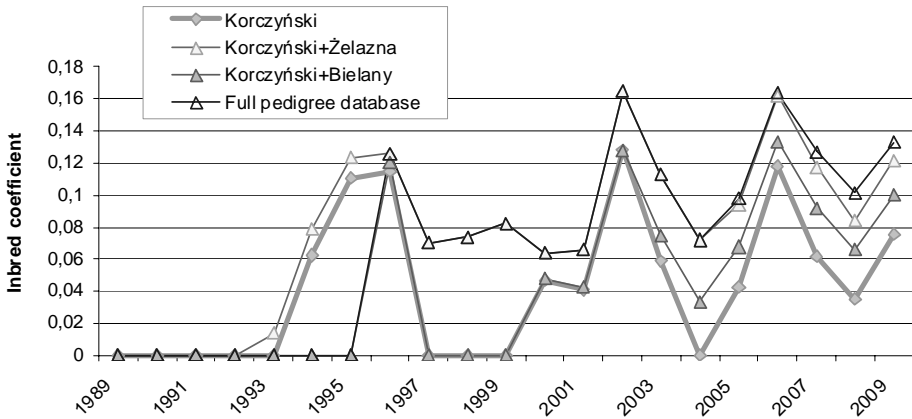


FIGURE 2. Differences in inbreeding coefficients of ram-lambs using gradually enhanced pedigree databases

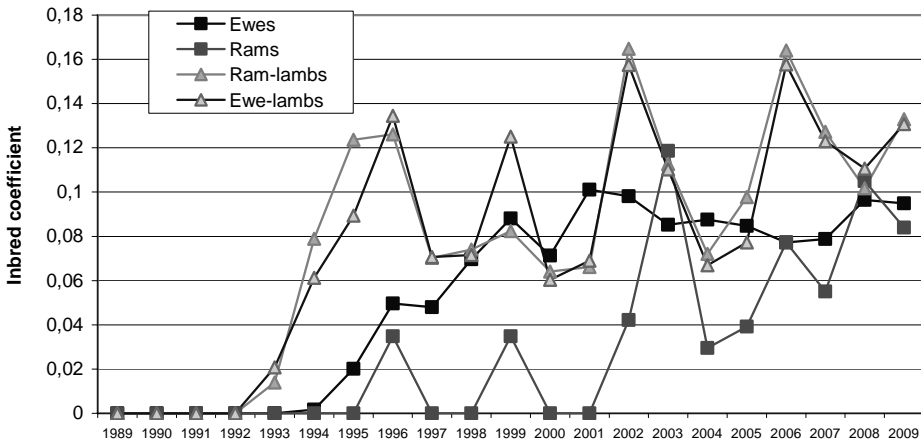


FIGURE 3. Mean inbreeding of ewe-lambs and ram-lambs born in the years 1989–2009 and mean inbreeding parents of the successive lamb crops

The mean inbreeding of ewes and rams taking part in the successive reproduction seasons is shown on Figure 3.

In some sheep, especially in the first few years, the pedigree data was incomplete. The highest inbreeding coefficient was observed in ewe number 100000611937, reaching 21.3%. The mean relationship of ewes with rams selected for mating varied from 2.2% to 32%. The mating strategy in

successive reproduction seasons applied by the breeder was usually advantageous, as shown in Figure 4.

The inbreeding level of lambs was growing continuously since the establishment of the flock, as shown in Figure 3. The substantial increase of inbreeding in years 1996, 2002 and 2006, resulted from using one ram in a few successive reproduction seasons, and by high relationship

of rams with the ewe flock (Fig. 4). In the year 2009, the mean inbred coefficient reached the level of 13.3% for ram-lambs and 13.1% for ewe-lambs. The mean and

maximum inbreeding coefficients for lambs are presented in Table 2. The rate of inbreeding increase for lambs was estimated as 0.67% per year.

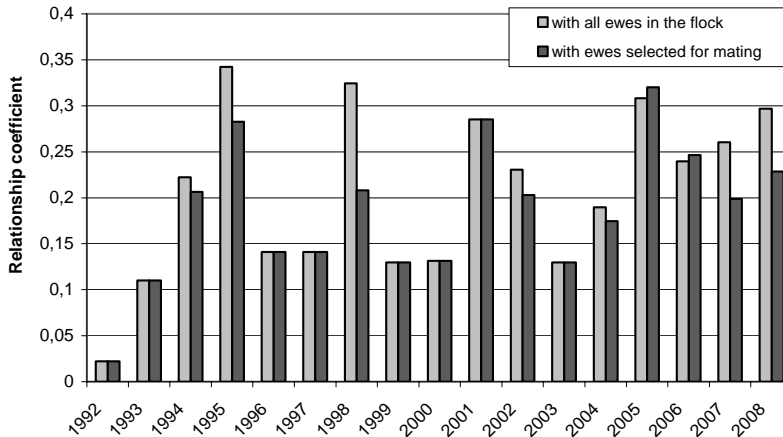


FIGURE 4. Relationship between rams and all ewes in the flock/ewes selected for mating

TABLE 2. Mean inbreeding coefficients for lambs in years 1989–2009

Year of birth	Full pedigree database							
	Ram-lambs			Ewe-lambs			Average for all lambs	
	N	$\bar{x} \pm sd$	Max	N	$\bar{x} \pm sd$	Max	N	$\bar{x} \pm sd$
1989	3	0	0	2	0	0	5	0
1990	4	0	0	3	0	0	7	0
1991	3	0	0	5	0	0	8	0
1992	/	/	/	/	/	/	0	/
1993	6	0.014 ± 0.021	0.014	6	0.021 ± 0.027	0.055	12	0.017 ± 0.024
1994	10	0.079 ± 0.061	0.141	13	0.061 ± 0.061	0.141	23	0.069 ± 0.060
1995	11	0.124 ± 0.093	0.146	14	0.089 ± 0.053	0.146	25	0.104 ± 0.069
1996	19	0.126 ± 0.063	0.183	16	0.135 ± 0.052	0.202	35	0.130 ± 0.052
1997	18	0.070 ± 0.016	0.094	14	0.071 ± 0.017	0.084	32	0.070 ± 0.016
1998	13	0.074 ± 0.021	0.097	16	0.072 ± 0.017	0.097	29	0.073 ± 0.018
1999	10	0.082 ± 0.010	0.183	11	0.125 ± 0.054	0.183	21	0.102 ± 0.043
2000	8	0.064 ± 0.015	0.101	4	0.060 ± 0.004	0.064	12	0.063 ± 0.013
2001	17	0.066 ± 0.016	0.101	11	0.069 ± 0.018	0.101	28	0.067 ± 0.017
2002	16	0.165 ± 0.032	0.206	11	0.157 ± 0.039	0.206	27	0.162 ± 0.035
2003	20	0.113 ± 0.058	0.197	18	0.110 ± 0.059	0.197	38	0.112 ± 0.058
2004	21	0.072 ± 0.015	0.096	14	0.067 ± 0.016	0.096	35	0.070 ± 0.015
2005	19	0.098 ± 0.068	0.285	22	0.077 ± 0.014	0.108	41	0.087 ± 0.048
2006	23	0.164 ± 0.052	0.228	25	0.158 ± 0.058	0.228	48	0.161 ± 0.055
2007	34	0.127 ± 0.057	0.228	28	0.123 ± 0.058	0.228	62	0.125 ± 0.027
2008	39	0.102 ± 0.025	0.193	31	0.111 ± 0.037	0.193	70	0.106 ± 0.031
2009	41	0.133 ± 0.029	0.192	35	0.131 ± 0.028	0.192	76	0.132 ± 0.029

The  $\chi^2$  test allowed rejecting the hypothesis that inbreeding level had any negative impact on either mean or maximum litter size of ewes.

## DISCUSSION

The results of the present study indicate that the reproduction potential of the analyzed flock was very high. For instance, in 2007 the mean litter size in the whole population of Olkuska sheep was 2.21 (Hodowla..., 2007), while in Mr. Korczyński's flock, in the same year, reached the level of 3.44. Continuous and frequent occurrence of large litters consisting of even seven lambs, indicate that the gene with a major effect on ovulation rate has a high frequency in this flock.

The problem of inbreeding was not sufficiently studied in Olkuska sheep population. So far, the most precise evaluation was available for Żelazna flock (Martyniuk and Marchowiecka, 2007) and to some extent, for Mr. Korczyński's flock (Martyniuk, 2007). The present study provided insight into the situation of the later flock. The differences between estimates of inbreeding level, depending on the database used, were high. Similar results were obtained by Martyniuk (2007). The results indicate that only use of full pedigree information, including historical pedigrees from the 1980s, when separation of prolific Olkuska population took place, can give precise results. It is very important to extend such study to cover all flocks of Olkuska sheep, and gather as much information as possible to enable both precise estimations of genetic relationship within this population, and to develop management tools to control inbreeding.

It can be assumed that inbreeding in the whole Olkuska sheep population is rapidly increasing, as was suggested by Martyniuk (2007). In Mr. Korczyński's flock, inbreeding of lambs increased at a rate of 0.67% per year. It is suggested that the acceptable inbreeding increase is about 0.5–1.0% per generation (Nicholas, 1989), but this estimate is for commercial breeds with a very large population size. Inbreeding in Żelazna flock after fourteen years since its establishment, was 5.7% for ewe-lambs, and 5.1% for ram-lambs (Martyniuk and Marchowiecka, 2007). The low rate of inbreeding increase in Żelazna flock was achieved by optimization of mating plans that was practiced in this flock, since 1996. Although Mr. Korczyński's flock mating strategy was usually advantageous, due to the small flock size, increasing of inbreeding was rapid. In some years, continuous use of the same ram in a few successive reproduction seasons increased the problem.

The maximum individual inbreeding coefficient of animals born between 1989–2009 in Mr. Korczyński's flock was high, and reached 28.5% in two ram-lambs born in 2005. Those ram-lambs were sons of a very valuable ram 755-001-2404, which was used in the flock in four continuous reproduction seasons, from 2002 to 2005. Similar inbreeding coefficients were observed in the closed Booroola flock, where the maximum individual inbreeding coefficient was higher than 32% (Rzewuska et al., 2005). High individual inbreeding coefficients were also observed in the study by Boujenane and Chami (1997), where they reached a range of 32.8% in the Sardi, and 37.5% in Beni Guil sheep. In Żelazna flock, where the optimization of mating plans

was routinely performed, the maximum individual inbreeding coefficients was equal to 19.5%.

Any inbreeding impact on prolificacy of ewes was not observed. Similar results were reported from the study on Booroola sheep population (Rzewuska et al., 2005). Analysis of variance showed no effect of ewe inbred class on any of reproduction traits analyzed, including fertility, ovulation rate and litter size. In other breeds, where ovulation rate is not determined by a single gene, the impact of inbreeding depression was observed (Lamberson and Thomas, 1984; Lamberson et al., 1982; Wiener et al., 1992b; Ercanbrack and Knight, 1991).

## CONCLUSIONS

Mr Korczyński's flock has the most valuable genetic material of Olkuska sheep, with a strong impact on development and increase of the whole population of this breed. Therefore, a high level of inbreeding in this flock suggests that inbreeding could become a serious problem in the whole population. It is necessary to undertake concerted activities to analyze the range of inbreeding in the whole population. It is also important to provide breeders with the information about relationships among rams available for sale, with ewes in their flocks. Optimization of mating plans in larger flocks that produce breeding stock will result in reduction of inbreeding in the whole Olkuska sheep population.

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**Streszczenie:** *Analiza przebiegu wzrostu inbrodu w wysokoplennym stadzie owcy olkuskiej.* Celem pracy było oszacowanie dynamiki wzrostu inbrodu w stadzie owcy olkuskiej należącym do prywatnego hodowcy, Pana Tomasa Korczyńskiego. Materiał doświadczalny obejmował 65 maciorek i 21 tryków oraz ich potomstwo, złożone z 661 jagniąt urodzonych w latach 1989–2009. Stado charakteryzowało się bardzo wysoką użytkowością reprodukcyjną. Średnia plenność kształtowała się na poziomie  $3,15 \pm 0,63$ . Poziom inbrodu w stadzie był wysoki. Średni poziom inbrodu jagniąt wzrastał stopniowo aż do roku 2009, kiedy osiągnął 13,3% u tryczków oraz 13,1% u maciorek. Nie obserwowano wpływu inbrodu na plenność matek. Wyniki badań wskazują, że inbred jest poważnym problemem w populacji owcy olkuskiej.

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## Litter size, puppy weight at birth and growth rates in different breeds of dogs

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**Abstract:** *Litter size, puppy weight at birth and growth rates in different breeds of dogs.* In this study was compare litter size, sex distribution and body weight at birth and during first 7 weeks of life in puppies 7 breeds, which can be classified into 4 size groups: miniature: Yorkshire Terrier, small: Shetland Sheepdog, Welsh Corgi Pembroke medium: Polish Mountain Hound, Bouvier des Flandres, large: Great Dane and Newfoundland. In analyzing growth rates in different breeds was used the average weights at subsequent weeks were calculated as percentages of adult weights and calculated as multiplicity of birth weight. All results were statistically tested with Anova and Bonferroni Tests. Litter size was bigger consequently with adult dogs body mass and difference between breeds were significant. Sex distribution was found significantly uneven in Yorkshire Terrier breed – the number of females was almost twice as big as the number of males. Body weight at birth compared to adult weight of respective breed was found significantly different among the breeds. This relation was highest in the miniature Yorkshire Terrier and small breeds, and lowest in large breeds: the Great Dane and the Newfoundland. Growth rate as percentage of adult weight in consecutive weeks of age was greatest in the smallest breed – the Yorkshire Terrier, only slightly lower in small, and distinctly lower in medium-sized and large breeds. Growth rate as multiplicity of birth weight was fastest in medium breed Polish Hound and large Great Dane, slowest in Yorkshire.

*Key words:* Dog, growth curve, puppy, litter size.

### INTRODUCTION

The most striking feature of the domestic dog (*Canis familiaris*) is its enormous diversity in size and shape, not found in any other species, either domestic or wild one. Size varies as much as from 500 grams and some 15 cms in the smallest specimens of the Chihuahua breed to 120 kgs in the Spanish Mastiff or 120 cms height in the Irish Wolfhound. Within this range virtually any size, height and shape can be found. One can hardly believe that it is still one species.

Recent studies, employing new methods of molecular genetics, found that the retrogen encoding fibroblast growth factor 4 (*fgf4*) is responsible for shortening legs (chondrodysplasia) in many short-legged breeds (Parker et al. 2009). Chase et al. (2002) identified 6 quantitative loci, influencing dog size and morphology. Mutations within IGF-1 (insulin-like growth factor 1) were found of crucial importance during puppy growth; they also influence metabolism in adult dogs (Sutter et al. 2007).

It was also show that small and miniature breeds are usually homozygous in three IGF-1 haplotypes. Research suggest that a single mutation appeared at very

early stage in domestic dogs development, and it is commonly found in small breeds. Yet the matter seems to be more complicated, as small breeds come in different sizes, and even within one breed there is still some variation in size, even though duration of pregnancy is similar in all breeds (63-65 days) and puppies are born at same stages of development.

Body weight at birth can vary from 100-150 grams in miniature breeds to 200-300 grams in medium sized, and 400-800 grams in large and giant ones. Interestingly, although weights of adult specimens of different breeds vary up to 40 times, they are only 10 times different at most. It may be expected then that puppies of different breeds develop and grow at different rates as it has been already demonstrated by Hawthorne et al. (2004). This rate is much slower in large breeds. Rainbird et al. (1990) who compared growth rates in breeds of similar size: the Labrador and the Briard, and the Newfoundland and the Great Dane, found that growth rates may be different even in this case.

In this study was compared growth rates from birth until 6 weeks of age in puppies of similar-size breeds and their development in the first weeks of life. Additionally, the size, sex distribution and number of stillborns in each litter were reported.

## MATERIAL

Body weights were collected in puppies of 7 breeds, which can be classified into 4 size groups:

Miniature: Yorkshire Terrier (Yorkie),  
Small: Shetland Sheepdog (Sheltie),  
Welsh Corgi Pembroke (Corgi),  
Medium: Polish Mountain Hound (Polish Hound), Bouvier des Flandres (Bouvier),  
Large: Great Dane (Dane), Newfoundland.

Detailed numbers of litters and puppies are shown in Table 1.

Puppies were electronically weight at birth and then every 7 days until they reached 7 weeks of age (49 days). They were born in kennels registered with the

TABLE 1.

Breed	Body weight (kgs) <sup>1</sup>	Number of litters	Number of puppies	Males	Females	Stillborns <sup>2</sup>
Yorkie		19	52	18	34	11
Sheltie	5 – 10	10	44	23	21	1
Corgi	10 – 12	10	40	21	19	0
Polish Hound	20 – 25	12	86	40	46	2
Bouvier	27 – 40	15	86	43	43	0
Newfoundland	50 – 70	12	106	61	45	5
Dane	50 – 70	10	87	44	43	6
Total	–	88	501	250	251	25

<sup>1</sup> Weight of adult dogs as specified in their respective breed standards.

<sup>2</sup> All puppies dead or died within 48 hours after birth.



Polish Kennel Club and housed with their respective breeders. Mother milk had been the only food until 4 weeks of age, when commercial foods were introduced. Commercial foods were originally fed two times a day and then the number of meals increased gradually to five per day in the 7<sup>th</sup> week.

## METHODS

Average litter size was compared and SD calculated within each breed. The significance of breed differences was established with Bonferroni Test. Fraction Test was used to estimate the influence of breed on sex distribution and percentage of stillborns in the litters.

For each breed it was calculated the percentage of average puppy weight at birth to the weight of adult dog of this breed (average of the values taken from the breed standard), as well as SD, maximum and minimum values. Results were verified with Anova Test and the significance of obtained differences was established with Bonferroni Test.

In analyzing growth rates in different breeds was used the average weight at birth, calculated as described earlier. Similarly, average weights at subsequent weeks were calculated as percentages of adult weights, and the results were statis-

tically tested with Anova and Bonferroni Tests.

Next we calculated multiplicity of body mass, achieved by puppies in the 24<sup>th</sup> and the 42<sup>th</sup> day of life and determined respective growth curves, enabling us to calculate the actual day each breed achieves multiplicity of its body weight at birth. Average daily growth was established as geometric mean, and verified as earlier.

## RESULTS AND DISCUSSION

As far as litter sizes are concerned, significant differences among the breeds was found (Table 2).

The smallest litters were those of the Yorkshire terrier, followed by the small breeds, while the largest were those of large breeds: the Newfoundland and the Great Dane. Litters of the Polish Hounds were relatively big and no litter smaller than 4 puppies was observed. The differences between the three smaller breeds and the two large ones were statistically significant and such was also the difference between the Yorkshire Terrier and the Polish Hound. These results are in accordance with the earlier studies by Willis (1989) and others, who found that litter size in different breeds of dog was closely linked to their height at withers,

TABLE 2. Average litter size

Breed	Average litter size	SD	Minimum	Maximum
Yorkie	3.7	1.1	2	5
Sheltie	4.4	2.0	2	7
Corgi	4.4	1.7	2	7
Polish Hound	7.2	1.6	4	9
Bouvier	5.7	2.3	2	10
Newfoundland	8.2	3.2	3	14
Dane	8.7	4.6	1	14

which obviously correlates with body weight.

There were no differences in distribution of sex in the litter of different breeds (Table 3), the only exception being the Yorkshire Terrier – in this breed significantly fewer males than females were born (Table 1). This may suggest that sex-linked lethal genes are present in the population.

Weights at birth as percentages of adult weights are shown in Table 4. Yorkshire puppies were relatively biggest, followed by Corgis and Shelties, whereas Newfoundlands and Danes were relatively smallest. In most cases the differences between breeds were statistically significant, except for those between the Yorkshire Terrier and the Corgi, and the Newfoundland and the Great Dane. Similarly, all four size groups – miniature, small, medium and large – showed significant differences. The smallest puppies were those of large breeds and the Bouvier. In all cases total weights of litters were lower than 15% of adult weights. Only in the Polish Mountain Hound there they were close to this value. However, all results were lower than those reported by Ściesiński (2003), who claims that the

total weight of a litter of 5–6 puppies was around 15% of their mother's weight.

Growth rate in consecutive weeks of age was greatest in the smallest breed – the Yorkshire Terrier, only slightly lower in the Corgi and the Sheltie, and distinctly lower in medium-sized breeds, i.e. the Polish Hound and the Bouvier. Large breeds – i.e. the Dane and the Newfoundland – showed the lowest growth rate. As seen on the Figure 1, those differences were increasing in consecutive weeks.

Three weeks after birth puppies of large breeds (Great Dane and Newfoundland) achieved same percentage of adult body weight as Yorkie puppies at birth. From the 4<sup>th</sup> week on the highest fraction of adult body weight was that of the Corgis. At the 7<sup>th</sup> day we found significant differences between the four groups: miniature (Yorkshire Terrier), small (Corgi and Sheltie), medium (Polish Hound) and large (Newfoundland and Great Dane). At the 14<sup>th</sup> day there was also a difference between breeds of the medium-sized group, i.e. the Bouvier and the Polish Hound, which – albeit small – was statistically significant. In other breeds the differences remained same as earlier. On the 21<sup>st</sup> day the two small breeds showed

TABLE 3. Significance of differences in sex distribution as shown with Fraction Test

Breed	Yorkie	Sheltie	Corgi	Polish Hound	Bouvier	Newfoundland	Dane
Yorkie	--	0.03*	0.02*	0.06	0.02*	0.004**	0.05*
Sheltie		--	1	0.25	0.386	0.32	0.4
Corgi			--	0.23	0.37	0.30	0.39
Polish Hound				--	0.30	0.087	0.34
Bouvier					--	0.179	1
Newfoundland						--	0.22
Dane							--

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

TABLE 4. Weight of puppies as percentage of adult weight

Breed	Mean puppy weight (%)	SD (%)	Minimum (%)	Maximum (%)	Mean weight of litter <sup>3</sup> (%)
Yorkie	3.6	0.7	2.7	4.7	13.3
Sheltie	2.5	0.7	1.3	4.0	11.0
Corgi	3.1	0.5	2.0	4.0	13.6
Polish Hound	2.0	0.7	0.9	4.0	14.4
Bouvier	1.6	0.2	1.3	2.2	9.1
Newfoundland	1.0	0.2	0.6	1.5	8.2
Dane	1.1	0.2	0.7	1.8	9.6

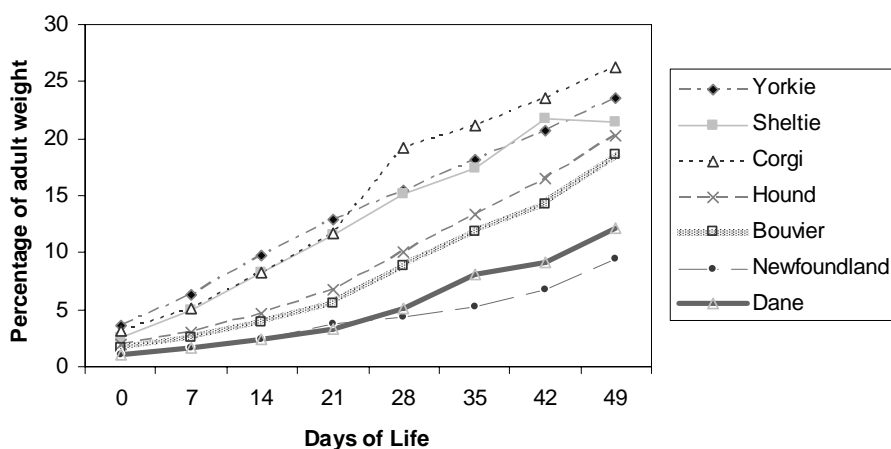


FIGURE 1. Increase of body weight (growth rate) during first 7 weeks of life (%)

same values as the Yorkshire terrier and no significant differences between them have been found since then, whereas differences between the three size groups remained significant. On the 35<sup>th</sup> day Great Dane puppies became significantly heavier than Newfoundlands. In the following weeks weight differences became more pronounced, both within the groups and between them. Till the eight week a single tendency was observed: the fastest growth rate is that of small breeds, the slowest

– of large breeds. Similar results were obtained earlier by Hawthorne et al. (2004), who compared growth rates of 12 breeds and found that miniature breeds finished their intensive growth period at 11 weeks, small and medium-sized at 14–16 weeks, and giant breeds at 22 weeks. The slowest growth rate was that of the English Mastiff (10.8% a week), the fastest – of the English Springer Spaniel (18.3% a week). Trangerud et al. (2007), comparing growth rates in Newfoundlands, Lab-

<sup>3</sup> Average number of puppies as show in Table 1.

rador Retrievers, Leonbergers and Irish Wolfhounds, claim that in general growth rates in all breeds were faster in the first 100 days of life, the fastest being that of the Labrador Retriever, the smallest of the breeds studied.

Table 5 shows multiplicity of body weight at birth achieved by all puppies on the 24<sup>th</sup> and 42<sup>nd</sup> day after birth. On the 24<sup>th</sup> day it was the highest in the Polish Hound and the Sheltie, the lowest in the Yorkshire Terrier. Two breeds: the Polish Hound and the Great Dane multiply their weight at birth by ten times. In the first breed growth rate was fast and stable, whereas Great Danes were originally growing relatively slowly, and then their growth rate accelerated markedly. Almost equally fast growth rate was

observed in Shelties. Breed differences were statistically significant.

More detailed results are shown on Table 6.

The fastest and the most stable growth rate was that of the Polish Hound. Great Danes started their growth at slow pace, which accelerated markedly from the 30<sup>th</sup> day on. Interestingly, the second large breed, the Newfoundland, showed growth rate only slightly faster than the Yorkshire Terrier. Shelties were growing steadily and fast. There were significant differences between all breeds with the exception of the Bouvier and the Newfoundland. Similar results were reported by Rainbird and Kienzle (1990) in Great Danes and Newfoundlands, while Hawthorne et al. (2004) found significant dif-

TABLE 5. Multiplicity of body weight at birth on the 24th and 42th day of life

Breed	Multiplicity on the 24th day	Multiplicity on the 42th day
Yorkie	3.98	5.74
Sheltie	5.38	9.45
Corgi	4.61	7.58
Polish Hound	5.39	10.83
Bouvier	4.16	7.25
Newfoundland	4.10	6.85
Dane	4.83	10.15
Mean	4.60	8.35

$P \leq 0.05$ .

TABLE 6. Multiplicity of body weight from birth to 42th day of life (6 weeks)

Multiplicity	Days of life	2×	3×	4×	5×	6×	7×	8×	9×	10×	11×
York		9	16	24	34	42					
Sheltie		7	12	18	23	28	32	36	40		
Corgi		10	17	22	26	30	38	42			
Hound		8	14	19	23	26	29	32	35	38	42
Bouvier		11	19	23	28	34	42				
Newfoundland		11	17	24	28	36	42				
Dane		9	19	24	29	31	33	32	36	42	

ferences among breeds of similar size: Newfoundlands differed significantly from Great Danes, English Mastiffs, St. Bernards and Irish Wolfhounds. Among medium-sized and large breeds Labrador Retrievers showed significant difference to English Springers and Beagles.

## CONCLUSION

Litter size was markedly larger in large breeds, slightly smaller in medium-sized and small breeds, and the lowest in the miniature breed. Differences in litter size were statistically significant, especially between large breeds and the miniature one.

In Yorkshire Terrier breed was found uneven sex distribution in the litters – the number of females was almost twice as big as the number of males. It is possible that a lethal sex-linked allele is present in this breed, yet this hypothesis requires further studies.

Body weight at birth compared to adult weight of respective breed was found significantly different among the breeds. This relation was highest in the miniature Yorkshire Terrier and small breeds, and lowest in large breeds: the Great Dane and the Newfoundland. Growth rate as percentage of adult weight in consecutive weeks of age was greatest in the smallest breed – the Yorkshire Terrier, only slightly lower in small – Sheltie and Corgi, and distinctly lower in medium-sized and large breeds. Contrary, when comparing growth rates as multiplicity of birth weight we found it was fastest in the medium-sized breed – the Polish Hound, almost as fast in the Great Dane, and it was slow in the Yorkshire Terrier.

In breeds of similar sizes we did not find much similarity in growth rates.

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**Streszczenie:** Porównanie wielkości miotu, masy ciała przy urodzeniu i tempa wzrostu różnych ras psów. Porównano wielkość miotu, rozkład płci, masę ciała po urodzeniu i przyrosty w pierwszych 7 tygodniach życia szczeniąt 7 ras psów podzielonych na 4 grupy: miniaturowe: yorkshire terier, małe: owczarek szetlandzki i welsh corgi pembroke, średnie: gończy polski i bouvier des flandres i dużych: dog niemiecki i nowofundland. Do porównania przyrostów u różnych ras użyto średniej masy ciała po urodzeniu i w kolejnych

tygodniach życia obliczonej jako odsetek masy psa dorosłego. Porównano też tempo przyrostu w badanym okresie jako wielokrotność masy urodzeniowej. Wyniki poddano analizie Anova i testem Bonferroniego. Wielkość miotu była tym większa, im większa była masa ciała dorosłych psów, a różnice między rasami były istotne. Rozkład płci istotnie odbiegał od równomiernego w rasie yorkshire terier – liczba suczek była niemal dwukrotnie większa niż piesków. Masa ciała po urodzeniu w porównaniu z masą psa dorosłego danej rasy różniła się istotnie między rasami. Była relatywnie najwyższa u rasy miniaturowej yorkshire terier i ras małych, a najniższa u ras dużych dog niemiecki i nowofundland. Podobnie przyrosty szacowane jako odsetek masy psa do-

rosłego w kolejnych tygodniach życia szczeniąt były najwyższe u yorkshire terierów, jedynie nieco niższe u ras małych i znacząco mniejsze u ras średnich i dużych. Natomiast przyrost szacowany jako krotność masy urodzeniowej był najszybszy u rasy średniej polski gończy i dużej dog niemiecki, a najmniejszy u yorkshire terierów.

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## **An analysis of incidents caused by captive cats in the world during 1990–2006 period on the basis of web database**

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**Abstract:** *An analysis of incidents caused by captive cats in the world during 1990–2006 period on the basis of web database.* On the basis of Internet database the incidents with captive cats were analysed. During 1990–2006 period 289 cases were recorded which resulted in 322 human victims in nearly 40 countries with the overrepresentation of USA. The incidents were caused by individuals of 14 wild cat species, but the effect of tiger (*Panthera tigris*) also as a number of fatal incidents was greatest of all. Incidents were recorded in all places where captive cats were kept: zoos, circuses, private estates, breeding centres, movie industry facilities etc. It seems that the main causes of cats aggression towards man was improper human behaviour, too close approaching or entering cage which had effect on animal territorial behaviour. These findings to the great degree confirmed previously obtained by the authors.

*Key words:* Wild cat, incident, aggression, zoological garden, circus.

### INTRODUCTION

The incidents involving wild animals have been well described since a long time. Especially problem of so-called man-eaters (e.g. the lion and tiger) gained public interest with a quite sensational dimension. Memoirs of famous hunters who killed cats-man eaters became best-sellers (e.g. Corbett, 1945). Even today there is problem with big cats killing humans in some countries. For example in

Tanzania lions (*Panthera leo*) have killed more than 563 persons and injured at least 310 during 1990–2005 period (Packer et al., 2005). As regards the tiger (*Panthera tigris*) very bad reputation as man-eaters have individuals living in the various regions e.g. Sunderbans (India/Bangladesh). This is rather slippery question taking into account conservationists effort to save this critically endangered animals (Seidensticker et al., 1999).

The behavioural problems with captive cats is quite another story. We witness the great popularity of keeping various wild animals as pets, among others also wild cats (Serpell, 1999). Today probably there are as many as 15,000 private-owned big cats only in USA (Handwerk, 2003). There are also many others kept in circuses zoological gardens and related institutions around the world. Despite the tameness and often friendly behaviour towards man there are still basically wild animals. Breaking from the human control they become potentially dangerous. This aspect of human-animal relations was poorly documented in up to date studies.

This paper is continuation of previous study concerning incidents caused by all captive animals and reported by international press and Internet databases

(Kaleta, 2006). 481 cases resulted in 530 human victims during the fifteen years period all over the world. In above-mentioned study the captive cats and elephants turned out to be the most dangerous for man. Captive cats were blamed for 50% of incidents and human victims. Therefore, in the present study authors focused on the cats.

## MATERIAL AND METHODS

The analysis was carried out in 2008. The source of information was Internet site [www.bigacatresuce.org](http://www.bigacatresuce.org) operated by big American sanctuary keeping 100 individuals from 14 cat species. The database comprised press reports which chronologically covered the whole years. The period 1990–2006 was selected for analysis. The full description of each case should be as follows: time and place of incident (day, month, year, city, institution), species of cat, situational context, result (injury or killing), characteristics of human victim.

## RESULTS

Taking into account above-mentioned criteria the 289 cases were selected which resulted in 322 victims. Incidents were recorded in 33 countries beyond Africa and in some number of unidentified countries in Africa zoos. The increasing of incident numbers was observed in succeeding years from 7 in 1990 to the 28 in 2006.

12 species of wild cats showed aggression towards man and caused the incidents: tiger (*Panthera tigris*), lion (*Panthera leo*), leopard (*Panthera pardus*),

jaguar (*Panthera onca*), snow leopard (*Panthera uncia*), cheetah (*Acinomyx jubatus*), cougar (*Felis concolor*), serval (*Felis serval*), ocelot (*Felis pardalis*), jungle cat (*Felis chaus*), caracal (*Felis caracal*), bobcat (*Lynx sp.*) (taxonomy based on Nowak, 1999, sixth edition). Incidents were also caused by the hybrids *P. tigris* x *P. leo*.

It should be kept in mind that probably occurred also incidents not included to the database and not all cases mentioned in database were fully described.

The number of analysed cases caused by particular cats and places of incidents is shown in Table 1. It shows that the tiger to the great degree exceeded the effect of the other species. Relatively high number of incidents was also recorded in lion, lower in leopard and cougar. The contributions of the other species were very small. The difference between species was highly significant (ANOVA,  $p = 0.00$ ).

It is striking that many cases were not reported from zoological gardens or circuses but from the “other places”. To this category belonged private-owned animals kept as pets, cats kept in various sanctuaries and shelters, wildlife parks, breeding centres and also animals working for entertainment industry. Also no wonder that the greater number of incidents was recorded in USA. Firstly, the private ownership of wild animals is not so restricted in USA as in European countries. Secondly, there is very good press coverage of such sensational topics in American media, so even small incident with wild animals can be reported. In USA incidents with captive cats were recorded in 37 states with the clear prevalence of Florida (25 cases) and Texas (22



TABLE 1. The number of incidents involving captive cats in the world in 1990–2006 period

Species \ Site	Number of incidents					
	Zoological garden	Circus	Other place	USA	Other countries	Total
Tiger	38	31	74	93	50	143
Lion	21	19	24	21	43	64
Lion and tiger kept together	–	–	3	3	–	3
Leopard	13	3	7	17	6	23
Jaguar	6	–	3	3	6	9
Snow leopard	3	–	–	1	2	3
Cheetah	1	–	2	3	–	3
Cougar	6	–	23	27	2	29
Serval	–	–	1	1	–	1
Ocelot	1	–	–	–	1	1
Jungle cat	–	–	1	1	–	1
Caracal	1	–	–	1	–	1
Bobcat	–	–	5	5	–	5
Hybrid lion x tiger	–	2	1	1	2	3
Total	90	56	143	178	111	289

cases). Outside the USA incidents were described in 30 countries of the world with prevalence of People’s Republic of China (13 cases).

**1. Causes of incident**

On the basis of particular cases description the authors tried to reconstruct the cause of incidents. The classification comprised eight main categories of situation when the accident took place:

- EN – man enters the pen (e.g. handler, staff member, service worker etc.).
- ESC – animal escapes from the enclosure and attacks (usually unknown) man.
- APP – animal reaction to approaching cage by unknown human, placing hand in the cage, trying to pet animal etc.
- LED – incidents when animal was used as attraction in parades or walks being lead on leash. The victim is usually bystander.

TR – incidents (mainly in circuses) during the training session or performance. The victim is trainer.

PET – incidents with animals kept as pets breaking from control. The victim is owner or bystander.

MO – incidents taking place during the work in entertainment industry (movie, commercial etc). The victim could be any person involved in the work during sessions.

OTH – others, no clearly described situation in which attack is recorded.

This diversification of cat attack cases resembled classification obtained in previous paper (Kaleta, 2006) It was found that APP and EN were the most important hypothetical causes of incidents with captive cats (see Table 2). However, the difference between causes as a whole was not significant (ANOVA,  $p = 0.605$ ).

TABLE 2. Number of incidents with cat aggression evoked by particular situations in 1990–2006 period

Species	Cause of incident							
	EN	ESC	APP	LED	TR	PET	MO	OTH
Tiger	28	12	30	6	18	9	20	20
Lion	13	5	16	2	9	7	3	9
Lion and tiger kept together	–	1	–	–	–	1	–	1
Leopard	1	3	10	–	1	2	1	5
Jaguar	2	1	4	–	–	–	2	–
Snow leopard	–	–	2	–	–	–	–	1
Cheetah	–	1	–	–	–	–	1	1
Cougar	4	2	5	1	–	8	4	5
Serval	–	–	–	1	–	–	–	–
Ocelot	1	–	–	–	–	–	–	–
Jungle cat	–	–	–	–	–	1	–	–
Caracal	–	–	1	–	–	–	–	–
Bobcat	1	–	–	–	–	4	–	–
Hybrid lion x tiger	–	1	–	–	–	–	–	2
Total	50	26	68	10	28	32	31	44
%	17	9	24	3	10	11	11	15

It is worth to mention that the description of 15% causes did not allow to classify the situational context of aggression.

In the case of APP humans unknown to animals (e.g. visitors in zoo) should be blamed. They often closely approached cage behind the safety barriers, tried to pet animal or in rare cases climbed into enclosure. For cats, mostly territorial animals this is highly disturbing situation. These persons were treated as intruders and restricted living space forced animal to response aggressively because of flight distance and/or personal space encroaching (Hediger, 1968).

In the case of EN the same motivation for aggressive behaviour is possible. Moreover, familiar persons entering the cage may be sometimes seen as social rivals. Previous animal abuse (e.g. puni-

shment) could be also important factor influencing animal behaviour.

Tiger and to the lower degree lion caused incidents belonging to the all classification categories. Particularly great number of incidents with tigers were recorded during movie production and in circuses. This cats also relatively frequently escaped and attacked humans. On the basis of media account there is often very hard to reconstruct the situation in these cases. It is well-known fact that escaped wild animals are usually very frightened, stressed, therefore prone to attack (Hediger, 1968).

In the previous paper approaching or entering the cage turned out to be also the most important causes of incidents with cats. (Kaleta, 2006). The difference was that contrary to the present study EN

was ranked as a first cause and APP as the second.

## 2. Human victims

In the present study contact of men with the captive cats resulted in 322 human victims see Table 3). Incidents caused injuries of various severity and 70 deaths. A sequences of cat attack were often similar: either pouncing on, dragging and mauling human body or simply biting on hand or leg. Deaths were result of aggression of individuals belonging to five cat species: tiger (greatest number of victims), lion, leopard, jaguar and cougar. Tigers procured also the most cases of injury.

A quarter of all victims were young persons (under 18 years in age). In the group of human victims unfamiliar per-

sons numbered 50%, animal handlers – 16%, – trainers – 15%, cat owner and/or his/her family members – 12%. The rest of group comprised of various persons involved in works related to cat keeping and husbandry. The result of incident was significantly dependent on the cat species (ANOVA,  $p = 0.01$ ).

In the previous study 262 human victims and 42 deaths were recorded. Nearly 30% of victims were the young persons. Tiger was also ranked as the most dangerous animal in this case (Kaleta, 2006).

To sum up, present investigation to the great degree confirmed the previous results and showed that there is some substantial risk in the man's contact with captive cats. Probably man's overconfidence about an animal "tameness",

TABLE 3. Number of victims involved in captive cats attacks during the 1990–2006 period

Species	Incident result				Age of victim		Country		Total
	Injury	Death	Unknown (result not described)	Absent (harmless incident)	Young (under 18)	Adult	USA	Other countries	
Tiger	115	40	1	1	39	118	101	56	157
Lion	48	21	2	–	13	58	25	46	72
Lion and tiger kept together	4	1	–	–	2	3	5	–	5
Leopard	20	4	2	–	8	18	17	9	26
Jaguar	7	3	–	–	4	6	4	6	10
Snow leopard	3	–	–	–	–	3	1	2	3
Cheetah	2	–	–	–	1	1	2	–	2
Cougar	31	1	2	1	12	23	33	2	34
Serval	3	–	–	–	2	1	3	–	3
Ocelot	1	–	–	–	–	1	–	1	1
Jungle cat	1	–	–	–	1	–	1	–	1
Caracal	1	–	–	–	1	–	1	–	1
Bobcat	5	–	–	–	3	2	5	–	5
Hybrid lion x tiger	2	–	–	–	2	–	1	1	2
Total	243	70	7	2	88	234	199	123	322

ignorance as regards its behaviour could bring about these incidents.

However, it is need to see at these incidents from the broader perspective. The number of incidents involving captive cats (and the other animals as well) seems to be completely insignificant when confronted with other sources of human casualties. For example in only one year there was 43000 persons dying in car accidents, 14000 murdered and 520 casualties resulted from fires in USA (various sources cited by Kaleta, 2006). But as keeping exotic pets becomes more and more popular and private wild-life breeding centres will probably thrive (because of greater threat of extinction i the case of many animal species) there is risk that number of such incidents will grow. Since the legislation in some countries is permissive as regards keeping wild animals the better education for visitors in zoos and similar places and for prospect wild cat owners is needed.

## CONCLUSIONS

1. The study based on Internet database showed that during sixteen years there were 289 documented cases of captive cats incidents with 322 human victims in around 40 countries.

2. In spite of relatively great number of cat species (14) involved, the tiger (*Panthera tigris*) turned out to be clearly the most dangerous which caused the greatest number of fatal incidents. These incidents were recorded in various places (zoos, private owner estate, movie industry etc).

3. It seems that the improper human behaviour towards animal was the real cause of many incidents. In the present

study the approaching cage too close by visitors and entering cage by for example the caretakers evoked cat aggressive reactions. Ethological interpretation indicates the encroachment the personal space and/or fight distance of animal and in some cases probably also some other aspects of territorial behaviour.

4. The results of this study to the great degree confirmed previous data obtained by authors.

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**Streszczenie:** *Analiza wypadków spowodowanych przez dzikie kotyzymane przez człowieka na świecie w latach 1990–2006 na podstawie bazy internetowej. W oparciu o bazę internetową przeanalizowano wypadki, do których doszło przy udziale kotów trzymanyh w niewoli. W okresie pomiędzy 1990 a 2006 rokiem odnotowano 289 takich przypadków, w wyniku których ofiarami stało się 322 ludzi w około 40 krajach (z dużą*

przewagą wypadków w USA). Incydenty spowodowały osobniki kotów należących do 14 gatunków, ale wpływ tygrysa (*Panthera tigris*) okazał się największy, także jeśli chodzi o wypadki, które zakończyły się fatalnie. Incydenty odnotowano praktycznie we wszystkich miejscach, w których trzymano dzikie koty: w ogrodach zoologicznych, cyrkach, na posesjach prywatnych, w centrach hodowlanych, miejscach związanych z przemysłem filmowym itd. Wydaje się, że najważniejszymi przyczynami agresji kotów względem człowieka były nieodpowiednie zachowania ludzkie, polegające na nadmiernym zbliżaniu się do klatki, lub

na wchodzeniu do niej, co miało wpływ na zachowanie terytorialne zwierząt. Te dane potwierdzają wcześniej uzyskanie przez autorów.

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## Identification of quantitative trait loci for body weight and body weight gain in two growth-differentiated mouse lines

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**Abstract:** *Identification of quantitative trait loci for body weight and body weight gain in two growth-differentiated mouse lines.* A genome-wide quantitative trait locus (QTL) analysis was performed in two lines L and C derived from four mice strains (A.CA, C57BL/6, BALB/c, BN/a). This lines differed in body weight, behaviour, length of reproduction period, rate of ageing, degree survivability. Five statistically significant QTL for body weight at 56 day, body weight gain from 12–21 and from 21–56 were detected. Lines C and L are unique tools for investigating genetic factors that regulate body weight.

*Key words:* body weight, quantitative trait loci, mouse, lines, growth.

### INTRODUCTION

For many years the efforts of animal breeders were to improve meat quality of livestock. Breeders control the quality of traits using selection. Most economically important traits have a quantitative nature and are determined by QTL (Quantitative Trait Loci). The knowledge of QTL affecting body weight will facilitate the selection. The investigation for quantitative traits is performed on lines of animals representing phenotypic extremes for choosen trait. Phenotypic differences

between selected lines are caused by changing of the allele frequency.

In experimental crosses between lines, farm animals are used, however mice are still the most common organism in this type of studies for obvious reasons like economy and short generation interval (Kirkpatrick and al. 1997).

The unique lines of mice have been bred by investigators from the Department of Genetics and Animal Breeding, Warsaw Agricultural University (SGGW). The lines of mice were selected for 120 generations for body weight changes on 21st day of life. The light line (L) was selectively bred for low body weight contrary to the heavy line (C). These lines, after multigenerational selection, differed not only in the trait which was the criterion of selection but also in many other traits (Wirt-Dzięciołowska and Czumińska 2000). Phenotypic differences resulting from the selection of examined mice may result from changes in the frequency of genes responsible for the level of the selected trait. They may also result from the interaction between various variants of genes which appear in mice coming from antagonistically selected lines.

In the present study we used linkage analysis to detect QTL affecting body weight and body weight gain in crosses between both growth-differentiated mouse lines. The aim of our research was to find out the region of genome that are responsible for the phenotypic differences between lines L and C.

## MATERIAL AND METHODS

### Animals:

Animals used in the present study originated from crosses between mice from the light line (L) and the heavy line (C) derived from a cross of four in-

bred strains: A/St, C57BL/6, BALB/c and BN/a. (Radomska i wsp. 1970a, b, Sławiński 1974). Criterion of selection was body weight on 21 day of life. After 120 generations 10 females from line C were crossed with 10 males from line L to produce F1. The F1 progeny were intercrossed to obtain F2. Body weight was recorded on 236 F2 mice on 12, 21, 56, 90, 150 day after birth. The body weight gain was calculated between 12-21, 21-56, 56-90, 90-150 days. The mice were housed in a conventionally conditioned room with temperature  $22 \pm 2^\circ\text{C}$  and 60% humidity. The mice were allowed *ad libitum* access to standard pelleted died

TABLE 1. List of the microsatellite marker loci

Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)	
<i>D1Mit3</i>	11	<i>D2Mit238</i>	28	<i>D3Mit164</i>	2.4	<i>D4Mit315</i>	0.0	<i>D5Mit346</i>	1.0
<i>D1Mit18</i>	29.7	<i>D2Mit102</i>	52.5	<i>D3Mit21</i>	19.2	<i>D4Mit288</i>	28.6	<i>D5Mit13</i>	20
<i>D1Mit46</i>	43.1	<i>D2Mit226</i>	96	<i>D3Mit173</i>	29.5	<i>D4Mit152</i>	40.0	<i>D5Mit394</i>	34
<i>D1Mit42</i>	78	<i>D2Mit265</i>	105	<i>D3Mit45</i>	78.5	<i>D4Mit234</i>	71	<i>D5Mit239</i>	58
<i>D1Mit200</i>	80	<i>D2Mit200</i>	107	<i>D3Mit147</i>	79.4			<i>D5Mit168</i>	78
<i>D1Mit15</i>	87.9								
<i>D1Mit511</i>	109.6								
<i>D1Mit155</i>	112								
Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)	
<i>D6Mit1</i>	2.8	<i>D7Mit178</i>	0.5	<i>D8Mit155</i>	1	<i>D9Mit48</i>	34	<i>D10Mit168</i>	9.0
<i>D6Mit228</i>	37	<i>D7Mit267</i>	11	<i>D8Mit287</i>	8	<i>D9Mit311</i>	65	<i>D10Mit115</i>	38.4
<i>D6Mit254</i>	60.5	<i>D7Mit147</i>	37	<i>D8Mit65</i>	22.5			<i>D10Mit134</i>	59
<i>D6Mit15</i>	74	<i>D7Mit40</i>	53	<i>D8Mit271</i>	57				
		<i>D7Mit68</i>	60	<i>D8Mit318</i>	57				
Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)	
<i>D11Mit78</i>	2.0	<i>D12Mit136</i>	13	<i>D13Mit88</i>	21	<i>D14Mit14</i>	10	<i>D15Mit12</i>	4.7
<i>D11Mit8</i>	46.17	<i>D12Mit214</i>	38	<i>D13Mit128</i>	48	<i>D14Mit165</i>	52	<i>D15Mit122</i>	34.2
<i>D11Mit301</i>	69	<i>D12Mit133</i>	56	<i>D13Mit77</i>	73			<i>D15Mit161</i>	69.2
Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)		Marker and map position (cM)	
<i>D16Mit9</i>	4.0	<i>D17Mit133</i>	10.4	<i>D18Mit64</i>	2.0	<i>D19Mit59</i>	0.5	ZFY	19
<i>D16Mit174</i>	43	<i>D17Mit152</i>	37.7	<i>D18Mit202</i>	22	<i>D19Mit19</i>	26	DXM140	62
<i>D16Mit114</i>	44.5	<i>D17Mit244</i>	55.7	<i>D18Mit184</i>	41	<i>D19Mit53</i>	43	DXM197	
<i>D16Mit50</i>	53.5								
<i>D16Mit106</i>	71.45								



(labofeed H manufactured by Morawski CO, Poland). Food and water were provided *ad libitum*. The animals received human care and ethical treatment. The experiment was approved by the Local Ethics Committee.

### Genotyping:

DNA was extracted from tails with NucleoSpin® Tissue kit (Macherey-Nagel-08/2004/rev 03). 78 microsatellite markers located on all autosomal and X, Y chromosomes were chosen for our investigation (Table 1). Special attention was paid to the polymorphism level of the four initial inbred strains, the uniformity of spacing in chromosomes (at every 20–30 cM) and the difference in the size of allele while the markers were choosing. The PCR reaction was performed in the standard conditions established on the basis of data obtained from the State Committee for scientific research grant No. 4PO5A04517. PCR products were electrophoresed on 4–5% agarose gel.

### QTL analysis:

SAS program was used to calculate allele frequency, Fisher test and chi-square test. The associations between phenotypes and genetic markers in the F2 population were determined by Qxpak (Perez-Enciso and Misztal 2004). The significance of each potential association was measured with likelihood ratio statistic LRS. LOD score were obtained by dividing the LRS by 4,605.

## RESULTS AND DISCUSSION

Basic statistics for body weight are presented in Table 2. The significant differences between animals from line C and line L were found. Mice from line C (on 21 day) were over twofold heavier than mice from line L. The difference in mean body weight between the mice from line L and C on 150 day was lower but still high.

Result for all QTL identified are presented in Table 3. 32 QTL for 8 traits were detected. No significant linkages for body weight gain from 56 to 90 days were identified. The trait with the greatest number of QTL was body weight gain from 12 to 21 days. No conclusive statistical evidence of QTL by sex interaction was found.

