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Physiological and histological effects of herbicides in fish

BARTOSZ BOJARSKI[1], MATEUSZ JAKUBIAK[2], MAŁGORZATA WITESKA[1]
[1]Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities
[2]Faculty of Mining Surveying and Environmental Engineering, AGH University of Science and Technology

Abstract: *Physiological and histological effects of herbicides in fish*. Among pesticides used in agriculture, herbicides are used in the largest quantities. These substances enter the aquatic environment using many ways, and monitoring studies show their permanent presence in natural waters. Herbicides cause changes in many physiological parameters in fish, e.g. reduction of acetylcholinesterase activity in internal organs, increased activities of hepatic transaminases or oxidoreductive balance disturbances. Alterations in hematological parameters are also observed, usually indicating anemia and inflammatory process. The most frequently observed histological changes include hyperplasia and hypertrophy of gill epithelium and changes in liver microstructure, such as vacuolation of hepatocytes. Markers of fish exposure to herbicides are generally very sensitive but nonspecific. Analysis of metabolic, hematological and histological parameters of tissues can be helpful in determining fish poisoning with herbicides, but it should always be confirmed by monitoring studies.

Key words: intoxication, pesticides, fish health

INTRODUCTION

Herbicides are chemicals widely used to protect crops by controlling unwanted plants (weeds) in agriculture. They include very diverse group of pesticides – more than 200 active compounds in several thousand commercial formulas. Most herbicides are selective and their selectivity is related mainly to the technique and timing of application adjusted to the stage of development of protected and target plants. Another mechanism of herbicide selectivity derives from the different metabolism of weed and crop plant species. Most of contemporary herbicides are organic compounds (Woznica 2008). Herbicides applied in agriculture may reach aquatic environment using various routes. According to Sadowski et al. (2014), herbicide particles are leached with runoff together with soil particles or are introduced directly to the water due to misapplication (e.g. pouring away spray leftovers, sprayer washing). Monitoring of surface and ground water pollution with herbicides is an important issue in evaluation of the natural environment quality. Contamination of natural waters with herbicides was observed in Poland (Ignatowicz 2006, Sadowski and Kucharski 2007, Sadowski et al. 2014) and in many other countries, e.g. in Italy (Achilli et al. 1995), USA (Clark and Goolsby 2000), Portugal (Cerejeira et al. 2003) or Australia (Tran et al. 2007). Herbicides in water may adversely affect aquatic organisms. Exposure to herbicides causes various pathophysiological effects in fish including metabolic disturbances manifested as changes in enzymatic activity and other biochemical

alterations, histopathological lesions and hematological changes. The aim of this work was to review the literature concerning these issues.

PHYSIOLOGICAL EFFECTS

Acetylcholinesterase activity

In fish exposed to herbicides reduction in acetylcholinesterase (AChE) activity is often reported. According to Glusczak et al. (2007), 96 h exposure of *Rhamdia quelen* to glyphosate (0.2 and 0.4 mg·l^{-1}) resulted in a decrease of AChE activity in fish brain. *Carassius auratus* exposed for 48 h to 0.5 mg·l^{-1} of carbofuran also showed reduced brain and muscle AChE activity. Similar effect in brain of the same species was observed after 24 and 48 h diuron (0.5 mg·l^{-1}, 24 h) and nicosulfuron (0.5 mg·l^{-1}) treatments (Bretaud et al. 2000). In *Leporinus obtusidens* exposed for 96 h to 0.5 mg·l^{-1} of clomazone AChE activity was reduced in brain and cardiac muscle, while after 192 h – also in skeletal muscles and eye (dos Santos Miron et al. 2008). Similar results of exposure to the same herbicide (after 12, 24, 48, 96 and 192 h) were observed in brain and muscles of *Rhamdia quelen* (Crestani et al. 2007). Decrease in brain and muscle AChE activities were also reported after 96-hour exposure of *Leporinus obtusidens* to 10 mg·l^{-1} of 2,4-D (da Fonseca et al. 2008). Short-term (96 h) treatment of *Rhamdia quelen* to 600 and 700 mg·l^{-1} of 2,4-D resulted, however, in an increase in brain AChE activity, accompanied by a decrease in muscle (Cattaneo et al. 2008). Exposure of the same fish species to metsulfuron (400, 800 and 1,200 mg·l^{-1}) caused reduction of muscle AChE activity and increase in brain (dos Santos Miron et al. 2005). The disturbances of acetylcholinesterase activity considerably affect fish due to a key role of this enzyme in maintaining proper levels of the neurotransmitter acetylcholine in the central and peripheral nervous system. The literature data show that herbicides usually inhibit the activity of this enzyme. Inhibition of AChE activity can impair locomotion and equilibrium in exposed fish (Bretaud et al. 2000). Behavioral effects of waterborne atrazine, diuron and carbofuran observed by Saglio et al. (1996) and Saglio and Trijasse (1998) in *Carassius auratus* also seem to be associated with changes in AChE activity.

Aminotransferases activity

Exposure of fish to herbicides usually causes increase in activities of aminotransferases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Treatment of *Cyprinus carpio* to 0.005; 0.01 and 0.02 mg·l^{-1} of trifluralin resulted in elevated gill, liver and serum AST activities, while gill, liver and kidney ALT activities increased only at the highest herbicide concentration. Increased ALT activity was observed in *Rhamdia quelen* after 20 days of glyphosate (3.6 mg·l^{-1}) exposure (Soso et al. 2007). Exposure to paraquat (2 h at 1 and 10 mg·l^{-1}) caused an increase in ALT and AST activities in *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Silurus glanis* (Nemcsok et al. 1985). The increase in serum AST and ALT activity might have been related to release of enzymes from the cytosol of damaged

cells to the extracellular fluid indicating hepatocyte injury or necrosis (Poleksic and Karan 1999).

Metabolic parameters

Alterations in enzymatic activity observed in fish exposed to various herbicides were often accompanied by changes in other metabolic parameters. Exposure of *Rhamdia quelen* to 0.2 and 0.4 mg·l^{-1} of glyphosate for 96 h caused an increase in hepatic glycogen, lactate, protein and ammonia, while hepatic glucose level decreased. At the same time muscle glycogen and protein levels decreased, while lactate, glucose and ammonia concentrations increased (Glusczak et al. 2007). *Leporinus obtusidens* treated with 10 mg·l^{-1} of 2,4-D showed reduction in muscle glycogen and lactate, increase in protein content, while hepatic protein and lactate were reduced. The fish exposed to 1 and 10 mg·l^{-1}of herbicide showed also a decrease in plasma glucose (da Fonseca et al. 2008). Exposure of *Rhamdia quelen* for 96 h to 400, 600 and 700 mg·l^{-1} of 2,4-D resulted in a drop in hepatic and muscle glycogen, while lactate level decreased in liver and increased in muscle (Cattaneo et al. 2008). Exposure of *Prochilodus lineatus* to 10 mg·l^{-1} of Roundup increased plasma glucose after 24 and 96 h and decreased chloride level after 24 h (do Carmo Langiano and Martinez 2008). The listed metabolic parameters showed a decrease or increase due to exposure to herbicides. The variety of results obtained by various authors makes them difficult to interpret. Therefore, these physiological parameters do not seem to be good indicators of fish exposure to herbicides.

Oxidoreductive balance

Herbicides were also reported to disturb oxidoreductive balance in fish. In *Prochilodus lineatus* exposed to 10 mg·l^{-1} of Roundup activity of glutathione peroxidase (GPx) was reduced after 6 h, and after 24 h – also activity of superoxide dismutase (SOD) decreased. After 24 and 96 h activity of glutathione transferase (GST) increased. After 6 and 24 h of Roundup exposure hepatic glutathione content also increased (Modesto and Martinez 2010). Exposure of *Carassius auratus* for 96 h to 2.5, 5, 10 and 20 mg·l^{-1} of Roundup resulted in a decrease in brain and kidney SOD activity, while activities of hepatic GST, brain, kidney and liver glutathione reductase (GR) were reduced (Lushchak et al. 2009). A long-term (90 days) exposure to 0.376 mg·l^{-1} of clomazone caused a decrease in hepatic catalase (CAT) activity in *Leporinus obtusidens* (Moraes et al. 2009). Similar effect was observed after 96 and 192 h exposure to the same herbicide at the concentration 0.5 mg·l^{-1} (dos Santos Miron et al. 2008). *Rhamdia quelen* exposed to 0.5 mg·l^{-1} of clomazone showed reduction in hepatic CAT activity after 48, 96 and 192 h, while at 1 mg·l^{-1} of clomazone activity of hepatic CAT decreased already after 12 h of exposure and remained reduced until 192 h post exposure (Crestani et al. 2007). Activity of CAT decreased also in *Leporinus obtusidens* after 90 days treatment with propanil (1.644 mg·l^{-1}) (Moraes et al. 2009). On the other hand, *Carassius auratus* exposed for 96 h to 2.5, 5, 10 and 20 mg·l^{-1} of Roundup showed an increase in hepatic (at 2.5 and 5 mg·l^{-1}) and kidney (at all concentrations) CAT

activity (Lushchak et al. 2009). Paraquat (1 mg·l^{-1}, 24 h) induced an increase in ascorbic acid concentration in liver of *Channa punctata*, while the level of uric acid increased in kidney and decreased in gill (Parvez and Raisuddin 2006). These data show that herbicides may disturb the oxido-reductive balance in fish organism. It is not clear whether it is a result of their direct prooxidative activity or oxidative stress is a result of e.g. inflammation process caused by herbicide-induced tissue lesions. Analysis of available literature allows to conclude that one of the often studied oxidative stress indicator, catalase, seems to be a reliable indicator of chemical stress in fish caused by waterborne herbicides. Most authors observed a decrease of the CAT activity after exposure to this group of pesticides.