Loci located on 5, 6, 9 and 19 chromosomes had the highest peak of lod score. The QTL which was located on chromosome 5 influenced body weight on 56, 90 and 150 day of life and body weight gain from days 21 to 56 (Fig. 1). Database searching provides no evidences to confirm our results. However in the same region on chromosome 5 three genes: (*Shh*, *Lmbr1* and *Fgfr3*) which regulate body weight were identified. Gene *Shh* (*sonic hedgehog*) is the one of the major genes involved in the organogenesis. *Lmbr1* (*limb region 1*) is responsible for limb development, whereas mutations in

TABLE 2. Basic statistics for growth traits in line L and C

	<i>Female</i>				<i>Male</i>			
	L		C		L		C	
<b>Cecha</b>	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Body weight at 21 days (g)</b>	6.1	1.1	13.1	1.2	6.5	0.8	13.7	1.4
<b>Body weight at 150 days (g)</b>	22.9	2.7	42.1	2.9	26.0	2.9	47.1	4.0

TABLE 3. QTL detected for body weight and body weight gain

Trait	LG*	LRS (LOD)	p	The highest peak LOD score (cM)	a	SD	d	SD	Flanking Markers	CI 90%	CI 95%
Body weight at 12 day	15	11.05 (2.4)	0.016	17	0.16	0.16	-0.96	0.29	D15M12-D15M122**	1-28	1-26
	18	9.07 (2.0)	0.042	28	0.13	0.14	0.82	0.27	D18M202-D18M184	24-33	27-30
Body weight at 21 day	9	8.1 (1.8)	0.043	63	-1.09	0.38	1.11	1.85	D9M311-D9M48	48-65	60-65
	15	10.3 (2.2)	0.023	26	0.18	0.27	-1.72	0.53	D15M122-D15M12	17-37	20-33
	19	<b>13.5 (2.9)</b>	0.003	1	-0.63	0.18	0.39	0.35	D19M59-D19M19	1-26	1-26
Body weight at 56 day	2	8.77 (1.9)	0.066	107	1.68	0.60	-1.91	1.72	D2M200-D2M200	102-107	
	5	<b>15.44 (3.3)</b>	0.003	20	0.25	0.33	2.13	0.55	D5M13-D5M394	11-30	12-28
	6	8.14 (1.8)	0.090	30	-0.26	0.46	-2.73	0.97	D6M228-D6M1	27-31	
	19	12.97 (2.8)	0.004	3	-1.19	0.43	2.19	0.91	D19M59-D19M19	1-26	1-26
Body weight at 90 day	5	<b>13.37 (2.9)</b>	0.009	20	0.41	0.41	2.35	0.66	D5M13-D5M394	12-28	14-27
	6	8.97 (1.9)	0.060	28	-0.21	0.56	-3.56	1.19	D6M228-D6M1	22-34	
	12	9.35 (2.0)	0.037	29	-0.73	0.54	3.32	1.25	D12M214-D12M136	22-37	25-32
	19	7.86 (1.7)	0.048	26	-1.89	0.88	3.81	2.10	D19M19-D19M19	2-26	23-26
Body weight at 150 day	5	<b>13.55 (2.9)</b>	0.008	18	0.24	0.49	3.02	0.82	D5M13-D5M346	9-27	11-25
	12	7.38 (1.6)	0.095	25	-0.96	0.64	3.26	1.49	D12M136-D12M214	25-26	
	15	8.23 (1.8)	0.063	25	0.52	0.91	-5.05	1.76	D15M122-D15M12	20-30	
	19	9.67 (2.1)	0.020	2	-1.64	0.61	1.90	1.25	D19M59-D19M19	1-26	1-26
Body weight gain from 12 to 21	1	8.64 (1.9)	0.089	73	1.02	0.52	-2.15	1.61	D1M42-D1M46	70-78	
	2	12.32 (2.8)	0.012	41	0.27	0.19	1.19	0.56	D2M102-D2M238	1-77	29-53
	3	10.05 (2.2)	0.036	20	0.00	0.11	0.46	0.21	D3M21-D3M45	13-41	18-29
	4	8.13 (1.8)	0.090	37	-0.15	0.13	-0.41	0.30	D4M152-D4M288	34-39	
	6	<b>19.08 (4.1)</b>	0.000	31	-0.29	0.13	-0.84	0.27	D6M228-D6M1	1-72	6-59
	7	9.43 (2.0)	0.061	53	0.19	0.11	0.21	0.17	D7M40-D7M68	47-56	
	9	<b>14.34 (3.1)</b>	0.002	65	-0.81	0.26	0.61	1.28	D9M311-D9M311	1-65	1-65
	10	7.34 (1.6)	0.097	46	0.14	0.13	0.29	0.29	D10M115-D10M134	44-48	
	11	7.77 (1.7)	0.079	60	-0.07	0.22	-1.20	0.81	D11M301-D11M8	51-66	
	12	7.72 (1.7)	0.081	44	-0.19	0.12	0.08	0.28	D12M214-D12M133	27-56	
	15	<b>13.44 (2.9)</b>	0.005	35	-0.07	0.20	-0.86	0.35	D15M122-D15M161	21-50	24-47
19	<b>13.64 (3.0)</b>	0.003	1	-0.36	0.12	0.13	0.24	D19M59-D19M19	1-26	1-26	
Body weight gain from 21 to 56	2	8.16 (1.8)	0.089	97	1.20	0.48	-2.35	1.36	D2M226-D2M200	96-99	
	5	<b>14.6 (3.2)</b>	0.005	21	0.23	0.28	1.73	0.46	D5M13-D5M394	14-28	15-26
	10	8.58 (1.9)	0.053	50	-0.80	0.35	-1.39	0.78	D10M134-D10M115	44-59	

*Fgfr3* (Fibroblast growth factor receptor 3) cause the most common forms of human and animal dwarfism – achondroplasia.

Locus on chromosome 6 had a strong effect on body weight gain from 12 to 21days (Fig. 2). In the same way the identified QTL affected also body weight

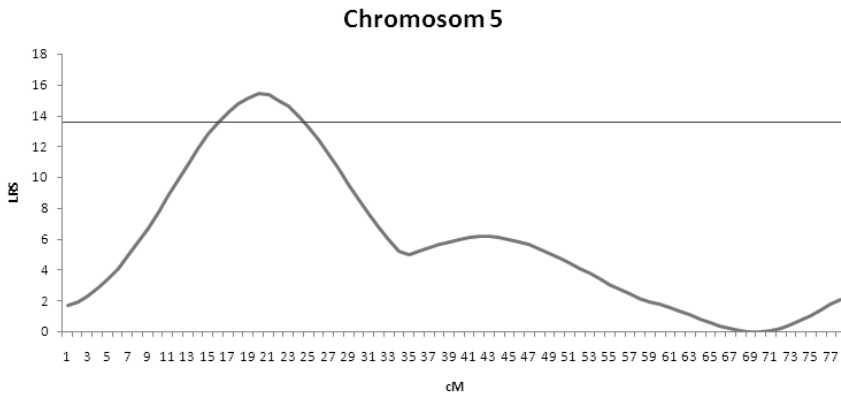


FIGURE 1. LOD score plot for Chromosome 5; Threshold is marked by solid line (significant – LRS>13,83 (LOD>3)). Plot of body weight at 56 day

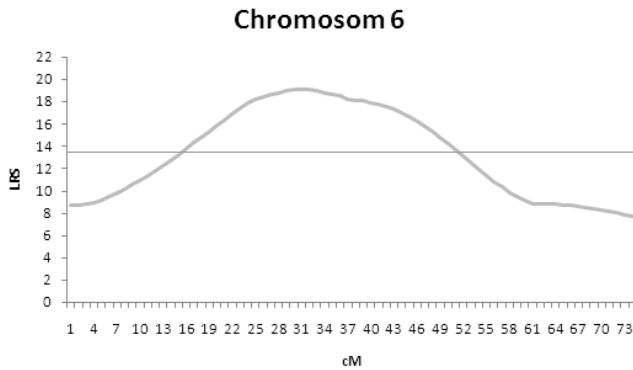


FIGURE 2. LOD score plot for Chromosome 6; Threshold is marked by solid line (significant – LRS>13,83 (LOD>3)). Plot of body weight gain from 12 to 21

at 56 and 90 days. There are some interesting QTL near locus indicated in this study. Roch et al. (2004) mapped QTL (28.5 cM) which regulate 10-week body weight. Keightley et al. (1996) identified locus (22 cM) for 6-week body weight.

Suto i Sekikawa (2004) also reported that the region on chromosome 6 (35.2 cM) influences body weight. The QTL on chromosome 6 was named as *Bwq2*. This locus regulate body weight not only on 40 day of life but also on 50, 60, 70, 80, 90, 100 days. *Bwq2* was statistically

suggestive for weight gain between days 21 and 30 and was significant for weight gain between days 30 and 40. The QTL identified in this study regulate body weight in the similar to *Bwq2* period of life. Moreover some interesting genes: *Ghrhr* (growth hormone releasing hormone receptor) (26 cM), *Ctnna2* (Catenin) (36 cM), *Tgfa* (Transforming growth factor alpha) (35.8 cM) are located near the identified QTL in the present study (<http://www.jax.org/>). Mice homozygous for mutation in *Ghrhr* gene have a dwarf

phenotype. The mutations in genes *Cttna2* and *Tgfa* also affect the size and body weight of mice.

For body weight at age of 21 days (trait which was the criterion of selection) we detected 3 QTL (9, 15, 19 chromosomes). Locus located on chromosome 9 regulates also body weight gain from 12 to 21 days. Database searching provide no information confirmed our results. In this region of chromosome 9 there are identified only QTL which regulate the body weight in the different period of life.

The literature data confirm our results for chromosome 15. Roch et al. (2004) mapped W3q7, Anunciado et al. (2001) identified locus which regulate the body weight at age of 21 days, Morris et al. (1999) indicated on 15 chromosome a suggestive body weight QTL. Cheverud (1996), Pomp (1997) and Reifsnnyder (2000) also mapped body weight QTL on 15 chromosome.

Locus detected on chromosome 19 affected body weight at age of 21, 56 and 150 days. The highest peak LOD score for QTL on chromosome 19 was obtained for body weight on 21 day and for weight gain from 12 to 21 days. There is a group of genes *Esrra* (*estrogen related receptor, alpha*), *Gng3* (*guanine nucleotide binding protein (G protein), gamma 3*), *Ltbp3* (*latent transforming growth factor beta binding protein 3*), *Mark2* (*Emk*) (*MAP/microtubule affinity-regulating kinase 2*) and *Men1* (*multiple endocrine neoplasia 1*) that are located near 91% CI of QTL identified in the present study. These genes affect growth at an early stage of life. *Men1* null mutant embryos were smaller in size compared to wild-type embryos (Bertolino et al. 2003).

Mice L and C present significant differences in body weight in prenatal and postnatal period. The differences in body weight between two lines have been observed throughout entire life.

In future analyses will be required a more detailed mapping studies for chromosomes 5, 6 and 19. The present study shows that phenotype differences observed in line L and C have a confirmation in genotype frequency. The next step in the study is to indicate strictly the region on chromosomes 5, 6 and 19 that affects body weight.

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**Streszczenie:** *Identyfikacja loci cech ilościowych dla masy ciała oraz przyrostów w liniach selekcyonowanych przeciwstawnie na masę ciała.* Poszukiwanie loci regulujących masę ciała prowadzono przy użyciu linii selekcyonowanych przeciwstawnie na masę ciała w 21 dniu życia. Różnice fenotypowe występujące pomiędzy dwoma liniami mogą być wynikiem zmian we frekwencji alleli genów odpowiedzialnych za poziom badanych cech. Do oceny zmienności genetycznej między myszami linii L i C zastosowano metodę analizy mikrosatelitarnej. Zmapowano pięć statystycznie istotnych loci regulujących masę ciała w 56 dniu życia przyrosty pomiędzy 12–21 oraz 21–56 dniem życia.

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## The conditions of Polish primitive horses' maintenance in some stables

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**Abstract:** *The conditions of Polish primitive horses' maintenance in some stables.* In this paper three stables for Polish primitive horses were analyzed for microclimate conditions. There were estimated following indicators: internal temperature, relative humidity, air velocity and intensity of light. The investigations were done during three periods: autumn, winter and spring. The studies shown that in examined buildings some parameters (especially temperature and level of light) were not compliant with Polish standards. But on the other side on these farms there were no serious problems with health of animals. It is very supposedly that it is connected with breed and the type of management. In these stables there were kept primitive horses, resistant to untoward weather's conditions. Additionally significant part of day the horses were kept outside so the influence of building was lower.

*Key words:* konik polski, microclimate, stable, Polish primitive horse.

### INTRODUCTION

The Polish primitive horses (koniki polskie) are the very special breed. This is primitive type of horses and they are closely relatives of tarpans. These animals are part of the program concerning the protection for endangered local breed (Żurowski, 2004). The genetic recourses conservation program is realized in Poland till 2000.

They are kept in two different ways. Very important for this type of horses is keeping them in preserves where they can live as the wild animals (Jaworski 2007). Second type of maintenance is keeping them in stables. In this situation the breeder should secure the best conditions as possible. He must remember about properly conditions inside the buildings – parameters of microclimate, properly system of maintenance and also about good feeding and prophylaxis etc. Of course very important is to give them possibility of pasture using. They should spend there a lot of time (Jaworski, Jaszczyńska 2004).

The conditions of microclimate plays a big role in welfare of animals. It also influence on health and productivity. Many healthy problems are connected with wrong conditions in building. So also in keeping the primitive breeds we should not forget about the principles of properly husbandry.

### MATERIAL AND METHODS

The investigations were carried out in two farms, where Polish primitive horses were kept.

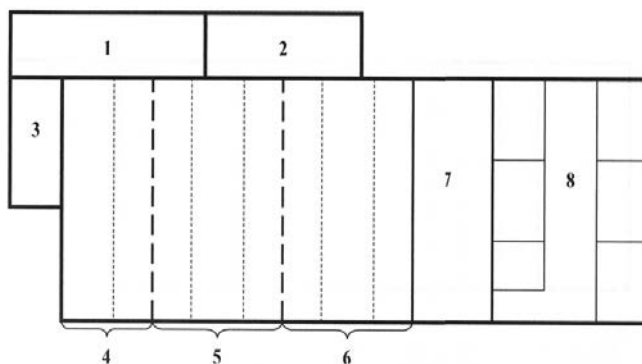
1. First farm with two stables where mares, foals and stallions were kept. The plan of first stable is presented on Figure 1 and the plan of the second is shown on Figure 2. On this farm there were 31 mares-mothers of polish horse, 6 of ponies' mares 18 one year old mares, 9 two years old mares, 4 reproductive stallions and 5 one years old stallion (include 3 stallions of ponies). All mares of polish horse are kept in loose housing system.

There was also pasture reserve for all horses.

2. On the second farm there were kept geldings and non – reproductive stallions – stable 3.

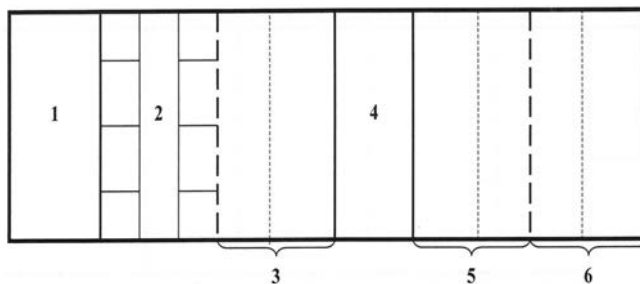
In all these stables straw was used as bedding.

To determine the conditions of microclimate there were estimate the following indicators: internal temperature, relative humidity, air velocity and inten-



- 1, 2 – loose housing stables for youths
- 3, 7 – farm accommodations
- 4, 5, 6 – loose housing stables for mares and mares with foals (covering the area 265.5 m<sup>2</sup>)
- 8 – boxes for stallion (covering the area about 84 m<sup>2</sup>)

FIGURE 1. The plan of the stable 1 in farm 1



- 1 – stable for weaned foals (covering the area about 53 m<sup>2</sup>)
- 2 – stable with boxes (covering the area about 90 m<sup>2</sup>)
- 3, 5, 6 – loose housing stables for mares and mares with foals (covering the area about 218.5 m<sup>2</sup>)
- 4 – the entrance to the attic

FIGURE 2. The plan of the stable 2 in farm 1



sity of light. The traditional equipment using in animal hygiene was used. The measurements were taken according to methodological basis of Polish standard (Kośła 2001).

## RESULTS

The results concerning the estimation of chosen parameters in autumn season are shown in Table 1.

The results concerning the estimation of chosen parameters in winter season are shown in Table 2.

The results concerning the estimation of chosen parameters in spring season are shown in Table 3.

The size of boxes were also measured.

### Building 1

Boxes for stallions – there were 3 boxes – 9.9 m<sup>2</sup>, 2 boxes – 10.8 m<sup>2</sup> and 1 box – 6 m<sup>2</sup>

TABLE 1. Microclimate parameters in autumn season in studied stables

Microclimate parameters	Stable 1		Stable 2	Stable 3	Polish standards
	Stallions	Mares with foals			
Internal temperature (°C)	7.9	8.8	8.9	11.6	5-28
Internal humidity (%)	79.7	79.3	81.6	75.3	Max 80
Air velocity (m/s)	0.04	0.04	0.08	0.04	0.3
Natural light (lx)	20.2	15.05	15	12.3	15-30

Stable 1 – first stable in farm 1 (Fig. 1); Stable 2 – the second stable in farm 1 (Fig. 2); Stable 3 – the stable in farm 2.

TABLE 2. Microclimate parameters in winter season in studied stables

Microclimate parameters	Stable 1		Stable 2	Stable 3	Polish standards
	Stallions	Mares with foals			
Internal temperature (°C)	-2.5	-2.5	0.13	2.8	5-28
Internal humidity (%)	56.6	60.4	74.5	57.4	Max 80
Air velocity (m/s)	0.06	0.1	0.1	0.08	0.3
Natural light (lx)	29	31.3	30	35	15-30

Stable 1 – first stable in farm 1 (Fig. 1); Stable 2 – the second stable in farm 1 (Fig. 2); Stable 3 – the stable in farm 2.

TABLE 3. Microclimate parameters in spring season in studied stables

Microclimate parameters	Stable 1		Stable 2	Stable 3	Polish standards
	Stallions	Mares with foals			
Internal temperature (°C)	12.4	11.3	14.9	13.4	5-28
Internal humidity (%)	74.6	75.9	74.4	67.5	Max 80
Air velocity (m/s)	0.04	0.05	0.1	0.04	0.3
Natural light (lx)	35	37	38	32	15-30

Stable 1 – first stable in farm 1 (Fig. 1); Stable 2 – the second stable in farm 1 (Fig. 2); Stable 3 – the stable in farm 2.

### **Building 2**

There were 8 boxes – about 8.4 m<sup>2</sup> each.

### **Building 3**

There were 12 cubicles – wide 1.9 m and length 2.7 m and 1 box – about 11 m<sup>2</sup>.

## DISCUSSION

The obtained results were compared with polish standards (Rozporządzenie Ministra Rolnictwa i Rowoju Wsi, DzU.167,1629); Kośla 2001; Kołacz, Dobrzański 2006).

During the **autumn** season (Tab. 1) in all stables the internal temperatures were consistent with level of polish standards. This parameter was on level about 8 to above 11.5°C.

The humidity (except a little higher value in stable 2) was on level lower than recommended 80%.

Also air velocity and intensity of light were

During the **winter** (Tab. 2) some parameters were beyond the recommended levels. In all stables the average temperature was below minimal level of standards. In the stable 1 there were even noticed value below 0°C. Probably this situation is a consequence of wrong state of windows, some of them were seriously damaged. Another problem was level of natural light – it was too high. Only in stable 1 the value of this parameter were close to maximum of standards. In others the estimated level was above recommended 30lx. It could be caused by the conditions of measurements (sunny days) and also the damaged windows could influence on foto-climate.

Other studied parameters – humidity and air velocity were up to standards. Although the level of humidity was a little

lower (about 60–70%) in this period than in autumn and spring. In low temperature this situation is better for animals than low temperature and high humidity conditions. (Kośla 2001)

During the spring measurements (Tab. 3) there were no problem with level of temperature – in all three stables this parameter was in the recommended level (between 11 and 15°C). Also air velocity and humidity were in proper ranges. During this season there was only one problem – the level of light. Similarly to the winter period there was also noticed higher value of this parameters in all stables. In all cases the level of light were above recommended 30lx.

In buildings another element could influence on animals' welfare. This is the size of boxes and cubicles. In studied stables a big number of horses were kept in loose housing, but some were kept in different type of maintenance. All boxes and cubicles had right constructions and size, adapted to this kind of horses.

According to data from veterinarian the examined group of horses were in quite good health conditions. There were only a few cases of bronchitis. But to improve the welfare of these herds the owner should take care about state of windows. According to information from breeder in imminent future some investments will be done.

## CONCLUSIONS

1. Too low temperature in winter season in all stables was estimated.
2. Too high level of light in spring was noticed.
3. Others indicators of microclimate were up to recommended level.

4. The recommendation for owner is to repair and make tight the windows to improve the temperature conditions, which have big influence on welfare.

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**Streszczenie:** *Kondycja utrzymania koni polskich ras prymitywnych w niektórych stajniach.* W pracy oceniono warunki mikroklimatu w trzech stajniach, zlokalizowanych w dwóch gospodarstwach, hodujących koniki polskie. Oszacowano następujące wskaźniki mikroklimatu: temperaturę, wilgotność, ruch powietrza i natężenie światła. Badania przeprowadzono w trzech okresach: jesienią, zimą i wiosną. Otrzymane wyniki porównano z normami obowiązującymi w Polsce. Okazało się, że niektóre parametry odbiegają od przyjętych norm (tyczyło się to przede wszystkim temperatury i natężenia oświetlenia). Z drugiej strony okazało się, że w badanych gospodarstwach nie stwierdzono problemów ze stanem zdrowia koników. Prawdopodobnie wynika to z tego, że koniki polskie są rasą prymitywną, odporną na niesprzyjające warunki pogodowe, a ponadto większość dnia spędzały one poza budynkiem.

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## The habitat preferences of amphibians in the catchment area of the Zagożdżonka River

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**Abstract:** *The habitat preferences of amphibians in the catchment area of the Zagożdżonka River.* The habitat preferences of amphibians in the catchment area of the Zagożdżonka River. The aim of the study was to describe habitats of amphibians in the small river Zagożdżonka catchment, situated in Central Poland, in the area of Koziernicki Landscape Park. The occurrence of amphibians was recorded in relation to water and terrestrial habitat features. The observations were carried out at 16 transects. The presence of 7 species of amphibians, common to lowlands was recorded. Higher number of species was stated in ponds or reservoirs without running water, with rather small size. The most common species were *Rana esculenta* complex and *Rana temporaria*. The breeding of *Triturus cristatus*, the endangered species within Europe territory, was recorded at four transects. Authors suggest to continue research on amphibians in this area to find what is the influence of human activities on this group of animals.

*Key words:* amphibians, habitat, Zagożdżonka catchment, Koziernicki Landscape Park.

### INTRODUCTION

Decline in amphibians populations is the process observed during last few decades, both globally and locally (Blaustein, Wake 1990, Stuart et al. 2004). Currently, the Global Amphibian Assessment consider 427 species as “critically endan-

gered”. While in wider perspective main threats of this animals consist of emerging diseases, climate change, increased ultraviolet-B radiation, or long-distance transmission of chemical contaminants, in local scale the direct changes of habitat and habitat fragmentation seem to be major factors. This rule applies to all organisms connected to water habitats (Barbault, Sastrapradja 1995). Generally amphibians due to their two-staged life cycle need two kinds of habitats to fulfill their life requirements: aquatic and terrestrial, so threats to either habitat can affect their populations. The small river catchment with its habitat variety can be a good example of how such dual threats influence this group of animals.

### MATERIAL AND METHODS

Investigation has been carried out in a small agricultural river basin of Zagożdżonka – left tributary of Vistula river ( $A = 82 \text{ km}^2$ ), located in the center of Poland (ca 100 km south of Warsaw). The Zagożdżonka river basin is research area of Department of Water Engineering and Environmental Restoration of Warsaw

University of Life Sciences. Majority of investigated area is covered by agricultural land with loose rural housing, some parts covered by forest belong to the Kozienicki Landscape Park. The dominant soil type are sandy soil, ranging from almost pure to loamy sands. In depressions areas like river beds, peaty soils can be found. Agricultural land use should be considered as extensive, with small farms and diversity of crop fields, meadows and pastures.

To study habitat preferences of amphibians, water bodies and watercourses with closest surrounding terrestrial habitat within the Zagożdżonka catchment were described and investigated for amphibians occurrence. 16 transect were designated as representative for different habitats. Each transect was 100 m long and covered terrestrial habitat next to water. The type of habitat, dominating plant species were described for each transect. The amphibians occurrence was recorded by the use of direct observations of adults, larvae and eggs, netting and vocal recordings. Each transect was visited at least three times (one time at dark). The field observations were carried out from the beginning of April to the end of June 2009, to cover the higher breeding activity of amphibians (Duellman 1994).

## RESULTS

The description of habitats occurring along investigated transect is shown in table 1. Running water (river Zagożdżonka and river Mirenka) was the base for water habitats of 7 transects, while ponds were present at 9 transects. The size of the ponds varied from 0.1 ha (transect 1) to 25 ha (transect 15). There were only one transect located

close to habitat with high human pressure – Pionki Staw Górny close Pionki Town. Other transects were situated in more natural habitats like forest areas (transects 2-4 Królewskie Źródła, 11, 15-16), village neighborhood (transects 6, 10, 11, 12) and extensive agricultural areas (transect 5-9).

During the whole observation period seven amphibian species were recorded: *Rana esculenta complex*, *R. temporaria*, *R. arvalis*, *Bufo bufo*, *Hyla arborea*, *Triturus vulgaris* and *T. cristatus* (Table 2). The most common and most numerous species was *R. esculenta complex*, recorded at 14 transects (6 transect – I class, 4 transects – II class, 4 transects – III class). The highest population number (III class) was observed in ponds of different size (from 0,6 ha – transect 13 to 25 ha – transect nr 15). The second common was *R. temporaria*, found at 11 transects. Other species occurred in less than 7 transects. Only for frogs species (*Rana*) the III number class was observed. The higher number of species (5) was recorded at transect nr 10 Sałki. 4 species were found at three transects: Królewskie Źródła taras (transect nr 2), Helenów 2 (transect nr 14) and Ług Tczowski (transect nr 15). The less suitable habitats for amphibians seem to be at transect nr 1 Pionki Staw Górny, transect nr 5 Czarna Jaz and transect nr 16 Ług Tczowski las, where only one species was recorded (*Rana esculenta complex*).

## DISCUSSION

Only 7 species of amphibians were recorded in all 16 transects inside Zagożdżonka river catchment. One may expect more out of 12 lowland amphibians species occurring in Poland.

TABLE 1. Habitat description of investigated transects within Zagożdżonka catchment

Number	Name of transect	Water habitat	Terrestrial habitat
1	Pionki Staw Górny	17.5 ha water reservoir. 50% of shore is built with concrete. Used for angling. <i>Phragmites australis</i> , <i>Elodea canadensis</i>	Next to Pionki Town, surrounded by gardens, asphalt road, pastures.
2	Królewskie Źródła taras	Backwater of Zagożdżonka. Shallow water with dense vegetation: <i>Phragmites australis</i> , <i>Typha angustifolia</i> , <i>Lemna minor</i>	<i>Alnus glutinosa</i> forest, mixed pine-deciduous forest
3	Królewskie Źródła molo	Zagożdżonka river	<i>Alnus glutinosa</i> forest, mixed pine-deciduous forest, meadows. <i>Phragmites australis</i> , <i>Caltha palustris</i>
4	Królewskie Źródła rzeka	Zagożdżonka river	<i>Alnus glutinosa</i> forest, mixed pine-deciduous forest, meadows. <i>Phragmites australis</i> , <i>Caltha palustris</i>
5	Czarna jaz	Zagożdżonka river	<i>Salix caprea</i> , <i>Alnus glutinosa</i> , <i>Caltha palustris</i> . Meadows and pastures
6	Płachty oczka wodne	small ponds 0.1 ha, connected with river when high water level occurs. Shadowed by <i>Alnus</i>	Meadows and pastures, farm buildings
7	Płachty Mirenka	small river Mirenka (tributary to Zagożdżonka). Narrow with sandy bed.	Ruderal plants, meadows, bushes
8	Mirenka Most	small river Mirenka (tributary to Zagożdżonka). Narrow with sandy bed.	Ruderal plants, meadows, bushes, asphalt road, <i>Alnus</i> forest
9	Mirenka Sławek	small river Mirenka (tributary to Zagożdżonka). Narrow with sandy bed.	agricultural land, meadows, pastures
10	Sałki	Small pond inside the village (0.1 ha). <i>Lemna minor</i> , <i>Lysimacha nummularia</i>	Some trees, bushes, asphalt road, buildings
11	Staw Jagodny	13.6 ha shallow pond with peatbog	Close to Helenów village, forest and meadows
12	Helenów 2	0.95 ha pond with peatbog <i>Phragmites australis</i> , <i>Typha angustifolia</i> , <i>Lemna minor</i> , <i>Nymphaea alba</i>	close to farm buildings, surrounded by forest.
13	Kuczki	0.6 ha pond	close to Kuczki village, bushes and some trees, ruderal plants
14	Czarny Lasek	1 ha peatbog with variety of water plants	birch and pine forest
15	Ług Tczowski	25ha peatbog	<i>Sphagnopsida</i> , <i>Drosera rotundifolia</i> , <i>Eriophorum</i> , <i>Calla palustris</i> , <i>Sanguisorba officinalis</i>
16	Ług Tczowski las	0.7 ha shallow pond	mixed pine-deciduous forest

TABLE 2. Occurrence of amphibian species along transects. I – 1-14 individuals, II – 15-49 individuals, III – more than 50 individuals

Number	Name of transect	Species							Total number of species
		<i>Rana esculenta</i> complex	<i>Rana temporaria</i>	<i>Rana arvalis</i>	<i>Bufo bufo</i>	<i>Hyla arborea</i>	<i>Triturus vulgaris</i>	<i>Triturus cristatus</i>	
1	Pionki Staw Górny	I							1
2	Królewskie Źródła taras	I	III	III	I				4
3	Królewskie Źródła moło		I	I	I				3
4	Królewskie Źródła rzeka		I	I	I				3
5	Czarna jaz	I							1
6	Plachty oczka wodne	I	I	I					3
7	Plachty Mirenka	II	I	I					3
8	Mirenka Most	I	I		I				3
9	Mirenka Sławek	I	II						2
10	Sałki	II	I		I	I		I	5
11	Staw Jagodny	III				I			2
12	Helenów 2	III	I			I	I		4
13	Kuczki	III				I		I	3
14	Czarny Lasek	II	I					I	3
15	Ług Tczowski	III	I	I				I	4
16	Ług Tczowski las	II							1
Number of transect where species occurred		14	11	6	5	4	1	4	

Dabrowski and Struzynski (2006) report four more species from neighboring area of Kozienski Landscape Park (*Bombina bombina*, *Bufo viridis*, *B. calamita* and *Pelobates fuscus*). The lack of those species at transects we investigated is caused by their different habitat requirements. Our work did not cover ponds surrounded by sandy slopes covered by vegetation. The fire bellied toad (*B. bombina*) was expected to live at least in some of investigated transects (e.g. transect nr 11 – Staw Jagodny, transect nr 13 Kuczki, transect nr 15 – Ług Tczowski). The character of water bodies (rather shallow with a lot of water plants) suggests right

habitat for this species. Probably more field observations should be done to record this species or population density is very low, what causes difficulties in observations. All other recorded species can be considered as common species for central part of Poland (Juszczak 1987). Green frogs (*Rana esculenta* complex) were recorded at almost every transect (14 of 16), brown frogs (*R. temporaria* and *R. arvalis*) respectively at 11 and 6 transects and common toad (*Bufo bufo*) were recorded in 5 transects. Green tree frog *Hyla arborea* and great crested newt *Triturus cristatus* were recorded at 4 transects. Common newt



(*Triturus vulgaris*) was observed only at one investigated transect. The presence of great crested newt at 4 transects shows good habitat conditions for this species. It is very important, because this species is listed in Annex II of Habitat Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and wild fauna and flora) and needs to be protected by Special Areas of Conservation within Natura 2000 network. The breeding of this species was recorded in rather small ponds (0,1ha to 1ha) and one observation was made in 25 ha pond (transect nr 15). Great crested newt requires special terrestrial habitat features: it was observed only at transects close to forest or at least bushes (transects nr 10 and 13-15). This species was recorded by the use of eggs observation and netting. The variety of habitats existing in the Zagożdżonka catchment seem to fulfill all the amphibians needs in terms of water and terrestrial habitat. More research should be done in this area to describe the potential influence of humans on this group of animals.

## CONCLUSIONS

- Zagożdżonka river catchment creates suitable habitat conditions for majority of lowland amphibians species occurring in Poland.
- The most numerous and common species were *Rana esculenta complex* and *Rana temporaria*.
- There are suitable habitats for *Triturus cristatus* – species endangered in Europe. In Zagożdżonka catchment this species breeds in small pond surrounded by trees and bushes, preferably close to forest areas.

- The highest number of species was observed in small ponds with extensive agricultural or forest surrounding.
- The studies on amphibians habitat preferences in Zagożdżonka catchment should be continued to find more relationship with human activities, like agriculture intensification or house and roads investments.

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- Streszczenie:** *Preferencje siedliskowe płazów w rejonie odłowu rzeki Zagożdżonka.* Przedmiotem badań były preferencje siedliskowe płazów w typowej zlewni małej nizinnej rzeki, jaką jest lewy dopływ Wisły – Zagożdżonka, która płynie w bezpośredniej bliskości Kozienickiego Parku Krajobrazowego. Badano występowanie płazów

w różnego rodzaju ciekach i zbiornikach wodnych na terenie zlewni w zależności od siedlisk wodnych i lądowych. Ogółem obserwowano płazy na 16 transektach. Stwierdzono występowanie 7 gatunków płazów, pospolitych na nizinach. Większym bogactwem gatunkowym wykazują się zbiorniki z wodą stojącą, o niewielkiej powierzchni. Najpospolitsze gatunki, które występowały w zdecydowanej większości badanych siedlisk to żaba wodna (*Rana esculenta complex*) i żaba trawna (*Rana temporaria*). Stwierdzono występowanie w 4 miejscach traszki grzebieniastej (*Tritu-*

*rus cristatus*), gatunku zagrożonego wyginięciem w skali Europy. Autorzy sugerują dalsze obserwacje płazów na tym terenie w celu określenia na ich populacje wpływu działalności człowieka.

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## Characteristic of population and working of earthdogs in Poland in 2001–2007

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**Abstract:** *Characteristic of population and working of earthdogs in Poland in 2001–2007.* The aim of this paper is to describe the population and working abilities of earthdogs in Poland during 7 years period: from 2001 to 2007. In Poland 15 breeds are considered as earthdogs: 9 Dachshunds and 6 Terriers. Data concerning number of registered dogs of each breed, reproductive characteristics and the results of field trials for earthdogs were analyzed. The most popular breed among all earthdogs is Dachshund wire hair standard and among all Terriers: German Terrier. The population of all Dachshunds breeds was decreasing from year to year. Terrier population was increasing slightly, mainly due to huge interest in Jack Russell Terrier. Both trends are significant. Other Terriers seem to have stable populations. The most numerous breeds have also the highest reproductive characteristics. The highest number of litters and puppies born was observed for German Terrier and Dachshund wire hair standard. Each year more Dachshunds than Terriers participated in earthdogs field trials and obtained better grades. The use of earthdogs should be promoted as a useful tool to reduce the fox population in Poland.

*Key words:* earthdogs, working dogs, terrier, dachshund.

### INTRODUCTION

Earthdogs is the group of breeds that are used in hunting predators digging burrows, like fox, badger and lately raccoon

dog. The tradition of breeding and working with hunting dogs goes far back in Polish history (Redlicki 2003). During last decade the huge increase of fox population can be observed in Poland. This seems to be a major threat for small game populations. Using earthdogs while hunting fox is very helpful to achieve significant reduction of its population (Kamieniarz and Bresiński 2000). To value the hunting ability of earthdogs the field trials are organized, where dogs work on so called “artificial burrow” (Brabletz 2005).

### MATERIALS AND METHODS

This paper describes the population of earthdogs in Poland during 7 years period: from 2001 to 2007. Data concerning number of registered dogs of each breed were obtained from Polish Kennel Club and analyzed. Also working abilities were describe on the base of data concerning results of field trials for earthdogs obtained from Main Board of Polish Hunting Association. The analysis concerns period from 2001 to 2006, because in 2007 the regulations on field trials changed, therefore results were not parallel. The data were basically compared for all Terriers

breeds together and all Dachshunds breeds together. In many characteristics also certain breeds were taken into account. Two trends were analyzed:

- population trend in terrier group (number of registered individuals per year),
- trends in breeding performance (number of litters per year).

To check the statistical significance of trend the Chi square test was performed (Ruszczyk 1980).

## RESULTS

During the analyzed period the population of all earthdogs breeds registered by Polish Kennel Club (ZKWP) was decreasing in individuals number from year to year (Table 1). In 2001 there was 5339

dogs registered and after six years there was 3944 dogs registered. This trend was observed for almost all breeds despite their quantity. The exceptions are Parson Russell Terrier and Jack Russell Terrier – those populations were increasing rapidly – from 64 individuals in 2001 to 129 individuals in 2007 and from 2 individuals in 2001 to 297 individuals in 2007 respectively. Also slight increase in number of individuals was observed in population of Dachshund “rabbit” both wire and smooth hair. The total number of all terrier breeds was increasing from 2001 to 2007 and total number of Dachshunds was decreasing (from 3766 individuals in 2001 to 2225 individuals in 2007). Those trends proved to be highly significant.

The most numerous breed was Dachshund wire hair standard (with mean of 962

TABLE 1. Number of earthdogs individuals registered by Polish Kennel Club in following years ( $P \leq 0.01$ )

Breed\year	2001	2002	2003	2004	2005	2006	2007	Mean
German Terrier	497	472	419	457	469	454	424	456
Border terrier	3	8	11	20	28	38	46	22
Fox Terrier smooth	301	303	277	268	299	281	285	288
Fox Terrier wire	321	316	284	239	281	236	234	273
Parson Russell Terrier	64	75	65	82	92	110	129	88
Welsh Terrier	385	329	377	345	423	365	354	377
Jack Russell Terrier	2	25	69	107	157	237	297	128
<b>Terriers total</b>	<b>1573</b>	<b>1528</b>	<b>1502</b>	<b>1518</b>	<b>1749</b>	<b>1721</b>	<b>1769</b>	<b>1632</b>
Dachshund long hair „rabbit”	315	323	257	215	199	156	167	233
Dachshund long hair miniature	593	617	603	553	525	379	338	515
Dachshund long hair standard	402	266	194	258	244	155	125	235
Dachshund smooth hair „rabbit”	30	48	41	35	39	45	46	41
Dachshund smooth hair miniature	310	275	219	233	199	154	131	217
Dachshund smooth hair standard	577	637	557	504	431	367	358	490
Dachshund wire hair „rabbit”	85	82	124	99	95	104	114	100
Dachshund wire hair miniature	227	257	206	238	248	229	222	232
Dachshund wire hair standard	1227	1162	1026	967	846	782	724	962
<b>Dachshunds total</b>	<b>3766</b>	<b>3667</b>	<b>3227</b>	<b>3102</b>	<b>2826</b>	<b>2371</b>	<b>2225</b>	<b>3026</b>
<b>All breeds total</b>	<b>5339</b>	<b>5195</b>	<b>4729</b>	<b>4620</b>	<b>4575</b>	<b>4092</b>	<b>3994</b>	<b>4649</b>

individuals registered per year). Its population decreased rapidly during seven years and in 2007 was almost twice lower than in 2001. Among terriers the highest number of registered individuals was noted for German Terrier population with average of 456 dogs per year and slight decrease during seven years. The same process was observed for Fox terriers (both smooth and wire) and Welsh Terrier.

No such differences were observed for breeding characteristics (Table 2). The number of litters during following years varied between breeds. For all terrier breeds together this characteristic was higher each year, and reached 207 litters in 2007, while in 2001 almost 50% less – 126 litters. For German Terrier, Fox Terrier Smooth and Welsh Terrier this number was rather stable with average

TABLE 2. Number of litters in following years of earthdogs breeds registered by Polish Kennel Club. A – mean numbers of litters per year, B – number of puppies born during whole observed period (2001–2007), C – mean number of puppies per litter, D – % of males born during whole observed period (2001–2007) ( $P \leq 0.01$ )

Breed/year	2001	2002	2003	2004	2005	2006	2007	Total	A	B	C	D
German Terrier	36	44	45	60	57	48	44	334	47.7	1413	4.2	49.71
Border terrier	0	1	1	2	2	5	5	16	2.3	72	4.5	42.14
Fox Terrier smooth	24	21	29	29	28	26	30	187	26.7	733	3.9	48.14
Fox Terrier wire	22	28	20	16	16	20	10	132	18.9	502	3.8	51.57
Parson Russell Terrier	8	5	11	8	9	9	18	68	9.7	301	4.4	56.86
Welsh Terrier	35	47	32	43	31	35	39	262	37.4	1122	4.3	51.71
Jack Russell Terrier	1	2	17	18	29	51	61	179	25.6	853	4.8	53.71
<b>Terriers total</b>	<b>126</b>	<b>148</b>	<b>155</b>	<b>176</b>	<b>172</b>	<b>194</b>	<b>207</b>	<b>1178</b>	<b>168.3</b>	<b>4996</b>	<b>4.2</b>	<b>50.55</b>
Dachshund long hair „rabbit”	28	21	12	17	12	14	14	118	16.9	336	2.8	50.43
Dachshund long hair miniature	46	40	42	43	44	40	39	294	42.0	1129	3.8	49.29
Dachshund long hair standard	15	13	13	14	11	6	14	86	12.3	380	4.4	46.71
Dachshund smooth hair „rabbit”	4	3	4	3	3	5	5	27	3.9	89	3.3	32.86
Dachshund smooth hair miniature	30	31	29	24	30	22	21	187	26.7	699	3.7	47.71
Dachshund smooth hair standard	44	29	32	31	31	26	31	224	32.0	1061	4.7	49.14
Dachshund wire hair „rabbit”	11	12	12	10	7	12	11	75	10.7	226	3.0	49.71
Dachshund wire hair miniature	30	32	41	32	22	29	24	210	30.0	706	3.4	45.57
Dachshund wire hair standard	98	81	68	80	59	68	62	516	73.7	2622	5.1	46.71
<b>Dachshunds total</b>	<b>306</b>	<b>262</b>	<b>253</b>	<b>254</b>	<b>219</b>	<b>222</b>	<b>221</b>	<b>1737</b>	<b>248.1</b>	<b>7248</b>	<b>4.2</b>	<b>46.46</b>
<b>All breeds total</b>	<b>432</b>	<b>410</b>	<b>408</b>	<b>430</b>	<b>391</b>	<b>416</b>	<b>428</b>	<b>2915</b>	<b>416.4</b>	<b>12244</b>	<b>4.2</b>	<b>48.3</b>

of 47, 26 and 37 litters respectively. Fox Terrier wire was the only terrier breed with decrease of number of litters in following years – from 22 litters in 2001 to 10 litters in 2007. The highest increase of number of litters was noted for Jack Russell Terrier – from 1 litter in 2001 to 61 litters in 2007. The highest mean number of litters per year among all terriers characterized German Terrier (47.7), the lowest was noted for Border Terrier (2.3). During the analyzed period 4996 terrier puppies were born with average 4.2 individuals per litter and secondary sex ratio 1:0.98. The mean number of puppies per litter was higher than 4 in all terrier breeds except Fox Terriers (smooth – 3.9 and wire – 3.8).

The same trend was observed for all Dachshund breeds in total. The total number of litters decreased from year to year and reached 221 in 2007, starting in 2001 with 306 litters. The total of 7248 puppies were born with average 4.2 individuals per litter and secondary sex ratio 1:0.87. The number of litters was rather stable for each breed. Slight decrease of this characteristic was observed for Dachshund wire hair standard (from

98 litters in 2001 to 62 litters in 2007), Dachshund smooth hair standard (from 44 litters in 2001 to 31 litters in 2007) and Dachshund long hair „rabbit” (from 28 litters in 2001 to 14 litters in 2007). The highest number of litters during all analyzed years was noted for Dachshund wire hair standard (516). For other three breeds it was more than 200: Dachshund wire hair miniature (210 – 30 litters per year), Dachshund smooth hair standard (224 – 32 litters per year) and Dachshund long hair miniature (294 – 42 litters per year). The less numerous was Dachshund smooth hair „rabbit” with only 27 litters (average 3,8 litters per year). For all Dachshund breeds there were slightly more males born, only for Dachshund smooth hair „rabbit” the secondary sex ratio was 1:2.05 (n = 89). The less numerous litters occurred in Dachshund long hair „rabbit” (average 2.8 puppies per litter), the most numerous litters were noted in Dachshund wire hair standard (average 5.1 puppies per litter).

Dachshunds were also more numerous than Terriers in earthdogs field trials (Fig. 1). The number of trials was rather constant during all years and varied from

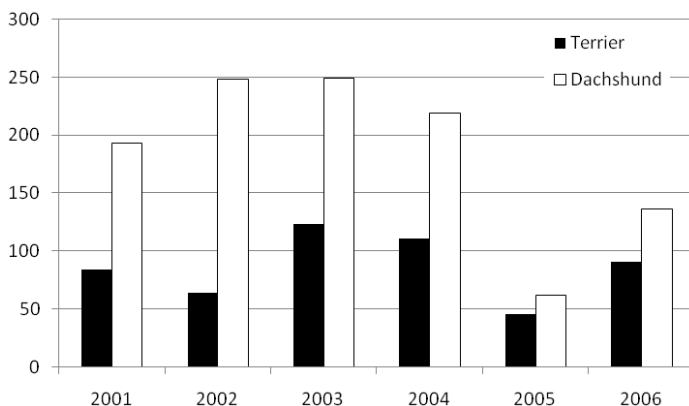


FIGURE 1. Number of Terriers and Dachshunds participated in earthdogs field trials in following years

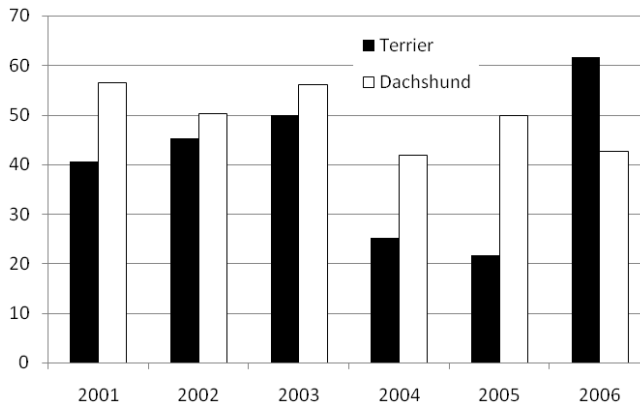


FIGURE 2. Percentage of dogs with 1<sup>st</sup> grade diploma of earthdogs field trials in all number of participating dogs in following years for Terriers and Dachshunds

10 in 2005 to 19 in 2003, with average of 15 per year. In each year there were more Dachshunds (1107 during 6 years) than Terriers (520) participating. During whole observed period the most numerous Terrier participating in earthdogs field trials was German Terrier – 375 individuals and among Dachshunds – the most numerous was wire hair type. In almost each year more Dachshunds obtained 1<sup>st</sup> grade diploma (as percentage of all participating Dachshunds) than Terriers (Fig. 2). The exception is 2006 when more than 60% of participating Terriers obtained this grade, comparing to 42% for Dachshunds.

## DISCUSSION

The data obtained from Polish Kennel Club show that the most popular breed among all earthdogs is Dachshund wire hair standard. This type has not only the best working abilities but also is concerned as a perfect pet animal. Due to its size it can be kept even in small apartments (Dembiniook and Brabletz 2003, Ściesiński 2003). Kramer (1996) descri-

bes Dachshunds wire hair as the most popular type, then long hair and smooth. If the size is concerned – the less numerous was “rabbit” type and the most numerous – standard type. The “rabbit” thou is the type that from year to year increases in number of registered individuals, in contrast to other Dachshunds that show the population decrease. The most popular in Terrier group (III group of FCI) among earthdogs was German Terrier. It is important to underline that due to its character and temperament this breed can only be kept as working dogs rather than pets in apartments. Its population was rather stable during observed period, as well as both Fox Terriers and Welsh Terrier. The distinct trend of increasing population can be observed in Border Terrier, Parson Russell Terrier and especially in Jack Russell Terrier. This last breed seems to become very popular among owners in Poland, but usually as pet animals rather than hunting dogs (Chwalibóg 2002). It is concerned as a family dog with force to different kinds of activities, like agility. All those trends concerning popularity are also visible in

reproductive characteristics. The number of Terriers participating in earthdogs field trials can be surprisingly lower than Dachshunds. The reason for this may be that many owners of Dachshunds don't hunt and participate in field trials for fun or to increase the value of their dog by obtaining working diploma. At the same time the majority of Terriers are working dogs, participating in real huntings. On the other hand there is a higher percentage of Dachshunds obtaining 1st grade diploma. This may be the result of participation of "new" breeds, like Jack and Parson Russell Terrier or Border Terrier that don't have working ancestors and therefore obtained poor grades and lower results for total Terriers group.

The situation of Polish hunting grounds is changing due to increased number of predators like badger and raccoon dog, with outstanding increase of fox population. This influences the decrease of populations of small game species like hare, rabbit, partridge or roe deer. The reduction of predators can be achieved by wider use of earthdogs during hunting (Kamieniarz and Bresiński 2000). This ensures the reduction of higher percentage of adults and females comparing to hunting without earthdogs. This important role of earthdogs should be popularized among hunters mainly dealing with arable lands hunting grounds.

## CONCLUSIONS

1. There were differences in number of registered by Polish Kennel Club earthdogs breeds. The most numerous among Terriers was German Terrier and among Dachshunds – Dachshund wire hair standard.

2. The population of all Dachshunds breeds was decreasing from year to year. Terrier population was increasing slightly, mainly due to huge interest in Jack Russell Terrier. The same trends were noted for number of litters for both groups. This trends are highly significant. Other Terriers seem to have stable populations.

3. The most numerous breeds have also the highest reproductive characteristics. The highest number of litters and puppies born was observed for German Terrier and Dachshund wire hair standard.

4. Each year more Dachshunds than Terriers participated in earthdogs field trials. Dachshunds also obtained more 1<sup>st</sup> grade diplomas than Terriers

5. There is a need to analyze possible causes of observed trends in further research.

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**Streszczenie:** *Charakterystyka populacji i pracy polskich ras norowców w latach 2001–2007.* Celem pracy było scharakteryzowanie populacji i wyników konkursów polowych psów ras norujących w Polsce w latach 2001–2007. Do takich ras zaliczamy w Polsce 9 ras jamników i 6 ras terierów. W pracy przeanalizowano dane uzyskane ze Związku Kynologicznego w Polsce dotyczące zarejestrowanych psów i ich charakterystyk rodzonych oraz z Polskiego Związku Łowieckiego dotyczących wyników konkursów pracy psów norujących. Wśród jamników najpopularniejszą

rasą okazał się jamnik standardowy szorstkowłosa a wśród terierów terier niemiecki. Zaobserwowano stały, istotny statystycznie spadek populacji jamników oraz nieznaczny wzrost liczebnościowy wśród terierów. W tej drugiej grupie liczebność większości ras jest stabilna, na uwagę zasługuje bardzo dynamiczny wzrost populacji Jack Russell Teriera. Najwięcej miotów i najwięcej urodzonych szczeniąt obserwowano u najliczniejszych ras.