Hematological parameters

The basic indicators of chemical stress in fish include hematological parameters. Velisek et al. (2009) revealed that short-term (96 h) metribuzin exposure of *Cyprinus carpio* (175.1 mg·l^{-1}) caused a decrease in Hct, Hb, MCV, and WBC values. Sub-chronic exposure of the same fish species to the terbutryn (2, 20, and 40 mg·l^{-1}) led to an increase in RBC, while MCV and MCH values were significantly reduced (Velisek et al. 2010). Short-time (96 h) Roundup exposure (3, 6, 10, and 20 mg·l^{-1}) of *Leporinus obtusidens* decreased RBC and Hct values and Hb level (Glusczak et al. 2006). Ramesh et al. (2009) showed that acute atrazine treatment (18.5 mg·l^{-1}, 24 h) caused reduction of RBC count and Hb content compared to control group, whereas WBC increased. Hussein et al. (1996) revealed that the exposure of *Oreochromis niloticus* and *Chrysichthyes auratus* to 3 and 6 mg·l^{-1} of atrazine resulted in a decrease of RBC, Hb and Hct as compared the control group in both species. Also, there were significant changes of MCV, MCH and MCHC for both species. The investigators noticed that *Chrysichthyes auratus* were much more affected by atrazine exposure than *Oreochromis niloticus*. Dobsikova et al. (2011) exposed *Cyprinus carpio* to 13 mg·l^{-1} of Gardoprim Plus Gold 500 SC (corresponding to 2.25 mg·l^{-1} and 3.75 mg·l^{-1} of terbuthylazine and S-metolachlor, respectively) for 96 h. Exposure to the preparation caused a decrease of Hct and WBC values, as well as lymphocyte count. Studies conducted by Modesto and Martinez (2010) demonstrated an increase of Hct, RBC, and WBC in *Prochilodus lineatus* exposed to Roundup Transorb® (5 mg·l^{-1}) after 24 and 96 h of exposure. Increase in the percentage of lymphocytes and decreased percentage of neutrophils after 96 h exposure were also observed. Kreutz et al. (2011) revealed that RBC and WBC were decreased in the blood of *Rhamdia quelen* from the glyphosate-exposed fish (96 h; 0.73 mg·l^{-1}) as compared to the non-exposed fish. However, Hct level did not change. Crestani et al. (2006) revealed that clomazone caused a decrease of Ht value after 96 h of exposure at a concentration of 0.05 and after 192 h at 0.05 and 1.0 mg·l^{-1} in *Rhamdia quelen*. After 192 h of purification the Hct level in treated fish (0.5 and 1.0 mg·l^{-1}) were similar to control values. Butachlor administration led to various hematological alterations in *Labeo rohita*. RBC decreased in fish exposed to tested herbi-

cide at concentration of 0.5 to 1.0 mg·l^{-1} for 72 and 96 h of exposure, as well as in fish exposed to concentration of 0.75 to 1.0 mg·l^{-1}for 48 h. The Hb concentration decreased significantly in fish after 96 h at 0.5 to 1.0 mg·l^{-1} and after 72 h at 1.0 mg·l^{-1}. Hct was reduced in fish treated with herbicide at concentration of 0.75 and 1.0 after 48 h of exposure and in groups of 0.5–1.0 mg·l^{-1} after 72 and 96 h of exposure. The WBC was increased in all tested concentrations of butachlor and times of fish exposure (Ghaffar et al. 2015). Experiments conducted by Bojarski et al. (2015) revealed that exposure of *Cyprinus carpio* to pendimethalin at concentration of 2.5 µg·l^{-1} resulted in significant increase of Hb content and MCHC value after 7 days of treatment. Higher concentration (25 µg·l^{-1}) led to decrease of Hb level after 1 day, decrease of Hct and increase of MCH after 3 days and increase of RBC and Hct values and Hb content after 7 days of herbicide exposure. Ethofumesate (0.11 µg·l^{-1}) caused increase of WBC after 3 days of exposure, while longer treatment (7 days) led to increase of RBC and Hct values and Hb content. The same herbicide at higher concentration (1.1 µg·l^{-1}) caused increase of Hb level and MCHC value after 3 days of exposure. In fish exposed to mixture of both herbicides (2.5 µg·l^{-1} of pendimethalin + 0.11 µg ·l^{-1} of ethofumesate) increased RBC and Hct values after 1 as well as 3 days of exposure were observed. Fish exposed simultaneously to pendimethalin and ethofumesate at higher concentrations (25 and 11 µg·l^{-1}, respectively) exhibited similar hematological alterations: RBC and Hct were increased after 1 and 3 days of exposure, while MCHC was decreased (1 day), and Hb content was increased (3 days). In contrast to above, longer exposure (7 days) resulted in reduction of RBC and Hct values and increase of MCV and MCHC. Also, exposure to molinate of *Anguilla anguilla* at sublethal concentration of 11.15 mg·l^{-1} for 96 h showed the effects in hematological indices. Hct, Hb, RBC and WBC decreased only during recovery period (72 and 96 h) after exposure (Sancho et al. 2000). Based on the literature data it can be concluded that the fish organism usually shows anemic response as a result of herbicide pollution which probably results from direct action of herbicides on circulating erythrocytes and shortening of their life span. Sometimes observed increase in the values of red blood parameters may be related to stress reaction. The increase in WBC observed by some authors may be a result of inflammation process caused by the action of waterborne herbicides on fish organs.

HISTOLOGICAL EFFECTS

Gills histopathology

Fish exposure to herbicides may also lead to pathological changes in various internal organs, including kidney (Jiraungkoorskul et al. 2002) and brain (Deivasigamani 2015). However, majority of studies concerning the influence of these substances on fish organism focus on the structure of gills and liver. Very often histological lesions are observed in gills being the site of first contact with environmental pollutants. They relatively quickly react to changes in environmental conditions and the presence of toxic substances in water. In the gills