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## Entomopathogenic nematodes in agriculture – potential threat to protected beetle species

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**Abstract:** *Entomopathogenic nematodes in agriculture – potential threat to protected beetle species.* Entomopathogenic nematodes are the most important insect parasites, therefore they are used in the production of biopreparations. Application of nematode based biopreparations in biological methods of pest control has many positive and negative features, some of them, however, raise some doubts. Particularly doubtful features are the selectivity in colonising and killing potential hosts by nematodes. Apart from pests, the ecosystems to which entomophilous nematodes have been introduced are inhabited by beneficial insects and those that are legally protected. One of such species is the hermit beetle (*Osmoderma eremita*), very endangered and strictly protected species according to the Bern Convention, listed in the second and fourth appendix to the Habitat Directive and mentioned in many European Red Books e.g. in Red List of Nearly Extinct and Endangered Animals in Poland and in Polish Red Book of Animals. The marbled rose chafer *Protaetia lugubris*, in spite of its rarity it is not the protected species. Its habitat preferences are similar to those of the hermit beetle, which makes it potentially endangered in the same way as *O. eremita*.

*Key words:* entomopathogenic nematodes, biological plant protection, EPN, protected Coleoptera, *Osmoderma eremita*, *Protaetia lugubris*.

### INTRODUCTION

Many methods including most popular chemical insecticides are used to control

various insect plant pests. Chemicals are highly effective, kill the pests quickly but may possess toxic and mutagenic properties (Bauman 1996). At present, a tendency is observed of searching alternative ways of pest control (Pezowicz, Sandner 1983). Biological methods commonly considered safe for the environment are such alternative ways (Franz, Krieg 1975). Species of the families Steinernematidae and Heterorhabditidae (Rhabditida) called entomopathogenic nematodes (Brzeski, Sandner 1974; Poinar 1979) are important in controlling the density of insects. Entomopathogenic nematodes are the most important insect parasites, therefore they are used in the production of biopreparations. Application of nematode based biopreparations in biological methods of pest control has many positive and negative features (Gaugler 1988; Webster 1980), some of them, however, raise some doubts. Particularly doubtful features are the selectivity in colonising and killing potential hosts by nematodes. Apart from pests, the ecosystems to which entomophilous nematodes have been introduced are inhabited by beneficial insects and those that are legally protected. Beetles are the

best studied group of nematodes' hosts. As given in Poinar (1975), nearly 50% of papers on nematodes found in the class Insecta are devoted to the order Coleoptera. Various families are being infected (Luckmann, Poinar 2003) but particularly interesting cases are those concerning rare and protected species. Recognising their enemies and natural mortality would help to better evaluate their risk status.

One of such species is the hermit beetle (*Osmoderma eremita*), very endangered and strictly protected species according to the Bern Convention, listed in the second and fourth appendix to the Habitat Directive (Szwalko 2004) and mentioned in many European Red Books e.g. in Red List of Nearly Extinct and Endangered Animals in Poland (Pawłowski et al. 2002) and in Polish Red Book of Animals (Szwalko 2004). Since many years it has been strictly protected in our country. The hermit beetle *Osmoderma eremita* is a representative of the family *Scarabaeidae* inhabiting nearly all Europe. It achieves 40 mm of length and 2.5 g body mass (Stebnicka 1978; Hedin, Ranius 2002). Despite its widespread occurrence all over the continent it has never been reported as numerous species. In places of its occurrence it forms local island populations associated mainly with deciduous and mixed tree stands (Ranius 2000). Polish population of the hermit beetle is estimated at several thousand individuals (Szwalko 1992), recent studies, however, have suggested that it might be more numerous (Oleksa et al. 2003; Szwalko 2004).

The marbled rose chafer *Protaetia lugubris* is smaller (17–24 mm) than the former beetle. In spite of its rarity it is not the protected species. Its habitat preferences

are similar to those of the hermit beetle (both species are often reported from the same feeding grounds) which makes it potentially endangered in the same way as *O. eremita*.

## MATERIAL AND METHODS

Thirty five larvae in the L3 growth stage and 20 coccolites of *O. eremita* and *P. lugubris* were collected in May 2005 from south-western part of the Cedynia Landscape Park within the Mieszkowickie Forests complex (UTM: VU54). Most of those that had fallen out from cut-off trunks of the locust tree were already dead – dried in the sun or killed by mites and ants. Touchwood from the surrounding and from tree hollows in the locust trees was also collected to serve as a substrate for culture. From this material 10 imagines of *O. eremita* and 7 of *P. lugubris* were obtained and released in the place of their finding (Kucharski, Żmihorski 2006). Part of the beetles laid eggs before release since several dozen new larvae were found in the rotten wood. These were further cultured in the same material.

## RESULTS AND DISCUSSION

The larvae showed unexpectedly high mortality which was quite a riddle. Most died in the L3 stage or at the stage of pupae. Finally, only 2 imagines of *O. eremita* and 10 of *P. lugubris* were obtained from this generation. The hermit beetles died not later than a week after pupation, before, however, they had normally taken food, defecated and maintained daily cycle of activity. Adult individuals of *P. lugubris* died not earlier than after 3–6 months having reproduced before.

Dissection of dead individuals showed the reason of their high mortality. In all cases a large number of entomopathogenic nematodes that filled the whole body cavity of the beetles were observed. From two dissected females of the hermit beetle 1766 and 880 nematodes *Steinernema feltiae* (Fig. 1) were obtained, respectively. From one female of the marbled rose chafer 2305 nematodes of the same species were obtained. Part of these nematodes was preserved as an inoculum for further cultures. No *Steinernema* were found in 3 dead larvae collected directly in the field in 2005. The larvae probably died of desiccation.

Reported observation is one of a few in which nematode species could be identified attacking both rare species from the family Scarabaeidae. Ranius in his paper (2005) mentioned a possibility of killing the hermit beetle by entomopathogenic nematodes. Nematodes, despite their wide range of hosts, can only sporadically be found in insects infected in their

natural habitats. The main reason of such situation is rapid growth cycle of nematodes in their host and fast decomposition of their prey's body. From among many species of Insecta susceptible to nematodes, natural infections were found only several times e.g. in species such as: *Zabrus tenebrioides*, (Coleoptera: Carabidae) (Poinar 1979), *Agriotes lineatus*, (Coleoptera: Elateridae) (Poinar, Veremcuk 1970), *Melolontha hippocastani* and *M. melolontha*, (Coleoptera: Melolonthidae) (Poinar et al. 1971), *Heliothis punctigera*, (Lepidoptera: Noctuidae) (Poinar 1979), *Helicoverpa zea* (Lepidoptera: Noctuidae) (Khan et al. 1976).

Observed nematodes may remain in the environment for years able to attack all growth stages of beetles (except for eggs). In the culture of *P. lugubris* and *Netocia metallica* (species related to *P. cuprea*) the parasite was kept for over three years (at its constant passaging) and retained high pathogenicity towards many representatives of Cetoniinae,

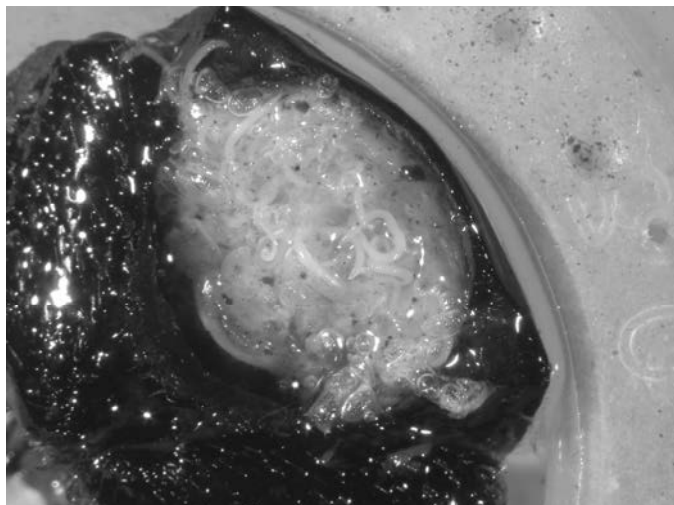


FIGURE 1. *S. feltiae* nematodes filling cavity of the thoracic part of hermit beetle (*O. eremita*)

Lucanidae, Tenebrionidae, Geotrupidae, Carabidae and even to spiders (Kucharska, Kucharski, unpublished). Having in mind rare occurrence of appropriate habitats for and limited dispersion of *O. eremita* (Hedin, Ranius 2002), colonisation of the same tree hollows by entomopathogenic nematodes may strongly limit local populations of the beetle. As shown in up-to-date observations, the presence of old trees with hollows of appropriate exposition to solar radiation does not itself guarantee survival of the hermit beetle in a given environmental patch. Noteworthy is also the possibility of attacking by nematodes other touchwood eating Scarabaeoidea including very rare and protected species like: *Lucanus cervus*, *Ceruchus chrysomelinus*, *Protaetia aeruginosa*. Nematoda may decrease survival of representatives of these species to a degree much larger than it is now expected. Further field observations and laboratory experiments are needed. Additional problem is that we know little on spreading and maintenance of introduced nematode species and on the possibility of their transfer to habitats colonised by rare and protected insect species. Timper et al. (1988) found a possibility of nematode transfer over large distances by infected butterflies *Spodoptera exigua*. In our culture infected imagines of the beetles lived for several days. Infection of new habitats sometimes several kilometres apart is thus quite possible.

## CONCLUSIONS

Application of nematode based biopreparations in biological methods of pest control can be potential threat and one has to consider limitation of the use of

biopreparations in areas close to habitats valuable for nature protection (e.g. parks with old tree stands with hollows). This is worthy of special attention in view of the more common use of biopreparations in the national agriculture.

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**Streszczenie:** *Nicienie entomopatogeniczne w rolnictwie – potencjalne zagrożenie dla chronionych gatunków chrząszczy.* Nicienie entomopatogeniczne należą do najważniejszych pasożytów owadzych, dlatego też znalazły swoje zastosowanie w produkcji bioprepatów. Stosowanie biopreparatów na bazie nicieni w metodach biologicznych posiada szereg cech pozytywnych i negatywnych, jednak niektóre cechy budzą zastrzeżenia. Szczególnie wątpliwymi są wybiórczość i selektywność zasiedlania i zabijania potencjalnego żywiciela przez nicienie. W ekosystemach do których wprowadzono nicienie entomofilne, oprócz szkodników, zagrożone są również owady pożyteczne oraz te objęte ochroną gatunkową. Jednym z gatunków zarażanych przez nicienie jest pachnica dębowa (*Osmoderma eremita*), gatunek o kategorii „bardzo zagrożony i ściśle chroniony” wg Konwencji Berneńskiej, wpisany do drugiego i czwartego załącznika Dyrektywy Habitatowej oraz wymieniany w wielu europejskich Czerwonych Księgach. Podobnie dzieje się z węgą marmurkową (*Protaetia lugubris*) o preferencjach siedliskowych bardzo podobnych do tych wybieranych przez pachnicę, co czyni ją również zagrożoną.

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## Restocking of the narrow-clawed crayfish (*Astacus leptodactylus* Esch.) in central-eastern Poland

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**Abstract:** *Restocking of the narrow-clawed crayfish (Astacus leptodactylus Esch.) in central-eastern Poland.* The programme of restocking the narrow-clawed crayfish is directly connected with the rapidly progressing extinction of native crayfish species in Poland. No more than a dozen or so sites of this species remained in central-eastern Poland till the mid-ninetieth of the 20th century. Therefore, at the beginning of the 21st century a programme of the restitution of the narrow-clawed crayfish was set up. It consisted in hatching and rearing the crayfish in artificial conditions. Studies connected with reconstructing the stock of crayfish were conducted in several stages and consisted in making an inventory of waters useful for living of native crayfish. Waters devoid of American crayfish were taken into consideration. Water was characterized by optimal parameters for crayfish; potential hazards associated with water pollution were not identified. Altogether narrow-clawed crayfish were let out to 11 bodies of water located in 5 provinces. Several years later the success of reintroduction was tested to demonstrate adaptation of crayfish to new biotopes. Crayfishes were found in 7 out of 11 restocked water bodies. Works are continued.

*Key words:* *Astacus leptodactylus*, restocking, native crayfish, Poland.

### INTRODUCTION

Narrow-clawed crayfish (*Astacus leptodactylus*), belongs to biggest freshwater invertebrates in Poland. Stagnant waters – lakes, ponds, clay pits or dam reservoirs – are typical biotopes of this species. It

avoids flowing waters. Together with the noble crayfish it is ranked among our native species. However, as a result of infection of European waters by aquatic fungi *Aphanomyces astaci* (crayfish plague) (Holdich 1988), reclamations, intensification of agriculture, industrial pollution and supplanting by American crayfish it became a rare species occasionally noted in inland waters of Poland. An inventory made at the end of the 20th century demonstrated only several sites of the narrow-clawed crayfish in central-eastern Poland (Strużyński, Śmietana 1999; Strużyński et al., 2001; Śmietana et al., 2004). The same was true for other regions of Poland (Krzywosz et al., 1994; Białokoz et al., 1996; Gielo 1999). Therefore, the programme of the reconstruction of the narrow-clawed crayfish stock was set up in selected, small water bodies in the central-eastern part of Poland. It is realised through reintroductions of crayfish to selected waters and breeding the hatch of crayfish in controlled conditions (Strużyński, Niemiec 2001; Strużyński 2007). Similar attempts of crayfish breeding or intense programmes of the reconstruction of crayfish stock were also conducted in other parts of Poland (Pyka 1999; Mastyński et al., 2001; Śmietana et al. 2004; Mastyński, Andrzejewski 2005).

## MATERIAL AND METHODS

Works have been carried out since 1999 in Lublin, Łódź, Mazovian, Podlasie provinces and in Świętokrzyski region. The first stage consisted in surveying waters of central-eastern Poland for the presence of crayfish. In the second stage waters intended for reintroduction of the narrow-clawed crayfish (*Astacus leptodactylus*) were selected. Selected waters should meet the following criteria: a lack of crayfish of American origin – of striped crayfish (*Orconectes limosus*) and of signal crayfish (*Pacifastacus leniusculus*), the presence of invertebrates indicative of high water quality and a lack of potential sources of pollution (intensive farming or local industry) in the catchment basin. Altogether 11 sites whose parameters answered the adopted criteria were selected. Selected water bodies were used for different purposes. They included ponds built on watercourses and used for angling, ponds situated in parks and such constituting the element of landscape architecture. Small mid-forest restored water bodies managed by the State Forest Administration were also used for reintroduction. All water bodies intended for reintroduction were stocked with marsh crayfish coming from one site in Janowskie Forests. The crayfish were caught in the years 1999–2004 according to the agreement with the Polish Angling Association which is the user of these waters. Crayfish were caught with special traps with fish meat used as bait. To catch live crayfish the traps were set every year since August till the half of October. Crayfish caught alive were subjected to preliminary selection according to norms concerning material

of farm crayfish in the category – stock crayfish. The sex ratio was about 2:1 of males to females. Crayfish caught alive were transported without water in punnets meant for crayfish. The transport of crayfish from places of their live catching to sites of reintroduction was done in the evening and depending on distance it didn't last more than 5 hours. In the place of the reintroduction crayfish were let out individually by repeated immersing in water to fulfill „gill chamber”. Otherwise crayfish would have died as a result of suffocation.

Establishing and leading the hatchery together with rearing the hatched crayfish was the next stage of the programme. Crayfish hatchery and nursery functioned in a recirculation system equipped with the biological-mechanical system of filtration, aeration of water and temperature control with highly efficient system of air conditioners. The hatch of crayfish obtained in artificial conditions was reared for 4–8 weeks with the destiny to stock previously selected water bodies. In the case of the narrow-clawed crayfish the optimum place of its reintroduction was small dam reservoirs in forest complexes, park ponds or gravel/clay pits. Young crayfish were transported in 50 litre containers filled with water and places of their release were distant about 50–80 km from the nursery. Transport of crayfish didn't last more than 1.5 hours which ensured good condition of transported animals.

## RESULTS

As a result of works carried out during the first inventory stage, only 1 site of the narrow-clawed crayfish in Janowskie

Forests was found. This site (a recreational water body near Janów Lubelski) was a place, from where crayfish were recruited for their reintroduction in different areas of central-eastern Poland till 2004. Within the second stage of the programme 11 sites were restocked (Table 1). Crayfish were released to two dam reservoirs near Koprzywnica and Klimontów in the Świętokrzyskie region. In Mazovian province altogether 6 water bodies were restocked: 3 mid-forest lakes in the forest district Garwolin, one in the grounds of the Forestry Commission Kozienice, one former gravel pit near Grójec and one pond in Okuniew on the land of allotments. In Łódź province crayfish were let out into one water body situated in Spalski Forest – a dam reservoir on the Gać River. The last two places of crayfish reintroduction were ponds located in palace parks in Drozdowo and Ciechanowiec (Podlasie province). Hatchery and rearing of the narrow-clawed crayfish were intensified after 2005 which brought about 7000 young crayfish released later into dam reservoirs in the forest districts Garwolin and Kozienice. A survey performed in the year 2006 demonstrated adaptation of restocked crayfish in 7 water bodies

(Fig. 1). To protect reintroduced material the precise location of selected sites is not given in this paper.

## DISCUSSION

Apart from already mentioned criteria of selecting waters for crayfish reintroduction one should take into account the way of utilisation of a water body. In many cases waters are intensively used for fishery or angling which reduces the chance for development of reintroduced crayfish. Intense stocking with fry of predatory species like Wels catfish (*Silurus glanis*), perch (*Perca fluviatilis*) or unintended stocking with fry of a strange species (e.g. brown bullhead catfish – *Ictalurus nebulosus*) leads first to the limitation of the number and finally to the disappearance of native crayfish populations. All authors carrying studies on native species of crayfish are of the opinion that reintroduction is a chance of reconstructing their population and call for intensification of breeding in controlled conditions (Girsztowtt 1995; Girsztowtt and Jakucewicz 1995; Strużyński and Niemiec 2001; Strużyński 2001; Śmietana et al. 2004). Demonstrating the presence of the narrow-clawed crayfish

TABLE 1. Summary of the restocking program of *Astacus leptodactylus* in central -eastern Poland

No	Name/lokalization of water	Stocking effects + or -
1.	Pond in Drozdowo near Łomży	-
2.	Dam Reservoir in Ciechanowiec	+
3.	Pond in Okuniew near Sulejówek	-
4.	Dam reservoir in Spała	-
5.	Former gravel pit near Grójec	+
6–8.	Forest lake in the forest district Garwolin	+, +, +
9.	Pond of the Forestry Commission Kozienice	-
10.	Dam reservoir in Koprzywnica near Sandomierz	+
11.	Dam reservoir in Klimontów near Sandomierz	+

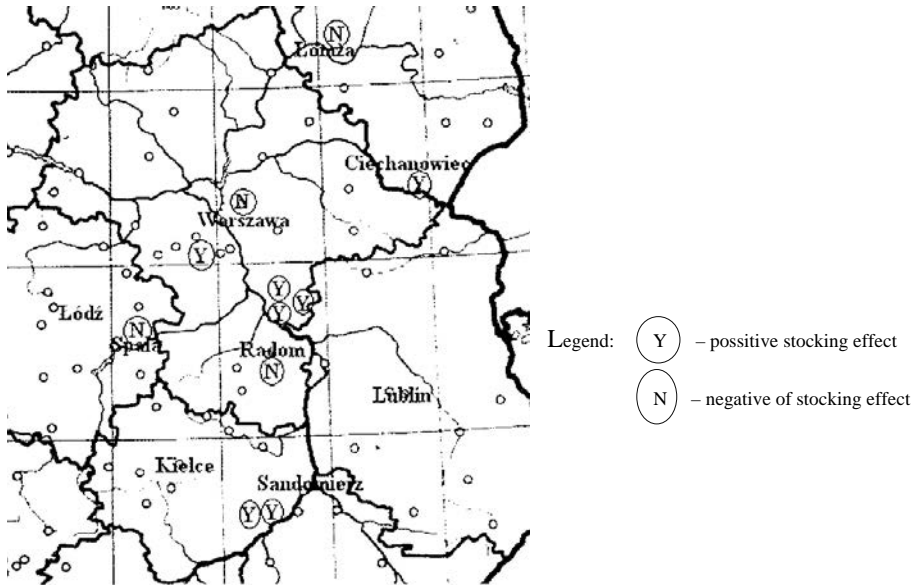


FIGURE 1. Restocking sites of the *Astacus leptodactylus*

in 7 out of 11 sites allows for concluding that works on restoration of this species should be continued and that there is a chance of reconstruction of the stock of native crayfish species.

## CONCLUSIONS

Based on obtained results one may expect that there is a possibility of creating „dens” of narrow-clawed crayfish intended for next local reintroductions. The results showed also that an ill-considered fishing economy could be a factor threatening rebuilt populations of the narrow-clawed crayfish. At the same time they indicate that small mid-forest water bodies originally meant as water retention reservoirs may very well play a role of habitats for native crayfish with the destiny to restock next, closely located bodies of water. For the purpose of further reconstruction of the marsh crayfish stock one should en-

large the network of small ponds with parameters optimal for crayfish. These water bodies should serve for controlled breeding of this species.

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**Streszczenie:** Odbudowa pogłowia raka błotnego (*Astacus leptodactylus*) w środkowo-wschodniej Polsce. Program odbudowy pogłowia raka błotnego w Polsce środkowo-wschodniej realizowany jest od 1999 roku. Rak błotny należy do rodzimych gatunków w wodach śródlądowych Polski. Prace prowadzone są w kilku etapach. Pierwszy dotyczył zinventaryzowania wód pod kątem występowania raków amerykańskich. Etap drugi polegał na typowaniu wód, optymalnych do reintrodukcji raka błotnego. Kryteriami wyboru wód był: brak raków amerykańskich, występowanie bezkręgowców wodnych charakterystycznych dla wód czystych lub mało zanieczyszczonych oraz brak w obrębie zlewni potencjalnych źródeł zanieczyszczenia. Równolegle z wyborem wód, rozbudowano wylęgarnie i podchowalnie przeznaczoną do uzyskiwania młodych raków błotnych, które po krótkim podchowiu wypuszczano do wybranych zbiorników wodnych. Łącznie wytypowano 11 stanowisk, do których wypuszczono raki błotne. Po upływie kilku lat rozpoczęto odłowu kontrolne, których celem była ocena efektywności prac. Raki wykazano w 7 zbiornikach wodnych.

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## The occurrence of the narrow-mouthed whorl snail (*Vertigo angustior*) in planned Natura 2000 sites in Masovian Province

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**Abstract:** *The occurrence of the narrow-mouthed whorl snail (Vertigo angustior) in planned Natura 2000 sites in Masovian Province. Vertigo angustior* is one of the smallest snails in Poland. Listed in the Annexe II to the Habitat Directive of the European Union it is a species subjected to particular forms of protection and its presence is an evidence of high environmental stability. The occurrence of this species in Masovian Lowland has not been checked since 1939. For this reason and in accordance with recommendations concerning new sites of Natura 2000 a study was undertaken which demonstrated 4 sites of *Vertigo angustior*.

**Key words:** *Vertigo angustior*, snails, Gastropoda, Masovian Lowland, Natura 2000.

### INTRODUCTION

Knowledge of the distribution in Poland of mollusks listed in the Annexe II of the Habitat Directive is insufficient. It is assumed that these species directly or indirectly associated with aquatic habitats and subjected to heavy human impact are, together with their habitats, strongly limited. Limitation is due to many threats associated with water pollution, disturbed water relations and water investments. Many mollusks living in Poland belong to common species. Some of them, however, are legally protected by the country law or by Habitat Directive. Therefore, 5 species of Polish snails and 2 species of bivalves are on the list of Natura 2000 and

mentioned in the Order of the Minister of Environment of 28<sup>th</sup> September 2004.

Snails of the family Vertiginidae are represented in Poland by 16 species belonging to the genus *Columella* (3 species), *Truncatellina* (3 species) and *Vertigo* (10 species) (Pokryszko 1990, Myzyk 2004, Wiktor 2004). Only four of these species namely (codes acc. to the II Annexe are given in brackets) *Vertigo geyeri* (1013), *Vertigo angustior* (1014), *Vertigo genesii* (1015) and *Vertigo moulinsiana* (1016) are protected by the Habitat Directive. *Vertigo angustior* is one of the smallest terrestrial snails in Poland; its presence on Masovian Lowland was not verified since 1939. The species is an important and valuable element of European fauna listed in the Annexe II of the Habitat Directive.

### MATERIAL AND METHODS

Studies were carried out in the area managed by the Regional Directory of State Forests in Warsaw and within planned sites of Natura 2000 network in Masovian Province. Material was collected and snails were caught from August till November in the years 2007 and 2008. In summer the population density of snails increases which gives a chance of obtaining better success in snail collec-

tion. Various methods of snail gathering were used depending on atmospheric conditions. Field collection consisted in picking up single individuals grazing on sedge and reed shoots. Snails were also collected under the canopy of alder wood by picking the snails from the last-year leaf fallout. Apart from direct collection of snails the samples of substratum and vegetation were also collected. They were later sorted in the lab. Collected snail shells were subjected to morphological assessment which helped to identify individuals to species using the description of morphological shell features given by Urbański (1957), Pokryszko (1990) and Wiktor (2004). Stereomicroscope Nikon SMZ1000 which allowed

photographic documentation and display of diagnostic features on a computer monitor was used during identification.

## RESULTS

As a result of studies carried out in 14 Forestry Commissions and 17 planned sites of Natura 2000 the narrow-mouthed whorl snail (*Vertigo angustior*) was found in four sites: in meadows of the central part of Kozienice Forest, in Podeblocie between Maciejowice and Trojanów, in Sołeckie Meadows near Piaseczno and in the Liwiec River valley between Paplin and Seklāk (Fig. 1). The sites had quite variable vegetation cover. Common element of these sites was a high water con-

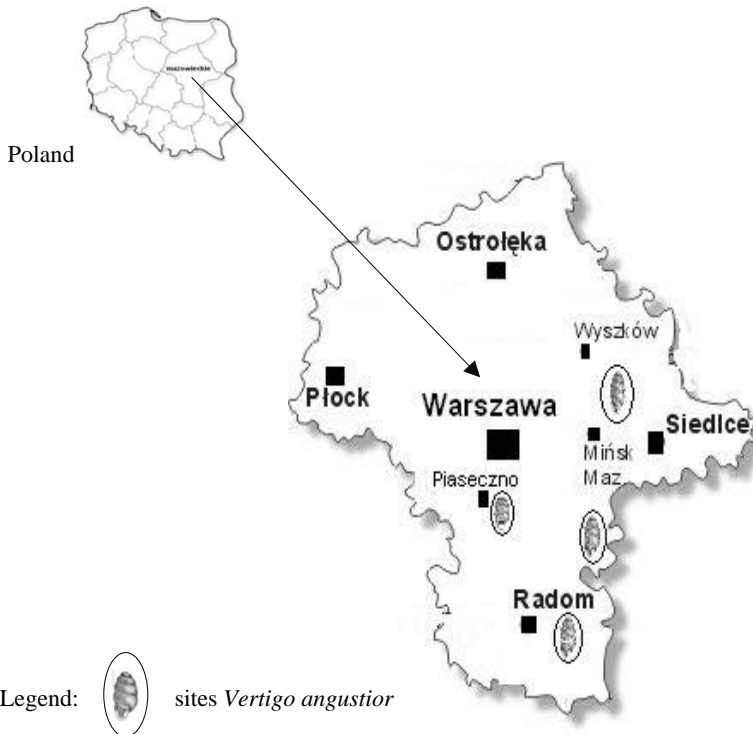


FIGURE 1. The sites of *Vertigo angustior* found in Masovian Province (an area of planned Natura 2000 sites)



tent with periodical spring flooding and extensive land use (as pastures or mown meadow). Snails during field observations were most often found on sedge shoots, on common reed and in places covered by the last-year tree leaves.

## DISCUSSION

None of the discussed sites was noted before as a habitat of *Vertigo angustior* though Jankowski (1939) found a site of this species in a manor park by the Okrzejka River (land estate Trojanów) situated 5 km from the site noted in this paper. Jankowski (1933) found also the same species near Piaseczno but did not give precisely the place of its finding. Sites of *Vertigo angustior* from Czerniaków, Drewnica and Zacisze given by Jankowski (1933) should also be considered historical. Unfortunately, Jankowski did not make a precise statement on these locations. Based on sites of other mollusk species reported in his paper one may guess that the site in Drewnica could be situated near ditches and peat holes, that in Czerniaków – near moat, pond or lake. For the vicinity of Zacisze Jankowski did not give precise location. It is highly probable that these sites are historical because they exercised a heavy urbanization for over 70 years. Barga-Więcławska (2008) mentioned a site of *Vertigo angustior* at the southern border of Masovian Province but, unfortunately, did not give its location.

## CONCLUSIONS

Based on obtained results one may conclude that *Vertigo angustior* is a very rare species characteristic for habitats of very

stable biotic composition. Therefore, only compact protected areas (natural reserves, habitat sites of Natura 2000) are able to support the described populations of *Vertigo angustior*.

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**Streszczenie:** Występowanie poczwarówki zwężonej (*Vertigo angustior*) w poanowanych ostojach Natura 2000 na Mazowszu. Poczwarówka zwężona (*Vertigo angustior*) należy do ślimaków lądowych z rodziny poczwarówkowatych (*Vertiginidae*). Jest ona jednym z najmniejszych ślimaków występujących w Polsce. W Europie uznana za gatunek rzadki i cenny przyrodniczo, z tego powodu została umieszczona w Załączniku II Dyrektywy Siedliskowej Unii Europejskiej. Siedliska, w obrębie których gatunek ten może występować, charakteryzują się bardzo stabil-

nym układem przyrodniczym. Tereny te są pod wpływem niskiej antropopresji co ma bezpośredni wpływ na utrzymywanie się populacji tego gatunku. Bardzo istotnym elementem jest stabilność stosunków wodnych, gdyż *Vertigo angustior* preferuje obszary okresowo podmokłe. Na obszarze województwa mazowieckiego ostatnie prace wykazujące ten gatunek były prowadzone w latach 30. XX wieku. Od tamtej pory z terenu Polski centralnej nie było jakichkolwiek danych dotyczących siedlisk poczwarówki zwężonej. Dopiero badania rozpoczęte i prowadzone w latach 2007–2009 pozwoliły na stwierdzenie tego gatunku na terenach planowanych ostoi Natura 2000 województwa mazowieckiego. W wyniku prac wykazano 4-stanowiska *Vertigo angustior* zloka-

lizowane: na Łąkach Soleckich pod Piasecznem w okolicach Warszawy, w okolicach wsi Molendy – centralna część Puszczy Kozienskiej, w Dolinie rzeki Liwiec pomiędzy wsią Seklak i Paplinem oraz w Podebłociu pomiędzy Maciejowicami i Trojanowem.

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## Evaluation of feeding value and yield of extensive pastures used in beef cattle grazing

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**Abstract:** *Evaluation of feeding value and yield of extensive pastures used in beef cattle grazing.*

Feeding value of green forage depends mostly on botanic composition of the pasture sward and stage of maturity of plants when the pasture is exploited. Delay in grazing and increase maturity of plants, results in their ageing – percentage of crude fiber, NDF and ADF increase, whilst crude protein and N-free extract decrease. The studies were conducted in the years 2006–2008 on two extensively exploited pastures, with different stocking rate, situated in the north-eastern Poland. The pastures were divided into four experimental areas on which the evaluation of the pasture yield, botanic composition of pasture green forage and feeding values was carried on. During pasture feeding (May – October) the samples of green forage were collected for examination of basic chemical composition and fiber fractions, analysis of botanic composition of the sward. Also, the evaluation of the pastures' yield was carried out. In the both farms, cows with calves were continuously grazed. The effect of vegetation period on participation of grasses and dicotyledonous plants in the sward was found. The yield of the pastures which was low (below 2 t DM/ha) was also changed together with the run of vegetation period. There was a relation of grasses and dicotyledonous with the vegetation period. Also the yield of the pastures was in relation to vegetation period. During all measurements, the forages were characterized by a high content of dry matter and crude fiber and by a low content of protein.

*Key words:* pasture, extensive grazing, beef cattle.

### INTRODUCTION

Pastures are often extensively exploited for beef production. It allows to reduce production costs, but lower yield of pastures and poorer feeding value of forage are achieved. However, extensive usage of pastures is helpful to protect many valuable species of plants and animals.

Feeding value of green forage depends mostly on botanic composition of the pasture sward and stage of maturity of plants when the pasture is exploited (Michna and Hadula, 1994; Kacperczyk and Kocorzyk, 1996). Delay in grazing and increase maturity of plants, results in their ageing – percentage of crude fiber, NDF and ADF increase, whilst crude protein and N-free extract decrease (Michna and Hadula, 1994; Mikołajczak, 1998; Gekara et al., 2001). Consequently intake and utilization of green forage by animals is lower.

Chemical composition and feeding value of green forage changes in time. At the beginning of pasture period there

is the higher content of crude protein and its gets lower in the following months. Amount of crude fiber changes the opposite way (Gekara et al., 2001).

Yield of pastures and their utilization by animals depends on many factors, such as grazing system, stocking rate, fertilization, botanic composition and environment condition (White and Wight, 1984; Ralphs et al., 1990).

## MATERIAL AND METHODS

The research was conducted in years 2006–2008 on the pastures in two farms specialized in beef cattle production. The farms are in north-east Poland: farm A near Dobre Miasto and farm B near Golgap. The pastures in the both of the farms were exploited extensively and cows with calves were continuously grazed. During the research the pastures were not fertilized and nor cultivated. There was different stocking rate in both farms: in farm A 0.83, in farm B 0.4 cows/ha of pasture.

The pastures in both farms were divided into four experimental areas. Evaluation of yield, botanic composition and nutritional value of sward was conducted through all the research years. In the period of grazing (May–October) the samples from experimental areas were analyzed four times (May, June/July, August and September/October) to check chemical composition and botanic composition of pasture sward (percentage of certain species in the sample). Sample of herbage were taken from five quadrates (1 m<sup>2</sup>) of each experimental area. Samples from each area were bulked and the collective samples was analyzed. Identification of individual plant families and individual

species was done using the standard botanical key (Szafer, 1959). The chemical composition (dry matter, crude protein, crude fiber, crude fat and crude ash) of the samples was defined according to standard methods AAOC (1990) and the ADF and NDF fiber fraction – Van Soesta et al. (1991). The evaluation of the pasture yield was done by Kostuch (1994) methods.

The influence of various factors on the results was analyses with SPSS software tools for Windows v. 12.0.

## RESULT AND DISCUSSION

**Botanic composition and yield of pastures.** Data from all the surveys is presented in the Table 1.

The grasses were the dominant species of the sward – over 80%. The percentage of legume was very low and increasing at the end of grazing season – up to 5%. The legumes are a good source of proteins, minerals and vitamins (Warda at al., 1996). Their low content in the sword were causing poor feeding value of green forage. Similar results, in a research on extensive pastures, were reported by Łozicki and Dymnicka (2003). Dobicki at al. (1999), after Mikołajczak, state that animals fully meet their nutrient requirements on the pastures with dominance of grasses with high nutritive value, and about 30% of another plants and legumes.

The analyses allowed to find significant differences in the botanic composition between the samples only in case of grasses and other species of dicotyledonous. The percentage of grasses was the highest in August while the other species of dicotyledonous was the lowest.

The examined pastures were characterized with low yield, which in any of

TABLE 1. Influence of vegetation period on botanic composition and yield of the pastures (n = 96)

Specification		Vegetation period			
		May	June/July	August	September/ October
Grasses (%) <i>Poaceae</i> (%)	LSM	84.30	83.76	90.45	83.13
	SE	1.95	1.95	1.95	1.95
	*	c	c	a,b,D	C
Complex Asteraceae (%) <i>Asteraceae</i> (%)	LSM	1.82	3.41	2.59	3.44
	SE	0.62	0.62	0.62	0.62
Legumes (%) <i>Fabaceae</i> (%)	LSM	2.48	3.87	1.63	5.17
	SE	1.29	1.29	1.29	1.29
Other species of dicotyledonous (%) <i>Dicotyledoneae</i> (%)	LSM	9.18	6.83	4.18	6.67
	SE	0.93	0.93	0.93	0.93
	*	C	c	A,b	
Horsetails (%) <i>Equisetaceae</i> (%)	LSM	0.97	0.42	0.03	0.06
	SE	0.27	0.27	0.27	0.27
Sedges and Rushes (%) <i>Cyperaceae et Juncaceae</i> (%)	LSM	1.25	1.72	1.12	1.54
	SE	0.81	0.81	0.81	0.81
Yield of pastures (t DM/ha)	LSM	1.17	1.71	1.53	0.99
	SE	0.14	0.14	0.14	0.14
	*	B	A,D	D	B,C

\*Differences at: a, ..., d –  $p \leq 0.05$ ; A, ..., D –  $p \leq 0.01$ .

the measurement did not achieved 2 tons per ha and was the lowest at the end of pasture period. The increase of yield from June to August was probably to be influenced by higher quantity of rain in that period.

The yield of pastures estimated in the research was lower than reported by Mikołajczak (1996) and Wasilewski (1993) for the pastures not fertilized with nitrogen.

The yield of pastures can be reduced also due to grazing system. Twardy (1996) and Prokopowicz and Wasilewski (1994) indicate that it is lower in case of continuous grazing than a paddock grazing.

The analysis of the data for the both researched farms proved no relation of botanic sword composition with the

farms and the research year (Table 2). There was a relation of grasses and other species dicotyledonous with the vegetation period. Also the yield was in relation to vegetation period.

The relation of the yield of pastures with the farm, proved in the research, resulted from different stocking rate in the both cases, as the forage intake varied. In the farm with lower stocking rate there was more undergrazed plants.

**Chemical composition of green forage.** Nutritive value of forage depends on its chemical composition. The data on chemical composition received from the surveys during the grazing period is presented in the Table 3.

All the surveys evidenced high content of dry matter and crude fiber, while the share of crude protein was low. These

TABLE 2. Influence of selected factors on botanic composition of sword (n = 96)

Specification	Factors			Interaction		X	S
	Farm	Year	Vegetation period.	Farm * year	Farm * vegetation period		
Grasses (%) <i>Poaceae</i> (%)	NS	NS	X	NS	X	85.41	0.97
Complex Asteraceae (%) <i>Asteraceae</i> (%)	NS	NS	NS	NS	X	2.81	0.31
Legumes (%) <i>Fabaceae</i> (%)	NS	NS	NS	NS	NS	3.29	0.65
Other species of dicotyledonous (%) <i>Dicotyledoneae</i> (%)	NS	NS	XX	NS	NS	6.71	0.46
Horsetails (%) <i>Equisetaceae</i> (%)	X	NS	NS	NS	NS	0.37	0.14
Sedges and Rushes (%) <i>Cyperaceae et Juncaceae</i> (%)	NS	NS	NS	NS	NS	1.40	0.41
Yield of pastures (tons dry matter/ha)	NS	NS	XX	X	X	1.35	0.07

Differences at: x –  $p \leq 0.05$ ; XX –  $p \leq 0.01$ ; NS – not significant.

TABLE 3. Influence of vegetation period on chemical composition of forage

Specification		Vegetation period			
		May	June/July	August	September/October
Dry matter (%)	LSM	25.26	28.67	24.06	27.43
	SE	1.39	1.39	1.39	1.39
Crude ash (%)	LSM	2.36	2.70	2.65	3.22
	SE	0.15	0.15	0.15	0.15
	*	D	d	D	A,b,C
Crude protein (%)	LSM	4.01	3.55	3.55	3.76
	SE	0.14	0.14	0.14	0.14
Ether extract (%)	LSM	0.77	0.73	0.67	0.73
	SE	0.03	0.03	0.03	0.03
Crude fiber (%)	LSM	6.14	8.09	6.84	7.37
	SE	0.49	0.49	0.49	0.49
	*	B	A		
N-free extract (%)	LSM	11.98	13.60	10.35	12.35
	SE	0.76	0.76	0.76	0.76
	*		C	B	
ADF (%)	LSM	8.83	10.70	9.60	10.13
	SE	0.63	0.63	0.63	0.63
NDF (%)	LSM	13.37	15.60	14.39	15.26
	SE	0.92	0.92	0.92	0.92
ADL (%)	LSM	1.73	2.06	1.84	1.95
	SE	0.13	0.13	0.13	0.13
	LSM	1.88	1.88		1.88
	SE	0.02	0.02	0.02	0.02

\*Differences at: a, ..., d –  $p \leq 0.05$ ; A, ..., D –  $p \leq 0.01$ .

parameters were worse than presented in tables of cattle feed (Normy, 2009; Table DLG, 1997). This indicates low nutritive value of the green forage.

High percentage of the dry mater and crude fiber increases the fill effect of the forage and restricts the intake. High percentage of crude fiber also reduces digestibility and utilization forage by animals. In case of nursing cows it causes lower milk production. Shortage of mother milk and low feeding value of green forage results in lower gaints of calves (Łozicki and Dymnicka, 2003).

Low content of crude protein in forage does not allow animals to intake them properly. Additionally, low level of the protein in feed reduces microorganism growth in a rumen and fiber digestion is worse (Bowman and Sanson, 1996).

During grazing periods changes in content of crude fiber and crude ash in the forage were observed. Content of other components and fiber fractions were at a similar level. The highest percentage

of crude fiber was at the turn of June and July. After dry period, many plants were dried up, and thus content of dry mater and fiber in the forage were increased.

Chemical composition was proved in the research to be in relationship with farm factors, year and vegetation period (Table 4). Influence of the farm particularities might result from different stocking rate, in the farm with the lower, the plants were undergrazed and herbage remained were drying up and was not desirable for the animals. As well, re-grow of the sword was reduced. Consequently chemical composition of the forage was less favorable. Sectors undergrazed at the beginning of the grazing period were lateron of lower feeding value, as well the animals unpreferred them. Resulting, yield of the pasture was decreased and effectiveness lowered utilization of the pasture by animals. It can point, that at the lower livestock density, mowing the part of the pasture at the beginning of the season for the purpose of hay is

TABLE 4. Influence of selected factors on chemical composition of sword (n = 96)

Specification	Factors			Interaction			X	S	N
	Farm	Year	Vegetation period	Farm * year	farm * Vegetation period	Year * Vegetation period			
Dry matter (%)	XX	NS	NS	NS	NS		26.36	0.69	96
Crude ash (%)	NS	NS	XX	XX	NS		2.73	0.07	96
Crude protein (%)	NS	NS	NS	XX	NS		3.72	0.07	96
Crude fat (%)	XX	XX	NS	XX	NS		0.72	0.02	96
Crude fiber (%)	XX	NS	X	NS	NS		7.11	0.24	96
N-free extract (%)	XX	NS	X	NS	NS		12.07	0.38	96
ADF (%)	X	NS	NS	NS	NS		9.82	0.31	96
NDF (%)	XX	NS	NS	NS	NS		14.66	0.46	96
ADL (%)	X	NS	NS	NS	NS		1.90	0.07	96

Differences at: x – p ≤ 0.05; XX – p ≤ 0.01; NS – not significant.

recommended to improve use of the turf. This way additional amount of hay will be obtained and at the same time plants growing again will have the maximum nutritional value. However such a way of using the pasture is recommended when appropriately a high amount of fallouts is appearing.

## CONCLUSION

The examined pastures were characterized by a low yield what is the stocking rate limiting factor. There were the grasses dominating in the sward. Share of legumes, improving the nutritive value of forage, was low. Analysis of the chemical composition of the forage proved its low feeding value. Green forage was characterized by a high dry matter and fiber content, whereas with the low protein content. These are limiting factors intake and utilization forage by animals.

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**Streszczenie:** Ocena wartości pokarmowej zielonki i wydajności ekstensywnie użytkowanych pastwisk w wypasie bydła mięsnego. Wartość pokarmowa zielonki pastwiskowej zależy w głównej mierze od składu botanicznego runi oraz fazy rozwojowej roślin w czasie użytkowania pastwiska, a na to wpływ ma między innymi sposób wypasu. Opóźnienie wypasu, wzrost wysokości porostu pastwiskowego, wiąże się ze starzeniem się roślin, a to powoduje, że w roślinach wzrasta zawartość włókna surowego, frakcji włókna NDF i ADF, spada natomiast poziom białka ogólnego oraz bezazotowych wyciągowych. Badania



prowadzono w latach 2006–2008 na dwóch ekstensywnie użytkowanych pastwiskach, o różnej obsadzie zwierząt, położonych w północno-wschodniej Polsce. Pastwiska podzielono na cztery powierzchnie doświadczalne, na których prowadzono ocenę wydajności pastwisk, składu botanicznego zielonki i jej wartości pokarmowej. W okresie żywienia pastwiskowego (maj–październik) czterokrotnie pobierano próbki zielonki do badania podstawowego składu chemicznego i frakcji włókna, analizy składu botanicznego runi oraz prowadzono ocenę wydajności pastwisk. Stwierdzono wpływ okresu wegetacyjnego na udział traw i roślin dwuliściennych w runi. Wydajność pastwisk, która była niska – poniżej 2 ton s.m./ha, także zmieniała się wraz z upływem okresu wegetacyjnego. Stwierdzono występowanie interakcji na poziomie gospodarstwo i okres wegetacji, a wydajność pastwisk i udział w runi traw

i astrowatych. Podczas wszystkich pomiarów zielonki charakteryzowały się wysoką zawartością suchej masy i włókna surowego, a niską zawartością białka. W trakcie sezonu pastwiskowego obserwowano istotne zmiany zawartości włókna i popiołu w zielonkach, natomiast zawartość pozostałych składników oraz frakcje włókna były na podobnym poziomie.

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## Effects of excretions/secretions produced by larvae of *Lucilia sericata* on growth of poultry isolates *Salmonella* Enteritidis *in vitro*

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**Abstract:** Effects of excretions/secretions produced by larvae of *Lucilia sericata* on growth of poultry isolates *Salmonella* Enteritidis *in vitro*. *Salmonella* Enteritidis is a major serovar associated with food – borne salmonellosis in humans. The increased level of antimicrobial resistance observed in *Salmonella* has become a public health concern. There is an urgent need for a new generation of antibiotics. The antibacterial properties of secretions collected from larvae of *Lucilia sericata* were examined *in vitro*. Studies revealed antibacterial activity of the larval excretion/secretion. The aim of this work was to define antibacterial potentials of maggot ES for the treatment of salmonellosis. In order to evaluate this, ES were screened for antibiotic properties against 10 pathogenic *S. Enteritidis* strains. The antibacterial activities of ES were tested using the agar well diffusion method and colony forming unit (CFU) assay. Based on the obtained results we can conclude that: (a) ES demonstrate bacteriostatic effect against *Salmonella*, (b) the highest antibacterial activity was revealed at 250 mg/L.

**Key words:** *Lucilia sericata*, maggot, excretions/secretions, *Salmonella* Enteritidis, antibacterial

### INTRODUCTION

*Salmonella enterica* serotype Enteritidis is motile, gram – negative, rod – shaped bacterium belonging to the *Enterobacteriaceae* family. It is human pathogen that has adapted to a wide range of hosts including poultry and rodents. The alimen-

tary tract is one of the most common sites of *Salmonella* colonization and transmission is often by the fecal – oral route. In last thirty years it became major serovar associated with food – borne salmonellosis. Salmonellosis includes several clinical manifestations such as enteric fever, bacteraemia and gastroenteritis [Lu et al., 2003]. Transmission of *Salmonella* to humans is usually by consumption of contaminated food. The most common sources of *Salmonella* Enteritidis are eggs and meat. It is the only human pathogen routinely transmitted in intact chicken eggs, while other serovars of *Salmonella* may contaminate eggs when egg shells are cracked. In recent years *Salmonella* Enteritidis was found in 1 per 10 hens stocks in western Europe [Petter – Guard, 2001].

The large – scale use of antimicrobials, including sulfonamides, streptomycin, kanamycin and  $\beta$  – lactams, in animals and humans enhances the level of antimicrobial resistance in *Salmonella* strains, which has consequences for public health [Foley and Lynne, 2007]. If bacteria turn out to be resistant to an antibiotic, this drug become useless in the future conventional treatment and alternative treatments is required. As a result,

there is an urgent need for a new effective generation of drugs. Several agents isolated from invertebrates have shown promise for possible new antibacterial drug development. In recent years, several reports have described antibacterial properties of the excretion/secretion isolated from larvae of *Lucilia sericata*. It has been demonstrated that nES (native excretion/secretions) consist of two molecular mass fractions. One fraction has a molecular weight of < 0.5 kDa and the other of 0.5–10 kDa [Kerridge et al., 2005]. Huberman et al. have identified three compounds extracted from the fraction with molecular weight of < 1 kDa: p – hydroxybenzoic acid (138 Da), p – hydroxyphenylacetic acid (152 Da) and cyclic dimer of proline (194 Da) [Huberman et al., 2007]. Investigations revealed that these factors within larval excretion/secretions are active against a range of bacteria, including Gram – positive *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pyogenes* and Gram – negative *Pseudomonas aeruginosa* and *Enterococcus* sp. [Bexfield et al., 2008]. The object of present paper was to investigate further the antimicrobial activity of larval ES from *Lucilia sericata* against Gram – negative bacteria *Salmonella* Enteritidis.

Based on the obtained results we can report that all 10 tested *Salmonella* Enteritidis strains were susceptible to all examined dilution of native excretion/secretions of *L. sericata*. However the antibacterial activity of nES were enhanced by its increasing concentrations. In this research relatively low dilution of nES were examined. That may be a reason why the MIC (minimal inhibitory

concentration) could not be established. Kerridge et al. (2005) were able to show that larval extracts in concentration of 40 mg/mL are active against Gram – positive (*S. aureus*) and Gram – negative (*P. aeruginosa*) bacteria [Kerridge et al., 2005]. According to that work, it has been shown here that the growth inhibition was dose – dependent and that the most significant antibacterial activity against *S. Enteritidis* were revealed by the highest concentration of nES of *L. sericata*.

## MATERIAL AND METHODS

To obtain larval secretion the following procedures were taken. Late 2<sup>nd</sup> and early 3<sup>rd</sup> stage larvae were washed successively with 70% ethanol and sterile ultrapure water. Then, washed larvae were transferred to sterile and ultrapure water at a density of 100 larvae per 10 ml ddH<sub>2</sub>O and incubated overnight. After incubation the water containing larval secretion were removed by pipette, filtered and lyophilized. Freeze – dried material was resuspended in ddH<sub>2</sub>O at chosen concentration prior to use in antibacterial assays. All tested pathogenic strains of *Salmonella* Enteritidis were clinical isolates, kindly provided by the Faculty of Veterinary Medicine Department of Preclinical Sciences Bacteriology, Warsaw University of Life Sciences. All cultures were stored at – 20°C in liquid Luria Bertani medium containing 20% glycerol. Samples were thawed when needed, transferred to MacConkey agar plates and incubated at 37°C.

For the well diffusion assay bacteria were grown in liquid LB medium at 37°C for 18 hours. Then the Müeller – Hinton agar was prepared and cooled to approxi-

mately 45°C and inoculated with 100 µl of bacteria. The agar was mixed and immediately transferred to Petri dishes. After solidification, 5 – mm – diameter holes were cut out of the agar and 100 µl of tested larval secretions were loaded into each well. Following concentration of larval secretion (ES) were tested – 0.5 mg/L, 5 mg/L, 50 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L 2500 mg/L, 5000 mg/L, and sterile ultrapure water was included as control. Plates were incubated at 37°C for 18 hours. Then the diameters of bacterial growth inhibition were measured.

In colony forming unit assay bacteria were grown as above. Then 1 ml of each bacterial culture was diluted in broth LB medium in order to gain concentration of  $10^{-7}$ . Following concentrations of ES – 5mg/L, 50 mg/L, 250 mg/L and 500 mg/L – were added immediately afterwards. In order to determine the number of surviving bacteria, after 30 minutes and 24 hours of exposure to ES, 100 µl of each sample were spread onto MacConkey agar plates, incubated overnight and thereafter CFUs were counted. As controls pure bacterial cultures were plated on MacConkey agar and incubated as above.

## RESULTS

The results of well diffusion assay are shown in Table 1. Larval ES demonstrated growth inhibition against 10 tested *Salmonella* Enteritidis strains. The growth inhibition was dose – dependent, as the antibacterial activity of nES were enhanced by its increasing concentrations. The most significant antibacterial effect is exhibited by ES at 5000 mg/L

(the highest number of strains with the highest zone of growth inhibition).

An alternative method, the colony forming unit assay was also tested, and the results are shown in Table 2. Incubation of ES at following concentrations 5 mg/L, 50 mg/L and 500 mg/L with *Salmonella* Enteritidis inhibited growth of 10 strains with the CFUs decreasing to 75% of the control (pure bacterial plates cultures) after 30 minutes. Between all tested concentrations of ES, only 250 mg/L significantly reduced the number of CFUs to 16% of the control after 30

TABLE 1. Inhibition of growth of *Salmonella* Enteritidis strains (n = 10) by excretion/secretion at ranging from 10 to 1000 µg/ml expressed as zones of well diffusion

Dose (µg/ml)	Mean zone of inhibition (in mm) ±SD
Control (H <sub>2</sub> O)	0
0.5	1.6 ± 1.64
5	1.7 ± 1.70
50	1.9 ± 1.19
100	2.0 ± 1.24
500	2.1 ± 1.28
1000	2.3 ± 1.25
2500	2.3 ± 1.33
5000	2.6 ± 1.50

TABLE 2. Bactericidal activity of various range of nES concentration. Values are expressed as mean of ten strains analyses (mean ± SD)

Dose (µg/ml)	Mean number of <i>Salmonella</i> colonies in 1 ml of nutrient medium after 30 minutes of exposure to nES (CFU/ml) ± SD
Control (H <sub>2</sub> O)	$3.8 \times 10^{10} \pm 4.5 \times 10^{10}$
5	$2.7 \times 10^{10} \pm 3.8 \times 10^{10}$
50	$2.7 \times 10^{10} \pm 3.8 \times 10^{10}$
250	$6.2 \times 10^9 \pm 1.2 \times 10^9$
500	$3.1 \times 10^{10} \pm 4.4 \times 10^{10}$

minutes incubation with *Salmonella* Enteritidis. After 18 hours incubation the bacterial growth was so significant that CFUs couldn't be counted.

## DISCUSSION

The beneficial effects of larvae of *Lucilia sericata* have been known for hundreds of years [Church, 1996]. The treatment with maggots was first introduced to clinical practice in the late 1920s by William Baer (professor of orthopedic surgery). In conventional medicine maggot debridement therapy (MTD) was used extensively during the 1930s and 1940s. The development of antibiotic therapy and introduction of improved surgical debridement resulted in declining use of MDT. With the recent findings of methicillin – resistant *Staphylococcus aureus* (MRSA) and vancomycin – resistant strains of *S. aureus* (VRSA) the MTD method re – emerged [Mumcuoglu et al., 2001].

Baer (1931) in his studies, noticed that maggots can decreased the number of bacteria in wounds. Shortly thereafter, research of Weil *et al.* (1933), Robinson and Norwood (1934) and Simons (1935) has confirmed the antibacterial effect of the ES of *Lucilia sericata* [Daeschlein et al., 2007]. In recent years, when studying excretion/secretions of maggots, bactericidal molecules with an activity against Gram – positive bacteria *Bacillus thuringiensis*, *Staphylococcus aureus* and *Streptococcus pyogenes* and growth inhibition of Gram – negative bacteria *Pseudomonas aeruginosa* and *Enterococcus* sp. has been described [Steenvoorde and Jukema, 2004].

The aim of current study was to determine the antibacterial activity of *Lucilia sericata* maggot secretions against poultry isolates *Salmonella* Enteritidis using the agar well diffusion method and colony forming unit (CFU) assay.

Based on the obtained results we can report that all 10 tested *Salmonella* Enteritidis strains were susceptible to all examined dilution of native excretion/secretions of *L. sericata*. The antibacterial activity of nES were enhanced by its increasing concentrations. In this research relatively low dilution of nES were examined. That may be a reason why the MIC (minimal inhibitory concentration) could not be established. Kerridge et al. (2005) were able to shown that larval extracts in concentration of 40 mg/mL completely inhibit growth of the Gram – positive (*S. aureus*) and slow down growth of the Gram – negative (*P. aeruginosa*) bacteria [Kerridge et al., 2005]. That research is in agreement with the previously published studies. In 1957 Pavillard and Wright reported that maggot ES had an antibacterial effect against *S. aureus*, but had no effect against *Escherichia coli*, *Salmonella* Typhimurium and *Proteus mirabilis*. The diversity between potency of nES toward Gram – positive and Gram – negative bacteria was demonstrated to be due to the differences in cell wall structure [Pavillard and Wright, 1957]. By contrast to prior work, it has been shown here, in the agar well diffusion assay, that the growth inhibition was only dose – dependent and that the most significant antibacterial activity against Gram – negative *S. Enteritidis* were revealed by the highest concentration of nES of *L. sericata*.

The CFU assay showed that nES has a bacteriostatic effects against examined *S. Enteritidis* strains. That results are in agreement with previous work. Antibacterial factors within nES have been shown to be less active towards Gram – negative bacteria than towards Gram – positive bacteria [Thomas et al.,1999]. Moreover their potency depend on its dilution [Steenvoorde and Jukema, 2004]. Results from the research conducted by Niemiec et al. [2008] revealed that nES inhibit the growth of *Salmonella*, but the growth of *E. coli* is unaffected. In contrast to these work, the most recent studies of Bexfield et al. [2008] imply that Gram – negative *E. coli* and *Bacillus subtilis* are more susceptible to purified ES than Gram – positive *S. aureus*. However the experiment carried out by us exposed that native maggot ES exert bacteriostatic activity against poultry isolates *Salmonella* Enteritidis. The CFU assay results revealed that all tested concentrations of native excretion/secretions of *L. sericata* inhibited growth of 10 examined *S. Enteritidis* strains with the CFUs decreasing to 75% of the control after 30 minutes. However, only one dilution at 250 mg/L between all studied concentrations of nES significantly reduced the number of CFUs to 16% of the control after 30 minutes incubation with *Salmonella* Enteritidis. Concentrations at 5 mg/L, 50 mg/L and 500 mg/L did not demonstrated significant growth inhibition. After 18 hours incubation the bacterial growth was significantly enhanced, so that CFUs couldn't be counted. The observed enhanced bacterial growth may be due to the high nutritional values of native ES, as well as salmonellas toxins,

which possess lytic activity and digest molecules within larval excretion/secretions. This conclusion is similar to Bexfield et al. [2004], who had incubated *E. coli* with purified ES, what have resulted in increased bacterial growth.

In order to detect and investigate the effect of the *L. sericata* ES on the growth of *S. Enteritidis* strains it is crucial to select the correct bioassay. Variations in dilution of the nES, times of exposure to nES, number of bacteria connected with the active molecules of nES and different types of nutrient media (solid vs liquid) – these are the factors that influencing the efficacy of used bioassays and may change the possible effectiveness of examined molecules [Millar and Ratcliffe, 1987]. Those issues have been recorded previously by Bexfield et al. [2004]. In their study three antibacterial assays were tested for their ability to established the antibacterial activity of nES. That were the zone of inhibition assay, the CFU assay and TB assay. Two of them were ineffectual and only the TB assay demonstrated antibacterial activity of maggot ES.

To summarize, this preliminary investigation has illustrated the antibacterial effect of excretions/secretions of larvae of *L. sericata* on growth of poultry isolates *Salmonella* Enteritidis. It has been proved that molecules in this excretions/secretions possess significant antibacterial activity against Gram – negative bacteria. A number of studies have shown that excretions/secretions of larvae of *L. sericata* can be administrated as a specific therapeutic agents in combination with today's antibiotics and improve their potency.

## CONCLUSIONS

To conclude, in the agar well diffusion method the ES of *Lucilia sericata* larvae at concentration from 0.5 mg/L to 5000 mg/L demonstrate antibacterial effect against *Salmonella* Enteritidis and in the CFU assay the highest antibacterial activity of ES was revealed at 250 mg/L.

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**Streszczenie:** Wpływ wydzielin sekretoryjnej larwy *Lucia suricata* na wzrost szczepów *Salmonella enteritidis*. *Salmonella* Enteritidis to powszechnie występujący patogen drobiu, odpowiedzialny u ludzi za odzwierzęce zakażenia przewodu pokarmowego. Wzrastająca lekooporność salmonelli na antybiotyki stanowi problem dla zdrowia zwierząt i ludzi. W związku z tym, poszukuje się nowych substancji o potencjalnym zastosowaniu klinicznym. Duże nadzieje wzbudzają związki antybakteryjne izolowane m.in. od bezkręgowców. Dotychczasowe badania potwierdzają silną aktywność antydnobnoustrojową wydzielin sekretoryjnych larw muchówek z gatunku *Lucilia sericata* (L.s.). Celem pracy była ocena wpływu biopre-



paratu z wydzielin sekrecyjnych (ES) larw *L. s.* na wzrost 10 szczepów *S. Enteritidis*. Określano wpływ ES na namnażanie się pałeczek w teście dyfuzji w podłożu agarowym oraz przez 30-minutową i 18-godziną inkubację z biopreparatem w podłożu bulionowym. W oparciu o otrzymane wyniki stwierdzono, iż: (a) w teście dyfuzyjnym preparat z wydzielin sekrecyjnych larw much *Lucilia sericata* wykazuje właściwości bakterio-  
statyczne względem *Salmonella* Enteritidis, (b) w teście rozcieńczeniowym preparat z ES o stęże-

niu 250 mg/L wykazuje działanie spowalniające wzrost względem *Salmonella* Enteritidis.

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## Influence of nanoparticles of silver/palladium alloy on chicken embryos' development

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**Abstract:** *Influence of nanoparticles of silver/palladium alloy on chicken embryos' development.* The objective of this study was to examine potential effects of silver/palladium alloy nanoparticles' hydrosols on growth and development of chicken embryo. Fertilized eggs (n = 75) were divided into 5 groups and injected in ovo; group I (control) – no treated; group II (placebo-1) – physiological saline with 1 injection; group III (Ag/Pd-1) – hydrocolloid of Ag/Pd nanoparticles – with 1 injection; group IV (placebo-2) – physiological saline with 2 injections; group V (Ag/Pd-2) – hydrocolloid of Ag/Pd nanoparticles with 2 injections. Nanoparticles of Ag/Pd alloy did not influence chicken embryo development. Injection *in ovo* with physiological saline increased activity of asparagine transferase in blood serum and caused hypertrophy of hepatocytes. Injection of nanoparticles Ag/Pd showed tendency to restore this negative effect.

**Key words:** chicken, embryo, nanoparticle, noble metals, DNA, DAPI.

### INTRODUCTION

The use of antibiotic based growth promoters in animal nutrition caused development of antibiotic resistant pathogen microorganisms, hence feed additives containing antibiotics are now prohibited in EU. Poultry species, having very high growth rate and kept in industrialized production systems, are susceptible

to microbial infections affecting health and performance of animals. A lot of research activities have been devoted to develop alternative feed additives (e.g. probiotics, herbs, preservatives), being not dangerous for consumer health and at the same time assuring profitability of poultry production. Nevertheless, the results from these investigations are so far uncertain.

Nanoparticles of noble metals, especially silver and their alloys exhibit unique biological properties being potential candidates for a new kind of non-antibiotic stimulators of growth. Nanoparticles of silver and probably palladium show antibacterial properties at the level of about 15 ppm (Sondi and Salopek-Sondi, 2004), but also are not toxic when given to animals (Sawosz et al., 2007). Moreover, nanoparticles of Ag are documented as anti-inflammatory agents or components of anti-inflammatory molecules, which do not destroy systemic homeostasis (Bhol and Schechter, 2005; Shin et al., 2007; Kemp et al., 2009). The unique structure of the crystal lattice of silver allows it to store atomic oxygen inside the octahedral holes of Ag (0) and probably influences oxygen level in environment

(Davies and Etris, 1997; Outlaw et al., 1991). Also, palladium has unique characteristics in relation to oxygen and its isotope is used in anti-cancer therapy (Sharkey, 1998; Finger, 1999).

Hypothetically, antimicrobial and anti-inflammatory properties and possibility to enrich cells with oxygen can stimulate growth and development of organism. Furthermore, it is likely that by using "nano-amounts" of these metals, toxicity could be minimized or eliminated and the risk of developing resistant microorganisms can be highly reduced.

The objective of this study was to examine potential effects of silver/palladium alloy nanoparticles' hydrosols on growth and development of chicken embryo.

## MATERIAL AND METHODS

Fertilized eggs ( $n = 75$ ,  $56 \pm 2.2$  g) from Ross Line 308 hens were obtained from hatchery Dembowka, Poland and stored during 4 days at  $12^{\circ}\text{C}$ . Then, the eggs were weighed and randomly divided into 5 groups, each with 15 eggs; group I (control) – no treated; group II (placebo-1) – physiological saline with 1 injection; group III (Ag/Pd-1) – hydrocolloid of Ag/Pd nanoparticles – with 1 injection; group IV (placebo-2) – physiological saline with 2 injections; group V (Ag/Pd-2) – hydrocolloid of Ag/Pd nanoparticles with 2 injections. Experimental solutions were given *in ovo* by injection to albumen (at  $2/3$  of egg's high from blunt ends) using sterile 1ml tuberculin syringe. Eggs were injected with 0.3 ml of physiological saline or hydrosol of Ag/Cu nanoparticles at the concentration of 50 ppm in group II and IV once and in groups III and V twice. The injection

holes were sterile sealed and eggs were placed in the incubator at standard conditions (temperature  $37.7^{\circ}\text{C}$ , humidity 60%, turn once per hour during 19 days.

At 20 day embryos were taken from shells and immediately sacrificed by decapitation, and embryos and selected organs were weighed and evaluated using Hamburger and Hamilton (1951) – HH standard. Blood was collected from neck vein and after 4 hours centrifuged at  $3000 \times 15$  min, in obtained serum activity of alanine aminotransferase (ALT) and asparagine aminotransferase (ASP) were examined using dry chemistry equipment Vitros DT 60 II, Johnson & Johnson. Liver slices were fixed in 4 % solution of paraformaldehyde and then placed in Phosphate Buffered Saline (PBS). After 24 hours samples were washed in PBS + 0.05% Tween 20 and in methanol from PBT and finally preserved in methanol. Prior to microscopic evaluation the samples were rinsed in the solution of methanol from PBT and PBT and then were freezed and sliced into  $9\text{--}10 \mu\text{m}$  by cryostat Leica CM 1900. The samples were placed on glasses covered with poly-L-lysine and incubated in 4',6-diamidino-2-phenylindole (DAPI) in 20 minutes at room temperature. DAPI is a fluorescent stain that binds strongly to DNA. Stained specimens were observed by a confocal microscope Olympus FV 1000 with UV 405 diode and magnification  $\times 1000$ .

Colloidal silver particles were obtained from Nano-Tech. Poland. The structure of nanoparticles was tested by transmission electron microscope JEOL model JEM-2000EX. Test samples were prepared by dispersing drop of nanoparticles onto a copper grid pre-coated with amorphous carbon (S147-4H-Agar Scientific).

The data were analysed using mono-factorial analysis of variance ANOVA and the differences between groups were tested by the multiple range Duncan test, using Statgraphics Plus 4.1. Differences with  $p < 0.05$  were considered significant.

## RESULTS

Microscopic pictures showed that nanoparticles of Ag/Pd had wide-ranging size (from a few to 100 nm) (Table 1) with polygonal and spherical shape. Nanoparticles injected *in ovo* to chicken embryos

did not influence body and selected organ weight (Table 2). Evaluation of chicken embryo morphology according to HH standard (Hamburger and Hamilton (1951)) also did not show any abnormalities in embryonic development of chickens from all groups. Single injection of physiological saline and also of Ag/Pd nanoparticles' hydrosol increased activity of asparagine aminotransferase in blood serum (Table 3). Double injection of Ag/Pd did not influence hepatic enzymes (ASP and ALT), comparing to the control group. Number of nuclei in samples of

TABLE 1. Size and content of nanoparticles observed in hydrocolloidal silver nanoparticle samples, using transmission electron microscopy in magnification 400 000 x

Size [nm]	2–3	3–4	4–5	5–6	6–7	> 7
Content [%]	15	52	18	6	2	7

TABLE 2 Embryos' body weight (g) and weight of selected organs (g/100 g body weight)

	Group						
	Control	1 injection		2 injection		ANOVA	
		Placebo	Ag/Pd	Placebo	Ag/Pd	SEM	P
	I	II	III	IV	V		
Body	45.9	46.1	44.8	42.6	45.0	1.876	NS
Heart	0.68	0.70	0.74	0.75	0.64	0.0430	NS
Spleen	0.05	0.05	0.03	0.09	0.03	0.0233	NS
Liver	1.60	1.63	1.63	1.64	1.50	1.44	NS
Bursa Fabricius	0.0850	0.0591	0.0963	0.110	0.071	0.02131	NS
Eye	1.098	1.013	0.8606	1.115	1.048	0.09904	NS

TABLE 3. Activities of aminotransferases in embryos' serum at 20 day of incubation and number of nuclei in liver samples stained with DAPI, observed by confocal microscope

	Group						
	Control	1 injection		2 injection		ANOVA	
		Placebo	Ag/Pd	Placebo	Ag/Pd	SEM	P
	I	II	III	IV	V		
Asparagine amino-transferase (U/l)	97.37 <sup>a</sup>	168.83 <sup>b</sup>	156.50 <sup>b</sup>	147.66 <sup>b</sup>	89.33 <sup>a</sup>	28.685	0.0158
Alanine aminotransferase (U/l)	24.50	29.25	29.60	24.66	22.33	2.980	NS
Nuclei, n	42.1a	53.1b	33.0c	47.1a	47.0a	4.25	0.0065

<sup>a,b</sup>  $P < 0.05$ .

liver, stained with DAPI and observed in confocal microscope, increased in placebo with 1 injection group and decreased in Ag/Pd with 1 injection group comparing to the control group (Table 3).

## DISCUSSION

Chicken embryo is a biological model independent from external influence, fast developing and easy to maintain. Moreover, it is well known and described in detail in the standard of Hamburger and Hamilton (1951). This model is used in medical, toxicological and also nutritional experiment as a primary investigation, often carried out prior to experiments with animals or humans (Valdel et al., 2003; Yoshiyama et al. 2005).

In our experiments we demonstrated that hydrosol of Ag/Pd, given *in ovo* in amount of 300  $\mu$ l, did not influence mortality, body weight, selected biochemical indices of blood serum and concentration of 8-oxodG in liver DNA of 20 days old embryos (Sawosz et al., 2009). The present results also did not show any effects on embryos survival, the embryos from all groups were properly developed, without any abnormalities. Hussein et al. (2005) in *in vitro* experiments with rat hepatocytes used nanoparticles of Fe<sub>3</sub>O<sub>4</sub>, Al, MoO<sub>3</sub> and TiO<sub>2</sub> and demonstrated significant detrimental effects at a level of 100–250  $\mu$ g/ml.

Alanine transaminase (ALT) is an enzyme being present in blood serum at normal condition, however, an increase of this parameter points on liver (or heart) damage by disintegration of cell membranes. Treatments with hydrosols of Ag/Pd nanoparticles at levels of 300  $\mu$ l and 2  $\times$  300  $\mu$ l had no harmless influ-

ence on hepatocytes' membrane structure. Asparagine transaminase (ASP) is an enzyme which high activity indicates dysfunction of mitochondrial membrane, especially, of hepatocytes. The present results showed that rather the process of injection in general than injection of nanoparticles influenced activity of ASP. However, treatments with double injection of Ag/Pd decreased activity of ASP to the level of control embryos. We can hypothesize that injection (group II, III, IV), even when was made very carefully, could be stressful for chicken embryo. However, double injection of Ag/Pd caused decreasing of ASP level to the level of control, what can suggest that this amount of Ag/Pd nanoparticles could protect liver, probably via oxidative stress resulting in apoptosis (Carlson, 2006) and not necrosis.

In the present experiments hepatocyte nuclear profile density was measure as a marker of hypertrophy of the liver cells. Injection of physiological saline, but not nanoparticles, increased hepatocyte nuclear profile density, however, without increasing liver weight. Injection of Ag/Pd hydrosol decreased the number of nuclei (density) in the liver, what confirm hypothesis, that single injection of 300  $\mu$ l Ag/Pd (increase of ASP activity and decrease hepatocyte nuclear profile density) may stimulate processes of apoptosis.

Some authors indicated non-toxic properties of Ag nanoparticles when used at low levels (Wen et al., 2007; Kim et al., 2008), but there are no corresponding experiments with Pd nanoparticles. However, in this preliminary study we did not observe any detrimental effects of hydrosol of Ag/Pd alloy nanoparticles injected *in ovo* to chicken embryo,

undoubtedly even this low level nanoparticle, used in experiment changed slightly homeostasis of organism.

## CONCLUSIONS

Nanoparticles of Ag/Pd alloy, did not influence chicken embryo development. Injection *in ovo* with physiological saline increased activity of asparagine transferase in blood serum, causing hypertrophy of hepatocytes. Injection of nanoparticles Ag/Pd showed tendency to restore this negative effect.

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**Streszczenie:** Wpływ nanocząteczek srebra na rozwój embrionu kury. Celem badań było określenie wpływu hydrokoloиду nanocząstek stopu srebra i palladu na wzrost i rozwój zarodka kury. Zapłodnione jaja ( $n = 75$ ) podzielono na 5 grup I stosowano iniekcję "in ovo", odpowiednio w grupach; grupa I (control) – bez iniekcji; grupa II (placebo-1) – sól fizjologiczna w pojedynczej iniekcji; grupa III (Ag/Pd-1) – hydrokoloიდ nanocząstek stopu Ag/Pd w pojedynczej iniekcji; grupa IV

(placebo-2) – sól fizjologiczna w podwójnej iniekcji; grupa V (Ag/Pd-2) – hydrokoloიდ nanocząstek stopu Ag/Pd w podwójnej iniekcji. Nanocząstki stopu Ag/Pd nie zakłóciły rozwoju zarodka kury. Podawanie in ovo soli fizjologicznej wpłynęło na zwiększenie aktywności aminotransferazy asparaginowej w surowicy krwi, obserwowano również tendencję w kierunku hipertrofii komórek wątroby. Zastosowanie nanocząstek Ag/Pd wpłynęło na przywrócenie stanu homeostazy hepatocytów.

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## Effect of milk diet on the selected indicators of immunity of rats

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**Abstract:** *Effect of milk diet on the selected indicators of immunity of rats.* The studies were conducted on 2 groups of the rats (10 animals in each group). Group I received milk diet and group II – synthetic one. The aim of the studies was to state whether the milk diet, and more strictly, biologically active components of milk affected the indicators of resistance of the rats, being the model animals for man. The following indices of immunity were determined: level of leukocytes together with the percentage participation of neutrophils and basophiles, the content of lymphocytes, the level of immunoglobulins IgG, IgE and IgM and IL-2 and IL-4 and also, the percentage of phagocytic neutrophils and monocytes and their phagocytic activity. Statistically confirmed differences between the mentioned above indicators of immunity were found only in case of significantly higher level of lymphocytes in the blood of the rats from the group, receiving milk diet as compared to their content in the blood of the rats, receiving synthetic diet.

*Key words:* immunity indicators, rats, milk diet.

### INTRODUCTION

Apart from the basic nutrients, i.e. protein, fat, lactose and minerals and vitamins, milk contains biologically active substances – functional compounds [Michaelidou and Steijns, 2006]; owing to this fact, it is considered as one of nutraceuticals [Steijns, 2001].

From among functional components of milk, the present proteins and peptides, affecting the immunological system are extremely valuable [Zimecki and Artym, 2005].

Lactoferrin, which belongs to whey proteins, plays a role of factor, stabilizing immunological system [Kliczka, 1994] and similarly as peroxidase, it is the element of non-specific cellular resistance [Schanbacher et al. 1997]. Lactoferrin has a stimulating effect on maturation of lymphocytes and initiation of immunological response what was documented in the studies of Zimecki et al. (1995) and Zimecki and Kruzel (2000). Lysozyme shows the cooperation with lactoferrin, what supports the immunological system [Kliczka, 1994]. Glycomacropeptide, deriving from casein reveals the activity, stimulating cellular resistance, increases phagocytosis and proliferation of macrophages [Li and Mine 2004]. Proline-rich polypeptide (PRP) which reveals a high resistance to proteolytic degradation (Zimecki 2008) possesses the capacity of inducing cytokines [Wieczorek et al. 1998, Zabłocka et al. 2001].

The aim of the present work was to determine how the milk diet affected the selected indicators of immunity in rats – model animals for man.

## MATERIAL AND METHODS

Milk administered to the rats in the experiment derived from the cows of Polish HF variety. Their milk yield amounted to 8000 kg and the content of fat, protein and the somatic cell count in the milk were, respectively, as follows: 4.67%, 4.54% and  $126.6 \times 10^3$ . The animals received the ratio standardized according to INRA in PMR system. The basic ration was prepared in the fodder mixing wagon and it consisted of maize silage, beet pulp, Meadow hay, wheat brans and extraction rape meal. The concentrates: soy meal and the mixture with mineral-vitamin mixture) were individually administered to the cows and their quantity in the diet was dependent on milk production. The rats were at random placed in single cages with the grated floor where they stayed for the adaptation period and during the experiment. The individual cages enabled the control of the taken feed. For the whole period of the experiment, the rats were found in the standard environmental conditions: temperature of  $20 \pm 1^\circ\text{C}$  and 12-hour cycle: light-darkness; humidity was equal to 50–70%. The animals had the unlimited access to drinking water.

After the adaptation period (5 days), the rats were divided into two experimental groups, 10 animals in each group and the specific experiment was commenced (21 days). The animals from the control group received synthetic diet, purchased in Labofeed company. The synthetic diet consisted of dehulled oats, maize, milk, soy meal, potato protein, linen semen and mineral and vitamin mixtures; the feed did not contain any pharmacological additives. The content

of the particular feed components was as follows: crude protein 22.0%; lysine 1.5%; crude fibre 5.0%; crude fat 4.0% and crude ash 6.5%. The experimental group received *ad libitum* milk during the whole period of the experiment. One rat received about 55 ml of milk per day, in average.

After completion of the experiment, the immunostimulation of the rats was employed with the aim to determine indices of immunity [LPS Sigma-Aldrich].

After euthanasia of the rats with ketamine rate (50 mg/kg of body weight) the samples of blood were collected to the test tubes, then, the blood was centrifuged and serum frozen ( $-20^\circ\text{C}$ ). Such prepared serum was stored until commencing the analytical tests.

The methods for determination of immunity indicators:

- Concentration of immunoglobulins IgG, IgE, IgM in the blood serum of the rats was determined by chemiluminescence method using Immunolete 2000 Analyzer;
- Concentration of interleukin IL-2 and IL-4 was determined by ELISA method using the kit of R&D System company according to the procedure, recommended by the producer;
- Indicators of non-specific immunity state was determined by flow cytometry method with the application of the kit of ORPEGEN Pharma PHAGOTEST (for determination of percentage content of neutrophils and phagocytic monocytes and the intensity of phagocytosis) using the flow cytometer of Becton-Dickinson company.

The obtained results were subject to statistical analysis – one-factor variance analysis was employed.

## RESULTS AND DISCUSSION

Leukocytes are the basic elements of immunological system.

The data contained in Table 1 represent the content of leukocytes and percentage participation of neutrophil granulocytes – rod-like neutrophils (PAL) and segmentocytes (SEG), basophilic granulocytes – basophiles (BAZO) as well as also, lymphocytes in the blood of the rats of the both groups, which received milk and synthetic diet.

From the determined elements of WBC system, the following ones are different than the reference values: lower content of leukocytes and higher percentage of neutrophils SEG in blood of the rats of the both groups and higher percentage of neutrophils PAL and the lower level of lymphocytes in group of the rats, receiving synthetic diet. The animal fed the milk diet were characterized by higher percentage content of leukocytes and lymphocytes (above the reference values) in relation to the rats, receiving the synthetic diet. Difference in the percentage content of lymphocytes in blood between the groups of the rats has been statistically confirmed. Significantly higher percent of lymphocytes in the blood of the rats from

the group, receiving the milk diet, indicates a positive effect of active milk substances on immunological system what may be a confirmation of the opinion of Schanbacher et al. [after Bernatowicz and Reklewska, 2003]. The stated that the peptides deriving from  $\alpha$ - and  $\beta$ -casein and peptides, released from whey proteins:  $\alpha$ -lactoglobulin and lactoferrin affect the blastogenesis of lymphocytes and their differentiation into T lymphocytes, participating in cellular immunity and B lymphocytes, participating in humoral immunity, responsible for production of antibodies against antigen [Gołab et al., 2002].

Table 2 contains the presentation of the levels of antibodies IgG, IgE and IgM and the level of interleukin-2. (The level of the IL-4 was lower in serum of the both groups of the rats as compared to the range of the sensitivity of Elisa 2000 reader).

In spite of the fact that any significant differences between the groups, receiving synthetic and milk diet in respect of the levels of the mentioned above immunity indicators in the blood of the rats, the discussed indices are higher in the rats fed the milk diet, excluding IgM. Somewhat higher IL-2 content in the blood of the rats from the mentioned group may result

TABLE 1. Morphological parameters of rat blood – elements of white cell system

Parameters	Diet		Reference values*
	I (milk)	II (synthetic)	
Leukocytes WBC ( $10^9/l$ )	<b>4.18</b> $\pm$ 2.081	<b>3.98</b> $\pm$ 1.674	6–17
Granulocytes:			
– Neutrophils (rods), PAL (%)	<b>0.40</b> $\pm$ 0.083	<b>0.60</b> $\pm$ 0.022	0–4.0
– Neutrophils (Segmentocytes) SEG (%)	<b>35.80</b> $\pm$ 14.515	<b>38.80</b> $\pm$ 14.786	10–30
– Basophiles, BAZO (%)	<b>0.00</b>	<b>0.00</b>	0–1.5
Lymphocytes LIMF (%)	<b>89.42A</b> $\pm$ 24.651	<b>54.66B</b> $\pm$ 27.573	65–85

The means in the rows, marked with letters A, B differ statistically for  $p \leq 0.01$ .

\*acc. to Wolfensohn and Lloyd (2003).

TABLE 2. The level of immunoglobulins: IgG, IgE, IgM, and IL-2 in blood serum of the rats

Parameters	Diet	
	I (milk)	II (synthetic)
IgG (g/l)	<b>2.23</b> ±0.041	<b>2.12</b> ±0.501
IgE (g/l)	<b>20.63</b> ±0.072	<b>18.41</b> ±0.063
IgM (g/l)	<b>0.48</b> ±0.282	<b>0.70</b> ±0.044
IL-2 (pg/ml)	<b>214.52</b> ±17,561	<b>200.43</b> ±14.233

TABLE 3. Phagocytic activity of neutrophils and monocytes in the blood of the rats

Cells	Parameter	Diet	
		I (milk)	II (synthetic)
neutrophils	percentage of phagocytic cells	<b>59.47</b> ±19.610	<b>47.84</b> ±13.849
	mean intensity of fluorescence (FU)*	<b>548</b> ±91.4	<b>491</b> ±25.6
monocytes	percentage of phagocytic cells	<b>20.42</b> ±3.530	<b>26.35</b> ±3.030
	mean intensity of fluorescence (FU)*	<b>327</b> ±19.7	<b>327</b> ±41.8

\*FU (fluorescence unit).

from the effect of lactoferrin, what is reported in the studies of Tome et al. [after Bernatowicz and Reklewska, 2003].

The percentage of phagocytic neutrophils and monocytes and their phagocytic activity in the blood of the both groups of the rats, is given in Table 3.

In spite of the lower level of neutrophils (Tab. 1), the basic function of which includes phagocytosis in the group of the rats, receiving milk diet, the percentage of phagocytic activity was higher in the discussed group as compared to the animals fed the synthetic diet. Schlimme and Meisel [1995] have reported that peptides, deriving from casein, enhance considerably the process of phagocytosis. In case of monocytes, higher content of phagocytic monocytes was found in the blood of the rats fed the synthetic diet; on the other hand, the phagocytic activity was the same in the both groups.

## CONCLUSION

The rats, receiving milk diet were characterized by significantly higher level of lymphocytes (exceeding the reference standards) as compared to the rats fed the synthetic diet. Any statistically confirmed differences between the remaining determined indicators of immunity, depending on the employed diet, were not found.

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**Streszczenie:** *Wpływ mleka na wybrane wskaźniki odporności u szczurów.* Badania prowadzono na 2 grupach szczurów (10 sztuk w grupie). Grupa I otrzymywała dietę mleczną a II – syntetyczną. Celem badań było stwierdzenie, czy dieta mleczna a ściślej składniki biologicznie czynne mleka wpływają na wskaźniki odporności u szczurów, zwierząt modelowych dla człowieka. Określono następujące wskaźniki odporności: poziom leukocytów wraz z procentowym udziałem neutrofilii i bazofili, zawartość limfocytów, poziom immunoglobulin IgG, IgE, IgM oraz IL-2 i IL-4 a także procent fagocytyujących neutrofilii i monocytów oraz ich intensywność fagocytarną. Różnice potwierdzone statystycznie między wyżej wymienionymi wskaźnikami odporności stwierdzono jedynie w przypadku istotnie wyższej zawartości limfocytów we krwi szczurów z grupy otrzymującej dietę mleczną w porównaniu z ich zawartością we krwi szczurów żywionych dietą syntetyczną.

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## Influence of nanoparticles of gold on chicken embryos' development

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**Abstract:** *Influence of nanoparticles of gold on chicken embryos' development.* The objective of this study was to examine potential effects of gold nanoparticles' hydrosols on growth and development of chicken embryo. Fertilized eggs (n = 120) were divided into 3 groups and injected in ovo; group I (control) – no treated; group II (placebo-1) – physiological saline with 1 injection; group III (NanoAu) – hydrocolloid of Au nanoparticles – with 1 injection. Nanoparticles of Au alloy did not influence chicken embryo development. Injection in ovo with physiological saline increased level of lactate dehydrogenase in blood serum and decreased embryos' body weigh.

*Key words:* chicken, embryo, nanoparticle, gold.

### INTRODUCTION

The level of nutrients in the incubated eggs may be too low to cover requirements of modern fast growing chicken embryos. Nutrition *in ovo* is a method of supplying extra nutrients into the egg in order to fully utilized genetic potential of developing embryos. There are only few studies regarding *in ovo* nutrition. Uni & Ferket (2004) administrated HMB ( $\beta$ -hydroxy- $\beta$ -methyl butyrate) to turkey eggs, resulting in increased body weight of hatched birds. However, the supply of nutrients *in ovo* does not guarantee

that nutrients will be transported into embryos as they can be scattered in different egg compartments. Consequently, carriers which can reach defined targets in the body are needed. In this context, nanoparticles of gold can be considered as potential carries of biological compounds (nutrients), provided that they are not toxic to the organism and do not cause any harmful effects on growth and development of embryos.

Gold is a noble metal with low chemical reactivity, it is a „friendly” compound for living tissues, commonly used to cover surgeon equipment and implants, reducing infections. Biological characteristics of gold nanoparticles (nanoAu) are related to number of overreactive external atoms in the crystal lattice of Au. Generally, more free atoms (i.e. smaller element of Au) gives higher activity of Au. NanoAu react with sulphuric groups in acid environment, as well as they interact with glycoproteins (Mukherjee, 2005). It is also known that nanoAu have affinity to connective tissue and affect blood vessels' development (Tsai, 2007), hence they are often used in medical treatment of rheumatic inflammation.

NanoAu are also used to label micro compounds for electron microscopy (Schroeder et al., 2003) and for labelling of antibodies detecting aflatoxins by immunochromatographic methods (Xiulan et al., 2005). Furthermore, nanoAu can be used in biosensors detecting small molecules, for example when conjugated with sulphuric groups (Wang, 2008). It has also been demonstrated that nanoAu enter cells by endocytosis, but placenta and blood-brain barriers can inhibit free passage of nanoparticles (Sadauskas et al., 2007).

NanoAu are potential candidates for accurate and nontoxic carriers of active compounds like drugs or nucleic acids (Han, 2007), and recently nanoAu have been shown to be precise carries of pharmaceuticals, effectively reducing concentrations and improving activity of used medicine (Murphy, 2008). Consequently, it is expected that nanoAu may be used as a carrier of nutrients into embryos. Nevertheless, prior to this application of nanoAu, it is necessary to evaluate whether nanoAu may have any toxic or harmful effects on developing organism. Therefore, the objective of the present experiment was evaluate effects of nanoAu on homeostasis of developing chicken embryo.

## MATERIAL AND METHODS

Colloidal gold nanoparticles were obtained from Nano-Tech, Poland, produced by non-explosive high voltage patented method with high purity demineralised water.

Fertilized eggs ( $n = 120$ ,  $66.9 \pm 3.6$  g) from Ross Line 308 were obtained from a commercial hatchery and stored during 4 days at  $12^\circ\text{C}$ . The eggs were weighed

and randomly divided into 3 groups, each with 40 eggs; control (without injection), placebo (injection with 500 ul of physiological salt) and nanoAu (injection with 50 ul of hydrocolloid of gold nanoparticles). Experimental solutions were given in ovo by injection to air sack after seven days of incubation. Eggs were incubated at standard conditions (temperature  $37.7^\circ\text{C}$ , humidity 60%) until 20 days and then were opened and embryos were immediately sacrificed by decapitation. Embryos were weight and evaluated using Hamburger and Hamilton (1950) stages, including detailed morphological evaluation of dissected organs. During decapitation blood was collected from neck vein to the 1.5 ml tubes. Serum was obtained by centrifugation (3000xg, 10 min). Concentrations of magnesium (Mg), calcium (Ca), potassium (P), triacylglyceride (TG) and cholesterol in VLDL, glucose and activities of asparagine transferase (ASP), alanine transferase (ALT), alkaline phosphatase (AP), level of lactate dehydrogenase (LDH) in serum were determined by dry chemistry methods using Vitros DT 60II equipment (Johnson & Johnson, USA).

The data were analysed using monofactorial analysis of variance ANOVA and the differences between groups were tested by the multiple range Duncan test, using Statgraphics Plus 4.1. Differences with  $p < 0.05$  were considered significant.

## RESULTS

Injection with hydrocolloid of Au nanoparticles and physiological salt increased mortality comparing to the control group. (Table 1). After 20 days of incubation body weight of placebo and nanoAu



groups were significantly lower ( $p \leq 0.05$ ) than in the control group, but weights of individual organs were not different. Furthermore, all embryos developed normally in accordance with standards of Hamburger i Hamiltona (1951).

The chosen biochemical parameters were not affected by nanoAu (Table 2). However, activity of LDH was significantly higher ( $p \leq 0.05$ ) in placebo group than in control and nanoAu groups.

## DISCUSSION

Chicken embryo, being a multi-tissue structure seems to be much better ani-

mal model than cell colonies (Baecher-Steppan et al., 1988; Datar et al., 2005). Nevertheless, injection of nanoparticles requires high precision technique, necessary to avoid any contaminations from surroundings, which may easily destroy homeostasis and cause mortality of embryos. In the present experiment, both injection of physiological salt and nanoAu increased mortality, however, in the nanoAu group mortality was lower than in the placebo group, probably indicating a beneficial effect of Au on immune response or an antibacterial effect.

There was no effect of nanoAu on chosen biochemical parameters, but the

TABLE 1. Mortality, body and selected organs weight

Parameter	Groups*			Anova	
	K	Pl	NanoAu	SEM	p-value
Mortality %	9	39.3	21.8	–	–
Liver [g]	0.906	0.912	0.896	0.0268	ns
Spleen [g]	0.027	0.023	0.026	0.0020	ns
Heart [g]	0.335	0.308	0.305	0.0231	ns
Body weight [g]	51.19a	49.52b	51.54a	0.5894	0.0420

K – control, Pl – placebo, NanoAu – nanoparticles of gold.

TABLE 2. Activity of elected biochemical parameters of blood serum

Parameter	Groups			Anova	
	K	Pl	NanoAu	SEM	p-value
Mg [mmol/L]	0.701	0.620	0.795	0.133	ns
Ca [mmol/L]	2.408	2.488	2.503	0.302	ns
P [mmol/L]	1.743	1.703	1.676	0.075	ns
Triacylglyceride TRIG [mmol/L]	0.803	0.892	0.775	0.096	ns
Very-low-density lipoprotein VLDL [mmol/L]	0.365	0.406	0.353	0.044	ns
Glucose GLC [mmol/L]	10.766	11.650	10.183	0.715	ns
Alkaline phosphatase ALKP [U/L]	555.200	586.200	579.000	45.362	ns
Aspartate transaminase AST [U/L]	151.167	177.800	211.333	31.823	ns
Alanine transaminase ALT [U/L]	14.250	9.600	12.200	2.714	ns
Lactate dehydrogenase LDH [U/L]	2495 a	4297.5 b	3395.17 a	370.327	0.0127

K – control, Pl – placebo, NanoAu – nanoparticles of gold.

<sup>a,b</sup>  $P < 0.05$ .

LDH level in blood was higher in placebo group than in the other groups. LDH is present in most tissues and in case of cell damage is released to blood. Probably an injection per se is stressful to the organism. It has been indicated (Anwar, 2003) that administration of cypermetrin at concentrations 50, 200 and 400 ppm, directly into the yolk, did not increase LDH concentration in the blood, while in the other experiment 100 ppm decreased the concentration. These different results are likely related to different injection techniques.

However, in case of nanoAu the stressful effect of injections might be restored by antimicrobial and redox regulating characteristics of nanoparticles (Guo et al., 2005), indicating protecting abilities of nanoAu.

## CONCLUSIONS

Hydrocolloid nanoparticles of gold had no negative effects on development and growth of chicken embryos. The injection of physiological salt increased level of LDH in blood decreased body weight and increased mortality of embryos after 20 days of incubation. However, nanoAu reduced the negative effects of injection, indicating protecting characteristics of gold nanoparticles.

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**Streszczenie:** *Wpływ nanocząteczek złota na rozwój embrionu kury.* Celem eksperymentu było zbadanie toksyczności hydrokoloиду nanocząstek złota na organizm modelowy jakim jest zarodek kury. Zapłodnione jaja (n = 120) podzielono na 3 grupy: I – kontrolną (bez iniekcji), II – placebo (iniekcja z soli fizjologicznej), III – badawcza (iniekcja z hydrokoloиду nanocząstek złota). Iniek-

cję in ovo przeprowadzono w 7 dobie inkubacji w odpowiednich grupach. Podawanie soli fizjologicznej wpłynęło istotnie na zmniejszenie masy ciała zarodka w 20 dniu rozwoju oraz zwiększyło koncentrację dehydrogenazy mleczanowej w surowicy krwi. Zastosowanie nanocząstek złota nie wpłynęło negatywnie na ogólny rozwój badanego organizmu.

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## **Analysis of chosen behavioral forms of Konik Polski horses from the Popielno Reserve**

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**Abstract:** The research included the herd of horses bred using a reservation system on the area of SBRE and HZZ PAN in Popielno. Research was conducted from January 2003 to January 2004, with 1 month intervals, for 24 hours. It was shown, that most of the time Koniks spend grazing. They grazed the longest during spring and autumn (64.0% and 65.7% of the time) and the shortest during summer (46.8% of time). They drunk relatively small. Analysis of the movements of Koniks shown, that the horses moved the most during summer and winter. They spent the most time resting in the summer, mostly standing. Adult horses lied down rarely, mainly in a breastbone position. Passing of urine and faeces was distributed evenly through the whole day and night.

*Key words:* horse, konik polski, behaviour.

### **INTRODUCTION**

Polish Koniks is a breed derived from tarpans. The Popielno reserve is the only place, where they are bred in living conditions mostly similar to natural ones. Due to such system, many valuable features of this breed were preserved (health, productivity, resistance), many interesting, natural behavior forms were recognized, distribution of their physiological activities during a day was learned, and so on.

### **MATERIALS AND METHODS**

The research was conducted Rusing the herd of polish Konik horses bred using

a reservation system on the area of SBRE and HZZ PAN in Popielno. The research was Research was conducted from January 15<sup>th</sup>, 2003 to January 29<sup>th</sup>, 2004. Horses were observed once a month, for 24 hours. The time and frequency of each behavioral form was registered. At the beginning the herd consisted of 8 horses (1 stallion, 5 adult mares and 2 foals). During the research the horses were inhabiting the north-east part of the reserve, bordered with Śniardwy and Warnoły lakes. The most common observation points were forest glades, so called “Wielka Łąka”, “Mała Łąka”, “Bieszczadki” and “Bieszczady”. There were three more herds in the area of the reserve in that time (herds of Nacios, Tytan and Tasznik).

### **RESULTS AND DISCUSSION**

The research consisted in observations of certain behavioral forms of Konik horses from the Popielono reserve, distinguishing the stallion, mares and summing them all together as adult horses.

#### **1. Feeding of adult horses**

Horses spent about 60% of time during a day for grazing. This time depended on the season of the year. They grazed the longest (65.7% of their time) in autumn, while in summer this time was the shortest

(46.8% of time). In summer horses were occasionally fed with hay but only when snow and frost made it impossible for them to find food on their own. Spring was the period for intensive grazing, especially for pregnant mares. Summer limited grazing due to high amount of troublesome insects and high temperatures. In the autumn Koniks had to stock up on fat in short time, therefore they spent a lot of time searching for food and eating.

Similar results were obtained by Kaproń et al. (2000), Kamieniak (2002) and Jaworowska (1967, 1982a, b). It was different in work of Sasimowski (1990), who had observed a shorter period of grazing of adult horses through a day. On the other hand, the research of Kownacki

(1978) has given higher results: the stallion and mares had spent respectively 70.1% and 69.6% of a day grazing. Jezierski (1988), who examined only the daylight activities, stated, that the mares grazed for 62.4% of their time. Boyd, who had examined a herd of Przewalski horses in Monoglia stated, that the horses had spent 47% of time grazing.

## 2. Thirst satisfaction

The research shown a small frequency of water drinking. This activity depended on a season of a year, temperature and kind of food eaten. In the winter horses drunk small amounts of water more often than in other seasons or they eaten snow. In spring they drunk less often. In summer they acquired water rarely, but

TABLE 1. Taking of food by Konik horses

Season of a year	Stallion			Mares			Adult horses overall		
	min/hour		% of a day	min/hour		% of a day	min/hour		% of a day
	Average	Standard deviation		Average	Standard deviation		Average	Standard deviation	
Spring	37	22.2	61.7	39.8	21.9	66.3	38.4	21.9	64
Summer	28.4	21.1	47.3	26.4	22.1	44	28.1	22	46.8
Autumn	40.9	20	68.2	39.1	20.5	65.2	39.4	20.4	65.7
Winter	37.7	20.5	62.8	37.4	20.6	62.3	37.6	20.9	62.7
Average throughout a year	36	22.3	60	37.2	22.1	62	37.1	22.2	61.8

TABLE 2. Thirst satisfaction by Konik horses from the Popielno reserve

Season of a year	Stallion		Mares		Adult horses overall	
	times/day		times/day		times/day	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Spring	2.3	0.4	2.3	0.6	2.3	0.8
Summer	2	1.8	0.4	0.3	0.6	0.6
Autumn	0	0	0	0	0	0
Winter	1.5	1.5	3.4	1.8	3.1	1.7
Average throughout a year	1.6	1.5	1.9	2.3	1.8	1.5

in bigger amounts. This activity was not observed in autumn.

Kownacki et al. (1978) have observed, that horses from the Popielno reserve had drunk water only once a day.

### 3. Forms of movement

The longest period of time for movement Koniks spent in winter and early summer. In winter it was probably caused by searching for food, while in summer the horses were searching for shelter from insects. The stallion shown more movement in spring, which was related to increased sexual activity, necessity of defending the herd from other stallions and searching for new partners.

Kownacki et al. (1978) have obtained different values: the stallion had spent 5.7% and mares 3.7% of a day for movement.

### 4. Comfortable behaviour

a) Koniks rested in a lying or standing position. The most of their time they spent resting in summer – about 47% of the time, while the least in autumn – 29%. Koniks lie mostly in a breast-bone position, rarely horizontally. The mares rested in the latter position more often than the stallion. What may seem interesting, horses lied the most often in winter (18.8%) and the least in summer (2%).

Kownacki et al. (1978) have noted, that the stallion had rested for 24.2% of the time, while the mares had spent 26.9% of time for this. According to Sasimowski (1987), horses rested the longest in winter and the shortest in spring. Boyd (1998) claimed, that Przewalski horses

TABLE 3. Time spent on movement by Konik horses

Season of a year	Stallion			Mares			Adult horses overall		
	min/hour		% of a day	min/hour		% of a day	min/hour		% of a day
	Average	Standard deviation		Average	Standard deviation		Average	Standard deviation	
Spring	47.7	11.8	3.3	33.7	7.2	2.3	36	8	2.5
Summer	76	7	5.3	74.3	4.6	5.2	74.6	5.05	5.2
Autumn	26.5	11.5	1.8	26.6	10.9	1.8	26	11.3	1.8
Winter	98	32	6.8	93	27	6.4	93.8	27.8	6.5
Average throughout a year	60.4	31.5	4.2	45.8	22.7	3.2	54.9	30.7	3.8

TABLE 4. Percentage distribution of the two forms of rest of Konik horses

Season of a year	Stallion		Mares		Adult horses overall	
	% of a day		% of a day		% of a day	
	Standing	Lying	Standing	Lying	Standing	Lying
Spring	33	2	29.2	2.2	31.5	2
Summer	47.4	0	47.5	3.3	47	1
Autumn	29	1	29	4	29.2	3.3
Winter	29.4	1	27.2	4.1	30	18.8
Average throughout a year	35	0.8	32	2.8	32.1	2.3

rested for about 6% of the day, mainly standing.

b) Faeces

Frequency of faeces during a year was coupled with a season of a year, which resulted in availability of variable food. In winter this frequency was high (9.0 times for the stallion, 7.6 times for mares). In spring this frequency reduced with the increase of availability of fodder rich in valuable mineral compounds.

Sasimowski (1990) observed similar values. Kownacki (1978) gave higher values, which were 12.8 times for a stallion and 6.7 times for mares.

c) Urination

In spring and summer Koniks urinated more often due to very juicy grass (9 times for the stallion and 3.7 for mares).

High frequency of this activity among foals was caused by taking of milk from their mothers. Stallion's urination was related to marking of territory.

Similar results were obtained by Sasimowski (1990). Abithigher values were reported by Kownacki et al. (1978), where stallion had urinated 12.8 times and mares 7.4 times a day.

CONCLUSIONS

1. Konik horses spent most of their time grazing. This activity was the longest in

TABLE 5. Frequency of faeces

Season of a year	Stallion		Mares		Adult horses overall		Foals	
	times/day		times/day		times/day		times/day	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Spring	4	2.2	3.9	0.8	3.9	0.4	3.2	0.6
Summer	7.5	0.5	6	0.6	6.2	0.5	4.6	0.3
Autumn	6.5	0.5	8.8	0.6	8.5	0.5	7.5	0.5
Winter	9	2	7.6	1.2	7.8	1.3	4	1
Average throughout a year	6.4	2.5	6.2	3.2	6.2	3.1	5.5	2.8

TABLE 6. Frequency of urination

Season of a year	Stallion		Mares		Adult horses overall		Foals	
	times/day		times/day		times/day		times/day	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
Spring	9	4.1	3.7	0.5	4.4	0.8	4.2	3.1
Summer	15	4	5	2	6.7	1	7.5	1.2
Autumn	5.5	1.5	3.7	0.7	3.9	0.8	4.3	0.3
Winter	4.5	1.5	2	0.6	2.4	0.7	2.7	1
Average throughout a year	8.5	5	3.6	2.1	4.4	3.3	4.9	2.9



spring and autumn and the shortest in summer.