of *Cyprinus carpio* exposed to 0.01 mg·l^{-1} of trifluralin for 14 days Poleksic and Karan observed hyperplasia of secondary lamellae and their partial fusion accompanied by folding of respiratory epithelium and hypertrophy of some chloride cells. At the concentration of 0.02 mg·l^{-1} trifluralin caused local lifting of secondary lamellae epithelium and its hyperplasia which resulted in lamellar deformation and fusion, while chloride cells were hypertrophic (Poleksic and Karan 1999). *Labeo rohita* exposed for 5 days to 0.18 mg·l^{-1} of atrazine showed increased gill mucosecretion, hyperplasia of primary lamellae and their merging, while in secondary lamellae separation of epithelium and lamellar fusion were observed. Destruction of pillar cells was also noted accompanied by vacuolation and necrosis of secondary lamellae epithelium (Jayachandran and Pugazhendy 2009). The effects of seven-day exposure of *Ctenopharyngodon idella* to 0.5 and 1 mg·l^{-1} of atrazine included local epithelial cell proliferation, epithelium lifting and fusion of secondary lamellae (Botelho et al. 2012). *Channa punctatus* exposed for 10 days to 1.21 mg·l^{-1} of alachlor showed deformed primary and secondary gill lamellae. Secondary lamellae were shortened and often fused, while their epithelial cells often showed vacuolation. Karyorhexis of pillar cells was also observed (Butchiram et al. 2009). According to Gosiewski et al. (2012), exposure to 15 mg·l^{-1} of Roundup caused edema, hypertrophy and hyperplasia of secondary lamellae epithelium. These changes were mode pronounced after 10 days of exposure compared to 6 months. Hued et al. (2012) reported that short-term (96 h) treatment of *Jenynsia multidentata* with 5 mg·l^{-1} of Roundup resulted in edema, hypertrophy and separation of secondary epithelium. Higher concentration (10 mg·l^{-1}) caused focal separation of secondary lamellae epithelium. In the lamellae dilated capillaries and chloride cell hypertrophy were also observed. At 20 mg·l^{-1}of Roundup slight thickening of secondary lamellae occurred due to epithelial hyperplasia. At the highest concentration – 35 mg·l^{-1} increased mucosecretion and considerable epithelial hyperplasia were observed which resulted in lamellar fusion. Subchronic exposure (28 days) to the same herbicide at the concentration 0.5 mg·l^{-1} caused strong mucosecretion and shortening of secondary lamellae (Hued et al. 2012). After 48 h of exposure to 36 mg·l^{-1} of Roundup *Oreochromis niloticus* showed different thickness of primary lamellae epithelium, edema and lifting of secondary lamellae epithelium and leukocyte infiltration among the epithelial cells (Jiraungkoorskul et al. 2002); after 96 h – apical enlargement of secondary lamellae and considerable epithelium thickening in primary lamellae. The results obtained by Ayoola (2008a) revealed cellular infiltration between the secondary lamellae in *Oreochromis niloticus* exposed for 96 h to 2, 9 and 97 mg·l^{-1} of glyphosate, uneven epithelium thickness at 30 mg·l^{-1} and gill edema and necrosis at 310 mg·l^{-1}. The gills of *Clarias gariepinus* treated with glyphosate for 96 h Ayoola observed cellular infiltration (at 19, 42, 94, 207 and 455 mg·l^{-1}), congestion (at 42, 94, 207 and 455 mg·l^{-1}), hemorrhages (at 94, 207 and 455 mg·l^{-1}) and necrotic lesions (at 207 and

455 mg·l^{-1}) (Ayola 2008b). These data indicate that herbicide poisoning leads primarily to hyperplasia and hypertrophy of the gill epithelium, and thus probably may impair the functions of this vital organ: respiration, osmoregulation and secretion. Therefore, it can be assumed that the exposure of fish to herbicides may indirectly cause further physiological disorders such as hypoxemia, hypercapnia and osmotic disturbances.

Liver histopathology

Liver in which metabolism of xenobiotics takes place is another organ that often shows pathological lesions in intoxicated fish. Unfortunately, the data concerning hepatic histpathologies in fish due to herbicide exposures are scarce. *Cyprinus carpio* exposed for 14 days to 0.01 or 0.02 mg·l^{-1} of trifluralin showed a concentration-related vacuolation of hepatocytes, accompanied at higher concentration by karyopyknosis (Poleksic and Karan 1999). Treatment of *Rhamdia quelen* to 1 mg·l^{-1} of clomazone for 192 h also resulted in hepatocyte cytoplasm vacuolation (Crestani et al. 2007). In *Channa punctatus* subjected to 1.21 mg·l^{-1} of alachlor for 10 days hepatocyte vacuolation and cytoplasm damage were observed accompanied by blood vessel breakdown and alterations in hepatocyte organization (Butchiram et al. 2009). *Prochilodus lineatus* exposed to 7.5 mg·l^{-1} of Roundup for 24 h showed hepatic vessel congestion, cytoplasm degeneration and hypertrophic hepatocyte nuclei; after 96 h at the same concentration of Roundup – hepatocyte vacuolation, progressive degeneration and pyknosis accompanied by cholestasis. Vacuolation and cholestasis accompanied by karyorrhexis and hepatocyte hypertrophy were observed in fish exposed for 24 h to 10 mg·l^{-1} of herbicide (do Carmo Langiano and Martinez 2008). Histopathological alterations accompanied by increase of liver transaminase activities indicate that liver is a sensitive to herbicide toxicity. Lesions in this organ may result in disruption of xenobiotic metabolism and increased susceptibility to chemical stress, as well as disturbances in other metabolic functions. Therefore, microstructure of fish liver, as well as gills, seems to be a sensitive indicator of water pollution by herbicides.

CONCLUSIONS

Pollution of aquatic environment with herbicides becomes an increasing problem. The data shown in present review indicate that reactions of fish organism to intoxication with various herbicides are nonspecific which makes identification of contaminant very difficult or impossible and implies the necessity of environmental monitoring. These data indicate that toxic effects of herbicides depend on various factor such as type and concentration of herbicide, time of exposure and fish species. Comprehensive evaluation of the harmfulness of herbicides requires performing biochemical, hematological and histopathological tests probably supplemented with other indicators as immune parameters and reproductive indices.

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Streszczenie: *Fizjologiczne i histologiczne skutki działania herbicydów na ryby*. Pośród pestycydów stosowanych w rolnictwie herbicydy są wykorzystywane w największych ilościach. Substancje te przedostają się do środowiska wodnego na wiele sposobów, a badania monitoringowe wykazują stałą ich obecność w wodach naturalnych. Herbicydy powodują zmiany wielu parametrów fizjologicznych u ryb, np. zmniejszenie aktywności acetylocholinoesterazy w narządach wewnętrznych, zwiększenie aktywności aminotransferaz wątrobowych czy zaburzenie równowagi oksydoreduktancyjnej. Obserwuje się również zmiany parametrów hematologicznych, zwykle wskazujące na anemię i proces zapalny. Najczęściej notowanymi zmianami histologicznymi są hiperplazja i hipertrofia nabłonka skrzeli oraz zmiany w mikrostrukturze wątroby, takie jak wakuolizacja hepatocytów. Markery ekspozycji ryb na herbicydy są na ogół bardzo wrażliwe, ale niespecyficzne. Analiza parametrów metabolicznych, hematologicznych i histologicznych może być pomocna w diagnostyce zatrucia ryb herbicydami, ale zawsze powinna być potwierdzona badaniami monitoringowymi.

Słowa kluczowe: intoksykacja, pestycydy, zdrowie ryb

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Authors' address:
Bartosz Bojarski
Zakład Fizjologii Zwierząt
Wydział Przyrodniczy
Uniwersytet Przyrodniczo-Humanistyczny
w Siedlcach
ul. Prusa 14, 08-110 Siedlce
Poland
e-mail: bbojarski@o2.pl

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Study of the electrical characteristics and sheathing heat protection of the American mink (*Neovison vison*) hair coat

PAULINA CHOLEWIŃSKA, MARTA MICHALAK, PATRYCJA WACŁAWIK, DOMINIKA GRABOLUS
Faculty of Biology and Animal Science, Wroclaw University of Environmental and Life Sciences

Abstract: *Study of the electrical characteristics and sheathing heat protection of the American mink* (Neovison vison) *hair coat.* Recently, research methods from other areas in the zootechnics become more and more popular, for example using the research of electrical features in agricultural production, which are used in microbiology to evaluate food products. The use of the test of electrical properties, i.e. testing the behavior of the material in the electromagnetic field is a very sensitive and fast method. It offers a very attractive tool, not destroying the tested material, which results in lower costs and less workload than using traditional research. The aim of the study was to detect differences between the hair cover of American mink (*Neovison vison*) living and dead by means of an impedance test and heat resistance. The biggest differences in resistance were observed at frequencies of 10 and 20 Hz, where the resistance at 10 Hz for post mortem collected samples was 1.33E+09 Ω, while for samples from live mink –7.60E+06 Ω, the value for post mortem samples from mink was higher about 170-fold. At a frequency of 20Hz, the hair covering resistance of post mortem hair samples was 7 times higher than that of the samples from living ones. The differences between groups (P = 0.046), can be received may indicate changes in the structure of keratin that occurred posthumously. In addition, the investigation of heat insulation compared to electric properties showed no differences, and its duration was half as long.

Key words: electrical properties, impedance, heat resistance, American mink

INTRODUCTION

In recent years, in order to determine the variability of materials, it has been increasingly common to study their electrical characteristics, and the most frequently used properties related to the behavior of the material as a dielectric or electrical conductivity. Each material tested permanently embedded in the molecule or induced on its surface. Differences in the set of their inaccurate conduct in the electromagnetic field and conditions do not apply in practice (Jha et al. 2011, Skiechura 2012, Yousefi et al. 2014).

The study of the electrical properties of biological materials can be divided into studies in relation to changes in the electrical properties of the material and its physicochemical characteristics, as well as on the study of biophysical, biochemical and microstructural changes occurring in the material structure under the influence of the electromagnetic field (Samouëlian et al. 2005, Łuczycka 2009b).

Since it is possible to detect changes at molecular level with this type of ex-

amination, it can be used in the hair coat assessment. The evaluation of the skin and quality of the hair cover is based on examination of their surface and evaluation of the hair cover by assessing its density, length and diameter. Traditional studies require more work compared to the study of electrical characteristics (Jha et al. 2011, Święcicka et al. 2016).

In the case of fur animals, it is important to assess the quality of the hair cover properly. American mink (*Neovison vison*) was introduced to Poland in 1928, since then this species has been gaining in popularity among breeders. Currently, American mink accounts for about 70% of the population of fur animals kept in the country (Święcicka et al. 2016). Their hair cover is on averange between 16 and 22 mm long and the thickness of down hair varies from 10.6 to 12.4 μm, the hair cover should be as even as possible and short hair (Piórkowska and Kowalska 2014).

The aim of the study was to detect differences between the hair-plate of American mink from live and dead specimens by means of an impedance test compare to heat resistance test.

MATERIALS AND METHODS

Animal material

The animal hair coat material consisted of mink: 10–5 pcs. from live animal hair coat, 5 pcs. from post mortem hair coat. The animal were 3 years old, from a farm located in the Wielkopolska Voivodship. Samples were taken in summer from the hindquarters by machine shaving from each individual (post mortem samples were taken between 6 and 8 h after death). The weight of the samples taken was standardized to 0.4 g for each individual, while the heat protection was achieved by collective measurements (2 samples) of 722 g (+/– 6.45 g).

Testing of electrical characteristics

The samples were tested with Atlas Solich 0441 High Impedance Analyser. The frequency range of the device was from 10 Hz to 1 MHz. The samples were placed between the copper electrodes in a 3.9 mm thick chamber with an inner diameter of 38 mm, made of plastic (PAN). Measurements were taken at a constant temperature of 25°C and 70% humidity and repeated twice.

Heat resistance test

The examination was carried out in the Roof and Hair Cote Laboratory. Thermal protection was tested using the Matest thermal radiation material assessment apparatus. The thermal transmittance coefficient (*WPC*) was calculated using the formula:

$$WPC = GSC_p / GSC_o$$

where:
GSC_p – the density of the thermal flux passing through the test (kW/m^2);
GSC_o – the density of the thermal flux to be tested (kW/m^2);

$$GSC_p = \frac{M \cdot C_p \cdot R_1}{A \cdot \alpha}$$

$$GSC_o = \frac{M \cdot C_p \cdot R_2}{A \cdot \alpha}$$

where:
M – mass of the aluminium calorimeter sample: 7.16 (g);
C_p – heat specific aluminium: 900 (J/kg·°C);
A – surface area of calorimeter: 0.00049 (m^2);
α – absorption coefficient of the blackened calorimeter surface area: 0.95 (–);
R_1 – rate of increase of calorimeter temperature in the linear part of the graph (for calorimeter with sample) (°C/s);
R_2 – rate of calorimeter temperature increase in the linear part of the graph (for empty calorimeter) (°C/s).

Elaboration of results

The results were elaboration by Statistica 13.1. Normal distribution of results was evaluated by the Shapiro–Wilk test. Due to lack of normal decomposition, Wilcoxon's test for impedance and heat protection was used. Differences between groups were statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

In mammals, about 30 types of keratin can be distinguished, depending on the species of animal and its structure (nails, hair, horns). Hair is made up of 90–95% "hard" α-keratin, which is characterized by a multi-level structure. Since, it fills the interior of the hair to various degrees, it can be distinguished by several anatomic hair types: down, transient, spinal, wide and dead hair.

Depending on the degree of keratin filling, hair will vary in thickness, hygroscopic properties, strength, elasticity and resistance to mechanical factors, which will result in different physical and chemical properties. The factors influencing hair structure and keratin filling are nutrition, breed of animal, age and environment (Safari et al. 2005, Cardamone et al. 2009, McKittrick et al. 2012, Piórkowska and Kowalska 2014). Taking into account the factors influencing the changes in hair structure, it can be concluded that the study of impedance shows them. It should be taken into account that impedantometry is a very sensitive study, taking into account changes already at molecular level (Nelson 2008, Jha et al. 2011).

The study of impedance, electrical resistance in general, showed significant differences between post mortem collected samples and from live American mink hair coat (Nelson 2008, Łuczycka 2009a). Impedance is a composite size, defined in Ω. The impedance test was performed in the current flow frequency range from 10 Hz to 1 MHz. The values up to 1,300 Hz and from that value followed by a linear decrease (Figs. 1, 2).

At the frequencies of 10, 20, 40, 80 and 196 Hz it covers the post mortem collected hair from American mink was characterized by a higher resistance compared to hair from live animals ($P = 0.046$). The greatest differences in resistance were observed at frequencies of 10 and 20 Hz, where the resistance at 10 Hz for post mortem collected hair was 1.33E+09 Ω, while for live ones –7.60E+06 Ω (the value for post-mortem sampless was approximately 170-fold higher). At a frequency of 20 Hz, the hair cover resistance of the post mortem collected hair was 7 times higher than that of the living one. Additionally, higher resistance (about 3-fold) was

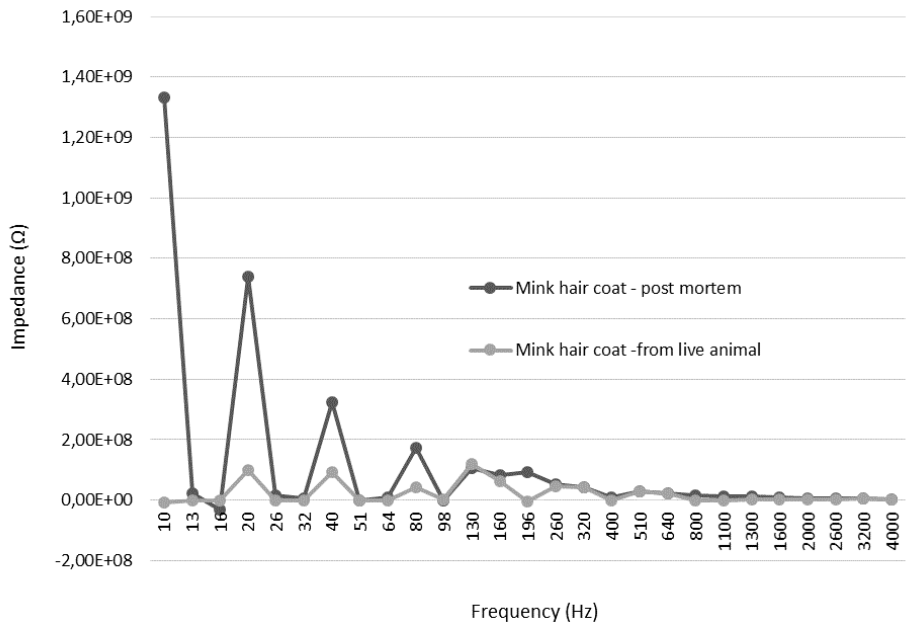

FIGURE 1. Impedance of hair coat from American mink (10–4,000 Hz)

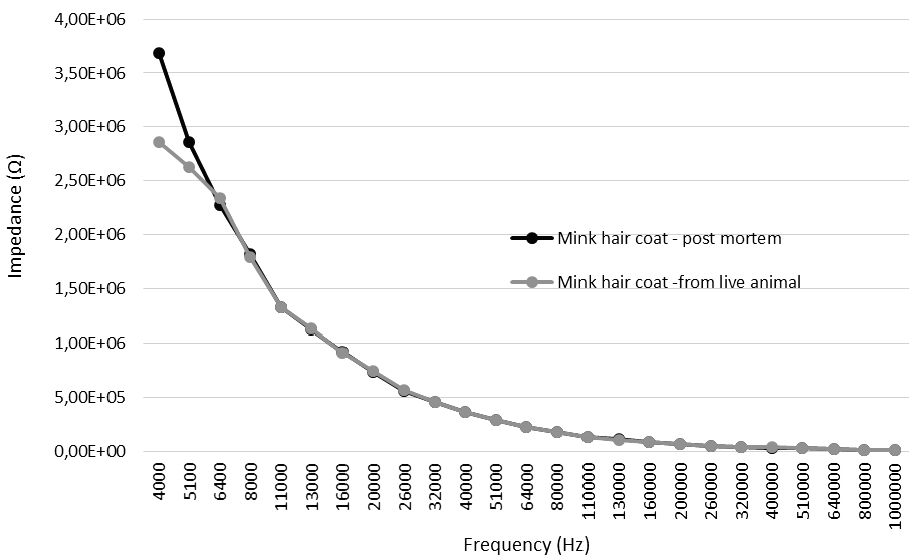

FIGURE 2. Impedance of hair coat from American mink (from 4,000 Hz to 1 MHz)

characterized by post mortem collected cover at frequencies of 40 and 80 Hz in relation to live animals, however, these differences were not as diametrical as at lower frequencies, which is visible in Figure 1. The results obtained may have been caused by changes in keratin structure – in this case probably the post mortem condition had a direct effect on hair cover condition.