2. The observed drinking frequency was low and related to a season of a year and kind of fodder consumed.

3. Analysis of movement of Koniks revealed, that horses spent for it most of their time in winter and early spring.

4. Koniks rested mostly standing and in summer. In autumn rest has taken only 29% of their time. Adult horses were lying rarely.

5. Urination and faeces were distributed evenly during a day. Their frequency depended on season of the year, kind of fodder consumed and age of animals.

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**Streszczenie:** *Analiza wybranych form zachowania konika polskiego z rezerwatu w Popielnie. Obserwacjom poddano osobniki dwóch stad. Obserwacje prowadzono od stycznia 2003 do stycznia 2004. co miesiąc i przez 24 godziny. Większość czasu zwierzęta spędzały na wypasie. Najdłuższe sesje wypasu były wiosną i jesienią, a najkrótsze latem. Koniki stosunkowo rzadko piły wodę. Zwierzęta te najintensywniej poruszały się latem i zimą. Wypoczywał latem w pozycji stojącej. Leżenie obserwowano rzadko, w pozycji mostkowej. Oddawanie kału i moczu odbywało się równomiernie w ciągu doby.*

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## The effect of a Thoroughbred filly participating in the Derby and its influence on her 3-year-old racing career

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**Abstract:** *The effect of a Thoroughbred filly participating in the Derby and its influence on her 3-year-old racing career.* The influence of the fillies' participation in the Derby on their three year old racing career has been studied. Careers of 198 three years old fillies born between 1987–2004 have been analyzed by comparing racing performance of fillies competing in the Derby and the Oaks, as well as of fillies which have not competed in the mentioned classic stakes. The best racing performances were achieved by fillies competing in both the Derby and the Oaks. This result may be caused by the fact that entered into both classics were the best race fillies, which were capable of dominating not only in their sex category, but also of competing against colts on the same level. Results show that the participation in the Derby influences the fillies' career in their three year old racing season after that race. Only a few achieve better results after taking part in the Derby, while a large number of three years old fillies achieve lesser results than prior to the race. Thus it can be said that competing in the Derby has a negative effect on the three year old racing career of fillies.

*Key words:* Thoroughbreds, racing, Derby, fillies.

### INTRODUCTION

The Derby is the most important race for three years old Thoroughbreds. Since its very beginnings it is a synonym of a great sporting event, the most important race in the racing calendar, as well

as great prestige. The participation in the Derby is debatable in the opinion of many owners and trainers. Some of them enroll the fillies into the race without fear, others do not regard it to be good for their further career. The confrontation with the best colts appears to be too exhausting for a three year old filly, especially since the race is held quite early in the season. Only exceptional individuals, very strong and physically developed, may cope with that race, as many authors point to colts being more valiant than fillies (Mota et al., 1998). The differences in racing performance between sexes normally reflect the different physiological characteristics of males and females under racing conditions (Jelinek, 1988).

A condition of participating in the Derby is proper, several months-long athletic preparation, with the greatest intensification of exercise in the Spring, which is a particular period for fillies. Natural physiological processes such as the oestrus can hamper training and have a negative effect on the physical condition and psychological disposition of the filly (Jorgensen et al. 1996; McDonnell 1997; Pryor and Tibary 2007). Another risk factor is that regardless of the place that the filly achieves in the race its hard

run, an uncompromising fight against the usually stronger colts and a long distance for a still young horse may turn out to be a too great burden, both physically and psychologically. Physical exploit of the filly in the Derby often ruins a further racing career. It was observed that the filly cannot later achieve top form and compete against the best. In extreme cases the participation in the Derby Stakes is her last start in the three year old season.

The aim of this study was to define the influence of the participation in the Derby of three year old Thoroughbred fillies on their further racing career during the three year old season and to compare the performance of fillies which participated in the Derby with those which did not run in this classic.

## MATERIAL AND METHODS

The research material consisted of 198 three years old Thoroughbred fillies, the best in terms of racing performance in their crops in Poland. Chosen were fillies which in the General Handicap achieved a weight of 75 kilos and above. The fillies were born in the years 1987–2004 and were trained by 26 trainers at the Warsaw Racetrack at Służewiec.

In order to define schemes of racing careers of the most valiant fillies, their population was divided into 4 groups:

1. Fillies which participated in the Derby but did not take part in the Oaks.
2. Fillies which participated in both the Derby and the Oaks.
3. Fillies which participated only in the Oaks.
4. Fillies which did not participate in both the Derby and the Oaks.

For each filly data about exploitation and racing performance was defined, such as earnings, number of starts, average earnings per start, number of wins, place and weight in the General Handicap after the three year old season, the filly's placing in the Derby and the Oaks, coefficient of success (the quotient of the total of a horse's earnings in a given crop and an average earning per horse in that given crop). A detailed analysis has been made of the racing careers of 60 three year old fillies which participated in the Derby, but not in the Oaks and fillies which participated in both the Derby and the Oaks.

The fillies' racing careers were taken from the Polish Racing Calendars (*Wiadomości Wyścigowe*) from the years 1990–2000 and official Racing Bulletins of the Polish Jockey Club from the years 2001–2007. In case of earnings won abroad the prize money was calculated into Polish Zlotys (PLN) based on archived currency rates of the National Bank of Poland (NBP). The value of the Polish Zloty in the years 1990–1994 was converted based on the Polish Zloty denomination act from the 7th of July, 1994 (*Dz.U. Nr 84, poz. 386 i z 1995 r. Nr 16, poz. 79*), on the basis of which 10 thousand "old" Polish Zlotys gained the value of 1 (one) "new" Polish Zloty. Additionally the value of earnings in 1990 was revised, having them recalculated by comparing them to amounts from 1991. This was done because in 1990 all race horses were the property of state studs and the amounts of the race prizes were symbolic. A ratio of the crop's total earnings from 1990 to the crop's total earnings in 1991 was determined. Then, using the determined ratio, the definite earnings of

the top filly in the 1990 Handicap were calculated. Using the data from 1991 it was calculated that the earnings of the top filly of the 1991 Handicap constituted for 10.91% of the entire amount of the crop's prize money. Using this percentage the corrected amount of earnings of the crop in 1990 was calculated.

The racing performance of 198 of the crop's best three years old fillies was statistically analyzed, depending on their racing career at the age of three: fillies participating in the Derby, in the Derby and the Oaks, in the Oaks and fillies which did not participate in the mentioned classics but achieved a 75 kg or more weight in the General Handicap at the end of the season. Racing performance was assessed based on four factors: total earnings won by the horse (in PLN), average earnings per start (in PLN), weight in General Handicap after the 3 years old season (kg), and the coefficient of success. The number of starts of each filly was also statistically analyzed. Due to the fact that the analyzed traits did not show a normal distribution, non-parametric Kruskal-Wallis and U Mann-Whitney tests were used.

In order to analyze the significant differences between dependant traits as performance defined by the total earnings, average earning per start and number of starts before and after the Derby, Wilcoxon's test was used. The coefficient of success was not taken into consideration because it would be difficult to calculate it for separate parts (before and after the participation in the Derby) of the racing season. When calculating the fillies' earnings before and after the Derby, the earnings won in the Derby itself were not taken into consideration and were not

assigned to either period (before or after the Derby) of the racing season, because their amount would have a great influence on the comparison of earnings of both periods.

## RESULTS AND DISCUSSION

The results of the analysis of the fillies' racing performance and number of starts, depending on their racing career as a three year old, show that the most valiant were fillies which participated in both the Derby and the Oaks, which is reflected by each of the analyzed racing indexes (Table 1). The performance of fillies which participated in only one of the two classic races was lower than of fillies participating in both, to which attest decisively higher values of average earnings per start and the coefficient of success. Differences between the average values of racing indexes were statistically highly significant. The reason for this may be the fact that entered into both races were the best performing fillies, capable of dominating not only in their sex category, but also of competing against the colts on the same level. In the study of Moritsu et al. (1994) the effect of sex was non-significant over a distance of 1200 m and significant over a distance of 1800 m. It should be assumed that the differences are even more apparent over a distance of 2400 m, a distance over which the Derby is held. Whereas Mota et al. (2005) found that males were significantly superior to females over all distances by an average of 0.3 seconds, which corresponds to a difference of 1.5 length at the finish line. It should be emphasized that fillies which did not participate in the Derby, but took part in the Oaks, despite achieving

TABLE 1. Median (percentile 50) of weight in the General Handicap, average earnings per start, coefficient of success and number of starts depending on the fillies' three year old racing career: fillies participating in the Derby, in both the Derby and the Oaks, in the Oaks and fillies not participating in any of the two classics, but achieving a weight of 75 kg or more in the General Handicap

Type of racing career	No. of fillies	Racing index									No. of starts		
		Handicap			Average earnings/start			Coefficient of success					
		Percentile			Percentile			Percentile			Percentile		
		25	50	75	25	50	75	25	50	75	25	50	75
Derby	14	77	<b>80</b>	81	838	<b>1805</b>	3815	0.96	<b>2.36</b>	3.65	5	<b>7</b>	8
Derby & Oaks	46	79	<b>82</b>	86	1944	<b>4074</b>	7556	2.73	<b>5.81</b>	9.26	6	<b>7</b>	8
Oaks	57	77	<b>78</b>	82	1124	<b>1833</b>	2805	1.70	<b>2.42</b>	3.74	6	<b>8</b>	9
Hcp $\geq$ 75	81	76	<b>78</b>	80	637	<b>1514</b>	2228	1.42	<b>1.90</b>	2.48	6	<b>8</b>	10

lower average weights in the Handicap, had higher averages of the coefficient of success and higher averages of earnings per start than fillies which participated only in the Derby, which may indicate that such a racing career is slightly more beneficial than risking the filly's start in the Derby. This could also result from the larger number of starts. Fillies which participated in the Derby or the Derby and the Oaks raced averagely less during their 3 year old racing career than fillies which did not participate in the Derby, which is understandable due to the hard run of the Derby race. Those differences were statistically insignificant.

The influence of the fillies' participation in the Derby on their later racing career at the age of three can be indirectly concluded from comparing their number of starts and average earnings per start before and after the Derby St. (Table 2 and Fig. 1).

The number of starts of 60 fillies, which participated in the Derby, was lower before their start in this race than after the Derby (Table 2). The reason for this were differences in the length of the

TABLE 2. Comparison of the number of starts before and after the Derby in the three year old racing career of fillies

Number of starts	Number of fillies	
	before the Derby	after the Derby
0	0	4
1	0	6
2	26	8
3	31	10
4	2	19
5	1	6
6	0	6
7	0	1
TOTAL	158	201

season before and after the Derby. Before the Derby (first Sunday of July) most of the fillies (95%) were raced twice or three times. After participating in the Derby the number of the fillies' starts was more diverse than before. The most numerous group of fillies (48.3%) raced 3 or 4 times, while 4 mares did not race any more in their three year old season.

Data from Figure 1 shows that among the 60 fillies participating in the Derby the average earning per start after participating in the Derby oscillated around

the value of this racing index before the start in this race, displaying however a much greater variance, which is shown by the diversified curve. It shows the effect of strict selection, when after the decisive trial in the Derby only a few fillies are capable of successfully continuing the tough, due to the participation in these stakes, racing season and to decisively stand out among their female peers. In the analyzed population there were 4 such fillies, whose average earnings per start after the participation in the Derby exceeded 20 thousand PLN: Triple Crown filly Dżamajka, Kombinacja, Maverera and Czerwona Róża (four highest peaks in Fig. 1). The racing careers of Dżamajka and Kombinacja after their win in the Derby were almost identical – a win in the Oaks, St. Leger and Wielka Warszawska, with the difference that

Kombinacja raced in Cologne, Germany prior to Wielka Warszawska St., but finished at a distant 9<sup>th</sup> place. Maverera, after placing third in the Derby, raced just twice – she was second in the Oaks in Warsaw and a winner of the Slovakian Oaks. Czerwona Róża finished the Derby at 4<sup>th</sup> place, while her next and at the same time last start in the season was the Oaks, where she won.

No statistically significant differences were noted between average earnings and average earning per start achieved by three year old fillies before and after the Derby. For that reason an assumption was made on the lack of relation between the results of the fillies before and after participating in these stakes. However a tendency of negative influence of the fillies' participation in the Derby on their further racing career can

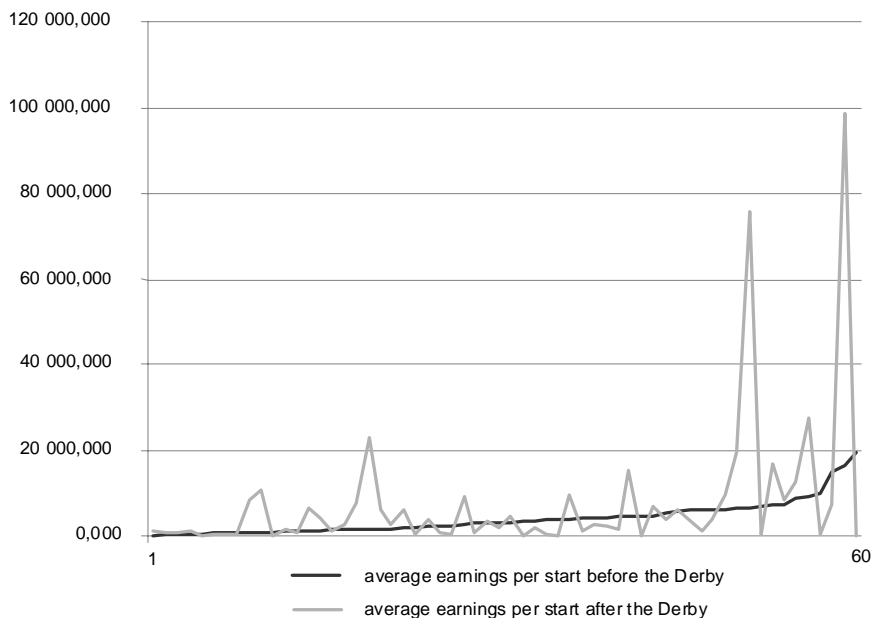


FIGURE 1. Comparison of average earnings per start before and after the Derby in the three year old racing career of 60 fillies at the Warsaw Racetrack in 1990–2007

be seen. Pointing to this is the comparison of the median and the percentile at 25 and 75 of earnings and average earning per start achieved by fillies before and after racing in the Derby, where a great variability of the fillies' results in the part of the season after the Derby can be observed. In the second part of the racing season only a few fillies achieve outstanding results (a visibly higher value of percentile 75 both in total earnings and average earnings per start achieved by fillies after the Derby as compared to the period before their start in this race), while a large number of three year olds achieved much lesser results than before the Derby, which is shown especially by the value of percentile 25 in the average earning per start, much lower than the respective value achieved by fillies before their start in the Derby. Significantly high statistical differences were noted only in the number of starts, which can be explained by the fact that the season before the Derby lasts only about 2 months, while after the Derby – as long as 4 months, which could also affect the shaping of racing indexes.

A good example of the different ways of realizing a racing career of three year old fillies are two leading fillies of the 1997 season – Jak Najlepsza and Zagara. The first one in the Spring part of the

season (before the Derby) raced 3 times and won 3 times. Her next race was the Derby, where in a hard competition against the colts, forced to exert maximum effort, she came second. In turn the second, late-maturing Zagara began her three year old racing career with two group races, placing 5<sup>th</sup> and 3<sup>rd</sup>, respectively. Her next two starts were the Solina St. (4<sup>th</sup> place) and Quattro race (1<sup>st</sup> place). Both fillies met on the 3<sup>rd</sup> of August in the Oaks St., where Zagara proved the better of the two, besting Jak Najlepsza at the finish line by almost a length. After the Oaks the carefully ridden Zagara competed in the Krasne St. where she placed third and at the end of the season she won in great style and by 3 lengths the most important comparative race of the season – Wielka Warszawska St. After that season Zagara was named Racehorse of the Year 1997. Whereas Jak Najlepsza after the exhaustive races of the Derby and the Oaks was entered into the St. Leger, where she placed fourth out of five horses. The filly did not start in any more races in that season.

Despite the fact that the Derby for Thoroughbred horses at Służewiec is held usually on the first Sunday of July and the Oaks at the beginning of August, meaning the period between the two is just one month, up to now the fillies have

TABLE 3. Median (percentile 50) of earnings, average earnings per start and number of starts of 60 best three years old fillies at the racetrack in the years 1990–2007 depending on the part of the season – before or after the Derby

Part of the racing season	No. of fillies	Total earnings			Average earnings per start			No. of starts		
		Percentile			Percentile			Percentile		
		25	50	75	25	50	75	25	50	75
Before the Derby	60	3325	<b>8610</b>	13515	1286.25	<b>3313.33</b>	5818.33	2	<b>3</b>	3
After the Derby	60	2399	<b>8160</b>	24645	663.44	<b>2826.50</b>	7440.63	2	<b>4</b>	4



won the Derby 28 times (from 1868), much more often than in European countries with a richer racing tradition (England – 6 times, France – 9 times). Also each year on the starting lists of the Derby in Poland, contrary to the countries of Western Europe, the fillies appear more often. This can result from the fact that the level of our breeding and racing differs so from the European standard, that the difference between racing class and physical condition of colts and fillies has been blurred and because of that the latter can successfully compete against theoretically stronger rivals and come out victorious. Another reason for this may be the poor financial condition of Polish racing, which prompts the owners of good fillies to enter them into the Derby and compete against the colts, looking for a chance to improve their financial result, even at the cost of a too great exploitation of the horse's organism. Yet another factor could be the fact that until the end of the 80s of the 20<sup>th</sup> century in Poland all horses racing at the Warsaw track were owned by the state, which meant a lack of interest and attention from the owner and gave the trainers a free hand in training and management. Perhaps the English racing system has a good solution to this matter, where the fillies are prevented from competing against the colts due to the Derby and the Oaks being set very close to each other in the racing calendar. In such a situation the owners of fillies usually give up on the much harder, demanding and more risky in terms of winning race and decide to participate in the Oaks. They realistically assess the chances of their fillies against the colts, as well as spare their strength for further starts in the season.

## CONCLUSIONS

1. Although the differences between the average indexes of racing performance of 3 year old fillies before and after the Derby did not prove to be statistically significant, it is possible to notice a negative influence on their further racing career. Only a few fillies achieved remarkable results after the Derby, while a major amount of fillies noted worse results than prior to their start in these stakes.

2. A comparison of racing careers of fillies which participated only in the Derby and those which participated only in the Oaks shows that although the latter achieved a lower handicap weight, they achieved a higher average of the coefficient of success and a higher average of earnings per start than fillies participating just in the Derby. This may suggest that such a career is slightly more beneficial than a risky start of the filly in the Derby.

3. Having compared the performance of fillies participating in the Derby, in both the Derby and the Oaks, only in the Oaks and good racing fillies, which have not participated in either of these classical races, it was noticeable that the best performing fillies were those participating in both the Derby and the Oaks. This result may be caused by the fact that entered into both classics were the best race fillies, which were capable of dominating not only in their sex category, but also of competing against colts on the same level.

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**Streszczenie:** *Wpływ startu klaczy pełnej krwi w Nagrodzie Derby na ich karierę wyścigową*

*w ciągu 3 lat.* Badano wpływ startu klaczy w Nagrodzie Derby na ich karierę wyścigową w wieku 3 lat. Analizowano kariery wyścigowe 198-trzyletnich klaczy urodzonych w latach 1987–2004, porównując dzielność wyścigową klaczy startujących w Nagrodzie Derby, w Derby i Oaks, w Oaks, oraz klaczy nie startujących w wymienionych klasykach. Najdzielniejsze okazały się klacze startujące zarówno w Nagrodzie Derby jak i Oaks. Może to świadczyć, że do obu tych gonitw zapisywane były klacze o najwyższej dzielności, zdolne nie tylko do dominowania w swojej kategorii płci, ale także do równorzędnego rywalizowania z ogierami. Wyniki przeprowadzonych badań wskazują, że start klaczy w Nagrodzie Derby w sposób znaczący wpływa na przebieg jej kariery wyścigowej po starcie w tej gonitwie. Tylko nieliczne klacze uzyskują wybitne wyniki po dobrym starcie w Derby zaś duża liczba trzylatek osiąga rezultaty o wiele słabsze niż przed startem w tej nagrodzie. Można więc mówić o negatywnym wpływie startu klaczy w Nagrodzie Derby na jej dalszą karierę wyścigową w wieku trzyletnim.

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## Activity of herds of Konik Polski horses free ranging in Biebrza National Park

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**Abstract:** *Activity of herds of Konik Polski horses free ranging in Biebrza National Park.* Behavior of three herds on specific, marshy terrains of Biebrza National Park were studied during July and August 2008. Observations of herds behavior have been carried out in three periods of the day. The following behavioral patterns were studied: ingestive behavior, resting behavior, eliminative behavior, comfort behavior, social behavior, play, sexual behavior, displacements. The largest time proportion had forage intake but only in August (on average 87% of the observance time) in relation to 35% in July. Differences in schedule of ingestive and resting activities between those two months seems to have a base in weather conditions. Temperatures in July were higher than those in August. High temperatures in July caused that horses were resting in almost 65% of their time, in August herds rest only in 13% of day time. High temperature greatly influences horse's behavior, by decreasing ingestive behaviors in favor of resting behaviors. General amount of social behavior, mainly aggressive reflexes and comfort behavior increase. Displacements are more common, when temperature outcomes are below 23°C. Horses use permanent tracks to get around within corral. Escaping tracks are the shortest ones, they are wide and easily walkable. Free ranged horses are not keen to gallop, they use it only in case of a threat. Main pace used by free ranging Konik Polski horses was walk.

*Key words:* Konik Polski horses, herd, behavior, Biebrza National Park.

### INTRODUCTION

Konik Polski is a breed of small and undemanding horses, which can be easily

kept under natural conditions. In spite of the fact that only 8% population is bred like this, free range system seems to become more popular these days. Various targets may be reached while those animals graze. First is giving animals a chance to express their natural features, behavior and abilities to adapt and to survive. Second is cheap carrying about landscapes and ecosystems variety. Third is protecting biodiversity.

One of the youngest breeding center of Konik Polski has been set up in Grzędy in the Biebrza National Park (BPN). In year 2004 twelve horses have been introduced to two hundreds hectares area of deciduous forest, raspberry thickets and marshy meadows with a special task to fulfill – keep local environment steady by reducing woody species expansion. Those natural conditions make an opportunity to enquire into unchanged by human horses' behavior. Under domestication the horse's environment have been restricted to the inside of stable or the limited areas of a grazing paddocks. Observing free ranged animals gives a chance to know their habits, to understand the animal's mental state and attempt to alleviate the initial causes of the psychological problems of stabled horses (vices). Careful studies of horses'

time budget may change our attitude towards breeding those animals under actual “normal” conditions.

The aim of this investigations is the analysis of behavioral observations of free ranging Konik Polski on specific, marshy terrains of Biebrza National Park.

## MATERIAL AND METHODS

The observations have been carried out in Grzędy in Biebrza National Park, during July and August 2008. The horses were kept in a free-roaming system on a fenced area of 210 ha of the Park with access to two watering-holes. The specificity of behavioral patterns and activity of the horses concentrated on behavior of herd as a whole. Due to that herd composition kept in Biebrza National Park was established. Observations of herds behavior have been carried out in three periods of the day: morning (from dawn to 11:00 am), afternoon (from 11:00 am to 4:00 pm) and in the evening (from 4:00 pm until dusk). The horses were observed in one hour intervals at various times of day. A total of 123.5 hours of behavior observations of Konik Polski herds were carried out, characterizing their daily activity (time budget). The observations were made from a distance of 5–20 m. The horses were used to the presence of the observers in their surroundings. The record of the horses’ behavior was made on an observation sheet designed in Horse Breeding Department of Warsaw University of Life Sciences – SGGW (Łojek, 2008). The sheet included the following behavioral patterns which have been defined into two groups: connected with time and with frequency of appearance. Behavioral patterns connected with time:

ingestive behavior [grazing, drinking, eating hay or shrubs, coprophagy, milking (foals)]. Resting behavior (activities while rest – standing on 4 or 3 legs, laying flank or on sternum). The proportion of time of the mentioned behaviors in the total time of the observations, depending on the time of day and herd, was determined. Behavioral patterns connected with frequency of appearance: eliminative behavior (urination, defecation, marking), comfort behavior (actions taken to express comfort as self grooming: scratching, shaking skin or head, licking, nibbling, rolling and mutual grooming), social behavior (actions taken inside the herd, connected with hierarchy), play (actions taken mainly by youngsters with no easily seen target beside developing skills), sexual behavior (number of attempts and matings), displacements – (number of displacements, distance, pace, trace and purpose). For the evaluation of the frequency of particular behaviors, the number of appeared cases in all horses was added up and divided by the number of observation hours – hour factor (Crowel-Davis et. al., 1985) and also by number of members in the herd – member factor. Temperature and relative humidity were measured and the amount of insects were also noted down to observation sheet by means of influencing horses’ behavior.

## RESULTS AND DISCUSSION

### Herds composition

During summer of 2008 in Biebrza National Park 3 family groups were kept:

Mrok’s herd. Peaceful and easy-going six years old stallion maintained a band of 12 mares in July and 14 in August. He was characterized by good herding

instincts, appropriate sexual behaviors. No keeping-territory behavioral patterns were observed. Harem took away from Hal in October 2007. Herd consisted of 10 adult mares in July: Tarfa (alfa mare, 4 years old), Terma (4), Hipika (4) with a foal born in 2008, Nawalnica (2), Lektura (2) and one year old - Natysza, Muszka, Nona, Noemi, Lida, Toyota, Hera. In August 2 one year mares joined the herd – Mira and Nawara (both by Trokar).

Trokar's herd. Distinctly territorial behaviors. Good herding instincts and normal sexual behaviors. Quite big herd in very good health condition consisted of 9 mares in July Niemcza (alfa mare, 6 years old), Niestoja (5), Tarta (5), Mantra with a foal (4), Tundra (4), Nocka with a foal (2), Nota (1), Mira (1), Nawara (1) and 7 in August after banishing daughters of Trokar Mira and Nawara to the Mrok's herd. The foal of Tarta was born in August.

Limanek's herd. Complaisant to other stallions. Not much experienced. Meagre herding instincts with no territorial claims. Normal sexual behaviors. Small herd consisted of 3 mares – Nikotyina (alfa mare, 3 year old) with a foal born in 2008, and 2 two years old mares – Netta i Nikotyina.

Bachelor group consisted of 3 one year old sons of Trokar: Talar, Tytan, Talizman.

### **Time budget**

Expressing all types of behavior, especially those connected with rest, enquires feeling comfort, that allows taking further basic life activities as foraging, watering, eliminate waist of metabolism, coat care, social and sexual behavior. If animal

does not feel comfortable it will not forage or groom, but will pay its attention to surround. Grazing is a behavioral pattern most often performed by adult equines, which is confirmed by a number of authors, including Tyler (1972), Duncan (1980) and Sasimowski et. al. (1990). The latter research team performed an observation of Konik Polski horses kept in the Roztocze National Park, from where originates most of the herd in the Biebrza National Park. Also in the case of the Biebrza National Park Konik Polski population it was found, that the largest time proportion had forage intake but only in August (on average 87% of the observance time) and in Trokar herd in July (60.5%) (Table 1). In this free roaming system the horses had two main places of residing where they spent nearly the entire day, using a small part of the 25 ha inner forest pasture (Wielka Łąka) located near the watering hole and residing in the small forest used also as a resting place (Kłycek). In July horses spent more of the observation time resting. Almost 65% of time was spent on this group of activity. Herds of Mrok and Limanek shared one terrain and even one 'core area'. Herds were spending major amount of time together and despite that fact, rest took them much more time than to Trokar's herd, which didn't have any close neighbours and didn't have to share its area. Mrok and Limanek seemed to be used to each other and, in spite of a few days of oestrus, that Hipika was in, they were very calm and peaceful.

Differences in schedule of ingestive and resting activities between those two months seems to have a base in weather conditions. Noted temperatures in July were higher than those in August (Table 2),

TABLE 1. Proportion of time spent on ingestive and resting behaviors in 3 herds depending on three periods of the day during July and August (%)

BEHAVIOR HERD	INGESTIVE				RESTING			
	Morning	afternoon	evening	mean /month	morning	afternoon	evening	mean /month
JULY								
Mrok	13	3	50	<b>20</b>	87	97	50	<b>80.0</b>
Limanek	25	0	65	<b>28.8</b>	75	100	35	<b>71.3</b>
Trokar	87.5	28.5	0	<b>60.5</b>	12.5	71.5	0	<b>39.5</b>
mean/period	41.8	1.5	38	<b>35.1</b>	58.2	98.5	62	<b>64.9</b>
AUGUST								
Mrok	100	70	90	<b>81.5</b>	0	30	10	<b>14.9</b>
Limanek	100	78	89	<b>83.3</b>	0	22	11	<b>16.6</b>
Trokar	0	62.5	100	<b>92.7</b>	100	37.5	0	<b>7.3</b>
mean/period	66.7	70.1	93	<b>87.0</b>	33.3	29.9	7	<b>13.0</b>

TABLE 2. Average temperatures in July and August 2008 depending on three periods of the day (°C)

	morning	afternoon	evening	mean	min.	max.
JULY	22	28	23	<b>26</b>	15	32
AUGUST	20	23	20	<b>21</b>	13	30

while mean humidity was rather equal in both months – 65% in July and 70% in August. It can be easily noticed that temperatures above 25°C (July, Table 2) influence horses' behavior by decreasing total amount of time spent on grazing and increasing interest in rest activities (Table 1). The influence of temperature on basic horses' activities can be easily noticed when comparison of day time periods is made. Mornings and evenings were generally cooler than afternoons.

The optimal period of forage intake can, in the free roaming system and in the wild, depend on many factors, such as temperature, time of year, migration of the herd in search of optimal water and forage sources, optimal time for other behaviors. These factors can in turn remain under the influence of environmental conditions and social factors.

Sasimowski et. al. (1990) found the dependence of the Konik Polski horses' day time grazing rhythm (kept in the wildlife reserve) on the time of year. This team also found that the periods of forage intake and the mean duration time of this activity of the two year olds was almost analogous to adult horses.

Before whole herd calm down and start resting, members of group pays great attention to each other and themselves. In those moments frequency of social and comfort behaviors increases. As it can be seen in table 3 higher temperatures in July greatly increased social behaviors which can be seen in number of events, hour factor (presents how many events were taken per one hour of observation), member factor (shows how many events were taken by one animal), what make established thesis more certain.

## Social behavior

Social behavior is defined to friendly and aggressive reflexes. Friendly, mainly mutual grooming, which was described above. In case of not enough space around, horses were showing off their irritation mostly by pinning ears. This pattern was most frequent in a group of aggressive reflexes and was taken firstly to signalize anger. Further steps were biting, kick threatening, kicking and were less frequent respectively. Most common reasons for aggression were: overcrowd during food intake, rarely during rest, accosting by younger horses. As Klimov (1988) claims, mares which gave birth to a foal promote in hierarchy. That kind of situation was recorded in August in Trokar's herd. Mare Tarta gave birth to a mare Taszka and for one week it sharply changed situation in a band. Mare was very stimulated and alerted. As it was presented in table 3 amount of social reflexes in herds was higher in July than in August for 69% (Mrok's and Trokar's herds) and 37% (for Limanek's herd). Again, temperature seems to be a main factor influencing herd's behavior, directly or indirectly affecting animals.

## Ingestive behavior

On July horses spent only 35% of their time on ingestive activities (Table 1), which was mainly grazing. Another

ingestive behaviors were eating shrubs, gnawing tree bark. Watering was rare. It was recorded only 3 times per 65 hours of observations, what gives 0,046 case per 1 hour. Those situations took place in a mornings. No case of coprophagy was noticed. On August total amount of time spent on food intake increased up to 87% (Table 1). Cases of watering were also more frequent – 9 times per 65 hours of observations, what gives 0.14 case per hour. They were mainly noticed on afternoon hours (11 a.m – 4 p.m) 66% of cases, 33% was the cases observed in evening (4p.m – dusk). No case of coprophagy was noticed. During grazing horses walked for some distances. Each selected a feeding station, bite off several times, chew and move to another feeding station. If herd was intake food on a meadow, horses were standing and moving randomly. Distances covered in that way were long and reached even 3 kilometers. Higher frequency of watering in August may be a result of a different time schedule in both months. Lower temperatures recorded during day allowed for more locomotion and ingestive behavior. Probably higher day temperatures in July forced horses to take those activities at night (dusk – dawn). A reverse situation was found by Crowel-Davis et. al. (1985), who discovered that the lowest frequency of water intake took place in the early morning (13.6%

TABLE 3. Number of activities, hour factor and member factor connected with social behavior in 3 herds in July and August 2008

	JULY			AUGUST		
	events	hour factor	member factor	events	hour factor	member factor
Mrok	125	3.85	8.93	87	3.22	5.44
Limanek	48	1.78	9.60	18	1.00	3.60
Trokar	26	3.47	2.17	18	1.38	1.64

of the observations), when the temperature was still low. The lower water intake during this period could have been influenced also by the presence of dew on the grass blades on the pasture. It is interesting to note that several times during long hours of observation no water intake was observed. Foals were sucking mares 0.7 times per hour and this activity took about 40 seconds. First trials of eating grass were observed in two weeks after a birth.

### Resting behavior

If animals felt comfortable enough to lie down, herd members rest recumbency or even lying flank. Observations showed that total amount of time spent on rest decrease with age. Also chosen rest position seems to be combined with age. Adult horses were resting mainly standing, sucklings – laying flank. That dependence was noted by many teams. Generally stallions did not lie down. However, there was one exceptional case recorded when stallion Limanek was lying recumbency.

High temperatures in July caused that horses were resting in almost 65% of their time, in August herds rest only in 13% of day time (Table 1). Comparing data from tables 2 and 4 it seems that temperature influences also a way of resting.

If outcomes were below 23°C and sun exposure was not big, horses rest standing for a while (3–15 minutes) between two grazing stations. Sasimowski et. al. (1990) found that the resting rhythm of Konik Polski horses maintained in the wildlife preserve is dependent on the time of year, type of given forage and age of the horses. In the spring and summer the horses rested much less in comparison to other times of the year. In turn Crowell-Davis (1994) links the resting-grazing cycle of the Welsh cob mares with the temperature of the surroundings. At dawn, around 5:00 am part or all of the ponies rested in their halting places. In the period right before or right after dawn the mares left their halting places and began to graze. In the late morning the mares searched for a shade and rest. In the middle of the day the mares alternately grazed and rested, except for the hotter days of July and August, when the temperature surpassed 25°C, when the mares searched for shade and from 9:00 am – 10:00 am to 4:00 pm – 5:00 pm spent most of their time resting. The discontinuing of grazing to spend long periods in the shade is probably a form of behavioral thermoregulation. When in the late afternoon or early evening the temperature fell, the mares returned to

TABLE 4. Percentage of time spent in different resting positions taken by a whole population (3 herds) of Konik Polski horses in Biebrza National Park in July and August 2008 (%)

RESTING PLACE	POSITION	JULY	AUGUST	AVERAGE
CORE ZONE	standing	84	23	53.5
	recumbency	3	1	2.0
	lying flank	1	0	0.5
MEADOW	standing	12	70	41.0
	recumbency	0	5	2.5
	lying flank	0	1	0.5



grazing. That way adult horses graze in the least stressing part of the day – when the temperatures are not so extreme – and they remain motionless in the remaining times of day.

Choice of a place for rest was probably determined by weather conditions: sun exposure, temperature, rain, wind and combined with those circumstances presence and activity of insects and also close neighborhood of other herds or other animal species.

### Eliminative behavior

Defecation and urination in all age groups of the Konik Polski horses in the Roztocze National Park researched by Sasimowski et. al. (1990) had a regular distribution during the day. Adult horses defecated an average of 3.2–10 times per day, while the 2 year olds – 2.5–5 times and the yearlings and sucklings – 1.7–7 times. An average frequency of urination of horses researched by Sasimowski et. al. (1990) amounted to 2.2–5.8 times per day in the case of adult horses, 2.8–5 in

the case of 2 year olds, 1.8–6 – for yearlings and suckling foals. In research done in the Biebrza National Park in 2008 age wasn't taken into consideration. The outcomes were as following: urination 0.3 per hour, defecation 0.4 per hour, what gives 7.2 case of urination per day and 9.6 case of defecation per day. Distribution was rather regular, however, an increase before and after rest was noticeable. Marking was easy to observe on Kłycek, where 2 herds have their resting place. When one of the stallions defecated, the other immediately came and defecated in the same place creating characteristic mounds. This was accompanied by a characteristic ritual, with stamping, pawing, squealing and even short fights.

### Comfort behaviors

Activities expressing comfort were presented by horses of Konik Polski population in Biebrza National Park during all day. However, increasing of the frequency of those activities took place in three situations: before and after rest, in

TABLE 5. Number of activities, hour factor and member factor connected with comfort behavior in 3 herds in July and August 2008

HERD	JULY			AUGUST		
	events	hour factor	member factor	events	hour factor	member factor
<b>COAT CARE BEHAVIOR</b>						
MROK	152	4.7	10.9	73	2.7	4.6
LIMANEK	45	1.8	9.0	21	1.2	4.2
TROKAR	30	4.0	2.5	39	3	3.5
<b>MUTUAL GROOMING</b>						
MROK	19	0.6	1.4	12	0.4	0.8
LIMANEK	6	0.2	1.2	3	0.2	0.6
TROKAR	11	1.5	0.9	6	0.5	0.6
<b>ROLLING</b>						
MROK	23	0.7	1.6	7	0.3	0.4
LIMANEK	11	0.4	2.2	3	0.2	0.6
TROKAR	6	0.8	0.5	3	0.2	0.3

case of higher temperature, when insects increased their activity.

### Coat care

Higher amount of coat care events in Konik Polski population in Biebrza National Park was observed in July (Table 5). Horses were scratching their coat, rolling and groom. Those activities were often connected with surroundings. Instantly, big amount of insects forced horses to shake their skins and heads to drive off irritating flies. Horses were putting a lot of attention to coat care before and after rest. Mainly by mutual grooming and rolling in a core area. On meadows – during foraging, if animals were distracting by insects they react to a stimulus and came back to food intake.

Mutual grooming pairs were not strict, however there was a repetition in selection of partners. There was only one recorded situation of mutual grooming, when stallion done it with a mare (Trokar and Niemcza). In all other cases, mutual grooming pairs consisted of mares. Mutual grooming by foals were an invitation to a play and never took longer than few seconds. This activity were taken mainly in core area. In cooler days also on a meadows, where horses rested. Again, on July there was more events observed (Table 5). Horses were rolling in core areas or in dust baths situated in a enters to a meadows. Creature sinked to a ground to roll over few times (2–11). Youngsters were rolling before and after rest, while adults were lying down only to roll and then got up. That dependence was already presented by Sasimowski (1990). Rolling was more common on July (Table 5), what was probably caused

by thermoregulation and bigger number of insects.

### Sexual behavior

In August four attempts to cover Hipika were observed and few serious fights between Mrok and Limanek in the same period. Attempts were accompanied with characteristic voices and prodding Hipika by Mrok. It should be noted, that peak of the natural breeding season occur in Poland in May-June, thus small number of observations of matings.

### Play

Observations show that adult horses were not interested in that kind of activity. If foal disturbed one and tried to play with, it was driven off. Then foal was trying to distract another herd member next in hierarchy. This conduct seems to has its base in heredity and is in accordance with Kaleta's (2007) thesis on playing purposes. One of them was said to be a training of social behavior.

Animal's age seems to influence not only length of these activities but also playing patterns. In Biebrza National Park newborns were focused on themselves. Foal was checking his own abilities. Thus, recorded activities were running, jumping, quick turns. All those behaviors were firstly done next to mother, more days passed, bigger distances were appearing between a foal and his mother. After 2 weeks animals were trying to imitate their mothers and they were taking first trials of grazing. That was also a moment when a suckling put its great attention to the youngest half siblings. If there was no peers in a herd, foal was seeking a contact with a one from another

band, if only it was available (Mrok's and Limanek's herds). When foal was about two months first, shy trials of different plays were taken. Foal was running straight on some member of a band, he was trying to put other attention to itself, by pawing and even biting. Young animals with similar rank hierarchy were interested in play. From now, animals were taking not only solitary plays but also activities in pairs or groups of three or four. In case of Mrok's and Limanek's herds members were often mixed for those moments. Newly appeared playing pattern was biting each others legs, manes, tails, mock fights and jumping on a play partner (no sex determined). Defined by McDonnell (2003) patterns seems to cover with a period of maturation at this early stage. They are suitable to a concrete moment of foal's life and they correspond to one's necessities. Firstly it is going to be a locomotive play, then object play, next – mock fights and at the end sexual-based patterns.

### Displacements

The most frequent reason for a displacement was following feeding stations. Those displacements could be very short and very long. Short ones were mainly covered inside one meadow and they were not consider as a displacements. Displacements during grazing consisted of 40% of observed displacements on July and August 2008 and should be consider as ingestive behavior, and is taken only in a walk. If horses were resting in core area they were forced to walk some distance to start other activity. And reversely if they were grazing on a meadow and decided to take some rest, they had

to walk to rest place. Displacements caused by a necessity of changing activity consisted of 33% of all displacements and were quite short, distances mainly to 100 meters. Trokar's core area (Wydma) was away for 300 meters from a meadow. This herd was covering this trace by trot. Beside that trace, horses did not trot. Gallop was observed in 9% of displacements and was caused by escaping. For this reason horses displaced in 9% of cases. The causes for escaping of the herd could be various. It was recorded, that herd started to stampede under the impact of falling bough. Featuring snap scare off horses, which were resting in a forest on Kłyceck. Horses were efficient and coordinated and started a gallop in one direction, after reaching nearest meadow horses graze for a few minutes and then came back to Kłyceck to continue rest. Last noticed purpose was watering – 19%.

Covered distances were depend on purpose of displacement and patches followed. Escapes were taken by the shortest pathways (50 m.), while during grazing longer pathways were preferred. Most frequently horses were following short traces – to 100 meters (44%). Distances covered to water hole were 42% of all distances to 100 meters long. Each of the distances of 100–500 m and 500–1000 m consisted of 19% of displacements. Distances above 1000 meters (19%) were taken only during grazing. Time spent to cover them reached even 5 hours. Time spent to cover the shortest traces took from 11 seconds to 1 minute.

Most frequently horses used walk for displacements – 86%. The trot was used in 5% and gallop in 9% of displace-

ments. Pace was depended on purpose of displacement i.e. gallop for escaping, walk for going to water hole, following grazing stations and changing activity in Mrok's and Limanek's herd. Trokar's herd used trot for last purpose. Following outcomes are similar to those given by Kaproń (2000), 91% for walk, 6% for trot and 4% for gallop.

Most displacements took place after 4 p.m. In July, when days were much hotter, horses took 37.5% of displacements at early mornings (to 8 a.m.). Afternoon locomotion activity was mostly dedicated to changing activity from graze to rest after 11 a.m. and from rest to graze before 4 p.m. Evening ones were those long-distanced grazing pathways (Table 6).

Horses were following previously treed out traces. Bushes and forests were full of those crossed tracks. There were wide, often used tracks and those used rarely. Pathways connecting core area and mostly used meadow were quite straight and short, horses were following them in a chain. Those, which was used for escape were the shortest ones and very wide to let animals run in a group. There was one situation noticed, when 2 herds (Mrok's and Limanek's) mixed up during runaway. The flock of horses was divided to 2 herds after reaching a meadow.

Tracks used for forage were long and vary. Some parts were very wide, there horses formed a random group and moved forward slower, other parts were narrow,

where horses moved in a chain, what was visibly faster. On meadows there was no easily noticed tracks. Horses were moving rather randomly, only on an entries to the forests or bushes, herds were compacting and walking on a pathway.

Alfa mare was deciding when and where to go. She was picking a pathway. She was followed by other mares in an order of hierarchy ranks. Stallions were generally walking after whole herd. However, Limanek did it in that way rarely. He was mainly walking firstly. After Mrok's mares. His mares was following him in appropriate order. Foals were following their mothers, youngsters were not keeping close. Hipis and Narkoty (Mrok's and Limanek's herd, respectively) were seeking of each other company. Neither Mrok nor Limanek tried to separate them.

## CONCLUSIONS

1. High temperature greatly influences horse's behavior, by decreasing ingestive behaviors in favor of resting behaviors. General amount of social behavior, mainly aggressive reflexes and comfort behavior increase. Displacements are more common, when temperature outcomes are below 23°C.

2. Horses use permanent tracks to get around within corral. Escaping tracks are the shortest one, they are wide and easily walkable.

TABLE 6. Percentage of displacements of a population of Konik Polski horses in Biebrza National Park on July and August 2008, according to in three periods of the day (%)

	MORNINGS	AFTERNOONS	EVENINGS
JULY	37.5	21.25	41.25
AUGUST	13.3	30	56.7

3. Free ranged horses are not keen to gallop, they use it only in case of a threat. Main pace used by free ranging Konik Polski horses was walk.

4. Most common reason to displace was occur to be following grazing stations.

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**Streszczenie:** *Aktywność stad koników polskich żyjących na swobodzie w Biebrzańskim Parku Narodowym.* Obserwowano aktywność trzech tabunów koników polskich utrzymywanych w warunkach wolnego wypasu w Biebrzańskim Parku Narodowym w lipcu i sierpniu 2008 r. Koniki utrzymywane były w ogrodzonej ostoi o powierzchni ok. 200 ha. Zachowanie tabunów obserwowano w ciągu 123.5 godz. w 3 porach dnia: rano (od świtu do godz. 11, w południe (od godz. 11 do 16) i wieczorem (od godz. 16 do zmroku). Obserwowane przejawy zachowania to zachowania pokarmowe, wydalnicze, pielęgnacyjne, społeczne, płciowe, odpoczynek i zabawa oraz przemieszczanie się tabunów. Pobieranie paszy miało największy udział w zachowaniu tabunów w sierpniu (87% czasu obserwacji), zaś w lipcu – odpoczynek (65% czasu obserwacji). Różnice w zachowaniu stad w tych dwu miesiącach mogły być związane z wyższą średnią temperaturą powietrza w lipcu (26°C), w stosunku do średniej temperatury sierpnia (21°C). Wpływ temperatury na zachowanie się stad był również widoczny gdy analizowano aktywność tabunów w poszczególnych okresach dnia (rano i wieczorem temperatura była zwykle niższa niż w południe). Wydaje się, że wzrost temperatury powietrza ponad 25°C powoduje zmiany w zachowaniu tabunu poprzez zmniejszenie czasu pasienia się kosztem odpoczynku. Wzrasta też liczba zachowań społecznych (głównie agresji) i pielęgnacyjnych. Tabuny przemieszczają się najczęściej przy temperaturze poniżej 23°C. Konie poruszają się zwykle stępem, po stałych szlakach. Galopem stado przemieszcza się jedynie gdy jest spłoszone, w stanie zagrożenia. Szlaki wykorzystywane do ucieczki są szerokie i łatwe do pokonania. Główną przyczyną przemieszczania się tabunów jest pasienie się.

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## Economic effects of racehorse stables management in Warsaw race track

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**Abstract:** *Economic effects of racehorse stables management in Warsaw race track.* Economic effects of racehorse stables management on the example of Warsaw race track in Służewiec in 2004–2006 were examined. The analysis of income as well as costs of keeping horses showed lack of profitability for this kind of activity. The profitability threshold would have been reached only if the sum of race prizes gained in races by the trained horses had been 6.43 times higher. The highest share in the structure of total costs in the subject stable were employees remuneration costs constituting over 50% of monthly costs connected with running the said stable.

*Key words:* thoroughbreds, race tracks, riding stable, costs.

### INTRODUCTION

Until the Second World War training of horses for races had taken place exclusively in private stables or in training centres, functioning at parent stud farms. After nationalization of the Służewiec racing track and the estate of other tracks in Poland after the Second World War, national stables were organized and trainers became employees of a national enterprise. As a result of political changes at the end of the 1980s a fundamental

change of the organization system of riding stables and localization of training centres took place. After privatization of riding stables, since 1993 trainers have undertaken their own business activity (public trainers) or they sometimes got hired in national or private stables (private trainers). A measurable effect of a race horse trainer are prizes won in races by his charges which, together with fees brought in for training by horse owners constitute an economic basis of stables functioning. In the past, average seasonal sums of wins per horse did not totally cover the costs connected with keeping horses in the race training, still, the perspective of possible victories in highly subsidised and prestigious prizes kept the owners' faith in economic success.

The aim of the work was the analysis of economic effects of racehorse stables management on the example of Warsaw race track in Służewiec. Research was to show what factors, and to what extent, shape the costs structure of keeping a race horse as well as in what part one can count on the repayment of borne expenses from race wins.

## MATERIAL AND METHODS

Riding stable in Warsaw was on the area of Tor Wyścigów Konnych (Służewiec Racehorse Track). Data concerning the costs of keeping the stables as well as income from the racing prizes were analysed from the period of April 2004 till December 2006. The stable functioning was based on the idea of stalls rental the price of which included using the training facilities and transportation surfaces of the facility. The lessee was obliged to pay the rent for all stalls which were at his disposal regardless whether they were occupied by horses or not. The stable included three buildings where 76 horses could be kept. All stalls were equipped with automatic watering and fixed mangers. Horses were kept on straw which was all exchanged once a week. During other days the display of straw in stalls was improved and, shall the need arise, part of manure was removed and replaced by a small amount of new straw. The stable had three carousels for walking the horse and a square for riding at a walk and trotting. The stable did not have paddocks, therefore horse movement was possible only when saddled or in the carousel. Due to the lack of the possibility of free movement the horses were kept in stalls for about 22.5 hours per day. Training of the horses took place on a working track with a sandy surface which had four runs and 1800-metre perimeter which was situated 250 metres from stable buildings. Working track, riding square as well as other common areas were administered by the Polish Horseracing Club which ensured keeping them in a way which enabled training of race horses. Therefore, there was no need

to have mechanical equipment to get rid of manure (it was stored near stables in three-walled silos) and to maintain training infrastructure. Unloading of feed and straw was hand-made. Straw, hay and oats were stored in the attic of the stables and in stalls which were not occupied by horses at that time or in stables corridors. Manure disposal was made by hand carts to the place of the storage, where it was collected by external entities. Stables supply took place on the basis of orders placed in advance which were serviced by private suppliers.

Costs connected with running a riding stable (costs of keeping race horses trained in the stable) were divided into the following categories:

- costs of bedding,
- costs of bulky feed (hay),
- costs of concentrate (oats),
- costs of supplementary feed,
- costs of prophylaxis and veterinary services,
- costs of shoeing services,
- costs of employees remuneration,
- costs of infrastructure.

Costs of keeping the horses are gross costs.

Income from won races in particular periods of time were given in net value, that means they were calculated by decreasing gross wins by the following elements:

- fees for registering the horses for races,
- remuneration for horse riders in the races,
- 5% prize gross for horse riders in the races,
- 10% prize gross for horse trainer.

Rate of costs borne on average monthly keeping of a race horse in relation to average monthly prizes won by a horse



in the races was calculated. Data concerning costs of keeping a riding stable in Służewiec racing track in Warsaw as well as income from racing prizes were gathered by using the questionnaire method and on the basis of accounting records of PKI SA. Analysis of gathered information was conducted by means of the comparative method.

## RESULTS AND DISCUSSION

What is noticeable is the high filling of horses in stables reaching from November 2005 and within 9 months of 2009 100% of available places (Table 1). It was certainly connected with the conditions of stalls rental and optimization of available places usage rate in Służewiec since stalls with no horses generated losses.