In addition, as resistance (in this case impedance) increases, the material becomes better dielectric properties, i.e. a material in which current is poorly conducted due to higher resistance. The most important property of the hair cover as a dielectric is the ability to accumulate electric charge, which causes it to act electrostatically on the human body (Jha et al. 2011, Vanhoof 2011). The electrostatic interaction of materials fibers has a very important aspect in the creation of materials with a pro-health effect and they are often based on a cover derived from animals. Studies on fiber electrostatics were included in the Callahan and Kornberg patent (1993), where natural materials (flax and wool) of dielectric nature showed a positive effect on the body by acting as an ion detector for electromagnetic waves emitted by skin layers. The obtained results indicate that the coat of dead mink is a better dielectric than that of living individuals, which suggests that contact with the hair coat with greater resistance may have a more beneficial effect on the dermal coatings than the hair follicles of the lower resistance.

On the other hand, the analysis of heat resistance showed no significant differences between the studied groups. The heat transfer coefficient (*WPC*) was 0.657 (+/–0.04) for the hair cover of live mink and 0.606 (+/–0.023) ($P < 0.05$) for the post mortem collected cover. Thus, it did not show the confirmation of impedance results. Both the results – the impedance testing and heat protection, should show changes in the structure of the coat due to differences in the filling with keratin. However, it should be taken into account that heat resistance is not so sensitive because it assesses the heat transfer through the hair shield, while the study of electrical properties is based on molecular changes of the material. The results of heat resistance are also a result of the stages of hair growth, because after the growth of the cores they act as an insulator against heat loss and they do not change their properties (Kuźniewicz and Filistowicz 1999, Mark and Bikales 2005, Cardamone et al. 2009, Jha et al. 2011).

Considering that the examination of electrical features allows to show changes at the molecular level, it can show more changes in the coat, where no thermal differences were observed in the performance of the heat resistance test (Safari et al. 2005, Nelson 2008, Banclari et al. 2016) has not yet been fully applied in the study of the properties and changes in the hair surface by means of impedance. During further studies, they may show its wider use in assessing the quality of the coat.

CONCLUSIONS

1. Testing of electrical characteristics (impedance) showed significant differences ($P = 0.046$) between the hair-plate of the American dead and living mink.

As a result, the hair of the dead mink had better dielectric characteristics than the living mink.

2. The heat resistance test did not reveal any significant differences between the hair cover of the American dead and living mink.

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Streszczenie: *Badanie cech elektrycznych i ciepłochronności okrywy włosowej wizonów amerykańskich* (Neovison vison). Coraz częściej w produkcji rolnej wykorzystuje się badania cech elektrycznych. Przykładem tego może być wykorzystanie ich w celu oceny mikrobiologicznej produktów spożywczych. Zastosowanie testu cech elektrycznych, czyli testowania zachowania się materiału w polu elektromagnetycznym, jest metodą bardzo czułą i szybką. Oferuje ona bardzo atrakcyjne narzędzie, nieniszczące badanego materiału, co skutkuje mniejszymi kosztami i mniejszym nakładem pracy w porównaniu z użyciem tradycyjnych badań. Wykonane badanie miało na celu wykrycie różnic między okrywą włosową norek amerykańskich (*Neovison vison*) żywych i martwych za pomocą badania jednego z parametrów cech elektrycznych – impedancji oraz ciepłochronności. Największe różnice w oporności wystąpiły przy częstotliwościach 10 i 20 Hz. Przy częstotliwości 10 Hz oporność okrywy włosowej od norek martwych wyniosła 1,33E+09 Ω, a okrywy żywych zwierząt –7,60E+06 Ω. Wartość dla okrywy włosowej norek martwych była większa ok. 170-krotnie. Przy częstotliwości 20 Hz oporność okrywy włosowej norek martwych była zaś 7-krotnie większa niż okrywy żywych zwierząt. Otrzymane różnice mogą świadczyć o zmianach w strukturze keratyny, jakie zaszły pośmiertnie. Mogą one także określić wpływ badanego materiału na powłoki skórne ludzi i przy przyszłym opracowaniu tej metody określenie jakości okrywy włosowej. Dodatkowo badanie ciepłochronności w porównaniu do badań cech elektrycznych nie wykazało różnic, a czas jego wykonania był o połowę dłuższy.

Słowa kluczowe: cechy elektryczne, impedancja, ciepłochronność, norka amerykańska

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Authors' address:
Paulina Cholewińska
Instytut Hodowli Zwierząt
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chelmońskiego 38c, 51-630 Wrocław
Poland
e-mail: paulina.cholewinska@upwr.edu.pl

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Effect of type of diet fed to growing-finishing pigs on production results and fattening efficiency

MARCIN DRÓŻDŻ, MARCIN SOŃTA, ANNA REKIEL, JUSTYNA WIĘCEK
Faculty of Animal Science, Warsaw University of Life Sciences – SGGW

Abstract: *Effect of type of diet fed to growing-finishing pigs on productivity and fattening efficiency.* The aim of the study was to compare productivity and fattening efficiency of growing-finishing pigs fed farm-produced vs. commercial mixtures on a farm specializing in producing fattening pigs. A total of 2,028 Danbred hybrid weaners with a mean initial body weight of 31.8 kg were placed in two bedding-free piggeries (1,008/1,020 pigs in groups 1 and 2) and divided into smaller groups (60 animals per pen). Pigs were fed continuously from automatic feeders (farm-grown feeds in group 1, commercial mixtures in group 2) and had constant access to water. Fattening lasted 90 days (phase I – 40 days, phase II – 50 days). Compared to the farm-produced feeds, the commercial mixtures for I and II fattening phase were more expensive by 4.95 and 6.52%. The energy to protein ratio of the farm-produced and commercial mixtures was higher for the former. The fattening and slaughter value results were very good in both groups, weight gains slightly better, and mortality slightly lower in group 1 compared to group 2. Unit revenue when feeding farm-produced feeds (group 1) was higher than that in group 2 (difference of 10.14 PLN per pig, 17.7%), which shows the appropriateness of taking measures to reduce feeding costs through the use of high value, cheaper farm-grown feeds. The present results justify the search for and the use of practical solutions, including better balanced diets and reduced feeding costs, as essential to improving the economic efficiency.

Key words: fattening pigs, feeding, production efficiency, revenue

INTRODUCTION

The utilization of low-value feeds, in particular low-protein feeds, is not appropriate for intensive fattening. High-lean pigs can be produced when feeding balanced diets with a high energy and nutrient concentration, which meet the requirements of growing pigs. Protein (lean muscle tissue) deposition in the body is contingent on its intake in feed as well as the energy to protein ratio of the diet (Kulisiewicz et al. 1995, 1996, Quiniou et al. 1995, 1996, Więcek et al. 2002, Rekiel and Olejniczak 2009).

Because feeding costs account for more than 60% of the total production costs for pigs, efforts are made to reduce them. One way to do so is to reduce the purchase of manufactured feeds or protein components for on-farm making of complete diets. It is worth considering to replace them with compound feeds prepared from farm-produced feed materials (cereals, leguminous plants). However, they should be supplemented with a few purchased components, which are necessary to balance the diets and meet the energy and nutrient requirements of a specific production group of pigs. This is

easiest to achieve with a group of growing-finishing pigs.

The aim of the study was to compare the production results and efficiency of fattening pigs with farm-produced and commercial mixtures, in a pig fattening farm.

MATERIAL AND METHODS

The study was conducted in an open-cycle private farm specializing in growing--finishing pigs production and located in central Poland. A total of 2,028 Danbred hybrid weaners with a mean initial body weight of 31.8 kg were placed in two piggeries (1,008 and 1,020 pigs) and divided into smaller groups. Animals were placed in four compartments, each of which held four pens. Around 60 pigs were grouped into each pen. Animals were kept on concrete slats without straw bedding. Pens were equipped with automatic feeders which were supplied from two external 13-tonne silos via a spiral conveyor. The silo assigned to building 1 was supplied with farm-produced feed (group 1) and that assigned to building 2 with commercial mixture, which was delivered in feed wagons (group 2). Feed was supplied continuously from tube feeders equipped with membranes maintaining a constant water level in the feeder pit, and water from available from automatic drinkers. Basic welfare standards were provided for the animals (Regulation of MARD 2003, Directive 2008/120/WE, Kondracki et al. 2014).

A 90-day fattening period was divided into phase I (40 days) and phase II (50 days). The farm-produced feeds, which were given to pigs from group 1, contained wheat and barley (Table 1), soybean meal (Table 2) and premixes, the proportion of which in the diets for phase I and phase II of fattening followed the manufacturer's recommendations. Tables 3 and 4 provide the composition and value of the farm-produced feeds.

TABLE 1. The results of analysis of purchased cereals (Agricultural Advisory Centre)

Parameters (%)	Wheat	Barley
Protein	11.1	9.5
Starch	61.5	55.1
Fat	1.4	2.2
Water	14	12.7
Carbohydrates	2.1	1.7
Fibre	2.3	3.7
Ash	1.3	2.3

TABLE 2. Characteristics of the value of HI-PRO soybean meal (Glencore Polska Sp. z o.o.)

Parameters (%)	HI-PRO soybean meal
Protein	46.0
Fibre	4.0
Fat	3.0
Moisture	12.0

In building 2 (group 2), animals received commercial mixtures: Grower M in fattening phase I and Finisher M in phase II (Table 4).

The cost of commercial mixtures was 1060 PLN/t ("Grower M") and 980 PLN/t ("Finisher M"). The farm-grown feeds cost less (grower diet – 1,010 PLN/t, finisher diet – 920 PLN/t). The tables provide averages values for groups.

TABLE 3. Composition, energy content and nutritive value of grower and finisher diets produced on the farm (own elaboration)

Item	Diet	
	Grower	Finisher
Wheat (%)	39.0	42.0
Barley (%)	38.5	41.5
Soybean meal (%)	20.0	14
Supplementary mineral mixture		
Rolmix W* (%)	2.5	–
Rolmix WT** (%)	–	2.5
Energy content and nutritive value of the diets***		
ME (MJ)	13.1	13.1
Crude protein (%)	17.2	15.8
Crude fibre (%)	4.35	4.70
Calcium (%)	0.80	0.77
Total phosphorus (%)	0.49	0.45
Digestible phosphorus (%)	0.26	0.23
Sodium (%)	0.18	0.18
Lysine (%)	1.02	0.87
Methionine + Cysteine (%)	0.54	0.48
Tryptophan (%)	0.21	0.19
Threonine (%)	0.61	0.54

* In 1 kg: lysine 14%, methionine 3.7%, Ca 12.9%, Na 7%, P 4.8%, vitamin A 600,000 IU, vitamin D_3 80,000 IU, vitamin E 4,500 mg, Cu 5,000 mg, Zn 5,500 mg, Mn 2,000 mg, Fe 4,500 mg, Se 10 mg, I 40 mg.
** In 1 kg: lysine 12.5%, methionine 2.5%, Ca 15.45%, Na 7.5%, P 3.0%, vitamin A 500,000 IU, vitamin D_3 100,000 IU, vitamin E 3,000 mg, Cu 5,000 mg, Zn 5,333 mg, Mn 3,226 mg, Fe 11,667 mg, Se 33 mg, I 55 mg.
*** Calculated.

RESULTS AND DISCUSSION

The productivity and fattening efficiency are presented in Table 5. The calculations did not include the costs of utilities, buildings and equipment depreciation, and labour.

The price difference between the diets fed to groups 1 and 2 was 50 PLN/t for fattening phase I and 60 PLN/t for fattening phase II. Compared to the farm-grown feeds, the commercial mixtures were more expensive by 4.95 and 6.52%, respectively. In the purchased and farm-produced feeds used in fattening phases I and II, the energy to protein ratio, expressed as the amount of crude protein (CP) per MJ ME, showed some differences: it was lower in commercial mixtures (12.63 and 11.69 CP/MJ ME) than in farm-produced feeds (13.13 and 12.06 CP/MJ ME). These differences might

TABLE 4. The energy content, nutritive value and ingredient composition of the commercial mixtures (manufacturer's information)

Item	Grower M	Finisher M
ME (MJ)	13.30	13.00
Crude protein (%)	16.80	15.20
Crude fat (%)	2.60	3.40
Crude fibre (%)	3.90	4.50
Crude ash (%)	4.00	3.90
Calcium (%)	0.67	0.55
Total phosphorus (%)	0.43	0.47
Sodium (%)	0.16	0.15
Lysine (%)	1.04	0.89
Methionine (%)	0.34	0.31
Methionine + Cysteine (%)	0.66	0.65
Mineral-vitamin PREMIX		
Vitamin A (IU/kg)	6 500	4 343
Vitamin D_3 (IU/kg)	1 650	1 004
Vitamin E (mg/kg)	105	33
Iron (mg/kg)	140	140
Copper (mg/kg)	18	10
Zinc (mg/kg)	113	54
Manganese (mg/kg)	75	17
Iodine (mg/kg)	1.00	0.50
Selenium (mg/kg)	0.30	0.20
Ethoxyquin (mg/kg)	0.09	0.06
Gallate (mg/kg)	0.01	0.01
Phytase (FTU/g)	+	300
Endo-1,4-beta xylanase (VU/g)	+	1 360
Acidifier (–)	+	–
Feed materials		
Grower M		Finisher M
wheat, barley, triticale, wheat bran, maize bran, soybean meal, rapeseed meal, maize DDGS, calcium carbonate, fatty acids and oils of plant origin, sodium chloride		wheat, barley, triticale, wheat bran, soybean meal, rapeseed meal, sunflower meal, maize DDGS, calcium carbonate, fatty acids and oils of plant origin, sodium chloride

TABLE 5. Fattening results and production efficiency (own elaboration)

Item	Group 1 (farm-produced feeds)	Group 2 (commercial mixtures)
Number of pigs at the start of experiment (head)	1 008	1 020
Number of pigs at the end of experiment (head)	992	997
Average initial mean body weight (kg)	31.5	32.2
Average body weight at the end of the phase I (kg)	70.5	70.2
Average final body weight (kg)	120.7	120.2
Mortality – phase I (head/%)	2 / 0.2	5 / 0.5
Mortality – phase II (head/%)	14 / 1.4	18 / 1.8
Total mortality (head/%)	16 / 1.6	23 / 2.3
Total feed intake – phase I (kg)	75 000	75 000
Total feed intake – phase II (kg)	180 000	172 000
Total feed intake (kg)	255 000	247 000
Average daily feed intake (kg)	2.84	2.72
Average daily gain – phase I (g)	975	951
Average daily gain – phase II (g)	1 004	1 000
Average daily gain fattening (g)	991	978
Feed conversion ratio – phase I (kg/kg)	1.91	1.94
Feed conversion ratio – phase II (kg/kg)	3.62	3.45
Feed conversion ratio during fattening (kg/kg)	2.88	2.81
Total production of live pigs (kg)	119 734.4	119 839.4
Cost of weaners (PLN)	302 400	306 000
Cost of mixtures – phase I (PLN)	75 750	79 500
Cost of mixtures – phase II (PLN)	165 600	168 560
Total cost (PLN)	543 750	554 060
Value of live pigs sold (PLN)	610 645.44	611 180.94
Total revenue (PLN)	66 895.44	57 120.94
Unit revenue (PLN per animal)	67.43	57.29

have resulted in 13 g (1.33%) better weight gains during fattening (group 1 compared to group 2), which, with 0.66 percentage point lower mortality and lower cost of farm-grown feeds yielded better unit revenue of 10.14 PLN (17.7%) for the group fed farm-produced feeds (group 1) compared to the group receiving commercial mixtures (group 2).

The experimental pigs willingly consumed the feed, which according to Tyra et al. (2015) is determined by the quality, taste (expressed as flavour and aroma), fibre level, and proper balancing of the feed. Constant access to water is also

essential. Fatteners from both groups achieved very good results expressed in weight gains. Studies performed by Bojko and Rekiel (2014), Goszczyński et al. (2016) and Taraska et al. (2016) confirm a very high genetic potential of hybrid pigs imported to Poland and fattened in Polish piggeries. This was reflected in a very good rate of growth, feed conversion ratio and meatiness of around 60%, regardless of the housing system. It is concluded from the present study that fattening success is conditional on meeting the nutrient requirements of animals by ensuring adequate intake of feed (*ad libitum* feeding, fattening phases) that meets the standards of a complete diet for growing pigs.