In Table 1 costs of bedding, hay, concentrate, supplementary feed and mineral-vitamin additives were presented, costs of shoeing services together with materials, stalls rental, treatments and veterinary prophylaxis, medicines and dressing means, employees gross remuneration as well as total costs of keeping horses in particular months in the period from April 2004 to December 2006 (in PLN). Taking into consideration level of the mentioned costs items and their share in total costs, they can be divided into 3 groups. The highest position in the structure of total costs were costs of employees remuneration, constituting usually over 50% of monthly costs connected with managing riding stables in Służewiec track (Figure 1). The reason of such a state of affairs is to be seen in the specificity of race horse riders work

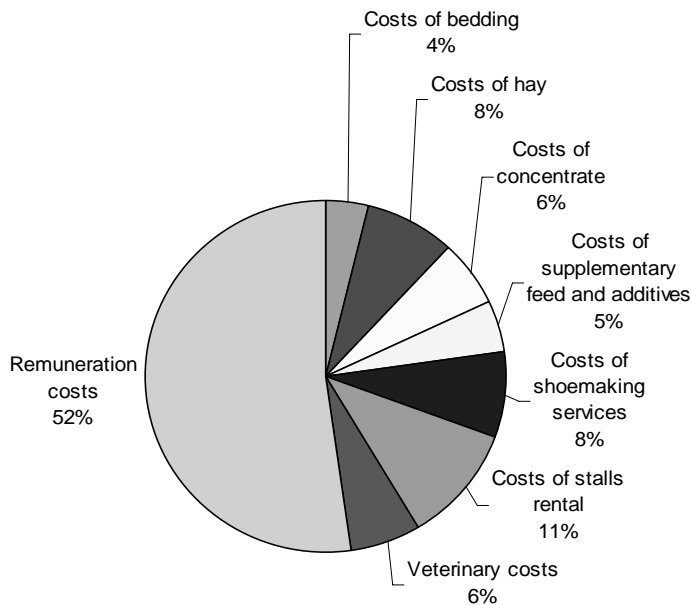


FIGURE 1. Structure of total costs in the subject stable  
Source: own study on the basis of PKI SA accounting records.

and stable personnel as well as a small number of persons willing to work in riding stables which results in difficulties in acquiring proper stable personnel and high work price. A relatively high position in the structure of total costs of running a riding stable in Warsaw track were also costs of stalls rental. Group of indirect costs consists of costs of concentrate and hay, veterinary and shoeing services. Cost of shoeing services in particular months of the examined period was on a stable level. Sandy surface of the training track in Warsaw enabled to shoe young horses as well as horses which were not in advanced training only for the front legs, which limited this item of costs. Significant changeability of costs connected with veterinary treatments in particular months of the examined period was observed. Big monthly fluctuations of costs connected with veterinary services resulted from the period of training or from the season. This phenomenon is to be connected, first of all, with the randomness of horses' injuries during the training, and second of all with vaccination timetable (2 times a year) as well as deworming of horses (3 times a year). The highest level of this type of costs was observed in the periods of training intensification or in the most important periods, in the highest subsidized races, especially of 3-year-old horses (June-August). During these periods the injury rate of the horses was increasing and as a result expenses for veterinary services connected with it. It needs to be emphasized that costs connected with treatment and veterinary prophylaxis are the least predictable and possible to plan ahead among all cost groups which make up the total keeping of a race horse.

The lowest share in costs was the cost of bedding as well as supplementary feed and mineral-vitamin additives. Supplementary feed and mineral-vitamin additives are nutrition components of high individual prices, that is why low level of costs connected with this item may seem slightly surprising. Still, it was connected with using relatively low nutrition norms concerning this type of feed since the stables in Warsaw received each year a significant number of young horses which were only receiving their racing training. Therefore, the intake of feed was lower among them than among mature horses which were in complete training. That is why they were given smaller supplementary feed portions.

Analysis of data included in Tables 1 and 2 shows that the feed costs ratio in relation to total costs for each horse was 0.19 monthly. Therefore, costs of feed were not the factor which mostly determined the level of total costs of running a stable, which is confirmed by Chrzanowski et al. research (2003), who, on the basis of costs of keeping and training the horses in two stables in the Warsaw track in season 1999 found out that nutrition costs were in one stable 24.7% and in the second – 30.9% of total costs of maintenance.

Table 2 includes data concerning income from race prizes won by the horses, total costs and total loss resulting from keeping a riding stable on the Służewiec track in particular months of the examined period (in PLN). Geographical location of Poland as well as lack of infrastructure enabling to carry out horse races in winter are main reasons of establishing, as typical, season of holding races, the period between April

TABLE 1. Costs of bedding, hay, concentrate and mineral-vitamin additives in particular months in riding stable in Warsaw in PLN

Item no.	Month	Average number of horses	Costs of bedding	Costs of hay	Costs of concentrate	Costs of supplementary feed and additives	Costs of shoemaking services	Costs of stalls rental	Veterinary costs	Remuneration costs
1.	April 04	66.0	2 257.20	4 920.30	3 989.70	4 004.96	4 831.20	8 556.66	3 819.58	37 478.61
2.	May 04	59.0	2 017.80	4 398.45	3 566.55	3 551.91	4 318.80	7 944.16	2 749.00	36 248.76
3.	June 04	61.0	2 086.20	4 547.55	3 687.45	3 571.15	4 465.20	8 132.06	6 732.00	33 989.00
4.	July 04	63.5	2 171.70	4 733.93	3 838.58	3 617.50	4 648.20	8 441.45	3 815.40	35 707.76
5.	August 04	64.0	2 188.80	4 771.20	3 868.80	3 617.26	4 684.80	8 201.31	2 963.00	34 424.00
6.	September 04	60.0	2 052.00	4 473.00	3 627.00	3 346.30	4 392.00	8 025.87	3 180.00	34 422.20
7.	October 04	59.0	2 017.80	4 398.45	3 566.55	3 249.68	4 318.80	8 194.02	5 440.45	36 182.60
8.	November 04	61.5	2 103.30	4 584.83	3 717.68	3 338.15	4 501.80	8 460.91	4 380.00	35 303.95
9.	December 04	62.5	2 137.50	4 659.38	3 778.13	3 297.67	4 575.00	8 088.16	3 414.00	38 733.56
10.	January 05	55.5	2 319.90	4 492.17	3 613.05	2 888.15	4 795.20	9 079.89	1 025.00	40 875.20
11.	February 05	55.0	2 299.00	4 451.70	3 580.50	2 794.60	4 752.00	8 087.94	1 143.20	37 457.00
12.	March 05	58.5	2 445.30	4 734.99	3 808.35	2 996.79	5 054.40	8 792.18	1 002.45	35 011.61
13.	April 05	52.5	2 194.50	4 249.35	3 417.75	2 781.97	4 536.00	7 644.13	1 243.00	36 044.00
14.	May 05	54.0	2 257.20	4 370.76	3 515.40	2 877.67	4 665.60	7 506.40	1 535.00	35 974.21
15.	June 05	60.0	2 508.00	4 856.40	3 906.00	3 106.47	5 184.00	7 132.94	1 357.18	35 378.00
16.	July 05	62.0	2 591.60	5 018.28	4 036.20	3 240.64	5 356.80	7 345.65	1 112.00	37 520.00
17.	August 05	64.0	2 675.20	5 180.16	4 166.40	3 300.75	5 529.60	7 392.39	3 082.01	39 068.00
18.	September 05	64.0	2 675.20	5 180.16	4 166.40	3 200.17	5 183.20	7 160.53	10 460.18	34 245.80
19.	October 05	64.0	2 675.20	5 180.16	4 166.40	3 199.58	5 302.40	7 521.11	1 672.24	33 606.46
20.	November 05	76.0	3 176.80	6 151.44	4 947.60	3 844.91	6 566.40	8 365.58	2 562.11	36 844.98
21.	December 05	76.0	3 176.80	6 151.44	4 947.60	3 733.57	6 516.20	8 514.13	1 129.20	38 203.98
22.	January 06	76.0	3 610.00	7 284.60	5 301.00	3 704.26	6 584.86	9 298.62	5 714.16	42 334.87
23.	February 06	76.0	3 610.00	7 284.60	5 301.00	3 676.66	6 602.16	8 327.14	9 598.70	43 815.59
24.	March 06	76.0	3 610.00	7 284.60	5 301.00	3 760.24	6 414.00	9 261.56	11 156.70	41 850.01
25.	April 06	76.0	3 610.00	7 284.60	5 301.00	3 797.35	6 389.30	7 681.16	3 469.62	38 340.01
26.	May 06	76.0	3 610.00	7 284.60	5 301.00	3 777.73	6 566.40	7 068.66	1 912.01	38 340.01
27.	June 06	76.0	3 610.00	7 284.60	5 301.00	3 896.33	6 701.90	7 256.56	12 696.20	44 063.25
28.	July 06	76.0	3 610.00	7 284.60	5 301.00	3 872.94	6 204.40	7 565.95	1 734.60	46 888.69
29.	August 06	76.0	3 610.00	7 284.60	5 301.00	3 783.04	6 500.38	7 325.81	4 971.08	37 175.55
30.	September 06	74.0	3 515.00	7 092.90	5 301.00	3 744.69	6 393.60	7 150.37	19 492.30	35 766.85
31.	October 06	68.0	3 230.00	6 517.80	5 301.00	3 383.96	5 875.20	7 318.52	7 408.65	31 491.15
32.	November 06	65.0	3 087.50	6 230.25	5 301.00	3 169.87	5 616.00	7 533.91	3 980.38	36 895.20
33.	December 06	76.0	3 610.00	7 284.60	5 301.00	3 693.83	6 566.40	7 624.66	4 540.41	31 845.44
<b>TOTAL (PLN)</b>		<b>x</b>	<b>92 349.50</b>	<b>186 906.44</b>	<b>145 524.08</b>	<b>113 820.73</b>	<b>180 592.20</b>	<b>262 000.39</b>	<b>150 491.80</b>	<b>1 231 526.30</b>
<b>ON AVERAGE (PLN/ MONTH)</b>		<b>66.3</b>	<b>2 798.47</b>	<b>5 663.83</b>	<b>4 409.82</b>	<b>3 449.11</b>	<b>5 472.49</b>	<b>7 939.41</b>	<b>4 560.36</b>	<b>37 318.98</b>
<b>ON AVERAGE (PLN/HORSE)</b>		<b>x</b>	<b>1392.903</b>	<b>2819.102</b>	<b>2194.933</b>	<b>1716.753</b>	<b>2723.864</b>	<b>3951.74</b>	<b>2269.861</b>	<b>18575.06</b>

Source: own study on the basis of PKI SA accounting records.

TABLE 2. Financial result in particular months in PLN

Item no.	Month	Income from race prizes	Total costs	Loss
1.	April 04	4 271.00	69 858.21	-65 587.21
2.	May 04	10 352.50	64 795.43	-54 442.93
3.	June 04	16 009.25	67 210.61	-51 201.36
4.	July 04	10 901.75	66 974.51	-56 072.76
5.	August 04	14 023.50	64 719.17	-50 695.67
6.	September 04	20 386.50	63 518.37	-43 131.87
7.	October 04	27 041.25	67 368.35	-40 327.10
8.	November 04	26 679.00	66 390.60	-39 711.60
9.	December 04	80.00	68 683.39	-68 603.39
10.	January 05	0.00	69 088.56	-69 088.56
11.	February 05	0.00	64 565.94	-64 565.94
12.	March 05	0.00	63 846.07	-63 846.07
13.	April 05	0.00	62 110.70	-62 110.70
14.	May 05	9 517.50	62 702.24	-53 184.74
15.	June 05	10 952.50	63 428.99	-52 476.49
16.	July 05	27 256.00	66 221.17	-38 965.17
17.	August 05	14 984.00	70 394.51	-55 410.51
18.	September 05	23 716.00	72 271.64	-48 555.64
19.	October 05	45 132.50	63 323.55	-18 191.05
20.	November 05	33 374.00	72 459.82	-39 085.82
21.	December 05	317.00	72 372.93	-72 055.93
22.	January 06	0.00	83 832.37	-83 832.37
23.	February 06	0.00	88 215.84	-88 215.84
24.	March 06	0.00	88 638.10	-88 638.10
25.	April 06	0.00	75 873.05	-75 873.05
26.	May 06	0.00	73 860.41	-73 860.41
27.	June 06	0.00	90 809.84	-90 809.84
28.	July 06	0.00	82 462.17	-82 462.17
29.	August 06	10 561.55	75 951.46	-65 389.91
30.	September 06	21 409.17	88 456.71	-67 047.54
31.	October 06	17 306.73	70 526.27	-53 219.54
32.	November 06	9 759.93	71 814.11	-62 054.18
33.	December 06	4 206.11	70 466.34	-66 260.23
<b>TOTAL (PLN)</b>		<b>358 237.74</b>	<b>2 363 211.43</b>	<b>-2 004 973.69</b>
<b>ON AVERAGE (PLN/MONTH)</b>		<b>10 855.69</b>	<b>3562.77</b>	<b>-60 756.78</b>
<b>ON AVERAGE (PLN/HORSE)</b>		<b>5403.284</b>	<b>35644.21463</b>	<b>-30240.93047</b>

Source: own study on the basis of PKI SA accounting records.

and December. The rest of the year, due to the lack of income from race prizes has to be financed from reserves established during the very season or other financing sources. Period from April 2004 till December 2006 encompassed only two typical racing seasons – the season in 2004 and 2005, whereas the 2006 season, due to the lack of a new organizer (the previous one STWK Służewiec Spółka Skarbu Państwa – Służewiec State Treasury Company – was declared bankrupt) did not start, as usually, at the turn of April and May, but as late as in August. Due to short time which was left till the winter season, the racing schedule encompassed a shortened racing list and an appropriately decreased pool of financial prizes. Consequently, year 2006 was especially difficult for race horses' owners due to the scanty repayment of maintenance costs from racing wins. Despite such a difficult situation the loss of number of horses submitted to the next racing season did not take place. It shows that the exceptionally unfavourable economic situation in 2006 did not significantly harmed horses' owners. The explanation of such a state of affairs can be found most of all in the general acceptance of the environment of the lack of profitability of keeping race horses and the need to finance means shortage from other sources as well as in the enormous numbness of the race horses breeding system in which decisions on decreasing the number of covered mares will be visible only after 2.5 years. Therefore, in case of the discussed seasons of 2006 and 2007 owners were left in a no-way-out situation since they had already had a specific number of horses ready to be trained whose value, in case of changing

their destination due to age and breed would constitute about 20% of breeding costs. Therefore, they had to agree with such conditions of season organization and expect new solutions in the following years.

Data included in Table 2 concerning the total economic effect of running the examined riding stables show that there is no economic justification for conducting this kind of activity. In none of the examined months, regardless of the racing season or noted income from prizes, that is in the “dead” winter season, not a single time did the examined stables reach a positive economic effect in the monthly settlement period which proves a stabilized unprofitability of riding stables. Without allotting bigger and bigger sums of money to prizes in the races it will be impossible to raise the costs return ratio of keeping horses in a racing training. The above mentioned findings were in accordance with research results of Chrzanowski et al. (2003) who in the season of 1999 in one of the stables in Służewiec decided that in two thirds of cases racing wins by thoroughbreds were lower than the costs of keeping and in the second one that half of the horses did not earn for their keeping. Also, keeping a riding stable outside Poland is financially unattractive which was confirmed by Gębska et al. (2008). In a stable in Ebreichsdorf the costs were 1.7 times higher than the sum of racing wins won by horses.

Comparison of income sums with the costs of running a stable (Table 2) allowed to find out that without external sources of support keeping a riding stable would not be possible since costs in the examined case were 6.43 times higher

than the sum of racing wins. Such an unprofitable financial result may result from very low winning rates which can be obtained as a result of a won race and not only horse or training quality.

## CONCLUSIONS

1. Managing a racehorse stable is an activity generating losses, in this case very high ones. Therefore, conducting horse selection in relation to their racing ability requires supporting this kind of activity.

2. Costs of stables management exceeded the income over six times. In the costs structure the main item is employees' remuneration. Due to high professional requirements expected from riding stables employees as well as the lack of the possibility of work mechanization in such stables, it is difficult to decrease them.

3. In order to cover costs connected with keeping race horses in the examined stables it would be necessary to increase racing prizes in the horse race track in Warsaw. Taking into consideration economic result of the examined stable in Warsaw Służewiec the prizes should be higher by 643%.

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**Streszczenie:** Wyniki ekonomiczne utrzymywania stajni przy torze wyścigów konnych. Analizowano efekty ekonomiczne prowadzenia stajni koni wyścigowych w warunkach warszawskiego toru wyścigowego na Służewcu w latach 2004–2006. Analiza przychodów oraz kosztów utrzymania koni wykazała brak rentowności tego rodzaju działalności. Próg rentowności zostałby osiągnięty dopiero w przypadku gdyby suma nagród wyścigowych uzyskana w gonitwach przez trenowane konie była 6,43 razy wyższa. Najwyższy udział w strukturze kosztów całkowitych w badanej stajni miały koszty wynagrodzeń pracowników stanowiące ponad 50% wysokości miesięcznych kosztów związanych z prowadzeniem badanej stajni.

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## Relationship between content of phospholipids in milk and stability of milk fat globule membrane and the effect of some factors on the frequency of their's destabilization

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**Abstract:** *Relationship between content of phospholipids in milk and stability of milk fat globule membrane and the effect of some factors on the frequency of their's destabilization.* The work involved examination of conditions affecting milk fat globule membrane (MFGM) destabilization. The investigations were conducted on 30 Polish Friesian cows. Milk composition including content of total milk fat and protein, phospholipids and free fatty acids were determined at the beginning of grazing period, during summer feeding and winter feeding season, in three stages of lactation and for three levels of daily milk yield. Daily milk yield affected significantly ( $p < 0.01$ ) phospholipids content and destabilisation MFGM frequency. The highest MFGM stability coincided with the lowest daily milk yield and the highest content phospholipids. The lowest rate of MFGM destabilization occurred in winter feeding season and was accompanied by the highest content of phospholipids. The rate of MFGM destabilization increased over 3 fold during the transition to grazing season and was very high in summer feeding seson.

*Key words:* stability MFGM, daily milk yield, season feeding, phospholipids.

### INTRODUCTION

In the initial investigations, Kuczyńska et al. (2000) found significant differences in the milk fat composition related to the susceptibility of milk fat globules destabi-

lization. All samples (particularly those of the bulk milk) subjected to analytical examination were collected in dairies. Thus, no detail information about feeding of cows, their daily milk yield or stage of lactation was available. In Poland, a considerable part of individual farmers tend to plan calvings in the spring. In the light of results indicating the influence of lactation stage on milk quality, its influence on the MFGM susceptibility to destabilisation should be also examined. Major effect on milk fat globules breakdown had a significant decrease in the concentration of milk phospholipids. The phospholipids, an integral part membrane, have both lipophilic and hydrophilic properties, and therefore contribute significantly to the stabilisation role of the membrane. The major types and their approximate percentages are: sphingomyelin –25%; phosphatidyl choline – 35.5; phosphatidyl ethanolamine – 30%; phosphatidyl inositol –5% and phosphatidyl serine –3% (Deeth, 1997). For a recent review of MFGM composition and role, see Dewettinck et. al. (2008). Thus, the present work was designed to evaluate the effect of lactation stage, daily milk yield and feeding season on the stability

of milk fat globule membrane, and then the role content of phospholipids in the stability of MFGM.

## MATERIAL AND METHODS

Cows ( $n = 30$ ) were selected from the herd according to previous milk performance testing records and allotted to three groups: with daily milk yield below 10 kg, from 10.1–20 kg and over 20 kg/day. All the cows were multiparous. In respect of the lactation stage they were divided into three groups: 21–100 days, 101–200 and 201–305 days of lactation. Milk samples were taken in three periods: 1 – at the start of grazing season (for 10 h/day at the beginning of May); 2 – during summer feeding (pasture sup-

plemented with a green forage) at the end of July; 3 – in winter feeding season. Compositions of diets in selected periods are presented in Tables 1, 2, 3. After machine milking, samples of 50 ml milk were taken to the bottles containing Mle-kostat CC. Then, they were transported to the Laboratory in the izotermic containers (at 4°C). Analytical examinations included: 1 – destabilisation of milk fat globule membrane measurements were performed using a Nikon ECLIPSE 90i microscope connected with a digital camera Nikon DS5-U1 and a computer image analysis system NIS-Elements AR (Nikon Corporation, Tokyo, Japan); 2 – estimation of somatic cell counts using Somacount 150, in order to eliminate cows with udder problems (all cows

TABLE 1. Composition of diet during winter feeding season for cows with average daily milk yield of 15 kg, and 600 kg body weight

Feed	kg	DM (kg)	UFL (kg)	PDIN (g)	PDIE (g)
Maize silage	32	9.0	8.6	447	712
Grass hay	3	2.6	1.8	212	220
Concentrate mixture*	2	1.7	1.9	135	177
Carrot	4	0.5	0.5	30	41
Brewer's grain	4	0.95	0.9	163	151

\*For every 3 kg milk over 15 kg/day – kg of concentrates DM – dry matter; UFL – feed unit for lactation; PDIN – protein digested in the small intestine supplied by rumen-undegraded; PDIE – protein digested in the small intestine supplied by rumen-undegraded protein and microbial protein from rumen fermented organic matter.

TABLE 2. Composition of diet at the start of grazing season (10 hours on pasture/day) for cows with average daily milk yield of 15 kg, and 600 kg body weight

Feed	kg	DM (kg)	UFL (kg)	PDIN (g)	PDIE (g)
Pasture (10 hours)	25	6.0	5.6	714	642
Maize silage	10	3.3	2.9	161	257
Grass hay	2	1.7	1.2	141	146
Concentrate mixture*	1	0.88	1.0	89	103
Brewer's grain	4	0.95	0.9	163	151

Explanation as in Table 1.



TABLE 3. Composition of diet during summer feeding season for cows with average daily milk yield of 15 kg, and 600 kg body weight

Feed	kg	DM (kg)	UFL (kg)	PDIN (g)	PDIE (g)
Pasture	30	9.9	6.8	876	816
Grass hay	2	1.7	1.2	141	146
Brewer's grain	4	0.95	0.9	163	151
Lucerne	10	1.9	1.6	268	189

Explanation as in Table 1.

were healthy); 3 – examination of basic chemical composition and free fatty acids on the MilcoScan FT 120; 4) separation of phospholipids by TLC method Touchstone (1995) followed by spectrometric measurements of their content. Results were analysed statistically using the SPSS package. Initially, effects of lactation stage, feeding season and daily milk on milk fat fat globule membrane stability were estimated by one way analysis of variance and regression analysis. Finally a multiple factor analysis of variance including regression for daily milk yield was performed according to the following general linear model:

$$Y_{ijk} = \mu + b(X_{ijk} - \bar{x}) + A_i + B_j + AB_{ij} + e_{ijk}$$

where:

Y – percentage of destabilisation MFGM;

$\mu$  – mean

b ( $X_{ijk} - \bar{x}$ ) – effect of regression on milk yield on sampling day;

$A_i$  – effect of lactation stage ( $i = 1, 2, 3$ );

$B_j$  – effect of feeding season ( $j = 1, 2, 3$ );

$AB_{ij}$  – effect of interaction between lactation stage and feeding season;

$e_{ijk}$  – random effect

## RESULTS AND DISCUSSION

Evaluation of milk fat globule membrane stability in milk samples from cows included into the investigation revealed

two different categories of cows: 1 – producing milk with MFGM susceptible to destabilisation and 2 – producing milk with stable MFGM. Table 4 shows the distribution of milk samples with destabilised MFGM depending on the feeding season. It appears that the lowest susceptibility to destabilisation occurred in winter feeding season. Then, an inclusion of pasture (for 10 h/day) induced over 3-fold increase in the number of milk samples with destabilised MFGM. Transfer to full time grazing resulted in its further increase. The summer mixed diet did not cause any significant change. Although, less cows produced milk with lower stability of MFGM was higher than at the beginning of grazing period, suggesting a higher degree of fat destabilisation. The beginning of grazing season makes a considerable change of feeding conditions for cows. There is a general agreement that diet and stage of lactation influence milk fat composition (Nałecz-Tarwacka et. al., 2008). Thus, it was assumed that destabilisation of MFGM occurring at the beginning of grazing season is rather a consequence of usually increased at this time calving rate, and resulting early lactation period. Preliminary examination of distribution of early lactation cows with stable or unstable MFGM throughout different feeding seasons indicated that milk from early lactation cows exhibited

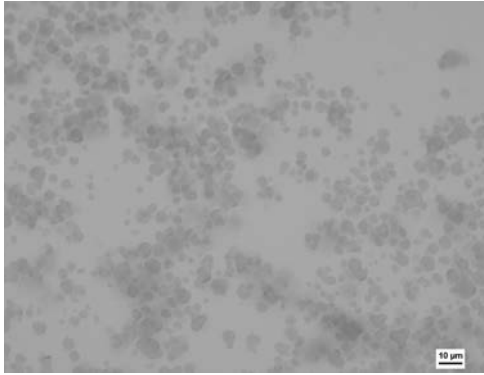


PHOTO 1. Destabilisation MFGM

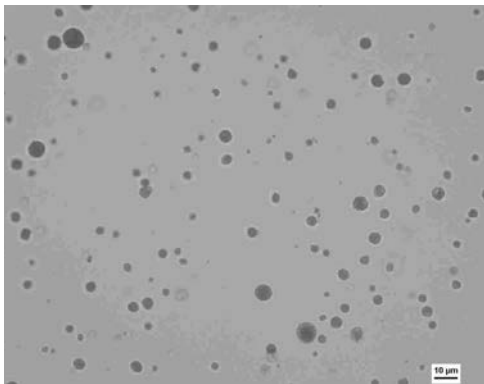


PHOTO 2. Stable MFGM

variable stability of fat globule membrane depending on feeding season (Table 5). Variable MFGM stability in milk originating from the same lactation stage suggest that other factors are responsible for the variation. It seems to confirm the results reported by Sharma et al., (1990) who did not demonstrate significant differences in milk fat composition between early, mid-, and late lactation. The effect of feeding season on MFGM susceptibility to destabilisation was evaluated at first by one way analysis of variance. The results verified in a multiple factor analysis are presented in Table 4. Composition of milk samples from different feeding seasons failed to demonstrate any significant differences in lipid composition, except phospholipids and free fatty acids content used as criterions of MFGM destabilisation rate together with microscopic examination, which was about three fold lower in winter than in summer feeding season ( $p < 0.05$ ). The equal proportion of early- and late lactation cows in this season excludes a pos-

TABLE 4. Effect of feeding season on the content of some milk components

Traits	Feeding season						Value F
	Transition to grazing season		Summer feeding		Winter feeding		
	LSM	SE	LSM	SE	LSM	SE	
Total milk fat (g/100 g milk)	4.32	0.14	3.97	0.18	4.28	0.15	1.19
Total milk protein (g/100g milk)	3.20	0.08	2.96	0.10	3.27	0.08	2.64
Free fatty acids (mg/100 g milk)	7.72	0.32	8.60	0.42	7.50	0.34	2.11
Phospholipids (mg/100 g milk)	0.780	0.02	0.750	0.03	0.790	0.02	0.48
Destabilisation MFGM (%)	9	0.06	10	0.08	3	0.06	5.68*

\*significantly at  $P \leq 0.05$ .

TABLE 5. Distribution of milk sampled from early lactation cows with stable and unstable MFGM depending on feeding season (%)

MFGM stability (%)	Transition to grazing season	Summer feeding	Winter feeding
Stable	23	50	64
Unstable	77	50	36

TABLE 6. Effect of daily yield on the content of some milk components

Traits	Milk yield						Value F
	< 10 kg		10.1–20 kg		> 20 kg		
	LSM	SE	LSM	SE	LSM	SE	
Total milk fat (g/100 g milk)	4.44	0.15	4.09	0.13	4.04	0.21	1.39
Total milk protein (g/100 g milk)	3.26	0.09	3.22	0.09	2.95	0.12	2.14
Free fatty acids (mg/100 g milk)	7.80	0.36	8.50	0.30	7.50	0.49	2.41
Phospholipids (mg/100 g milk)	0.795	0.02	0.820	0.02	0.710	0.03	3.87*
Destabilisation MFGM (%)	8.0	0.07	16	0.06	40	0.09	3.33*

Explanation as in Table 4.

sibility that lactation stage is a reason of the highest destabilisation of MFGM. The highest rate of MFGM destabilisation expressed in the highest percentage under microscope in summer feeding season coinciding with the highest free fatty acids and the lowest phospholipids content. Increased lipolysis coinciding with the destabilisation milk fat membrane globule suggest that the amounts of phospholipids could be insufficient to maintain integrity of MFGM. Such a suggestion in relation to phospholipids was reported by Deeth (1997). The decrease of total protein and fat content recorded in summer feeding season indicate to less favourable feeding condition compared with winter season. Such conditions may hasten the rate of lipolysis, confirmed by Jurczak (1994) on the large number of data. MFGM stability and content of some lipid components playing important role in milk fat globule membrane stability depending on daily milk yield is presented in Table 6. Polynomial

regression analysis of daily milk yield on destabilisation MFGM was significant ( $p < 0.05$ ). However, because the effect milk yield is biased with the lactation stage a multiple factor analysis of variance including regression of daily milk yield on MFGM stability was performed and it was found the effect of lactation stage on the susceptibility of fat globule membrane was insignificant.

## CONCLUSIONS

Daily milk yield affected significantly ( $p < 0.01$ ) phospholipids, globular protein content and destabilisation MFGM frequency. The highest MFGM stability coincided with the lowest daily milk yield and the highest content phospholipids. The lowest rate of MFGM destabilization occurred in winter feeding season and was accompanied by the highest content of lipid phosphorus ( $p < 0.05$ ). The rate of MFGM destabilization increased over 3 fold during the transition to gra-

zing season and was very high in summer feeding season.

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**Streszczenie:** *Zależność między zawartością fosfolipidów w mleku a stabilnością MFGM i wpływ wybranych czynników na częstotliwość ich destabilizacji.* W pracy badano wpływ stadium laktacji, wydajności mlecznej i sezonu żywienia na stabilność otoczek kuleczek tłuszczowych (MFGM- milk fat globule membrane) oraz zawartości fosfolipidów w mleku na częstotliwość występowania ich destabilizacji. Próbki mleka pobierano od 30 krów, ocielonych wiosną w 3 sezonach żywienia: w przejściowym na początku wypasu pastwiskowego, gdy krowy przebywały pierwsze 10 godz. na pastwisku, w letnim po 2 miesiącach pełnego wypasu pastwiskowego uzupełnianego zielonką zadawaną do żłobu i alkierzowego w okresie zimy. Destabilizację MFGM obserwowano pod mikroskopem. Badania analityczne mleka obejmowały ogólny skład chemiczny, zawartość fosfolipidów i wolnych kwasów tłuszczowych. Zaobserwowano, że krowy w okresie przejściowym z żywienia zimowego na letnie, przebywające pierwsze 10 godz. na pastwisku produkowały mleko ze zwiększoną 3-krotnie destabilizacją MFGM, która utrzymywała się w okresie letnim. Wyższą stabilnością MFGM charakteryzowało się mleko pochodzące od krów w sezonie żywienia zimowego, które zawierało wyższą zawartość fosfolipidów. Mleko pozyskiwane od krów z okresu żywienia alkierzowego wykazywało wyższą stabilność otoczek kuleczek tłuszczowych, było mniej podatne na lipolizę i posiadało wyższą zawartość fosfolipidów w porównaniu z okresem żywienia letniego.

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## PRNP gene polymorphisms in Polish population of East-Friesian milk sheep

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**Abstract:** *PRNP gene polymorphisms in Polish population of East-Friesian milk sheep.* The research was carried out on the flock of East-Friesian Milk sheep in Poland to genotype the prion protein gene (PRNP) polymorphisms. Blood samples were collected from 108 ewes and 7 rams and the DNA was isolated by the method of chromatography on silica mini-columns (A&A Biotechnology). The investigation of polymorphisms in the PRNP gene was done due to the KASPar® technology. Frequencies of alleles ARR, ARQ and AHQ were 0.335, 0.391 and 0.274, respectively. Six genotypes of PRNP gene were observed. The ARR/ARR genotype was the most valuable at statistically significant level and the frequency of ARR allele was higher in rams in contrary to ewes what may be important in case of its possibly high frequency in offspring. It may be confirmed by the low frequencies of ARQ and AHQ alleles ( $p \leq 0.05$ ) observed in rams in contrary to the ewes. No differences in the frequencies of other genotypes (except ARR/ARR) due to the sex of animals were observed. The breeding work should be continued to increase the frequency of ARR allele. The selection of best rams with the ARR/ARR as the flock rams as well as the elimination of ARQ allele in flock ewes are preferred.

*Key words:* East-Friesian Sheep, PRNP, alleles and genotypes.

### INTRODUCTION

In 2001 the EU Parliament established the law rules concerning the prevention,

controlling and eradication of transmissible spongiform encephalopathies (no. 999/2001/EC) (7). In 2003, the decision of European Commission no. 2003/100/EC (8) imposed the obligation of creation the breeding systems aiming the increase of genetic resistance to scrapie in every sheep breed in Europe. Moreover, the regulation no. 260/2003/EC (9) determined the eradication of TSE in sheep and goats as well as regulated the trade of live sheep and goat as well as bovine embryos. Scrapie is the natural occurring form of transmissible spongiform encephalopathy (TSE) in sheep, as it is the BSE in cattle and Crauzfeldt-Jacob disease in human. Presumably, the prion protein (PrP) is responsible for the occurring of scrapie in sheep. Several polymorphisms in PRNP gene were observed at codons: 136, 154 and 171, which seems to be responsible for the genetic resistance or sensibility for scrapie (1, 2, 5).

Moreover, it has been approved that the ARR allele guarantees the lowest sensibility for scrapie. It was observed in Great Britain and the Netherlands, that VRQ allele is responsible for the high level of sensibility for scrapie in sheep. Whereas, ARR allele occurred rarely in

sheep presenting the clinical symptoms of scrapie. Therefore, the selection for ARR allele is the basic tool in eradication and controlling of scrapie in sheep (3, 4, 5). According to all these aforementioned reasons, the attempt of the evaluation of the frequencies of alleles and genotypes in the whole flock of East-Friesian Milk sheep was undertaken accordingly to the EU regulations.

## MATERIAL AND METHODS

The research was carried out on the sheep farm located in malopolskie voivodeship (Poland). The flock of East-Friesian Milk sheep contained 108 ewes and 7 rams. The blood samples were taken from the jugular vein (*vena jugularis*) to 9 ml test tubes S-Monovette (Sarstedt AG&Co., Numbrecht, Germany). The genomic DNA was isolated from collected blood samples which were stored in 4°C. Before the proper DNA isolation the blood samples were purified from the heme compounds and the products of erythrocytes lysis. The proper DNA isolation from leucocytes was conducted by the method of chromatography on silica mini-columns (A&A Biotechnology, Gdańsk, Polska, www.aabiot.com). Afterwards, the DNA samples were amplified and genotyped

due to the KASPar® technology (www.kbioscience.co.uk) and then searched for the four SNPs in the PRNP gene. The primers and SNP polymorphisms were presented in Table 1.

This method allowed to establish five alleles of PRNP gene: ARR, ARQ, AHQ, ARH and VRQ, which were used for preparing the distribution of alleles and genotypes within ewes and rams, separately. Afterwards, the results were applied in further studies. The range of the frequencies of alleles and genotypes between both sexes as well as the differences between sexes within the frequencies of alleles and genotypes were calculated with chi-square test using the SPSS v.12.0 software (10).

## RESULTS AND DISCUSSION

The results are presented in Table 1 and on the Figure 1. Within frequencies of alleles and frequencies of genotypes in PRNP gene were observed between ewes and rams at respectively, the highly significant ( $p \leq 0.01$ ) and significant ( $p \leq 0.05$ ) levels. Only three alleles (ARR, ARQ and AHQ) and its six genotype combinations were observed. The higher frequency of ARR allele in rams ( $p \leq 0.01$ ) and much lower frequencies of ARQ and AHQ

TABLE 1. Primers and genotyped SNPs in the PRNP locus gene

Locus	Name	Codon	Primers 3' to 5' (Forward/Reverse)	Genotyping method	SNP	Change	Place
PRNP	Prion protein	136	CACAGTCAGTGGAAACAAGCC/ CCCCAACCTGGCAAAG	PCR-KASPar®	AY909542:g.479C>T	C/T	exon 3
		154	CACAGTCAGTGGAAACAAGCC/ CCCCAACCTGGCAAAG		AY909542:g.534G>A	G/A	
		171	CACAGTCAGTGGAAACAAGCC/ CCCCAACCTGGCAAAG		AY909542:g.385A>G	A/G	
		171	CACAGTCAGTGGAAACAAGCC/ CCCCAACCTGGCAAAG		AY909542:g.386G>T	G/T	

TABLE 2. The frequencies of genotypes and alleles of PRNP gene in East-Friesian Milk sheep according to rams and ewes

Genotypes		Rams		Ewes	
		N	%	N	%
ARR/ARR	*	3	42.86**	7	6.48
ARR/ARQ		2	28.57	23	21.30
ARR/AHQ		2	28.57	30	27.78
ARQ/ARQ		0	0.00	24	22.22
ARQ/AHQ		0	0.00	17	15.74
AHQ/AHQ		0	0.00	7	6.48
Alleles					
ARR	**	10	71.43**	67	31.02
ARQ		2	14.29	88	40.74**
AHQ		2	14.29	61	28.24*

Significance at: \*P≤0.05; \*\*P≤0.01.

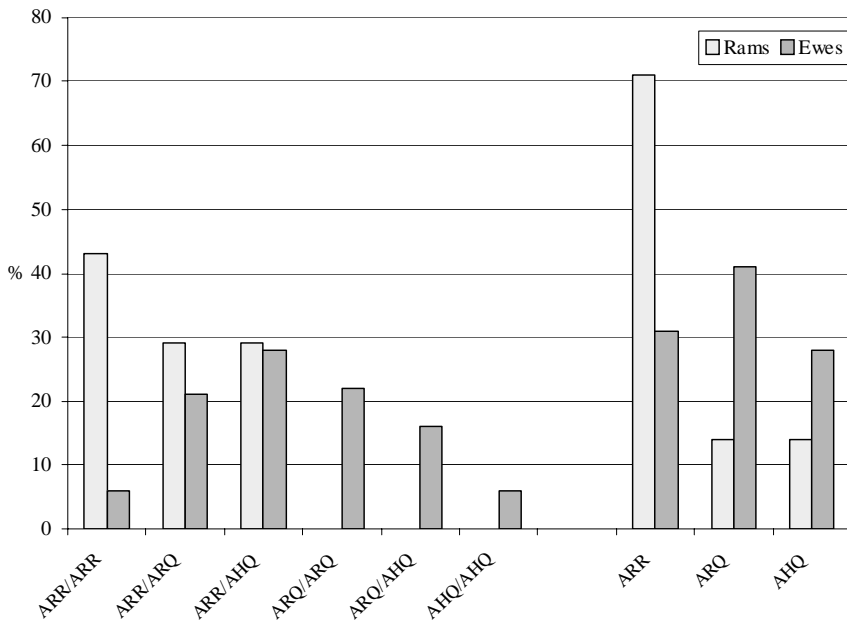


FIGURE 1. Distribution of the frequencies of genotypes and alleles of PRNP gene in East-Friesian Milk sheep according to rams and ewes (n = 115)

alleles in contrary to ewes may be found as the positive results. According to genotype frequencies, all rams presented at least one ARR allele in their genotypes. Also, in this group the frequency of

ARR/ARR genotype occurred at highly significant level ( $p \leq 0.01$ ) in contrary to ewes. No VRQ allele was observed in the flock, that allows to avoid the culling of any animal in the flock. Such distribution

of alleles and genotypes in PRNP gene has not appeared in other sheep breeds (1, 2, 3, 4, 5, 6), what indicates the possibility to manage the proper breeding work on this field.

Generally summing up, the significantly high frequencies of ARR/ARR genotype and ARR allele in rams in contrary to ewes may be considered as much more valuable, what augurs the increase of the these frequencies in offspring. This thesis is supported by the low frequency of both ARQ and AHQ alleles in rams (respectively, at  $p \leq 0.01$  and  $p \leq 0.05$  levels) in contrary to ewes. Moreover, the lack of statistical differences within all genotypes (except of ARR/ARR) between both sexes also approved the aforementioned thesis. Also the only rams with ARR/ARR genotype should be chosen as the reproductive rams in further breeding schemes.

## CONCLUSIONS

1. The highly significant ( $p \leq 0.01$ ) and significant ( $p \leq 0.05$ ) differentiation in frequencies of alleles and genotypes in PRNP gene were observed between ewes and rams, respectively.

2. The frequencies of ARR allele and ARR/ARR genotype in rams occurred at significant level (respectively, at  $p \leq 0.05$  and  $p \leq 0.01$  levels) in contrary to ewes. All rams presented at least one ARR allele in their genotypes.

3. Generally summing up, the breeding work should be continued to increase the frequency of ARR allele and ARR/ARR genotype by the selection of rams with the ARR/ARR and using them in breeding schemes.

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**Streszczenie:** Polimorfizm genu *PRNP* u wschodniofryzjskich owiec mlecznych w Polsce. Badania zostały wykonane w stadzie owiec wschodniofryzjskich zlokalizowanym w południowej Małopolsce na materiale 108 macioerek i 7 tryków. Przeprowadzono oznaczania alleli i genotypów trzęsawki na podstawie analizy DNA genomowego izolowanego z krwi, metodą KASPar®. Na podstawie przeprowadzonych badań stwierdzono wysoce istotne zróżnicowanie frekwencji alleli i istotne genotypów pomiędzy ocenianym w tym zakresie płciami u owiec wschodniofryzjskich. Wykazano wysoko istotnie lub istotnie wyższą częstotliwość występowania alleli ARR i genotypu ARR/ARR u tryków w porównaniu

z maciorkami, jak również brak osobników płci męskiej nie zawierających w genotypie allelu ARR. W związku z powyższym wskazane jest prowadzenie dalszej pracy hodowlanej prowadzącej do zwiększenia częstotliwości występowania allelu ARR i genotypu ARR/ARR poprzez wybór do dalszej hodowli tryków ARR/ARR i wykorzystanie ich do krycia.

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## Characterization of body constitution of old type Polish Merino ewes bred in mazowieckie voivodeship

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**Abstract:** *Characterization of body constitution of old type Polish Merino ewes bred in mazowieckie voivodeship.* The study was conducted in 2008 on the 5 flocks of the old type Polish Merino sheep, which were classified into the breed preservation programs. The flocks were located in the mazowieckie voivodeship at territory of 3 districts: plockie (3 flocks), ciechanowskie (1 flock) and grojeckie (1 flock). The ewes were at the age of 2–11 years (225 heads in total). The body measurements were done in 8 places on live animals and their body weight was also obtained. The study showed the huge variation of body constitution and body weight of the old type Polish Merino ewes according to the age and the flock. The results indicate the necessity of stronger focusing on the body constitution as well as the breeding conditions typical for that breed in the aim of the unification of body constitution and body weight of sheep which are classified to the programs of preservation of genetic resources.

*Key words:* old type Polish Merino, body measurements.

### INTRODUCTION

The choice of flocks of sheep breed included to the group of the old type Polish Merino was done in 2008. Five of these flocks were located in mazowieckie voivodeship. Also in 2008, all ewes and rams of Polish Merino were reviewed in flocks chosen by the Regional Offices of

Sheep and Goat Breeders Association. Only 56 flocks keeping 3265 ewes were classified into the breeding program of the old type Polish Merino sheep. The finding of appropriate number of breeding rams was the general problem, what was the cause to find out the better exterior patterns for that breed. Due to aforementioned reasons, such breed patterns was the main aim of this study conducted on 5 flocks located in mazowieckie voivodeship, in which the body measurements and body weights of ewes were established to characterize these traits in sheep of that type in population from mazowieckie region.

### MATERIAL AND METHODS

The study was conducted in 2008 on the 5 flocks of the old type Polish Merino sheep, which were classified into the breed preservation programs. The flocks were located in the mazowieckie voivodeship at territory of 3 districts: plockie (3 flocks), ciechanowskie (1 flock) and grojeckie (1 flock). The ewes were at the age of 2–11 years (225 heads in total) and were kept all the year round in stables with the feeding system based on on-farm feeds

production due to the norms. All ewes were weighted and the body measurements were done in 8 places (Niżnikowski i wsp. 2007):

- with the measuring cane: height in withers, body length, spread of chest and depth of chest;
- with the measuring tape: length of head, spread of head, round of foreshank and length of foreshank.

Obtained values were calculated including the effect of the year of birth of ewe as well as the effect of the flock. Moreover, the regression for the body weight of ewe was used in the statistical model for the body measurements. The statistical calculation was conducted in SPSS v.12.0 software (2004). The Duncan test was used to evaluate the differences between flocks and between years in which the ewes were born.

## RESULTS AND DISCUSSION

The effects of chosen factors and interactions on examined traits are presented in Table 1. The effect of year of birth of

ewe affected the body length ( $p \leq 0.05$ ), measurements of foreshank ( $p \leq 0.05$  and  $p \leq 0.01$  for the round and length, respectively) as well as body weight ( $p \leq 0.01$ ). Due to the body length the highest values were observed in ewes born in 1999–2001, 2004 and 2006 (Figure 1). The highest values of foreshank measurements (Figure 2) were observed in animals born in 1999–2001, whereas the highest value of the round of foreshank was presented in ewes born in 2008. The lowest body weight (Figure 3) was obtained in animals born in 2000 and 2008, what may indicate the different result in contrary to animals born in the corresponding years.

The effect of flock (Table 1) appeared to be stronger almost in all traits (excluding the lengths of head and foreshank). Analyzing the data gathered in Table 2, the highest height in withers was observed in the flock no. 3 in contrary to flock no.1. Due to the body length, the lowest value was observed in the flock no. 2 in contrary to the flocks no. 1 and 4. The spread of chest and depth of chest

TABLE 1. Effect of chosen factors on body measurements and live body weight in old type polish merino sheep

Traits	Effect of:			$\bar{X}$	S
	Year of birth	Flock	Body weight		
Height in withers (cm)	NS	X	XX	64.95	0.40
Body length (cm))	X	XX	XX	72.20	0.34
Spread of chest (cm)	NS	XX	XX	27.52	0.25
Depth of chest (cm)	NS	XX	XX	33.12	0.18
Length of head (cm)	NS	NS	XX	23.80	0.11
Spread of head (cm)	NS	XX	XX	11.68	0.75
Round of foreshank (cm)	XX	X	XX	8.57	0.50
Length of foreshank (cm)	X	NS	X	12.25	0.70
Body weight (kg)	XX	XX	–	58.58	0.80

X -  $P \leq 0.05$ ; XX -  $P \leq 0.01$ ; NS – non-significant.

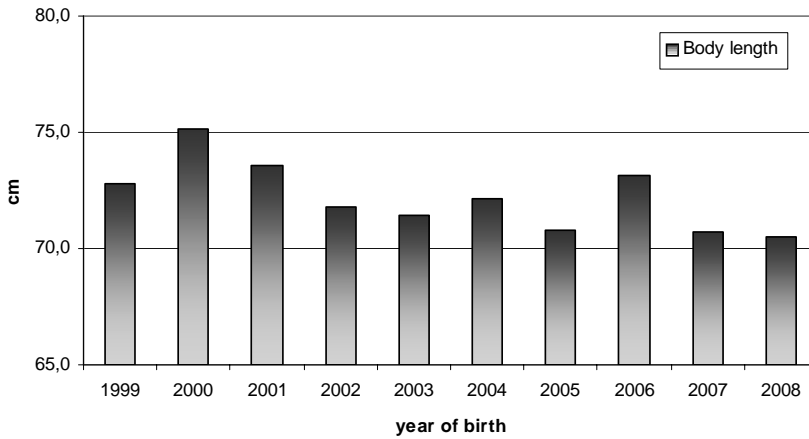


FIGURE 1. Body length of ewes of the old type polish merino sheep according to the year of birth

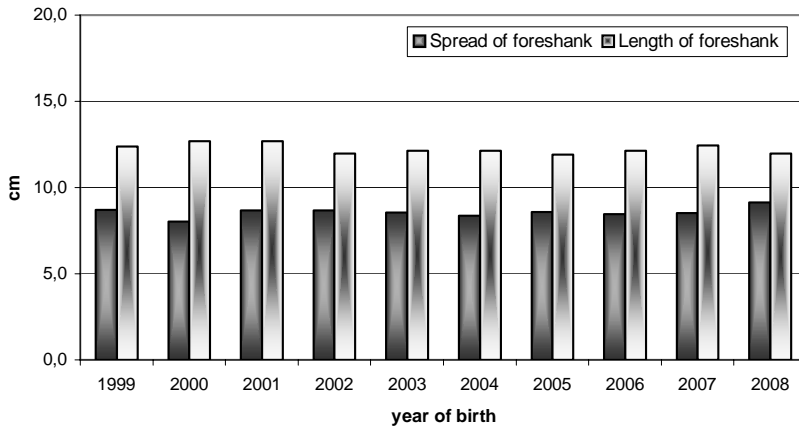


FIGURE 2. Spread and length of forshank of ewes of the old type polish merino sheep according to the year of birth

were the lowest in the flock no. 3 as well as the spread of head. The highest values of the round of foreshank were presented in the flock no. 3. The general analysis of the body measurements indicated the smallest body constitution of sheep kept in the flock no. 3 and this trend is not confirmed in body weight of animals from that flock. The lowest values of body weight were observed in the flocks of no. 2 and 3 (ciechanowski and grojecki mu-

nicipalities) and the highest in the flock no. 5 (plocki municipality). Summing up, the body constitution of Merino sheep in the mazowieckie voivodeship is highly varied, e.g. between the plocki municipality and the other, where homogeneity of these traits should be increased by the proper breeding work. Due to the fact, that the values of the aforementioned traits may be affected by the environmental conditions in several years as

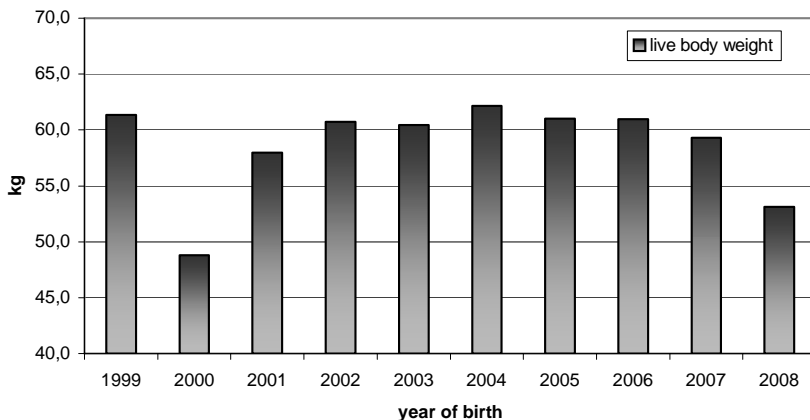


FIGURE 3. Live body weight of ewes of the old type Polish merino sheep according to the year of birth

TABLE 2. Parameters of the body conformation and body weight of ewes of old type Polish merino sheep in the studied farms

Traits		Farm 1 (A)	Farm 2 (B)	Farm 3 (C)	Farm 4 (D)	Farm 5 (E)
Height in withers (cm)	LSM	63.58	65.21	65.76	64.60	65.60
	SE	0.52	0.75	0.69	0.60	0.67
	*	C,E		A		A
Body length (cm)	LSM	74.32	67.97	71.80	74.52	72.40
	SE	0.44	0.64	0.59	0.50	0.57
	*	B,C,E	A,C,D,E	A,B,D	B,C,E	A,B,D
Spread of chest (cm)	LSM	28.63	28.52	25.92	27.40	27.12
	SE	0.33	0.47	0.44	0.38	0.42
	*	C,d,e	C,d,e	A,B,D,e	A,b,C	A,b,c
Depth of chest (cm)	LSM	33.63	33.45	32.55	33.58	32.38
	SE	0.23	0.34	0.31	0.27	0.30
	*	B,D	c,E	A,b,C	D,E	A,B,D
Length of head (cm)	LSM	23.75	23.21	24.35	23.97	23.74
	SE	0.14	0.20	0.19	0.16	0.18
Spread of head (cm)	LSM	11.37	12.78	9.88	12.22	12.14
	SE	0.98	1.42	1.31	1.12	1.27
	*	b,C	a,C,D,e	A,B,E	B	b,C
Round of foreshank (cm)	LSM	8.64	8.37	8.71	8.54	8.60
	SE	0.07	0.10	0.09	0.08	0.09
	*	b	a, c	b		
Length of foreshank (cm)	LSM	12.19	12.16	12.37	12.26	12.26
	SE	0.10	0.14	0.13	0.11	0.12
Body weight (kg)	LSM	60.50	54.27	52.07	61.41	64.64
	SE	1.05	1.47	1.29	1.20	1.32
	*	B,C,E	A,D,E	A,D,E	B,C,e	A, B, C, d
	N	56	35	42	52	40

\* - statistical significance at: a, ..., e -  $P \leq 0.05$ ; A, ..., E -  $P \leq 0.01$ .

well as in the several farms, probably, the uniformity of these factors should be the first step for the breeding programs. The commission which qualified the animals to the program in the whole Poland, focused on the general appearance and was not able to focus on every point of the exterior, which were determined in this study. Obtained results gives the opportunity to overall sight on the population of Merino sheep in old type bred in Poland and this population needs the breeding work in the aim of the unification of the body constitution.

## CONCLUSIONS

1. The huge variation of body constitution and body weight of the old type Polish Merino ewes according to the age and the flock was observed.

2. The results indicate the necessity of stronger focusing on the body constitution as well as the breeding conditions typical for that breed in the aim of the unification of body constitution and body weight of sheep which are classified to the programs of preservation of genetic resources.

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**Streszczenie:** *Charakterystyka budowy ciała macioerek merynosowych starego typu utrzymywanych w województwie mazowieckim.* Badaniami objęto stada macioerek merynosa starego typu z terenu woj. Mazowieckiego położone w powiatach: plockim (3 stada), ciechanowskim (1 stado) i grójeckim (1 stado) zakwalifikowanych do programu ochrony genetycznych zasobów tej rasy. Maciorki znajdowały się w wieku od 2 do 11 lat (łącznie 225 sztuk). Na zwierzętach dokonano oceny 8 pomiarów zoometrycznych oraz masy ciała. Wykazano znaczne zróżnicowanie typu budowy i masy ciała macioerek rasy merynos polski starego typu w zależności od wieku i stada. Wyniki prac wskazują na konieczność zwracania większej uwagi na typ budowy oraz warunki utrzymania tej rasy, celem ujednoczenia typu budowy oraz masy ciała zwierząt biorących udział w programie ochrony genetycznych zasobów.

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## Chemical composition and physico-chemical properties of meat of Ayam cemani hens and cocks

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**Abstract:** *Chemical composition and physico-chemical properties of meat of Ayam cemani hens and cocks.* The experimental material consisted of 30 slowly growing Ayam cemani chickens (15 males and 15 females), reared since hatching until the 18 week of life. The birds were fed the standard full – ration diets for broilers: starter – 22.1% of protein and 12.8 MJ ME, grower – 21% of protein and 13.2 MJ ME, and finisher – 20% of protein and 13.4 MJ ME. After slaughter, dissection was carried out and the samples of breast muscles and leg muscles from 9 females and 9 males were collected for analysis. In the muscles, the following determinations were performed: basic chemical composition (dry matter, crude protein, crude fat), content of haemic dyes and technological properties (48h after slaughter), pH, water holding capacity and termal drip. The mean content of dry matter in breast muscles of Ayam cemani hens amounted to 26.5% in females and 26.3% in males; in leg muscles – 26.3% in females and 25.8% in males. The breast muscles as well as leg muscles were characterized by lower level of dry matter in males. The breast muscles of females contained 24.5% of protein and of males – 24.8%, the leg muscles contained 21.4% and 21.6% respectively. The leg muscles were characterized by higher fat content (females 3.7% and males 3.6%) as compared to breast muscles (females 1.0% and males 0.6%) and contained 3-times more haemic dyes (157–57 ppm of haemine). A similar pH value was found in the breast muscles (5.5–5.6) as well as in leg muscles (6.0–6.2). Any statistically significant differences in chemical composition and physicochemical properties of the breast and leg muscles, depending on the sex, were not found.

*Key words:* Ayam cemani, meat, chemical composition, physicochemical properties.

### INTRODUCTION

In poultry meat production in Poland, only crossbreds of slaughter chickens and turkeys, highly specialized in this directions, are presently utilized. A high growth rate of body weight of the discussed birds, with the unequal development of the whole body, leads often to metabolic disturbance, in consequence, it is negatively reflected in their health state and first of all – from the consumer’s point of view – in meat quality. The age has an influence on dressing percentage, musculature and fatness of the carcass, i.e. on the effectiveness of production of poultry carcasses and poultry in elements (Grabowski 1993). Ayam cemani is a breed of very origin and interesting hens, being a local variety, coming from a black hen Ayam kedu, which is mostly managed by the inhabitants of villages: Kedu, Beji and Kahuripan in Kedu district, Temanggung Regency, province situated in the central Java. The word “ayam” means “hen”; the word “cemani” derives from Javanese language and means

“completely black” ([www.balitnak.litbang.deptan.go.id](http://www.balitnak.litbang.deptan.go.id)). The black colour concerns the whole body of hen, together with the comb, bells, beak, eye-balls, skin, legs, feathers and internal organs, bones and muscles. The population of Cemani in Indonesia is utilized for production of eggs and meat to the internal market. For Javanese people, it is also a ritual material; sometimes the birds are utilized in medicine, inter alia, in circulatory or respiratory system diseases (Muryanto 1991, Iskandar, Saepudin 2005). Ayam cemani in Europe are considered as decorative hens. Body weight of adult male at the age of 18 weeks is equal to 2.0–2.5 kg and that of female – 1.5–2.0 kg (B.D.R.G. 2006). In highly developed countries (Germany, France), production of slowly growing chickens supplies ca. 10–40% of poultry meat to the market and their popularity among the consumers is constantly increasing (Kijowski 2002). Prolongation of rearing period affect the concentration of chemical compounds in breast and leg muscles what has an influence on more attractive smell and taste and by this, on sensory qualities (Qinghua 1994, Fujimura et al. 1994). The hens with a black skin (Silky) being commonly met in retail sale e.g. in the USA, are highly popular due to the original appearance of the carcass, as compared to typical female broiler. In Poland, ostrich meat is the only one luxury poultry meat. It is characterized by a low caloricity and a relatively small content of cholesterol and by a favourable profile of fatty acids (Sales et al. 1996). In respect of amino acid composition, the ostrich meat is similar to beef and meat of broiler chickens (Sales, Oliver – Lyons 1996); it contains more

phosphorus, iron, copper and manganese (Sales, Oliver – Lyons 1996, Lombardi – Boccia et al. 2002, Lombardi – Boccia et al. 2005, Majewska et al. 2009).

The aim of the conducted experiment was to compare chemical composition and physicochemical properties of meat of Ayam cemani females and males.

## METHODS AND MATERIALS

The experiment was conducted In Poultry Farm of SGGW In Obory, on 30 Ayam cemani chickens (15 females and 15 males), reared since hatching until 18 week of live. The chickens were kept on the litter in standard conditions. The feed, employed in the experiment, did not differ in respect of the content of nutrients. During the first period of rearing until 21 day of life, the chickens were fed the standard diet of starter type, with 22.1% of protein, 12.87 MJ ME, 1.32% of lysine and 0.97% of methionine + cysteine. During the period from 22 to 118 day, grower diet contained 21.1% of protein, 13.2 MJ ME, 1.24% of lysine and 0.94% met. + cys. On the other hand, the content of the mentioned above components in finisher, being used until the end of rearing, amounted to 20% of protein, 13.4 MJ ME, 1.14% lysine and 0.89% met. + cys., respectively.

On 126 day (18 week), 9 males and 9 females from each experimental group, with body weight similar to the mean for a given sex, there were selected for slaughter, to further studies. The carcasses of the chickens were chilled by the air method at temperature of 4°C for 24 h, then dissection was carried out, on the ground of which dressing percentage was determined and the following calcu-

lations were performed: participation of breast muscles, leg muscles, abdominal fat and offals in the carcass. For analytical tests, nine samples each from breast and leg muscles of males and females were collected. After 48 h since slaughter, the following determinations were carried out: pH – according to PN – ISO 2917, water – holding capacity – by centrifuge method acc. to Wierbicki et al. (1962), the quantity of drip after thermal treatment (30 g of disinter grated meat was heated up in the beaker, covered with self – sticking polyethylene foil, in water bath at temperature of 72°C for 30 min.) and also, basic chemical composition (water, protein, fat and ash content) by standard methods (AOAC, 1995).

Significance of differences was statistically developed by variance analysis, calculated by the least square method in statistical programme SPSS 14.0 Pl for Windows. Values of the examined traits, as given in the tables, were expressed by the lowest square means (LSM) and standard errors of the means (SEM).

## RESULTS AND DISCUSION

In the period of 21–126 day of rearing, the highest body weight was obtained by Ayam cemani males. Statistical analysis revealed significant differences ( $P \leq 0.05$ )

in body weight at the age of 21 days as well as significant differences ( $P \leq 0.01$ ) at the age of 119 and 126 days (Table 1). On 126 day of life, differences in body weight between females and males amounted 660 g, in overage.

Feed conversion during the whole rearing period for females and males total amounted to 4.1 kg per 1 kg of body weight gain whereas the mortality rate was equal to 3.3%. In respect of dressing percentage, any significant differences between females and males were not found; it varied from 73.3 to 72.5%. Similarly, any differences in the participation of leg muscles between the groups were not recorded; on the other hand, the differences were found ( $P \leq 0.05$ ) in the participation of breast muscles. The highest participation of the breast as well as leg muscles was recorded in the group of females (Table 2). Gradually with the growth of the chickens and physiological changes, occurring in their bodies, the concentration of chemical compounds, being meat flavor sensors was also changing in their muscles; the maximum level of the mentioned substances is reached not earlier than after obtaining sexual maturity by the birds (Flechter 1999).

Any significant influence of sex on basic chemical composition of the meat

TABELA 1. Mean body weight kurcząt Ayam cemani (g)

Group	21 day	119 day	126 day
	LSM	LSM	LSM
♂	151.6 <sup>a</sup>	1853.9 <sup>A</sup>	1879.3 <sup>A</sup>
♀	128.4 <sup>b</sup>	1210.7 <sup>B</sup>	1218.0 <sup>B</sup>
SE	8.02	85.7	84.1

<sup>a,b</sup> Means with the different subscripts differ significantly at  $P \leq 0.05$ ,

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ .

of Ayam cemani chickens was not found. In the breast samples content of water amounted to 73.5–73.7% in average, that of protein 24.5–42.8%, of fat 0.6–1.0% and of ash 1.1–1.2%. On the other hand, in the leg muscles the content of water was equal to 73.7–74.2%, of protein 21.4–21.6%, of fat 3.6–3.7% and of ash 1.1% (Tables 3 and 4). Contrary to leg muscles, the breast muscles of the chickens contain significantly less fat and it is genetically determined trait. It lowers their sensory values but improves dietetic value. In Poland, the basic slaughter raw material for production of poultry meat includes the broiler chickens – four line

hybrids of meat hens where Dominant White Cornish (DWC) is the paternal line and White Rock (WR) is the maternal line. They are characterized by a very quick growth rate, excellent musculature, usually white skin and white feathers (Grabowski, Kijowski, 2004). The most known breeding lines include: Ross, Cobb, Hubbard. In the breast muscles of the mentioned chickens, the mean water content is 73.7–75.6% and ash content 1.0–1.3%. On the other hand, leg muscles contain as follows: 74.2–75.4% of water, 18.5–19.7% of protein, 4.6–7.2% of fat and 1.0–1.1% of ash (Pisarski et al. 2006; Pietrzak et al. 2005, 2006; Ristic

TABELA 2. Results of slaughter analysis of Ayam cemani chickens- males and females; %

Group	Dressing percentage	Muscles	
		Breast	Leg
	LSM	LSM	LSM
♂	73.3	15.2 <sup>b</sup>	21.3
♀	72.5	20.6 <sup>a</sup>	25.4
SE	0.44	1.18	1.99

<sup>a,b</sup> Means with the different subscripts differ significantly at  $P \leq 0.05$ ,

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ .

TABELA 3. Chemical composition and physico-chemical properties of breast muscles of Ayam cemani

Płeć	pH	Thermal drip [%]	Water holding capacity [%]	Dyes [ppm heamines]	Water [%]	Protein [%]	Fat [%]	Ash [%]
	LSM	LSM	LSM	LSM	LSM	LSM	LSM	LSM
♂	5.5	1.0	17.9	57.8	73.7	24.8	0.6	1.2
♀	5.6	0.9	19.2	50.9	73.5	24.5	1.0	1.1
SE	0.04	0.12	1.9	10.0	0.2	0.2	1.8	0.02

TABELA 4. Chemical composition and physico-chemical properties of leg muscles of Ayam cemani

Płeć	pH	Thermal drip [%]	Water holding capacity [%]	Dyes [ppm heamines]	Water [%]	Protein [%]	Fat [%]	Ash [%]
	LSM	LSM	LSM	LSM	LSM	LSM	LSM	LSM
♂	6.0	13.8	26.2	157.3	74.2	21.6	3.6	1.1
♀	6.2	12.1	36.9	150.9	73.7	21.4	3.7	1.1
SE	0.05	0.8	2.9	4.9	0.2	0.2	0.3	0.02

et al. 2005, 2008; Szkucik et al. 2009). When comparing chemical composition of meat of Ayam cemani chickens and the meat of traditional broiler chickens, we may notice that the first one contains more protein, with the simultaneous, relatively low fat content what is favourable from the nutritional point of view.

The contents of haemic dyes in the meat of Ayam cemani females and males were similar and they amounted as follows: in breast muscles 50.9–57.8 ppm of haemine and in leg muscles 150.9–157.3 ppm of haemine (Table 3 and 4). The breast muscles of the chickens were characterized by almost 3-times lower content of haemic dyes as compared to leg muscles; it results from the fact that they have the domination of white muscular fibres with a low content of myoglobine. The obtained values are many times higher than in the traditional meat of broiler chickens. Słowiński and Mroczek (1997) report that the content of haemic dyes in the leg muscles of broiler chickens is usually equal to ca. 65 ppm of haemine and in the breast muscles – ca. 25 ppm of haemine.

Colours of carcass and of fresh meat is an important commercial feature, evaluated at first by the consumers. The content of haemic dyes and the resulting colour of poultry meat is depending on many factors, first of all, on species of the birds, sex, age, method of nutrition, type of muscles and their life performance activity and the degree of bleeding. The colour is also affected by fat content, structure of muscular tissue and active acidity (pH) of meat (Grabowski, Kijowski 2004).

Technological value of meat, that is indirectly also quality of the manufactured products is dependent, inter alia,

on: pH, water holding capacity and amount of thermal drip. Irrespectively of the sex, the leg muscles of the chickens were characterized by higher capacity as compared to the breast muscles, what is connected with their higher pH (by 0.5–0.6 units in average). On the other hand, the amount of the drip during heat treatment of the leg muscles was significantly higher than in the breast muscles. The leg muscles contain more collagen which – after heat treatment – holds water worsely as compared to the muscular tissue proteins. Similar tendency was observed in the studies of Pietrzak et al. (2005, 2006).

## CONCLUSION

Ayam cemani are slowly growing chickens. In the studies, any statistically significant differences concerning chemical composition and physicochemical properties in breast muscles and leg muscles between females and males were not found. The chickens of the discussed breed may be successfully utilized in small – trade management. The meat of the mentioned birds is characterized by a high protein content and a low fatness – being desirable by the consumer.

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**Streszczenie:** *Skład chemiczny i właściwości fizykochemiczne mięsa kur i kogutów Ayam cemani.* Materiał doświadczalny stanowiło 30 kurcząt wolno rosnących Ayam cemani (15 kogutów i 15 kur) odchowywanych od wylęgu do 18 tygodnia życia. Ptaki żywiono standardowymi mieszankami pełnoporcjowymi dla brojlerów: starter – o zawartości 22,1% białka ogólnego i 12,8 MJ EM, grower – 21% białka i 13,2 MJ EM oraz finisz, o zawartości 20% białka i 13,4 MJ EM. Po uboju wykonano dysekcję, pobrano do analizy mięśnie piersiowe i nóg od 9 kur i 9 kogutów. W mięśniach oznaczono podstawowy skład chemiczny (suchą masę, białko ogólne, tłuszcz surowy), zawartość barwników hemowych oraz właściwości

technologiczne (48h po uboju) pH, wodochłonność i wyciek termiczny. Średnia zawartość suchej masy w mięśniach piersiowych u kur Ayam cemani wynosiła 26,5% u kur i 26,3% u kogutów, w mięśniach nóg u kur 26,3% i u kogutów 25,8%. Zarówno mięśnie piersiowe jak i nóg charakteryzowała niższa zawartość suchej masy u kogutów. Mięśnie piersiowe u kur zawierały 24,5% białka, kogutów – 24,8%, a mięśnie nóg odpowiednio: 21,4% i 21,6%. Mięśnie nóg charakteryzowały się większą zawartością tłuszczu (kury 3,7%, koguty 3,6%) w porównaniu do mięśni piersiowych (kury 1,0% koguty 0,6%) oraz zawierały 3-krotnie więcej barwników hemowych (od 157 do 57 ppm heminy). Stwierdzono podobną wartość pH zarów-

no w mięśniach piersiowych (od 5,5 do 5,6), jak i nóg (od 6,0 do 6,2). Nie wykazano statystycznie istotnych różnic w składzie chemicznym i właściwościach fizykochemicznych w mięśniach piersiowych i nóg zależnie od płci.

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## Wheat distilled dried grains with solubles (DDGS) as a replacer of extraction soil meal in nutrition of broiler

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**Abstract:** *Wheat distilled dried grains with solubles (DDGS) as a replacer of extraction soil meal in nutrition of broiler.* The experiment was carried out on 540 broiler chickens of Cobb 500, which were at random allocated to three nutritional groups: K, D<sub>1</sub>, D<sub>2</sub>. The content of wheat distilled dried grains with soluble (DDGS) in starter feed was the differentiating factor for the groups. Any negative effect on production results of the chickens was not found at any of the used levels of the dried distiller's grains. The application of wheat DDGS in the diet has affected lowering of the number of bacteria and pathogenic fungi and the increase of the number of "favourable" microorganism in the contents of alimentary tract. The obtained results indicate that in correctly balanced feed rations, distillers dried wheat grains with soluble (DDGS) may be a good replacer of extraction soy meal in the feed for broiler chickens.

*Key words:* broiler chickens, wheat DDGS, microflora of the contents of small and caecum intestine.

### INTRODUCTION

In many countries, including also Poland, the studies are conducted on the possibility of using distillers dried grains with solubles (DDGS) as a replacer of extraction soy meal in poultry nutrition. Dried distiller's cereal are most valuable as they are rich in protein, exogenous amino acids, phosphorus, vitamins of B group,

biotine and minerals (Koreleski and Świątkiewicz 2007, Min et al. 2008).

Modern production technology of high-proof ethyl alcohol (spirit) enables obtaining of dried distiller's grains with the considerably more favourable parameters, what causes that discussed product may be employed in poultry nutrition. After distillation of alcohol, the remaining product contains more protein than the initial raw material because the existing yeasts produce their own protein of a high biological quality. Moreover, dried distiller's cereal contain postfermentation residues of cereal grains, soluble substances and yeast cells and their metabolites, multiplied during fermentation process (Thacker and Widyaratne 2007). The content of nutritive components in DDGS is differentiated and it is dependent, inter alia, on a type of grain, plant, method of fermentation, temperature and time of drying. The assimilability of nutritive components is highly determined by the method of drying which affects protein denaturation and, by this, the availability of exogenous amino acids. The content of amino acids is also depen-



TABLE 2. Nutritional value of wheat distilled dried grains

	%
Dry mass	95.21
Crude protein	32.65
Crude ash	2.16
Crude fat	5.29
Crude fibre	14.64
EM MJ/kg calculated from*	8.474

\*Smulikowska (1996).

medium) in the intestinal contents, *Lactobacillaceae* (on medium APT), mesophilic heterotrophic bacteria (nutritive agar) and microscopic fungi (Sabouraud's medium), the plate method of Koch, with application of surface inoculation was employed. Then, macroscopic tests of the grown colonies and microscopic tests (on the ground of observation of living fungi and bacteria, stained by Gram method; using Nikon E600 microscope with camera) were performed. On the ground of morphological and physiological properties, the species of the isolated strains were determined according to Bergey's systematic (2001) for bacteria and acc. to Fassatiouva (1979) for microscopic fungi. Moreover, for each examined sample of faeces, coli/lacto index, as the ratio of the number of bacteria *Enterobacteriaceae* family, developed on medium acc. to Mc Conkey (Gram – negative *Escherichia coli* rods) and lactic acid bacteria from *Lactobacillaceae* family (Gram – positive rods from *Lactobacillus* genus), was calculated. Low value of coefficient is an evidence of favourable composition of microflora of intestinal ecosystem of the examined broilers. The obtained results were statistically developed, using variance analysis, calculated by the test square method,

in the statistical SPSS programme 14.0 PL for Windows. The submitted values of the examined traits were expressed by the lowest square means (LSM) and by standard error means (SEM).

## RESULTS AND DISCUSSION

Since 21 day of rearing, the highest body weight was obtained by the broiler chickens from K group (control) as compared to D<sub>1</sub> and D<sub>2</sub>. Significant differences ( $P \leq 0.05$ ) in body weight of males at the age of 35 and 42 days were found, depending on the administrated feed. The highest body weight on 35 day was characteristic of the males and females in group K (2384 g; 1962 g). The lowest body weight at the discussed age was found in case of male from D<sub>1</sub> group (2266 g) and females from D<sub>2</sub> group (1937 g). On 42 day of rearing, the highest body weight was recorded for the males from K group (3086 g) while in relation to females, it was D<sub>1</sub> group (2521 g) (Table 3).

The improvement of feed conversion was observed in case of the chickens fed the higher wheat DDGS content in the diet (Table 3). The mortality rate in the particular groups was equal from 3.0 to 5.5%; the lowest health cases were found in the experimental D<sub>1</sub> (3.0%) as compared to the control K group and D<sub>2</sub> (5.5%). Dale and Batal (2003) showed that 6% participation of DDGS in starter feed and 12% in grower and finisher did not have the influence on deterioration of production results. Similarly, Lumpkins et al. (2004) stated that it was possible to employ safely 6% DDGS in the starter – type feed and up to 15% in grower and finisher, without deterioration of produc-

TABLE 3. Performance of broiler chickens, in g

Item	Feeding group			
	K	D <sub>1</sub>	D <sub>2</sub>	
	LSM	LSM	LSM	SEM
Body weight, 21 days	1024	1005	1014	9.0
Body weight of male broilers, 35 days	2384 <sup>Aa</sup>	2266 <sup>Ba</sup>	2353 <sup>b</sup>	30.1
Body weight of female broilers, 35 days	1962	1948	1937	34.0
Body weight of male broiler, 42 days	3086 <sup>a</sup>	2938 <sup>b</sup>	3048	46.7
Body weight of female broilers, 42 days	2504	2521	2500	36.8
Feed conversion (kg) per 1 kg of gain	1.79	1.78	1.75	
Mortality (%)	5.5	3.0	5.5	

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ ;

<sup>a,b</sup> Means with the different subscripts differ significantly at  $P \leq 0.05$ .

tion results. Thacker and Widyaratne (2007) employed the mixture with different participation of wheat DDGS (0.5; 10; 15 and 20%) in nutrition of broiler chickens and they revealed that the participation of wheat DDGS in the feed for broiler up to 15% did not affect lowering of production results. Wang et al. (2007) stated that DDGS could be used up to 15% in the feed for broiler chickens only in case of careful balancing of the diet in respect of amino acids composition and energy; therefore, most of the authors suggest frequent physico-chemical analyses of DDGS products.

Microflora of the examined samples of the faeces from broilers, receiving the addition of wheat DDGS in the mixture, was differentiated in respect of the number of bacteria and fungi. Any favourable effect of DDGS wheat in the diet on lowering of the number of pathogens in small intestine was not found; on the other hand the considerable increase of lactic acid bacteria number in the experimental groups, as compared to the control group was recorded (Diagram 1). Lowering of the number of patho-

gens and improvement of the system of intestinal microflora was observed in caecum gut (Diagram 2). The application of wheat DDGS in greater ration in the diet has improved anti-fungal effect. A decline of the number of bacteria from *Enterobacteriaceae* family in caecum intestine of the chickens from the experimental groups suggests a favourable effect of wheat dried grains on obtaining a low coli/lacto ratio in alimentary system what affects the improvement of health state of the birds (Table 4). A low value of coli/lacto index in caecum gut of broilers, fed DDGS indicates the most favourable composition of microflora in the discussed animals and the effect of wheat DDGS on the growth of the number of lactic acid bacteria. In the connection with this fact the addition of wheat dried grains to the diet, with the aim to obtain favourable composition of intestinal microflora (especially in case of caecum gut). As it results from the conducted studies, DDGS has a favourable effect on intestinal microflora of broilers although there is a lack of such reports in literature.

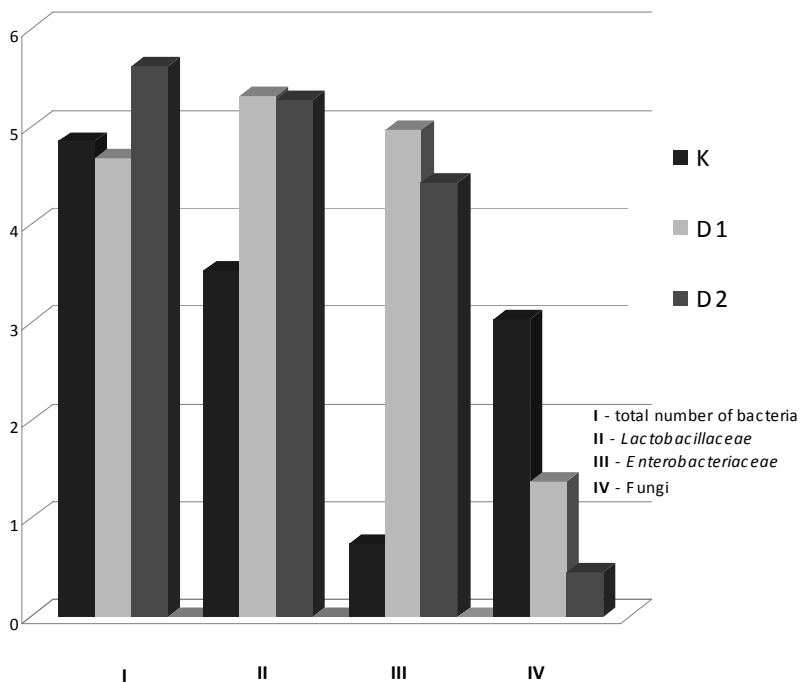


DIAGRAM 1. Total number of cfu (colony forming units) in 1 g of small intestinal chicken broilers content

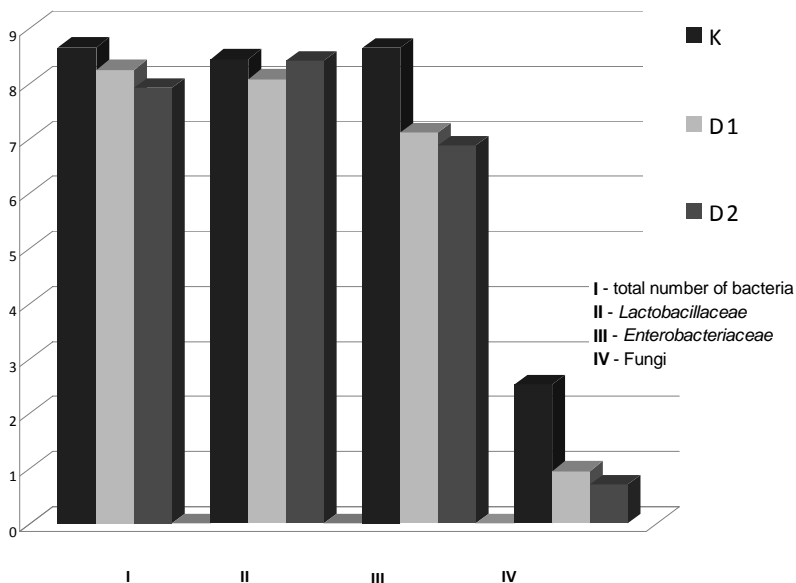


DIAGRAM 2. Total number of cfu (colony forming units) in 1 g of caecum intestinal chicken broilers contents

TABLE 4. The value of coli/lacto index for small and caecum intestine chicken broilers content

Intestine	Feeding group			
	K	D <sub>1</sub>	D <sub>2</sub>	
	LSM	LSM	LSM	SEM
Caecum	1.02	0.81	0.88	0.10
Small	0.14 <sup>B</sup>	0.28 <sup>A</sup>	0.43 <sup>A</sup>	0.06

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ .

## CONCLUSION

The application of dried wheat grains did not have a negative effect on production results of the chickens; the increased DDGS content in the diet lowered the number of bacteria and fungi and increased the number of “favourable” microorganisms.

Correctly balanced (fibre, energy, amino acids) feeding ration of DDGS may success fully constitute good energy – protein component in the feed for broiler chickens, being a reasonable method for its utilization.

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**Streszczenie:** *Pszenny suszony wywar gorzelniany (DDGS) jako zamiennik poekstrakcyjnej śrutu sojowej w żywieniu kurcząt brojlerów.* Doświadczenie przeprowadzono na 540 kurczątach brojlerach COOB 500, podzielonych losowo na 3 grupy żywieniowe: K, D<sub>1</sub>, D<sub>2</sub>. Czynnikiem różnicującym grupy była zawartość suszonego wywaru pszennego w mieszance starter. W doświadczeniu kontrolowano masę ciała ptaków (w 21, 35 i 42 dniu), spożycie paszy na 1kg przyrostu masy ciała oraz śmiertelność. W 42 dniu wybrano do uboju z każdej grupy po 6 kogutów i 6 kur o masie zbliżonej do średniej w danej grupie i pobrano wycinki jelit: cienkiego i ślepego. Porównano wyniki produkcyjne oraz ogólną liczbę bakterii i współczynnik coli/lacto w treści jelita cienkiego i ślepego kurcząt żywionych mieszankami z udziałem pszennego wywaru gorzelnianego (DDGS).

Zastosowanie wywaru pszennego nie wpłynęło negatywnie na wyniki produkcyjne kurcząt, zwiększona zawartość wywaru pszennego w diecie obniżyła liczbę bakterii i grzybów patogennych i zwiększyła liczbę „korzystnych” mikroorganizmów.

Uzyskane wyniki wskazują, że w prawidłowo zbilansowanych dawkach pokarmowych suszony wywar pszenney (DDGS) może być dobrym zamiennikiem poekstrakcyjnej śrutę sojowej w mieszankach dla brojlerów.

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## Results of rearing and technological traits of meat of slaughter chickens depending of their slaughter age

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**Abstract:** Results of rearing and technological traits of meat of slaughter chickens depending of their slaughter age. The aim of the studies was to compare production results and technological properties of the meat of chickens, slaughtered at the age of 35 and 42 days. The experiment was conducted on 320 broiler chickens of Cobb 500, which were kept on the litter, in standard zoohygienic conditions, in accordance with the standards, recommended by Cobb company. It was found that the prolongation of the rearing period affected the deterioration of production results (growth of mortality rate and feed conversion) but dressing percentage of 42 days – old chickens was significantly higher than that one of 35 days old birds. The period of rearing the chickens of Cobb 500, amounting to 35 days is sufficient as to receive the meat with good technological properties.

*Key words:* Cobb 500 chickens, production results, technological properties.

### INTRODUCTION

Production of broiler chicken is oriented toward obtaining greater and greater body weight, with the better feed conversion and shorter rearing period. During the recent several years, the considerable progress in poultry breeding has been performed. The new genetic sets of broiler chickens, characterized by a quick growth and high participation of breast muscles

and low level of abdominal fat in carcass have been introduced (Ricard et al. 1994; Rémignon et al. 1995; Le Bihan-Duval et al. 1998; Ristic and Steiner 2005). Owing to intensive nutrition of the chicken, the period of rearing was reduced from 56 to 42, and even to 35 days. Such high growth rate has, however, the affect on physiological traits of the chickens and quality of meat and its technological stability (Edwards 2000; Świerczewska et al. 2002; Michalczuk et al. 2004; Szkucik et al. 2007). One of the conditions of further development of poultry meat production includes ensuring of its high quality. It is expected by the consumers as well as by the enterprises, manufacturing poultry products. Technological value of meat, so indirectly the quality of its products is affected, inter alia, by pH, water holding capacity and the quantity of thermal drip (Brzóška et al. 2000). Lowering of the slaughter age of broiler chickens which – due to intensive feeding – obtain earlier the technology required body weight, may affect the quality of muscular tissue (Ristic and Steiner 2005; Szkucik et al. 2007). Due to these reasons, the studies were undertaken with the aim to compare the production results and technological

properties of meat of Cobb 500 chickens, slaughtered at the age of 35 and 42 days.

## METHODS AND MATERIALS

The studies were carried out in the experimental farm of SGGW, on 320 broiler chickens of Cobb 500, the birds were managed on the litter, in standard zoohygienic conditions, in accordance with the standards, recommended by Cobb company. The density rate was equal to 11.4 birds/m<sup>2</sup>. The chickens were fed the diets which contained the increased barley content (Table 1). During the run of the experiment, body weight of the birds (35 and 42 day) and death rate, were controlled. Finally, feed conversion per 1 kg of body weight gain and the European Yield Index (EYI) were calculated. On 35 and 42 day of rearing, 6 males and 6 females with the body weight similar to the mean in the flock, were selected for slaughter.

The chicken carcasses were chilled by the air method at temperature of 4°C for 24 h; then, dressing percentage (together with offals) was determined and the participation of breast muscles, leg muscles and abdominal fat in the carcass, was calculated. For analytical tests, 3 averaged samples from breast muscles and 3 samples leg muscles were prepared; the samples consisted of 4 disintegrated muscles (2 from males and 2 from females) After 48 from slaughter, the following determinations were carried out in the breast and leg muscles of the chickens: pH – acc. to PN – ISO 2917; water – holding capacity – by centrifuge methods acc. to Wierbicki et al. (1962) and the quantity of the drip after thermal treatment (30 g of disintegrated meat was heated up in the beaker, covered with the self – sticking polyethylene foil, in water bath at temperature of 72°C for 30 min.). The obtained results were statistically developed, using varia-

TABLE 1. Composition and nutritional value of feeding mixtures, %

Components	Starter	Grower	Finisher
Wheat	39.61	32.00	25.10
Barley	20.00	30.00	40.00
Soybean meal 46	30.89	29.83	27.19
Soybean oil	3.50	3.50	3.50
Limestone	–	0.67	0.19
Premix	6.00	4.00	4.00
<b>Nutritional value</b>			
ME (MJ/kg)	11.79	11.89	12.00
Crude protein	21.16	20.86	19.92
Fat	5.01	4.94	4.86
Crude fibre	3.03	3.17	3.28
Lysine	1.39	1.30	1.18
Methionine	0.58	0.57	0.55
Methionine + cystine	0.92	0.90	0.87
Threonine	0.88	0.82	0.77
Tryptophan	0.26	0.26	0.24

nance analysis, calculated by the least square method in statistical programme SPSS 14.0 Pl for Windows.

## RESULTS AND DISCUSSION

On the ground of production results, which have been submitted in Table 2, it was found that the broiler chickens, subjected to slaughter at the age of 35 days, reached good production results: EYI was found on a high level, i.e. 369 points. The chickens, slaughtered at the age of 42 days were characterized, what is evident, by the higher body weight (by 0.57 kg in average), higher feed conversion (by 0.29 kg/kg body weight gain, in average) but also, by higher death rate what affected lowering of EYI to 327 points. Mc Millan (2000) reports that a high body weight of the chickens leads often to health problems of the birds and decline of their immunity what could be the reason for higher number of deaths of 42 days – old chickens. Decisively worse production results were obtained by Pietrzak et al. (2005), according to whom body weight of Cobb 500 chickens, slaughtered at the age of 42 days amounted to 2.2–2.3 kg and feed conversion was found within the limits of 1.89–1.97 kg/kg of the gain. Most of slaughter

poultry was slaughtered at the time when the decrease of body weight gain rate had place. It was mainly determined by economic premises. The decline of the gain rate means deterioration of feed conversion index, deciding on the profitability of production (Grabowski and Kijowski 2004).

Prolongation of the time of chickens rearing from 35 to 42 days affected significantly the increase of dressing percentage (from 72.1 to 74.8%) which is a parameter, deciding on the effectiveness of production. It is greatly dependent on the breeding line of the chicks, nutrition, slaughter age and body weight of the birds. In the European Union countries, it is equal to 66–67% in average, without consideration of edible offals whereas with the edible offals, it increases above 70%. The participation of breast muscles in the carcasses of 35 and 42 days – old chickens was very similar and amounted to 30.8–30.9%, in average. In the carcasses of the chickens, slaughtered at the age of 42 days, significantly higher participation of abdominal fat (by 0.2 percent units, in average) as compared to 35 days old chickens (Table 3), was recorded. According to Pietrzak et al. (2005), who studied the effect of addition of different growth simulators to feed on the quality

TABLE 2. Production results of Cobb 500 broiler chicken

Age (days)	Body weight (kg)		Feed conversion (kg/kg)	Mortality (%)	EYC* (point)
	LSM	SEM			
35	2.30 <sup>B</sup>	22.43	1.74	1.87	369
42	2.87 <sup>A</sup>	23.79	2.03	2.81	327

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ ;

$$(*) \text{ EYC} = \frac{\text{mean body weight (kg)} \times \text{survival rate}}{\text{day of production} \times \text{feed conversion (kg/kg)}} \times 100$$

of meat and fat of Cobb 500 chickens, the participation of breast muscles in the carcass amounted to 21.0–24.2%, that of leg muscles: from 18.7 to 22.0% and of abdominal fat – from 1.9 to 2.3% Szkucik et al. (2007) stated that dressing percentage and participation of muscles and abdominal fat in carcasses of Cobb 500 chickens were not significantly changed gradually with the prolongation of the lengthening of time rearing (32, 38 and 44 days). On the other hand, Ristic and Steiner (2005) showed that the percentage participation of the most valuable elements of the carcasses, i.e. of breast and thigh parts, was dependent on breeding line of the chickens. The highest participation of the breast part was found in the broiler chickens of Ross 308 line and the participation of thigh part was higher in Cobb 500 and Cobb 800 broilers.

The results of determinations of technological properties of breast muscles and leg muscles of broiler chickens have

been given in Table 4. Irrespectively of the slaughter age of the chickens, the pH of the breast muscles was equal to 5.8 and that of leg muscles to 6.3. The similar results were obtained in the earlier studies, according to which pH of the breast muscles of Cobb 500 chickens was found on the level of 5.8–6.1 and that of leg muscles 6.3–6.7 (Pietrzak et al. 2005, 2006). In the breast muscles of the chickens, slaughtered at the age of 35 days, twice lower amount of thermal drip was recorded (1.6%) as compared to the muscles of the chickens, slaughtered at the age of 42 days (3.1%). The prolongation of the period of rearing did not affect significantly the water holding capacity of the breast muscles. The leg muscles of the chickens, slaughtered at the age of 35 days were also characterized by somewhat smaller quantity of thermal drip but worse water holding capacity as compared to 42 days old chickens. It may be also mentioned that the amount

TABLE 3. Results of slaughter analysis, %

Age (days)	Dressing percentage	Breast muscle	Leg muscles	Abdominal fat
35	72.1 <sup>b</sup>	30.9	18.4 <sup>B</sup>	1.6
42	74.8 <sup>a</sup>	30.8	21.0 <sup>A</sup>	1.8
SEM	0.69	0.65	0.48	0.19

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ ;

<sup>a,b</sup> Means with the different subscripts differ significantly at  $P \leq 0.05$ .

TABLE 4. Technological properties of breast and leg muscles of broilers

Age (days)	pH <sub>48</sub>		Water holding capacity (%)		Thermal drip (%)	
	BM	LM	BM	LM	BM	LM
35	5.8	6.3	30.4	33.4 <sup>b</sup>	1.6 <sup>B</sup>	6.5
42	5.8	6.3	31.8	40.3 <sup>a</sup>	3.1 <sup>A</sup>	7.2
SEM	0.05	0.11	1.13	1.27	0.09	0.50

BM – breast muscles, LM – leg muscles;

<sup>A,B</sup> Means with the different subscripts differ significantly at  $P \leq 0.01$ ;

<sup>a,b</sup> Means with the different subscripts differ significantly at  $P \leq 0.05$ .

of the drip during heat treatment of leg muscles was 2–4 times higher than in case of breast muscles. It results from the fact that thigh muscles contain more collagen which – after heat treatment – holds water more worsely in comparison to proteins of muscular tissue. The leg muscles of the chickens were, however, characterized by somewhat higher water holding capacity than the breast muscles what is connected with their higher pH (by 0.5 units, in average). Similar relationship between pH and water holding capacity of the chicken muscles was observed in the studies of Pietrzak et al. (2005, 2006).

## CONCLUSION

The period of rearing of Cobb 500 broiler chickens, amounting to 35 days, is sufficient in order to obtain the meat with good technological properties. Therefore, analysis of the costs of the chickens and conversed feed and EYI result should become a criterion for undertaking the decision on the period of rearing of the broiler chickens.

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**Streszczenie:** Wyniki odchowu oraz właściwości technologiczne mięsa kurcząt rzeźnych w zależności od wieku ubojowego. Doświadczenie przeprowadzono na 320 kurcząt Cobb 500 odchowywanych zgodnie z normami utrzymania brojlerów zalecanymi przez firmę COBB. W czasie trwania doświadczenia w 35 i 42 dniu odchowu kontrolowano wyniki produkcyjne oraz wybrano do uboju po 6 kogutów i 6 kur o masie zbliżonej do średniej w stadzie. W pobranych mięśniach piersiowych i udowych oznaczano: pH, wodochłonność i ilość wycieku po obróbce termicznej. Stwierdzono, że wraz z wydłużeniem okresu produkcyjnego

następuje pogorszenie wyników produkcyjnych (wzrost śmiertelności i zużycia paszy), wiek wpłynął negatywnie również na wyższą wodochłonnością mięśni kurcząt w 42 dniu odchowu. Czas odchowu kurcząt COBB 500 wynoszący 35 dni jest wystarczający, aby uzyskać mięso o dobrych właściwościach technologicznych.

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## Traditional carp farming in Poland as an example of sustainable animal production

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**Abstract:** *Traditional carp farming in Poland as an example of sustainable animal production.* Aquaculture is a very fast developing branch of food production, app. 9% per year. However, this fast development, usually focused only on one species being involved, might be very harmful for environment. As a result of growing concern regarding mainly ecological damages caused by aquaculture a concept of sustainable development in aquaculture was elaborated and set out with suggestion to be introduced into practice in upcoming future. A very good example for sustainable aquaculture is traditional carp production in earthen ponds in Poland. Carp ponds have very positive influence on environment as they accumulate large amount of biogens from supplying waters, creates very good habitats for many fauna and flora species. Ponds are also very well accepted by inhabitants, produce very good quality consumable fish and great amount of restocking material for lakes, rivers and other waters. But economic viability is the “weak point” of traditional pond aquaculture now and should be strengthen.

*Key words:* aquaculture, carp ponds, sustainable development.

### INTRODUCTION

Expression „aquaculture” means “*the rearing or cultivation of aquatic organisms using techniques designed to increase the production of the organisms in question beyond the natural capacity of the environment; the organisms remain*

*the property of a natural or legal person throughout the rearing or culture stage, up to and including harvesting”* (WE nr 1198, 2006). It is very fast developing branch of food production, due to some statistic, probably the fastest one. Official FAO statistics says, that since 1980 annual increase of production in aquaculture could be estimated between 8–10%, on average 8.8%. In 1950 global production of water organisms amounted app. 1 million tons, in 2004 it was 41 million tons and in 2008 world aquaculture provided 50 million tons of food for human consumption. At the moment about half of consumable fish comes from aquaculture (FAO 2006). FAO estimates also, that the present rate of aquaculture development must be maintained, because world demand for consumable water organism in 2030 will exceed 100 millions tons. The leading region in aquaculture is Asia, which produces itself almost 92% of all aquatic organisms, what it caused of course by very high natural increment in the region.

However, such very fast development of production might be very harmful for environment, what in case of fish production is already observed in sea fisheries.

Approximately 75% of global sea fish populations are overfished or even totally destroyed ([www.sustainaqua.org](http://www.sustainaqua.org)). Aquaculture, usually focused on one or only a few cultivated species, could also cause a very serious damages in environment. The problem is similar to what had happened in case of modern and intensive agriculture, accused of disappearance of many terrestrial fauna and flora species. In case of aquaculture the most spectacular example of very negative influence on natural ecosystems are mangroves forests in Asia, totally annihilated in some regions by very fast developing shrimp and lobster farms.

To avoid such problems in aquaculture the idea of sustainable development, generally described in Brundtland Report (WCED 1987), was also introduced into water organisms production. In FAO Code of Conduct for Responsible Fisheries (FAO 1995) the sustainable development in agriculture, forestry, fisheries sectors is described as such activity, which *“conserves land, water, plant and animal resources, is environmentally non-degrading, technically appropriate, economically viable, and socially acceptable”*. It is very important that all these three pillars must be in equilibrium, well balanced, because usually producers care about economic and technical side of their enterprise, less on social aspects, while environmental impact is not taken into consideration.

General rules for world sustainable aquaculture development were described in “Code of Conduct for Responsible Fisheries” (FAO 1995) and *“The Bangkok Declaration and Strategy”* (NACA/FAO 2000), and Europe is now the leading region in providing regulations and

research in this area (Roth et al. 2000). In 2000 Federation of European Aquaculture Producers (FEAP) prepared “Code of Conduct for European Aquaculture” ([www.feap.info](http://www.feap.info)), in 2004 European Commission issued “European Code of Sustainable and Responsible Fisheries Practices” (EC 2004). Several international projects were undertaken, incorporating scientist from many European countries, to evaluate already existing knowledge and elaborate new indicator for sustainable aquaculture development in Europe ([www.sustainaqua.org](http://www.sustainaqua.org), [www.feap.info](http://www.feap.info), [www.euroaquaculture.oinfo](http://www.euroaquaculture.oinfo), [www.easonline.org](http://www.easonline.org)). The most important of them are “Towards Sustainable Aquaculture in Europe” prepared by Consensus and published by European Aquaculture Society in 2008 (EAS 2008) and “Integrated approach for a sustainable and healthy freshwater aquaculture” elaborated and published in 2009 by SustainAqua (SustainAqua 2009). As a result, in new Operational Program for European fisheries for the years 2007–2013 supporting and promoting sustainability in aquaculture is very important issue for each EU country, with special attention on already existing examples of sustainable production. One of such examples are earthen ponds in Central and Eastern Europe.

The aim of the paper is to present very special and unique, multifunctional values of traditional carp pond production, a very good example of sustainable aquaculture.

## MATERIAL AND METHODS

The paper presents critical reviews of publications relating to different functions



and values of traditional carp production. Multidimensional role of carp farming was described. Environmental, social and economic values of carp farming were summarized.

## RESULTS

### **Environmental values of carp ponds**

Due to the Polish regulations carp ponds are fully artificial water bodies and are called “water devices” (Dz.U. 2001 Nr 115 poz. 1229). However, carp ponds are made of soil, natural material, and from biological point of view are similar to natural water bodies, with biological processes similar to littoral zone in lakes. Because water management in the pond is based on retention and accumulation, carp ponds accumulate very big amount of biogens (Tucholski 1994, Kolasa-Jamińska 1999). Knosche et al (2000) says that in intensively used rural areas 1 ha of carp ponds retains each year 3.8–8.36 kg of phosphorous, 96.5–559.8 kg of total nitrite and 1100–1600 kg of suspended solids. Zygmunt (2006) showed, that in small lowland river drainage area 1 ha of carp ponds accumulates on average 2.6 kg of phosphorous, 4.9 kg of nitrite and 186.5 kg of suspended solids. If we take into consideration, that total area of carp ponds water mirror in Poland is app. 50000 ha, the amount of bygones retained by the ponds is enormous.

Carp ponds have very positive influence not only on water quality but also on water flow in supplying river (Drabiński et Wieniawski 1992). Ponds usually are filled with water in late autumn, winter or early spring, when huge amounts of water are available, actually at this period of the year it is a “spare” water for

environment. Ponds play the same role as reservoirs, accumulating app. 1–1.5 million cubic meters of water. Water accumulated in the pond comes back to the environment in more stable way. Only 40% of it is released into the supplying river during fish harvesting, 60% comes by evaporation and drainage (Zygmunt 2006) and it is of course very clean water, filtrated or almost distilled.

Another very important feature of carp pond is water management. Cultivation of carp and other similar species does not need water flow. Because of this carp ponds might be build, to some extend of course, regardless to water supply. Basing on water not only from springs, streams or rivers but also seasonally available, like water from milting snow or heavy rains (Guziur 1997, Wojda 2006) pond-scould be located almost in any area (urban, rural, woodland, grassland) and like enclaves of natural environment give hiding places for many flora and fauna species, especially birds (Dobrowolski et al. 1995).

Looking at the dispersion of carp ponds in Poland it could be easily seen, that carp farms are located mostly in the areas where is lack of natural water bodies (Rudnicki et al 1965). The largest areas of ponds are in Southern and Central part of Poland, while in North part (Pomeranian and Warmia and Mazury Region) number of ponds is rather small. Carp ponds are very important compound of the environment, especially for avifauna. Also in the areas, where there are no good places for nesting, naturally looking ponds are preferred by many bird species (Bocheński 1960, Borowiec 1981, Bukaciński et Bukacińska 1991, Wasilewski et Huflejt 2003). On average

approximately 130 species of birds (1/3 of all existing in our country) are recorded on every carp farm, almost regardless to its area (Bukaciński et Bukacińska 1991). Only the largest pond complexes, larger than 500 ha, usually are represented by larger number of birds (Dobrowolski et al. 1995).

Environmental values of traditional carp ponds are attractive not only for birds but for many other living organisms (Zysk et al. 1999) Bieniarz et al. 2003). Siemińska et Siemińska (1967) investigating Gołysz Ponds and nearby surroundings recorded 2105 all together fauna and flora species, many of them protected by law. Such big carrying capacity of carp ponds is created by very special formula of ponds cultivation and fish production, developed and improved during hundreds years in very close correlation and dependence on natural conditions not by nature preservation regulations.

As it was already mentioned, carp production in earthen ponds takes place in natural-like environment. Growth of the fish depends very much on natural food, growing itself in the shallow water of the pond from sunlight and biogens deposited in the bottom and coming with supplying water. Cycle of natural food chain is very slow, so the growth of the fish is also slow. Usually it lasts 3 years to obtain consumable carp in ponds in Poland and the stocking density is very small, approximately one fish per 10 m<sup>2</sup>. As a result carp ponds usually have very large areas and are built in so-called "complexes". Because amount of natural food is limited, growing fish need new places for feeding. Each carp farm consists of ponds differing in size, depth,

usage time, flooding period etc. for different fish categories. Such form of carp production was elaborated almost 150 years ago by Tomasz Dubisch (Guziur et al. 2003) and in core principles is almost unchanged. From aerial view farm looks like a patchwork. The patchworkness is characteristic not only for the whole farm, but for every pond. In each carp pond open water can be distinguish, penetrated by sunlight, producing natural food and oxygen like living reactor. Next is ecoton zone on dyke banks, reedbads growing in the water around the pond, bushes on dykes and outside the ponds. Such "patch" of water, good quality, with diversity of habitats and food sources and resources somehow automatically creates very big carrying capacity of any environment (Zalewski 2002). Additionally carp ponds are build on low value grounds, not suitable for ordinary agriculture (Wojda 2006) where because of arid conditions carrying capacity and biodiversity is very low. The positive influence of carp ponds on biodiversity protection in such regions and generally for whole environment is invaluable.