Mortality in groups 1 and 2 was within normal limits and did not exceed 3%, although it was slightly elevated in the group receiving commercial mixtures. The majority of deaths occurred in the final month of fattening. Supplementation of water with a liquid acidifier prevented further losses. The beneficial effect of acidifiers during rearing of young pigs and growing animals was confirmed by many experiments, including Rekiel et al. (2017). They have the advantage of reducing digesta pH and thus forming an environment in which pathogens cannot multiply. This shortens the duration of diarrheas and makes them less frequent, which is of benefit to health and prevents mortality.

Based on the nutrient requirements of pigs (Grela and Skomiał 2015), growth potential of the experimental pigs was intermediate between high (1,000 g/day) and medium (850 g/day). The results obtained in both groups for the entire fattening period (Table 5) are considered very good (a difference of around 1.4 percentage points). A slightly poorer result was obtained for feed conversion ratio (Table 5), which was similar in both groups but exceeded 2.8 kg/kg. It can be assumed that a small change in the energy to protein ratio, resulting from increased per unit content of energy (e.g. by adding oil to the diet), could have a positive effect on FCR value.

The average final body weight of pigs (after 90 days of fattening) exceeded 120 kg and was similar in the groups, as was carcass meatiness. It was around 59% and most of the carcasses were graded "E" according to the EUROP system. These results are considered very good. They are comparable with, and event slightly better than the results reported by other authors (Rekiel and Olejniczak 2009, Bojko and Rekiel 2014, Taraska et al. 2016).

When comparing the fattening results and the production costs, it can be stated that the values obtained in the compared groups were similar. The total cost of farm-produced feeds for the whole fattening period was 241,350 PLN for group 1, while the cost of commercial mixtures was 248,060 PLN (group 2). The difference of 6,710 PLN appears to be small. However, if we assume that fattening will be conducted in both piggeries all year round, the annual savings from feeding farm-produced feeds will be at least four-fold higher.

CONCLUSIONS

The unit revenue, when the pigs were fed farm-produced feeds (group 1), was higher than in group 2. This shows that it is appropriate to take measures to re-

duce feeding costs through the use of high value, but at the same time cheaper farm-grown feeds that ensure very good production results. The results of the present study justify the search for and the use of practical solutions, including better balanced diets and reduced feeding costs, as essential to improving the economic efficiency.

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Streszczenie: *Wpływ rodzaju mieszanek stosowanych w żywieniu świń rosnących na wyniki produkcyjne i efektywność tuczu***.** Celem pracy było porównanie wyników produkcyjnych i efektywności tuczu przeprowadzonego w gospodarstwie specjalizującym się w produkcji tuczników, po zastosowaniu w żywieniu rosnących świń mieszanek własnych i z zakupu. Warchlaki mieszańców Danbred (2028 szt.) o średniej początkowej masie ciała 31,8 kg umieszczono w dwóch chlewniach bezściołowych (1008 i 1020 szt. – grupy 1 i 2). Zwierzęta podzielono na mniejsze grupy, po ok. 60 szt./kojec. Świnie żywiono z automatów paszowych w sposób ciągły, przy stałym dostępie do wody, stosując w grupie 1 mieszanki własne, a w grupie 2 mieszanki z zakupu. Tucz trwał 90 dni, w tym okres I – 40 dni i okres II – 50 dni. Pasze na I i II okres tuczu pochodzące z zakupu w porównaniu do własnych były droższe o 4,95 i 6,52%. Stosunek energetyczno-białkowy mieszanek własnych i z zakupu różnił się – był większy dla pasz własnych. Uzyskane wyniki tuczu i wartości rzeźnej były bardzo dobre w obu grupach, przyrosty nieznacznie lepsze, a upadki nieco mniejsze w grupie 1. Przychód jednostkowy przy żywieniu tuczników paszami własnej produkcji (grupa 1) był większy niż uzyskany w grupie 2 (różnica 10,14 zł/szt., 17,7%), co wskazuje na zasadność działań zmierzających do zmniejszenia kosztów żywienia poprzez stosowanie pełnowartościowych, tańszych mieszanek własnych. Wyniki badań wskazują na zasadność poszukiwania i stosowania rozwiązań praktycznych, takich jak lepsze bilansowanie mieszanek paszowych oraz zmniejszenia kosztów żywienia jako priorytetowych w aspekcie poprawy rachunku ekonomicznego.

Słowa kluczowe: tuczniki, żywienie, efektywność produkcji, przychód

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Authors' address:
Marcin Sońta
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: marcin_sonta@sggw.pl

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Postpartum progesterone profiles in cows with different milk yields

ZYGMUNT GIL, JUSTYNA ŻYCHLIŃSKA-BUCZEK,
KRZYSZTOF ADAMCZYK, JOANNA POKORSKA
Faculty of Animal Science, University of Agriculture in Krakow

Abstract: *Postpartum progesterone profiles in cows with different milk yields.* The aim of the study was to determine progesterone profiles in postpartum dairy cows depending on their milk yield. Analysis was also made of the effect of milk production level and milk progesterone concentration on date of first estrus in cows. The study was performed with two herds of Polish Black-and-White Holstein-Friesian dairy cows. Twenty six cows in the Wielkopolska herd and 17 cows in the Małopolska herd were investigated. The full lactation milk yield of the cows from these herds averaged 9,921 and 7,640 kg, respectively. The cows were kept in a loose-housing system and received TMR diets. Milk progesterone concentration was measured with the FT Multilyser analyser. Milk samples were collected in the morning and evening on days 12, 19, 21, 28, 35, 40 and 42 postpartum. Data on milk yield and milk urea concentration from the first and second test-day milking were taken from RW-2 recording reports. The herd had a statistically significant effect on progesterone concentration in cow's milk, which for all the tested days averaged 15.5 mg/l in the Małopolska herd and 24.5 mg/l in the Wielkopolska herd. The level of the cow's daily milk yield had no significant effect on the milk progesterone concentration. Large individual variation was observed between the cows in milk progesterone concentration. The milk progesterone content was negatively correlated with the number of days from calving to first estrus. Daily milk yield had a significant effect on the date of first estrus after calving.

Key words: dairy cows, progesterone profiles, milk yield

INTRODUCTION

Estrus detection continues to pose many problems to dairy cow farmers. Despite advancement of knowledge and technological progress, the correct detection of estrus is in many cases difficult. These difficulties are due not only to poor work organization but also to the incidence of silent estrus (Zduńczyk et al. 2005, Kozdrowski et al. 2006). In addition, a high percentage of cows come into estrus at night (Kozdrowski et al. 2005). Estrus is easier to detect in herds of cows housed in free-stall barns, where various activity meters (most often pedometers) are used for estrus detection (Holman et al. 2011, Bruyčre et al. 2012, Chanvallon et al. 2014, Rutten et al. 2014). However, these meters cannot be used in tie-stall barns, which is the predominant type of barn in Poland (Banaszkiewicz 2017).

Considering various problems associated with estrus detection, research is ongoing to find new, automated, and more importantly, efficient methods of estrus detection (Bruyčre et al. 2012, Saint-Dizier et al. 2012, Homer et al. 2013). In comparative studies on efficiency of different estrus detection methods, the authors very often use milk progesterone

concentration as the reference method (Morton et al. 2010, Holman et al. 2011, Bruyčre et al. 2012, Chanvallon et al. 2014). Today, the concentration of progesterone in cow's milk is increasingly measured with automated techniques (Saint-Dizier et al. 2012, Samsonova et al. 2014), including those used in field conditions (eProCheck® 2014).

Milk progesterone concentration is naturally dependent on the stage of estrous cycle, but cows exhibit differences in the level of progesterone (Friggens et al. 2008, Forde et al. 2012). The impact of the cow's milk production level and the associated energy balance on progesterone concentration in body fluids, including milk, is also relevant (Taylor et al. 2003).

The aim of the study was to determine progesterone profiles in postpartum dairy cows depending on their milk yield. Analysis was also made of the effect of milk production level and milk progesterone concentration on date of first estrus in postpartum cows.

MATERIAL AND METHODS

The study was performed with two herds of Polish Black-and-White Holstein-Friesian dairy cows. Twenty six cows in the Wielkopolska herd and 17 cows in the Małopolska herd were investigated. The full lactation milk yield of the cows from these herds averaged 9,921 and 7,640 kg, respectively. The cows were kept in a loose-housing system.