### **Social values of carp ponds**

Carp production, based in some cases even in 100% on natural food consumption, need large areas to provide growing fish enough food. To maintain ponds in proper condition very high employment is indispensable. On average carp farm employs 4 persons per 100 ha (Wolos et al. 2006). From producer's point of view it is very bad, because labor costs are the most expensive in any activity. But from social point of view such very high employment is very important, because people simple have work. Moreover,

carp farms usually located on agricultural west lands are the only opportunities to get any job, because such regions usually have very high unemployment, like “East Wall”, the eastern regions of Poland. Very important is, that about 95% of carp farms in Poland are small size enterprises, belongs to the SMSE category (Lirski 2007), where both men and women could get the job. Very often it is a real small family business.

Very important is also social acceptance of carp ponds and carp farms. Usually animal production (pigs, chickens or cattle) generates conflicts between farmers and neighboring inhabitants. In opposite, carp ponds are very well accepted, to such extend, that houses are built almost on ponds dykes, what could be easily observed in many regions in Poland. However, without permanent application of breeding actions, known in pond aquaculture as maintaining of “*pond culture*” (Stegman 1952), carp ponds will turn into wastelands in several years.

From social point of view very important are cultural values of carp ponds. The history of carp farming in Poland goes back at least to medieval times. Poland

was always the leading country in ponds construction and carp farming. First handbook on carp production was published in Poland in 1573 by Strumieński, several years later, in 1607, the next one was published by Stroynowski. One of the “fathers” of polish language, Mikołaj Rej, in 1568 wrote about fish pond farming, underlining its, what we call now, economic, environmental and social values. On many farms very old examples of pond devices or architecture could be found, but the most fascinating are nature monuments like old trees.

### Economic values of carp ponds

Sustainability sometimes is also defined as “*the ability of a society, ecosystem or any such on-going system to continue functioning into the indefinite future without being forced into decline through exhaustion or overloading of key resources on which that system depends*” (Frankic et Hershner 2003). Figure 1 presents the share of carp ponds in Poland in relation to age. Data were obtained by survey circulated through the present owners of the farms, literature and other documents. The numbers are estimated as it is almost impossible to

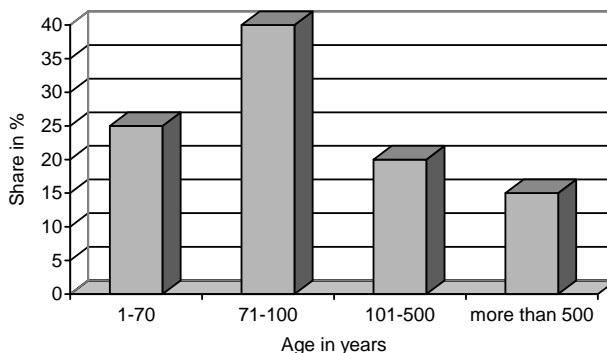


FIGURE 1. Share of carp ponds area in relation to age of the farm

say if the farm constructed in XII century has exactly the same area now.

The largest share of carp ponds in Poland is represented by farms constructed at the beginning of XX century, especially during interwar era. But the share of older ponds is very impressive to. It could be estimated that approximately 40% are ponds older then 100 years and about 15% or even more are ponds older then 500 years. It means that in some regions carp farming is the only solution to use the ground in economic way.

Average total carp production in Poland ranges from 15000 tons up to 20000 tons (Lirski et Myszkowski 2008). The fish are of very good quality, healthy and tasty, because they consume a lot of natural food (Wojda 2006). However, the economic viability of carp aquaculture is now questionable, what shows Table 1.

TABLE 1. Economic viability of different forms of fisheries in Poland (Wołos 2006)

Type of fishery activity	Net profit (in %)	Share of fish production in total income (in %)
Trout	+18.0	98
Carp	-2.8	94
Lake	+10.4	49
Lake + carp	+7.0	75

These data, gathered by Wołos et al. (2006) in 2005, were the first investigation on economic situation in fish production in Poland after economic transformation in 1989. They show that carp farming is in very bad condition, 2.8% below zero. Authors did not investigate the factors generating situation. One of the possible factors might be changes in consumer's preferences. For many years carp was the only valuable fish on polish table, especially during Christmas. Right now

one visit in supermarket shows, how rich is the offer in seafood store. The number of available fish, crustaceans, molluscs etc. is enormous, never before. Also consumers are looking for easy food, while carp is still offered as live fish, what for many people, especially young, is unacceptable (Cieśła 2008). As a result carp production was reduced from app. 20000 tons in 2000 to 15500 tons in 2008 (Lirski 2008, Seremak-Bulge 2008). Lower demand for market carp means lower production, and worth economic condition of farms. Many farmers try to improve their economic situation by introducing some alternative or additional species. Carp farms cultivate now about 20 native fish species, it is 1/3 part of all. Another way for supporting economic viability of the farm is multifunctional use of the farm by offering recreational fisheries, tourism, courses and conferences etc. However it seems absolutely impossible to replace carp production from the system, what shows data gathered at Fisheries Research Station Łąki Jaktorowskie, Warsaw University of Life Sciences – SGGW, presented in Table 2.

In the years 1996–1998 four different activities were carried out at the Research Station in Łąki Jaktorowskie:

- a) production of consumable carp,
- b) production of other consumable species (pike, pikeperch, crussian carp, wells),
- c) production of additional species for restocking purposes,
- d) recreational fisheries (angling).

Basing only on carp production, the final result of the farm would be very bad, more than 10% below zero, while all four activities together gave almost 10% net profit. However, the share of

TABLE 2. The share of different income sources in total income and economic viability of Fisheries Research Station Łąki Jaktorowskie in the years 1996–1998

Source of income	Share of consumable carp in total income (in %)	Net profit (in %)
a) carp	100	-13
b) carp + other species	95	-8
c) carp + other +restocking fish	79	+6
d) carp + other + restocking + angling	75	+9

consumable carp production was still very high, about 75%.

One of the most famous European multifunctional carp farms is Hungarian Aranypony (www.aranypony.hu). It offers consumable carp, other consumable fish, carp and other species for restocking purposes, angling, hunting, birdwatching, horse riding, conference rooms, restaurant, hotel services and shops. Even in such really multifunctional farm, the share of carp production is not less than 50% (owner's personal information). The question is how many such farms could be established in Poland, when about 10000 units declare their activity in aquaculture (Lirski 2007).

## CONCLUSIONS

1. Traditional carp ponds play very important, multifunctional role, especially for environment protection, and could be recognized as good example of sustainable animal production.

2. Economically viable carp production is the only way to maintain traditional carp ponds in good and proper condition, what guarantee subsistence of all their production and off production values.

3. Economic viability of carp ponds is challenging now and due to this special measures must be undertaken to improve the situation, for example by introducing

alternative species, tourism or recreational fisheries. In case of consumable fish transition from life carp to more processed fish seems to be essential.

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**Streszczenie:** *Tradycyjny chów karpia w Polsce jako przykład zrównoważonej produkcji zwierzęcej.* Akwakultura jest obecnie jedną z najszybciej rozwijających się gałęzi produkcji żywności. W ostatnim dwudziestolecu tempo przyrostu produkcji, mierzone rok do roku, wyniosło w ska-

li ogólnoświatowej 8,8%. Niestety ten szybki rozwój, często ukierunkowany tylko na jeden gatunek będący przedmiotem chowu, może być bardzo uciążliwy dla środowiska. Dlatego też w strategii rozwoju akwakultury w dwudziestym pierwszym wieku szczególnie nacisk kładziony jest na to, aby był on zrównoważony, czyli nie powodujący degradacji środowiska naturalnego, akceptowalny społecznie, o odpowiednim stopniu wyposażenia technologicznego oraz stabilny ekonomicznie.

W pracy omówiono różnorodne walory tradycyjnych stawów typu karpowego. Przeanalizowano ich funkcję społeczną i kulturotwórczą, przedstawiono wpływ stawów na ochronę i konserwację bioróżnorodności środowiska naturalnego oraz na jakość wody w zlewni i na bilans wodny w środowisku, w którym są zlokalizowane. Przeanalizowano także ekonomiczną opłacalność produkcji tradycyjnych gospodarstw karpowych.

Tradycyjne stawy karpowe spełniają wiele bardzo pozytywnych funkcji, są doskonałym przykładem zrównoważonej produkcji zwierzęcej, szczególnie w zakresie ochrony i konserwacji

środowiska naturalnego. Pod tym względem jest to wręcz modelowy przykład zrównoważonej produkcji. Najsłabszym ogniwem tradycyjnej gospodarki karpowej jest obecnie strona ekonomiczna, na krawędzi opłacalności produkcji, która wymaga wsparcia, np. poprzez promocję ekologicznej wartości produkowanych ryb, dywersyfikację oferty handlowej poprzez wprowadzanie alternatywnych w stosunku do karpia gatunków ryb, rozwój przetwórstwa oraz otwarcie na różnorodne formy turystyki (np. rybactwo rekreacyjne, ścieżki edukacyjne, obserwacja ptaków).

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## Development of digestive tract and swim bladder in larvae and juvenile stages of the Central American *Cichlidae* family on the example of T-bar cichlid (*Cryptoheros sajica*, Bussing 1974)

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**Abstract:** *Development of digestive tract and swim bladder in larvae and juvenile stages of Central American Cichlidae family on the example of T-bar cichlid (Cryptoheros sajica, Bussing 1974).* The aim of the work was to investigate the development of digestive tract and filling of swim bladder in larval fish and juvenile individuals of T-bar cichlid. For tests, larvae and juvenile individuals from 1<sup>st</sup> to 30<sup>th</sup> day post hatching (dph) were selected and subjected to standard histological procedure. It was found that the endogenous feeding period in T-bar cichlids lasted since 1 to 4 dph, the endo-exogenous feeding period – since 4<sup>th</sup> to 10<sup>th</sup> dph and the exogenous feeding period was commenced since the 10<sup>th</sup> dph. In the examined species, the juvenile period was commenced together with the complete development of stomach and stomach glands, what occurred on 20<sup>th</sup> dph. The presence of swim bladder in T-bar cichlid was found on 1<sup>st</sup> dph. The discussed species have a closed bladder (*physoclisti*). Air duct is present until filling of swim bladder (5<sup>th</sup>–7<sup>th</sup> dph).

Key words: T-bar cichlid, digestive tract, swim bladder.

### INTRODUCTION

Fish from *Cichlidae* family are widely popular in the inland waters of the Central and Southern America, Africa and Madagascar, India and the Near East. It is a big family, including about 1400 species (Chakrabarty 2004). Numerous

*Cichlidae* species are ornamental species cultivated in aquariums; the other ones include valuable consumption fish such as Nile tilapia (*Oreochromis niloticus*) (Fujimura and Okada 2007) or Mayan cichlid (*Cichlasoma urophthalmus*) (Chavez-Sanchez et al. 2000).

The knowledge of embryo and larval development of various fish species has a practical application in aquacultures and fish biology. Cahu and Zambonino Infante (2001) found that study of digestive tract development is especially important for understanding digestion physiology and process of larvae nutrition.

The aim of the work was to investigate digestive tract development and filling of swim bladder in larvae and juvenile individuals of T-bar cichlids. The mentioned species may serve as a model of embryo and larvae development for substrate-brooding of the Central American cichlids.

### MATERIAL AND METHODS

The studies were conducted in laboratory of Division of Ichthyobiology and Fisheries of Warsaw University of Life Sciences. The research material consist-

ed of T-bar cichlid larvae derived from one parental pair. The spawning was at 26–27°C. Larvae were reared at temperature of 26–27°C. Fish were *ad libitum* fed with *Artemia nauplii* since 3<sup>rd</sup> day post hatching (dph) and then, since 15<sup>th</sup> dph with *Tubixex* sp. and commercial diet Tetra Min (Tetra, Germany). Larvae and juvenile individuals were collected since 1<sup>st</sup> to 30<sup>th</sup> day of life, 5 individuals each 24 hours. The fish were anaesthetised using MS 222 (tricaine methane sulfonate, Sigma) and then, preserved in Bouin-Hollande liquid. The preserved fishes were immersed in paraffin and cut into sections of 5 µm thickness, which were obtained using microtome Leica RM 2265 (Leica Microsystems, Nussloch, Germany). The performed preparations were stained by AB/PAS method (Alcjan blue and Schiff reagent) (Pearse 1985). Observations under microscope were conducted using Nikon Eclipse 90i and cooperating camera Nikon Digital Sight DS-U1 (Nikon Corporation, Tokyo, Japan). Photographs were made using programme NIS-Elements AR.2.10 (Nikon Corporation, Tokyo, Japan).

## RESULTS

After spawning, parental pair took care of fish eggs until hatching of larvae what occurred after 3 days. On the day of hatching, the total mean length of larvae was equal to 2.49±0.07 mm. Larvae began their active swimming between the 5<sup>th</sup> and 6<sup>th</sup> dph (total mean length 3.41±0.07 mm).

In 1<sup>st</sup> dph old fish, the yolk sac was distinctly visible and consisted of many fat droplets and yolk plates (Fig. 1A). The yolk sac was surrounded by yolk syncy-

tium layer. During endo-exogenous feeding period (4<sup>th</sup>–10<sup>th</sup> dph), volume of the yolk sac was gradually decreasing. By the end of the mentioned period, the yolk sac was not macroscopically visible. The complete, microscopic absorption of yolk occurred up to 15<sup>th</sup>–18<sup>th</sup> days after hatching. At the moment of hatching, digestive tract of larvae was non-differentiated, simple duct, situated on the yolk sac.

**Mouth, Pharynx and Oesophagus.** Mouth of larvae on 1<sup>st</sup> dph was closed and in oesophagus between the epithelium cells, single mucous cells were observed (AB/PAS-positive). On the 2<sup>nd</sup> day post hatching, patency of mouth had place. During the endo-exogenous feeding period, oesophagus was prolonged and also, longitudinal folds were created in it. The number of mucous cells, as observed in oesophagus increased together with age. Taste buds, teeth on oral jaws and pharynx teeth were visible since 9<sup>th</sup> dph. During exogenous feeding period (10<sup>th</sup> dph), the number of mucous cells and taste buds in mouth and pharynx was increased.

**Stomach.** Between 6<sup>th</sup> and 7<sup>th</sup> dph, the primary stomach, being the enlargement of oesophagus, was observed (Fig. 1B). It was covered with monolayer cylindrical epithelium whereas outer layer was constituted by thick muscle tissue. Since the 9<sup>th</sup>–10<sup>th</sup> day post hatching, stomach glands became to differentiated in the stomach. On the 10<sup>th</sup> dph, the presence of sphincter was found in larvae and since the 15<sup>th</sup> dph – the central part of stomach, i.e. blind sac was developing. Fully developed stomach with three parts: cardia, blind sac and pyloric stomach were observed in juvenile individuals on the 20<sup>th</sup> dph (Fig. 1C).

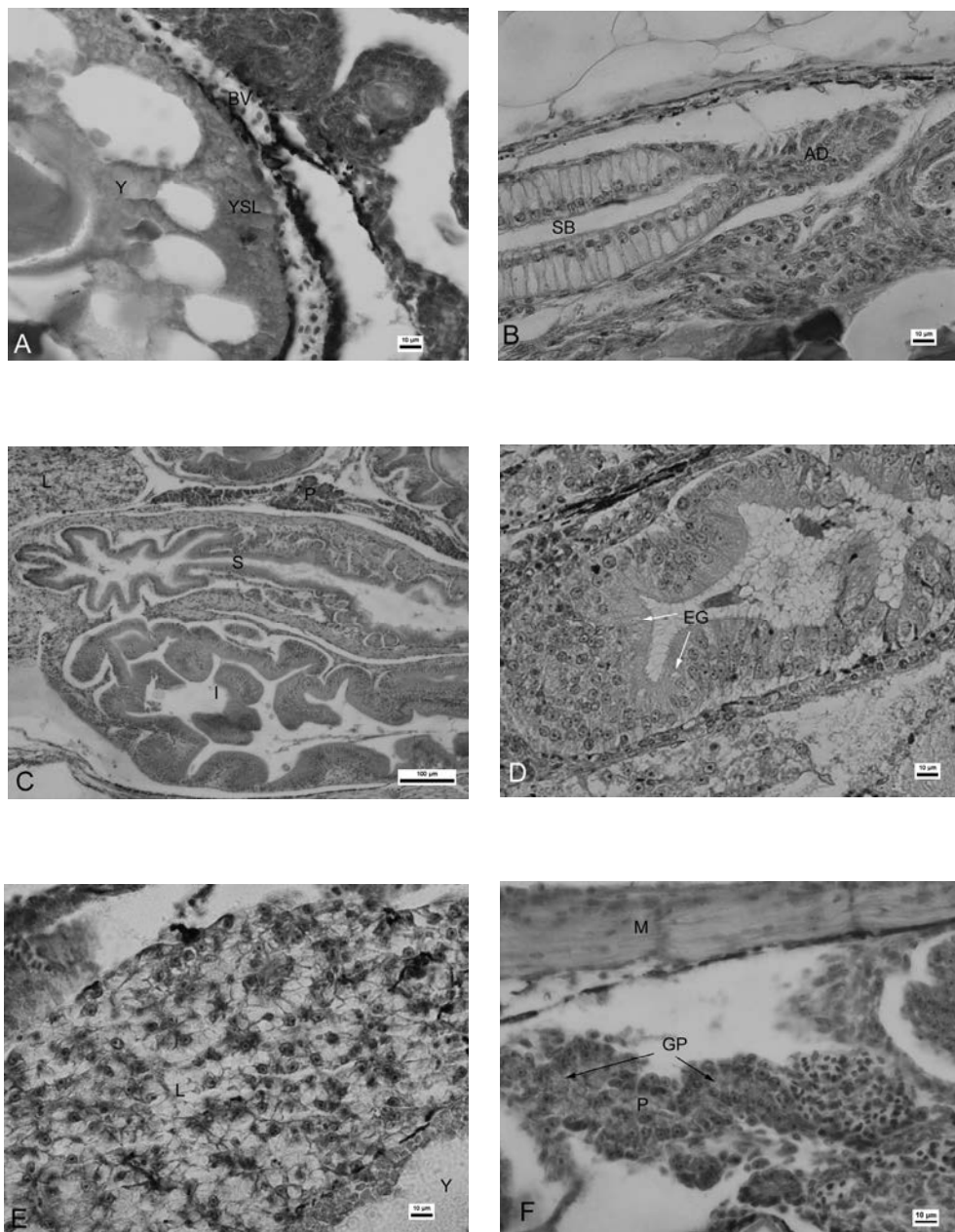


FIGURE 1. Digestive tract of T-bar cichlid: A) The yolk sac (Y), blood vessels (BV) and yolk syncytium layer (YSL), 1<sup>st</sup> dph, B) Primary stomach (PS) and oesophagus (OE), 6<sup>th</sup> dph, C) Fully developed stomach (S), intestine (I), liver (L) and pancreas (P), 20<sup>th</sup> dph. D) Hind gut and vacuoles with eosinophilic granulatuin (EG), 5<sup>th</sup> dph, E) Liver (L) and the yolk sac (Y), 6<sup>th</sup> dph; F) Exocrine part of pancreas (P) with granules of proenzyme (GP), muscle (M), 6<sup>th</sup> dph

**Intestine.** In the day of hatching (1<sup>st</sup> dph), intestine was a simple duct, covered with non-differentiated epithelium. On the second dph, the presence of monolayer cylindrical epithelium in the intestine was found. During the these period of larval development, any presence of mucous cells in the gut was not observed. In the 5<sup>th</sup> day post hatching, the division of intestine into three segments was found, i.e.: forward gut, middle gut and hind gut. Differences between the particular parts of gut resulted from digestive processes and nutritional components and occurrence of valve (septum) separating the middle gut from the hind part. The first folds in the gut were observed between the 5<sup>th</sup> and 6<sup>th</sup> dph and the first mucous cells (AB/PAS positive) were found between the 4<sup>th</sup> and the 5<sup>th</sup> dph. In perinuclear areas of enterocytes in the hind gut, a small number of vacuoles with eosinophilic granulation was observed since the 5<sup>th</sup> dph (Fig. 1D). The presence of vacuoles in the perinuclear areas of enterocytes in the middle part of the gut was also found between 8<sup>th</sup>–12<sup>th</sup> dph. They were not numerous and quickly disappeared during development. During the exogenous feeding period, the increase of the number of mucous cells was observed in epithelium of all parts of the gut. Since 10<sup>th</sup> dph, when sphincter was formed, the development of pyloric appendices was recorded. They were short, often branched and there were AB/PAS positive mucous cells in their epithelium.

**Liver and pancreas.** Liver and pancreas were observed since the day of hatching of T-bar cichlids. Hepatocytes of liver were characterized by distinctly visible nuclei and the presence of lipid vacuoles in cytoplasm. During the endo-

exogenous feeding period (4<sup>th</sup>–10<sup>th</sup> dph), the liver was found in the abdominal – back part of the body, after yolk sac, under the gut; on the other hand, pancreas was situated between the generating loops of the gut. Since 6<sup>th</sup>–7<sup>th</sup> dph, granules of glycogen (PAS-positive) were observed in cytoplasm of hepatocytes (Fig. 1E) and at the same time, the presence of granules (PAS positive) of proenzyme was found in exocrine part of pancreas (Fig. 1F). During the exogenous feeding period (10<sup>th</sup> dph), the liver was situated in forward – abdominal position in relation to stomach.

**Swim bladder.** At the moment of hatching of T-bar cichlid larvae, the presence of swim bladder was found; its lumen was lined with high columnar epithelium (Fig. 2A). Air duct, which connects swim bladder with digestive tract, was visible for the endogenous feeding period till the moment of filling the swim bladder (Fig. 2B). Filling of the swim bladder (inflation) occurred between 5<sup>th</sup> and 7<sup>th</sup> dph. After inflation, the increase of the swim bladder lumen as well as degeneration of air duct (Fig. 2C) was observed. Since the 7<sup>th</sup> day post hatching, the differentiation of gas gland cells in abdominal wall of the swim bladder was found.

## DISCUSSION

On the ground of the conducted studies it was stated that T-bar cichlid larvae had a similar process of development as other American cichlids (Meijide and Guerrero 2000; Martinez-Palacios and Ross 2004; Gagliardi-Seeley and Itzkowitz 2006). The period of change in the way of nutrition, i.e. from endogenous into

exogenous feed is often specified as a critical one. It is characterized by a high mortality rate in wild living populations as well as in those rearing in the

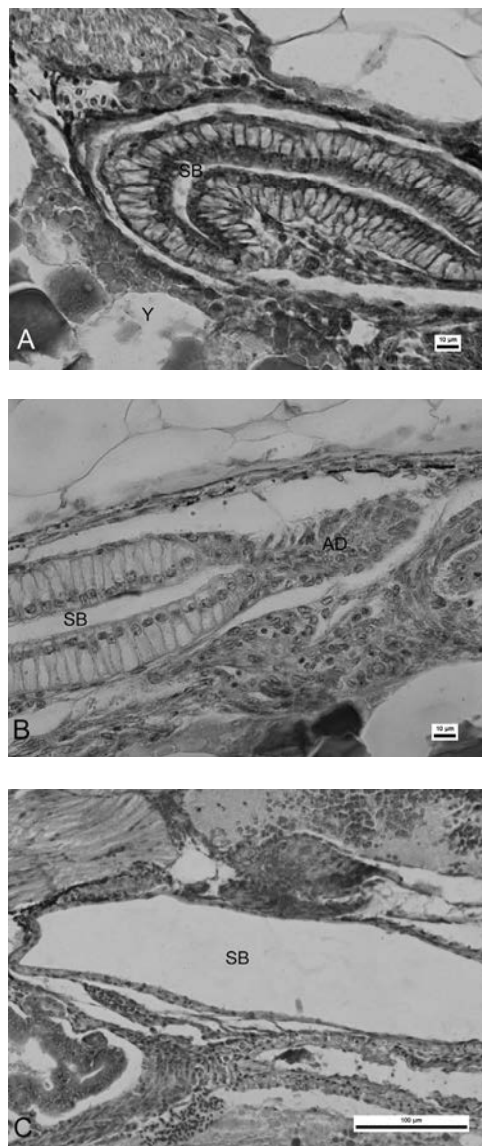


FIGURE 2. Swim bladder of T-bar cichlid: A) swim bladder (SB) and the yolk sac (Y), 1<sup>st</sup> dph; B) Air duct (AD) and swim bladder (SB), 5<sup>th</sup> dph, C) Filled swim bladder (SB), 7<sup>th</sup> dph

controlled conditions (Sarasquete et al. 1995; Gordon and Hecht 2002; Yúfera and Darias 2007). During development of T-bar cichlid larvae, the critical period did not occur probably due to the fact that the discussed larvae had a developed stomach before the complete absorption of reserved substances, accumulated in yolk sac. In the macroscopically examined larvae, the yolk sac was not visible between the 9<sup>th</sup> and 10<sup>th</sup> dph but full absorption of its contents occurred between 15<sup>th</sup> and 18<sup>th</sup> dph. Similar phenomenon was observed by Mejjide and Guerrero (2000) in *Cichlasoma dimerus* larvae. The yolk sac was not observed in external body structure of larvae of the discussed species between the 7<sup>th</sup> and 8<sup>th</sup> day post hatching. The full absorption of reserve material which was found in the yolk sac occurred, however, on 11<sup>th</sup> dph. On the other hand, macroscopic emptying of the yolk sac in pike-perch (*Sander lucioperca*) occurred in 6<sup>th</sup> dph, the remaining part of reserve substances was absorbed until 17<sup>th</sup> day of life (Ostaszewska 2005). The yolk sac in many fish species was completely or almost completely resorbed when mouth and anus were open (Yúfera and Darias 2007). In red porgy (*Pagrus pagrus*), opening of mouth and anus was overlapped with absorption of the yolk sac content what occurred in 3<sup>th</sup> dph (Darias et al. 2007). In California halibut (*Paralichthys californicus*), opening of mouth and resorption of the yolk sac had place between 1<sup>st</sup> and 2<sup>nd</sup> dph (Gisbert et al. 2004).

In the moment of hatching, T-bar cichlid larvae had a simple and non-differentiated digestive tract similarly as larvae of other fish species (Sysa et al. 2006; Darias et al. 2007; Sánchez-Amaya et

al. 2007; Yúfera and Darias 2007). The mouth of the examined fishes was closed at the moment of hatching similarly as in *Cichlasoma cyanoguttatum* (Fishelson 2005) and *Cichlasoma dimerus* (Meijide and Guerrero 2000) and became open in 2<sup>nd</sup> dph.

Mucous cells in oesophagus of T-bar cichlids were present since the moment of hatching similarly as in clownfish (*Amphiprion percula*) (Gordon and Hecht 2002). Morrison et al. (2001) found the presence of mucous cells in African Nile tilapia (*Oreochromis niloticus*) in the 2<sup>nd</sup> day after hatching. The presence of mucous cells in oesophagus was observed in 1<sup>st</sup>–2<sup>nd</sup> days after opening of mouth in many species (Ostaszewska 2005; Kozarić et al. 2008). In the gut of T-bar cichlid larvae, mucous cells appeared in 4<sup>th</sup>–5<sup>th</sup> dph; they occurred at the moment when the larvae started intake of exogenous feed. The presence of mucous cells in the gut of Nile tilapia (Morrison et al. 2001) and in European catfish (*Sirulus glanis*) (Kozarić et al. 2008) was found in a similar period.

The perinuclear vacuoles with eosinophilic granulations were found in cytoplasm of enterocytes of the hind part of the gut in T-bar cichlid larvae since 5<sup>th</sup> dph similarly as in other fish species (Gordon and Hecht 2002; Ostaszewska 2005; Sysa et al. 2006; Sánchez-Amaya et al. 2007). The mentioned authors suggest that the discussed vacuoles are a result of absorption of proteins by pinocytosis which is an alternative way of protein digestion in situation when a digestive tract in larvae has not been fully developed and functional (Sarasquete et al. 1995). Perinuclear vacuoles, as observed in enterocytes of the middle part of the gut in

T-bar cichlid larvae in 5<sup>th</sup> dph may be an evidence of extracellular digestion of fat by lipase and of fatty acid absorption to enterocytes (Sarasquete et al. 1995; Ostaszewska 2005; Chen et al. 2006). Sysa et al. (2006) observed the first symptoms of digestion and fat absorption in enterocytes of the nase (*Chondrostoma nasus*) on 9<sup>th</sup> dph and Zaiss et al. (2006) found it in larvae of shi drum (*Umbrina cirrosa*) between 5<sup>th</sup> and 11<sup>th</sup> dph.

The primary stomach in T-bar cichlid larvae was observed in 6<sup>th</sup> dph. Ostaszewska (2005) found the presence of the primary stomach in pike-perch larvae in a similar period, i.e. in 5<sup>th</sup> dph. The first stomach glands were observed in T-bar cichlid larvae in 9<sup>th</sup> dph whereas those fully developed were recorded in 15<sup>th</sup> dph. Stomach glands in pike-perch (Ostaszewska 2005) and in shi drum (Zaiss et al. 2006) were observed in a similar period. In other species, the presence of stomach glands was found at later period of larvae development e.g. in *Pagrus auriga* in 16<sup>th</sup> dph (Sánchez-Amaya et al. 2007), in *Pseudosciaena crocae* in 21<sup>th</sup> dph (Mai et al. 2005), in *Sphoeroides annulatus* (García-Gasca et al. 2006) after 32<sup>th</sup> dph. In *Amphiprion percula* larvae, stomach glands were earlier differentiated, i.e. in 5<sup>th</sup> dph (Gordon and Hecht 2002).

The first symptoms of liver functioning, that is, storage of glycogen in the examined fish was observed between 6<sup>th</sup> and 7<sup>th</sup> day post hatching. The first symptoms of liver activity in pike-perch larvae were recorded in a similar period (Ostaszewska 2005). In European catfish, accumulation of glycogen and fat in hepatocytes occurred between 7<sup>th</sup>–9<sup>th</sup> dph (Kozarić et al. 2008) whereas Daria

et al (2007) observed storage of glycogen in hepatocytes of red porgy larvae after 4<sup>th</sup> dph. The first granules of proenzyme, evidence -secretory activity of pancreas in its exocrine part, were observed between 6<sup>th</sup> and 7<sup>th</sup> days post hatching. Darias et al. (2007) observed first granules of proenzyme in exocrine pancreas of red porgy larvae in 6<sup>th</sup> dph. Ostaszewska (2005) found the first symptoms of pancreas activity in pike-perch larvae in a similar period.

The *Perciformes* belong to the group of *physoclisti*. Swim bladder of the mentioned fish is characterized by a lack of connection with digestive tract (Doroshev and Cornacchia 1979) or by the presence of air duct connecting swim bladder with digestive tract. The discussed duct is present only for a certain period of larval life (Trotter et al. 2004). The moment of commencing the filling of swim bladder is often correlated with the beginning of exogenous feed intake by the larvae and the complete absorption of reserve substance of yolk sac (Marty et al. 1995). Filling of swim bladder in T-bar cichlid larvae occurred between 5<sup>th</sup> and 7<sup>th</sup> day post hatching. In the these period, yolk sac was resorbed in a half and the larvae took their exogenous feed similarly as Nile tilapia larvae (Fujimura and Okada 2007).

Morrison et al. (2001) observed air duct connected swim bladder with digestive tract in the day of hatching Nile tilapia larvae, using histological preparations. Zilberg et al. (2004) confirmed the presence of air duct in the day of hatching in case of another cichlid – angelfish (*Pterophyllum scalare*). The mentioned duct disappeared on the next day. Doroshev and Cornacchia (1979) did not observed

air duct or another link between digestive duct and swim bladder in Mozambican tilapia larvae (*Oreochromis mossambicus*). The present studies indicated that T-bar cichlid larvae have the air duct present since the moment of hatching until filling of swim bladder i.e. 5<sup>th</sup>–7<sup>th</sup> dph.

## CONCLUSIONS

The period of endogenous feeding of T-bar cichlid lasted until 4<sup>th</sup> dph, the period of endo-exogenous feeding – since 4<sup>th</sup> to 10<sup>th</sup> dph; the period of exogenous feeding was commenced since 10<sup>th</sup> dph. The juvenile period of the examined species began together with the formation of stomach and stomach glands what occurred in 20<sup>th</sup> dph.

T-bar cichlid belongs to the group of *physoclisti* (closed swim bladder). The air duct is present since hatching till the moment of filling the swim bladder (5<sup>th</sup>–7<sup>th</sup> dph).

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- i osobniki młodociane od 1 do 30 dnia od wyklucia (dph), które poddano standardowym procedurom histologicznym. Stwierdzono, że u pielęgnicy sajica okres odżywiania endogennego trwał od 1 do 4 dph, okres odżywiania endo-egzogenego od 4 do 10 dph, natomiast okres odżywiania egzogenego rozpoczął się od 10 dph. U badanego gatunku okres młodociany rozpoczynał się wraz z pełnym ukształtowaniem żołądka i gruczołów żołądkowych co następowało 20 dph. U pielęgnicy sajica stwierdzono obecność pęcherza pławnego w dniu wyklucia. Są to ryby zamkniętopęcherzowe. Przewód powietrzny obecny jest do momentu napełnienia pęcherza pławnego (5–7 dph).

**Streszczenie:** *Rozwój układu pokarmowego oraz pęcherza pławnego u larw i stadiów młodocianych pielęgnicy środkowoamerykańskich na przykładzie pielęgnicy sajica (Cryptoheros sajica, Bussing 1974). Celem pracy było prześledzenie rozwoju układu pokarmowego oraz napełnienia pęcherza pławnego u larw i osobników młodocianych pielęgnicy sajica. Do badań pobierano larwy*

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## Regional differentiation of costs and profitability of milk production in Poland

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**Abstract:** *Regional differentiation of costs and profitability of milk production in Poland.* Numerical material has been got from 158 milk farms located in four regions in Poland. Average productive and economic results of farms in regions have been compared. Average area of agricultural land in investigated farms was 33.9 hectares. The biggest farms were in Pomorze and Mazury Region and the smallest ones in Małopolska and Pogórze Region. The highest milk yield per 1 cow was achieved in Wielkopolska and Śląsk Region (5777 l) whereas the smallest one in Małopolska and Pogórze Region (4836 l). In investigated farms consumption of concentrated feed ranged between 0.28 and 0.36 kg per production of 1 litre of milk. The highest agricultural income per 1 hour of work and 1 farm have been achieved in Pomorze and Mazury Region. The biggest costs of labour were incurred in Małopolska and Pogórze Region and there agricultural income, value of production and gross margin without payments per 1 hour of labour were the lowest.

*Key words:* milk cows, region, farm, cost, agricultural income.

### INTRODUCTION

Milk production is an important factor (element) which has effect on volume of animal production in our country. Differentiation of size of farms in Poland influences on standard of production, size of expenditures, costs and incomes.

Characteristic natural conditions and organizational and economic conditions

affect size of use of productive potential and its regional differentiation [Krasowicz, Kopiński, 2006].

Productive and economic results of farms specialized in milk production depending on region were compared in this article.

### MATERIAL AND METHODS

158 farms which keep milk cows located in four regions in Poland were investigated in 2006. Numerical material was collected from farms where accounting was carried on within the FADN<sup>1</sup>. Investigations were carried out at:

- 22 farms in Pomorze and Mazury Region,
- 27 farms in Wielkopolska and Śląsk Region,
- 76 farms in Mazowsze and Podlasie Region,
- 33 farms in Małopolska and Pogórze Region.

Individual costs of feed nutrients (protein and energy), direct costs of milk production in regions and economic efficiency of milk production expressed by value of gross margin and agricultural income have been calculated. Presented

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<sup>1</sup>FADN- Farm Accountancy Data Network.

results are the weighted average for the region.

Using real data analysis of connection between a region where activity took place and economic results of milk production was carried out.

## RESULTS OF RESEARCHES

Information about investigated farms by regions is presented in the Table 1. Average area of agricultural land at investigated farms amounted to 33.9 hectares and it was bigger by 26.3 hectares than average area of agricultural land per 1 farm in Poland (7.6 hectares, 2006) [GUS, 2007]. There were the biggest farms in Pomorze and Mazury Region and the smallest ones in Małopolska and Pogórze Region.

Small farms where an area amount to 1–5 hectares prevail in polish agriculture. Farms where an area amount to above 20 hectares account for 6% and use 35% of the area of agricultural land. The biggest number of small individual farms is in southern and south-east parts of the country. There is more favourable

agrarian structure in northern Poland [Krasowicz, Kopiński, 2006].

Quality of land used at farms in Pomorze and Mazury Region is the highest (soil valuation coefficient – 1.02 points). There were weaker lands at the rest of investigated farms

There was the biggest area of grassland in the structure of area of agricultural land at farms in Małopolska and Pogórze Region (Table 1).

Considerably bigger share of area of grassland in the area of agricultural land was in 18 milk farms investigated by Lipińska and Gajda [2006] and it fluctuated from 42.4% to 52.3%. But pastures prevailed in the area of grasslands at smaller farms and meadows at bigger farms. Individual farms where pastures and meadows have a big share in the area of agricultural lands amount to 20% of total number of farms in Poland. [Prokopowicz, 1997]. Big share of pastures and meadows at farms limits to a certain degree a possibility to choose the direction of production because it is possible to produce only feed on grasslands.

TABLE 1. Information about investigated farms by regions

Item	Average by farm	Region			
		Pomorze and Mazury	Wielkopolska and Śląsk	Mazowsze and Podlasie	Małopolska and Pogórze
Total number of farms	158	22	27	76	33
Area of agricultural land (ha)	33.9	61.4	35.9	28.4	26.6
Share of area of grassland within the area of agricultural land (%)	25.4	27.0	26.2	19.5	36.8
Soil valuation coefficient of agricultural land (point)	0.81	1.02	0.71	0.69	0.89
Cows as annual average (heads)	20	24	23	21	11
Milk yield (litre)	5627	5235	5777	5588	4836
Cow culling coefficient (%)	15.5	14.7	16.7	15.8	14.6
Milk sale price (PLN/litre)	0.99	0.99	0.96	1.02	0.94

Source: own calculations.

2 cows were kept at farms which have the smallest area of land and 75 at farms which have the biggest area of land. Average number of cows was the biggest in Pomorze and Mazury Region (24 cows).

This is characteristic for Polish agriculture that in spite of changes has been taking place there are small scale of production and dispersion of this production. There are 874 thousand milk farms in Poland and the average herd of cows amounts to 3 heads [Krasowicz, 2008].

At the investigated farms average milk yield per 1 cow was higher by 1400 -liters than average milk yield at individual farms in country (average milk yield in Poland – 4074 liters) [Skarżyńska and co-workers, 2008]. The highest average milk yield per 1 cow was achieved in Wielkopolska and Śląsk Region and the lowest one in Małopolska and Pogórze Region.

Milk sale price was over 1 PLN per 1 litre only in farms in Mazowsze and Podlasie Region.

There is big regional differentiation of milk sale prices. Farmers in Opolskie Voivodship and Podlaskie Voivodship received the highest prices but the prices were slightly higher than 1 PLN per 1 litre of milk. The lowest prices (0.75–0.83 PLN/litre) have been received by farmers in Voivodships as Podkarpackie, Małopolskie, and Świętokrzyskie where yield of marketable agricultural produce was low [Szajner, 2006].

Cows at investigated farms were fed on grass from pastures of good quality and on valuable silages and silages made from hay (Table 2). According to the rule of milk cows' feeding which says that it is possible to fully meet a demand of nutritional requirement of cows using

maximum bulky feed. Cattle are genetically adjusted to digest this kind of feed. Costs of milk production are decreased thanks to using grass from pastures for summer cows feed. Lower cost of summer cows feed result from the situation that animals feed themselves so farmers do not need to mow grass and transport it to a cowshed every day. According to Ostrowski and co-workers [2000] decreasing of costs of feeding without decreasing of milk yield is only possible when a pasture is used well to provide constant supply of fresh feed.

To achieve profitable and high production of milk cows need to be fed on concentrated feed and mineral components and vitamins. Feed ration must include proper concentration of feed components (carbohydrates, proteins, fiber, vitamins, mineral salts). Complete feeds are necessary to obtain big efficiency of animal production [Strzetelski, Osieglowski, 1998].

Concentrated feeds are at first position in hierarchy of costs of production however cost of purchased complete feeds is higher by 200 % than cost of cereals from own production. So that in Poland economics of milk production depends not only on milk prices but also on a level of intensity of production and rate of dependency from feed market [Okularczyk, Borecka, 2003].

At investigated farms concentrated feeds in feed ration amounted to average 9.7% and ranged from 3.3% (Małopolska and Pogórze Region) to 20.6% (Wielkopolska and Śląsk Region). The use of concentrated feed have been increasing together with an increase in milk yield because requirement for feed which has bigger concentration of energy per 1 kg

TABLE 2. Structure of feed consumption (dt/cow)

Item	Average at milk farms	Region			
		Pomorze and Mazury	Wielkopolska and Śląsk	Mazowsze and Podlasie	Małopolska and Pogórze
Number of investigated farms	158	22	27	76	33
Purchase feeds, including:					
Concentrated feed	8.69	6.09	9.51	10.01	5.39
Mineral additives and feed additives	0.25	0.16	0.22	0.30	0.20
Dried roughage	0.64	0.09	0.08	1.14	0.18
Succulent bulky feed	8.26	6.31	3.58	7.74	20.64
Liquid bulky feed	9.28	0.00	0.21	16.63	5.94
Own feed from manufacture, including:					
Concentrated feed	8.71	8.62	11.09	7.79	8.87
Potatoes	0.73	0.84	0.32	0.82	0.87
Own feed from non manufacture, including:					
Fodder beet	0.84	1.70	0.16	0.42	2.50
Other fodder roots	0.12	0.00	0.00	0.16	0.35
Grass	60.33	130.85	44.77	45.20	52.80
Hay	7.65	7.40	8.23	6.11	13.49
Silage and silage made from hay	77.11	85.02	67.37	85.10	48.56
Own by-products, including:					
Straw	4.85	4.50	4.13	4.56	7.69
Beet leaves	0.55	0.38	1.44	0.15	1.05
Silage made from beet leaves	2.99	1.13	4.82	3.44	0.74

Source: own calculations.

dry matter increases together with an increase in milk yield.

A share of bulky feed in feed ration for cows was the highest at farms in Małopolska and Pogórze Region and the lowest one at farms in Wielkopolska and Śląsk Region.

Comparing different groups of farms to average production it was found that the highest total value of production was achieved at farms in Mazowsze and Podlasie Region.

Value of milk production had decisive influence on value of gross margin at all farms (Table 3). Weight of weaned

calves ranges from 51 kg (Pomorze and Mazury Region) to 67 kg (Małopolska and Pogórze Region).

Direct costs at farms in Pomorze and Mazury Region were lower than average direct cost at all investigated farms.

Feed costs dominated in structure of direct costs at investigated farms. Own feed prevailed at farms in Małopolska and Pogórze Region but in other regions value of own feed and value of purchased feed were almost on the same level. Farms bought mainly complete feed, complementary feed, seed extracted, mineral additives, feed additives and dried pulp.

TABLE 3. Production, costs and gross margin without payments of milk production at investigated farms (in PLN per 1 milk cow)

Item	Average at investigated farms	Region			
		Pomorze and Mazury	Wielkopolska and Śląsk	Mazowsze and Podlasie	Małopolska and Pogórze
<b>Total value of production, including:</b>	6116	5783	6225	6357	5393
milk	5425	5184	5570	5668	4510
weaned calves	455	402	394	449	655
culled cows	236	197	261	240	228
<b>Total direct costs</b>	2286	2062	2478	2422	1763
Herd exchange	403	348	404	442	364
Purchase feed	756	603	898	846	359
Own feed	820	763	840	840	776
The rest of direct costs, including:	307	348	336	294	264
Specialized costs	137	137	146	138	114
medicines and veterinary service	163	204	187	151	122
Gross margin without payments	<b>3830</b>	<b>3721</b>	<b>3747</b>	<b>3935</b>	<b>3630</b>

Source: own calculations.

Farmers used mostly grass and hay from meadows and pastures, grass silages and maize silages as their own feeds. They also used by-products as mention: straw and sugar and fodder beet leaves as green fodder or silage to feed cows. But farmers used by-products in minimum amounts what was correct because particularly beet leaves are not recommended to use in cow feeding (Table 2). Costs of medicines and veterinary services were a significant part among the rest of direct costs.

Value of gross margin grew as individual capacity was growing. The highest value of gross margin was achieved at farms in Mazowsze and Podlasie Region. According to Seremak-Bulge [2006] Podlaskie Voivodship was the real power of milk production. Every third litre of milk and every fifth of cube of butter produced in Poland comes from Podlaskie Voivodship. 20% of polish cheeses and cream was produced there.

20% of polish milk was bought in this Voivodship.

Growth of direct costs including growth of feed costs appeared in the group of farms which bought the biggest amount of feeds and achieved the highest capacity this resulted in the decrease in profitability. According to Bilik [1997] growth of milk production to the level of 6600 liters per year caused growth of profitability. However, in polish conditions growth of milk production over this level results in considerably bigger costs of feeding, mainly because of the use of expensive concentrated feeds and in this way profitability of this production can fall down. What happened at investigated farms.

Consumption of concentrated feed ranged from 0.28 to 0.36 kg per 1 litre of milk production at investigated farms. Consumption of concentrated feed per 1 litre of milk, when average milk yield

was 7853 kg, amounted to 0.3 kg at farms investigated by Ostaoja-Solecki and Blicharski [1998].

The cost of 1 MJ of energy was at similar level but the cost of protein was at different level in groups of investigated farms.

Okularczyk and Borecka [2003] found out that costs of 1 MJ of energy from concentrated feeds of own production were twice as low as the costs of energy from concentrated feeds (average cost of 1 MJ amounted 0.043 PLN in own feeds and 0.074 PLN in purchased feeds). The

situation with costs of protein was similar. The cost of 1 kg of protein from concentrated feeds of own production was lower by 170% on average than the cost of 1 kg of protein from purchased concentrated feeds (average cost amounted to 2.37 PLN in own feeds and 4.27 PLN in purchased feeds).

Measures of economic proficiency of milk production presented in Table 4 show regional differentiations. The level of agricultural income per 1 hour of work is a synthetic measure of efficiency. Considering that index farms were distinctly

TABLE 4. Measures of economic proficiency of milk production at investigated farms

Item	Average at investigated farms	Region			
		Pomorze and Mazury	Wielkopolska and Śląsk	Mazowsze and Podlasie	Małopolska and Pogórze
Consumption of concentrated feeds per 1 litre of milk (kg/l)	0.31	0.28	0.36	0.32	0.29
Consumption of protein (nBO) per 1 litre of milk (kg)	0.60	0.21	0.44	0.53	1.07
Consumption of NEL per 1 litre of milk (MJ)	7.94	8.87	6.70	8.51	8.14
Feed costs (PLN/l)	0.28	0.26	0.30	0.30	0.23
Costs of 1kg of protein (nBO) (PLN)	0.47	1.23	0.68	0.57	0.22
Costs of 1 MJ (PLN)	0.04	0.03	0.04	0.04	0.03
Total value of production (PLN/litre)	1.09	1.10	1.08	1.14	1.12
Direct costs (PLN/litre)	0.41	0.39	0.43	0.43	0.37
Gross margin without payments (PLN/litre)	0.68	0.71	0.65	0.70	0.75
Total work expenditure (h/litre)	0.02	0.02	0.02	0.03	0.04
Total value of production (PLN/h of work)	44.19	51.37	51.59	45.97	26.58
Gross margin without payments (PLN/h of work)	27.67	33.05	31.05	28.45	17.89
Agricultural income without payments (PLN/farm)	39240	47981	43046	40994	24711
Agricultural income without payments (PLN/h of own work)	14.22	20.45	18.49	15.31	10.23

Source: own calculations.



different. The highest agricultural income without payments and the highest Gross margin per 1 hour of work and agricultural income per farm was achieved in Pomorze and Mazury Region. The highest work expenditures were at farms in Małopolska and Pogórze Region and there were the lowest agricultural income per 1 hour of work, value of production and gross margin without payments.

Growth of milk production scale had a positive influence on efficiency of work expenditures so the best results were achieved at farms where population of cows and milk production were the biggest and labour consumption was the lowest.

According to numerical data milk production could pay for expended work at investigated farms. Standard rate amounted to 9.02 PLN/h in year 2006 [Skarżyńska and co-workers, 2006].

## CONCLUSION

According to the researches carried out and achieved results following observation and conclusions were made:

1. Low quality of soil and big share of meadows and pastures in structure of agricultural land in investigated farms causes that the farms deal with milk production. The shortage of feeds at farms was filled in with purchased feeds in order to achieve high milk production per cow per year.

2. Value of production per 1 cow amounted to 6116 PLN, total direct costs – 2286 PLN and the result was that gross margin amounted to 3830 PLN. The highest value of gross margin was achieved at farms in Mazowsze and Podlasie Region.

3. Bigger share of own feeds at farms in Małopolska and Pogórze Region caused that direct costs were lower and gross margin was higher.

4. Agricultural income and value of production and gross margin without payments per 1 hour of work were the lowest at farms in Małopolska and Pogórze Region. Farms of that Region kept smaller number of cows.

5. Measures of economic proficiency of milk production were the most advantageous at farms in Pomorze and Mazury Region.

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**Streszczenie:** *Regionalne zróżnicowanie kosztów i zysków z produkcji mleka w Polsce.* W niniejszym artykule przedstawiono wyniki produkcyjno – ekonomiczne 158 gospodarstw producentów mleka, położonych w czterech regionach Polski. Średnia powierzchnia użytków rolnych w badanych gospodarstwach wynosiła 33,9 ha. Największą przeciętną roczną produkcję mleka od krowy osiągnięto w Wielkopolsce i Śląsku (5777 l), natomiast najniższą w Małopolsce i Pogórzu (4836 l). W analizowanych gospodarstwach w strukturze kosztów bezpośrednich dominowały koszty pasz. W gospodarstwach Małopolski i Pogórza przeważały pasze własne, a w pozostałych regio-

nach wartość pasz własnych i z zakupu była na zbliżonym poziomie.

Wysoki udział pasz z zakupu w grupie gospodarstw położonych w Wielkopolsce i Śląsku doprowadził do wzrostu kosztów bezpośrednich i obniżenia opłacalności. Zużycie paszy treściwej w badanych gospodarstwach wahało się od 0,28 do 0,36 kg na produkcję 1 litra mleka.

Najniższy: dochód rolniczy, wartość produkcji i nadwyżkę bezpośrednią bez dopłat w przeliczeniu na godzinę pracy uzyskano w gospodarstwach Małopolski i Pogórza. Gospodarstwa tego regionu posiadały mniejszą liczbę krów. Wzrost skali produkcji mleka pozytywnie wpływa na efektywność nakładów pracy, dlatego w gospodarstwach o największym pogłowie krów i wysokiej produkcji mleka oraz najmniejszej pracochłonności osiągnięto najlepsze wyniki. Mierniki sprawności ekonomicznej produkcji mleka osiągnęły najbardziej korzystne wielkości w gospodarstwach Pomorza i Mazur.

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