Progesterone concentration was measured in milk sampled in the morning and evening on days 12, 19, 21, 28, 35, 40 and 42 postpartum. Progesterone concentration was measured with the FT Multilyser analyser.

Data on milk yield (kg) and milk urea content from the first and second test-day milking were taken from RW-2 recording reports.

The day of first estrus after parturition was determined from automatic interpretation of changes in locomotor activity (pedometers), visual observations, and milk progesterone concentration.

The data were subjected to analysis of variance according to the following model:

$$Y_{ijkl} = \mu + G_i + M1_j + M2_k + \epsilon_{ijkl}$$

where:
μ – overall mean;
G_i – effect of i-th barn/herd (Wielkopolska farm, Małopolska farm);
$M1_j$ – effect of j-th daily milk yield during the first test-day milking ($\leq$ 35 kg, $>$ 35 kg);
$M2_k$ – effect of k-th daily milk yield during the second test-day milking ($\leq$ 35 kg, $<$ 35 kg);
ϵ_{ijkl} – random error.

Differences between the means were tested with the Scheffe test. In addition, coefficients of Pearson's correlation were calculated between values of the variables. Statistica 10 was used for the statistical calculations.

RESULTS AND DISCUSSION

There was a statistically significant ($P < 0.05$; $P < 0.01$) effect of herd on progesterone concentration in cow's milk (Table 1). In cows from the Wielkopolska herd, this hormone was almost twice as high on all test days as in cows from the

Małopolska herd. When considering the effect of herd, it should be noted that in both cases the cows were kept in a similar rearing system and were fed TMR diets, but differed in milk yield per lactation, which was higher by almost 2,300 kg in cows from the Wielkopolska herd. This factor may possibly explain the effect of herd on cow's milk progesterone concentration, although no statistically significant effect of daily milk yield on milk progesterone concentration was observed (Table 1).

Rabiee et al. (2002) found no effect of daily milk yield on plasma and milk progesterone concentrations. In turn, Stronge et al. (2005) showed a negative linear correlation between cow's daily milk yield and milk progesterone concentration. An inverse relationship was also reported by Reksen et al. (2002).

In our study, milk progesterone concentration showed wide variations, as evidenced by the high standard deviation in relation to the mean (Table 1). Also Friggens et al. (2008) called attention to the high individual variation between the cows for milk progesterone concentration.

Another parameter tested in our study was the number of days from calving to first estrus (Table 1). It averaged 32.8 days and was higher for cows from the Wielkopolska herd (34.8 days) compared to cows from the Małopolska herd (28.3 days). The daily milk yield, measured at the second test-day milking, had a highly significant effect on the number of days between calving and first estrus. In the higher yielding cows, first estrus after calving started later. No significant effect of the milk progesterone level on the date of first estrus after calving was observed.

Yet another parameter measured in our study was the cow's milk urea concentration (Table 1), which is an indicator of energy balance. Milk urea concentration was 214.4 mg/l in the milk from first test-day milking and 234.1 mg/l in the milk from second test-day milking. It was significantly (almost twice) higher in the milk of cows from the Wielkopolska herd, but fell within the normal range. In addition, daily milk yield had a statistically significant effect on the milk urea content, especially for milk from the second test-day milking (Table 1).

Table 2 shows the coefficients of correlation between the analysed parameters. As for the level of progesterone, relatively high and statistically significant coefficients of correlation were found between milk progesterone concentrations on different days after calving and the mean from these days. The highest coefficients were noted between milk progesterone concentration on days 21 and 28, and the mean (0.85 and 0.90, respectively). A negative correlation was found between the milk progesterone concentration and the number of days from calving to first estrus, in relation to both the individual days of the postpartum period and the mean progesterone concentration on these days.

The progesterone concentration was weakly correlated with the milk urea concentration – the coefficients of correlation can at best be described as average (Table 2). The progesterone concentration also showed a weak correlation with daily milk yield, which confirms the previous observation that daily milk yield of the cows had no statistically significant effect on the milk progesterone concentration.

TABLE 1. Effect of herd and daily milk yield on progesterone level, number of days to estrus, and urea level in cow blood

Item	Total	Herd		Daily milk yield			
				ML1		ML2	
		Małopolska	Wielkopolska	≤ 35 kg	> 35 kg	≤ 35 kg	> 35 kg
N	32	10	22	9	23	9	23
P12	20.4 ±11.0	15.3 ±5.5	22.8 ±12.1	17.4 ±7.6	21.6 ±12.0	20.1 ±11.1	20.6 ±11.1
P19	20.9 ±10.0	15.5^{a} ±5.9	23.4^{a} ±10.6	19.6 ±6.9	21.4 ±11.1	17.3 ±7.6	22.3 ±10.6
P21	20.2 ±9.8	14.0^{b} ±5.4	23.0^{b} ±10.2	16.7 ±5.9	21.5 ±10.8	19.7 ±10.0	20.3 ±9.9
P28	21.9 ±12.8	12.6^{A} ±4.6	26.1^{A} ±13.2	17.7 ±10.5	23.5 ±13.5	20.6 ±10.6	22.4 ±13.8
P35	23.0 ±10.7	17.3^{c} ±5.3	25.6^{c} ±11.6	23.2 ±11.3	23.0 ±10.7	25.1 ±9.1	22.2 ±11.4
P40	21.8 ±9.9	16.4^{d} ±5.8	24.2^{d} ±10.5	19.9 ±7.7	22.5 ±10.7	23.2 ±8.6	21.2 ±10.5
P42	23.5 ±10.8	17.3^{e} ±6.7	26.3^{e} ±11.2	20.2 ±10.0	24.7 ±11.0	26.4 ±14.2	22.3 ±9.2
P	21.7 ±8.0	15.5^{B} ±3.8	24.5^{B} ±7.9	19.2 ±7.3	22.6 ±8.3	21.8 ±8.1	21.6 ±8.2
R	32.8 ±11.6	28.3 ±9.3	34.8 ±12.2	30.0 ±11.5	33.8 ±11.8	23.6^{F} ±9.0	36.3^{F} ±10.7
M1	214.4 ±84.9	129.9^{C} ±64.0	252.8^{C} ±62.9	168.0 ±98.6	232.5 ±73.5	185.9 ±77.8	225.5 ±86.6
M2	234.1 ±84.8	126.9^{D} ±39.1	282.9^{D} ±44.8	177.4 ±83.6	256.3 ±76.0	187.7^{G} ±72.5	252.3^{G} ±83.7
M	224.2 ±79.6	128.4^{E} ±45.7	267.8^{E} ±45.7	172.7 ±84.3	244.4 ±69.5	186.8^{H} ±67.5	238.9^{H} ±80.4

P12, P19, P21, P28, P35, P40, P42 – milk progesterone content (ng/ml) on successive days after calving; P – mean milk progesterone concentration (ng/ml); R – number of days from calving to first estrus; M1 – milk urea concentration (mg/l) at first test-day milking; M2 – milk urea content (mg/l) at second test-day milking; M – mean milk urea concentration (mg/l); ML1 – daily milk yield (kg) at first test-day milking; ML2 – daily milk yield (kg) at second test-day milking; values designate the mean ± standard deviation; values marked with the same small letters differ at $P < 0.05$; values marked with the same small letters differ at $P < 0.01$.

TABLE 2. Correlations between progesterone level and number of milking days, and between urea level in blood and daily milk yield of the cows

Traits	P12	P19	P21	P28	P35	P40	P42	P	R	M1	M2	M	ML1	ML2
P12	1.00													
P19	0.41*	1.00												
P21	0.54**	0.75**	1.00											
P28	0.33	0.69**	0.79**	1.00										
P35	0.21	0.24	0.52**	0.69**	1.00									
P40	0.24	0.27	0.45*	0.60**	0.74**	1.00								
P42	0.24	0.30	0.42*	0.55**	0.43*	0.77**	1.00							
P	0.57**	0.70**	0.85**	0.90**	0.74**	0.77**	0.71**	1.00						
R	–0.24	–0.18	–0.20	–0.29	–0.28	–0.40*	–0.30	–0.36*	1.00					
M1	–0.02	0.15	0.27	0.41*	0.39*	0.38*	0.42*	0.39*	0.17	1.00				
M2	0.13	0.21	0.26	0.47**	0.42*	0.43*	0.38*	0.44*	0.24	0.76**	1.00			
M	0.06	0.19	0.28	0.47**	0.43*	0.43*	0.43*	0.44*	0.22	0.94**	0.94**	1.00		
ML1	0.06	0.02	0.10	0.13	0.04	0.08	0.01	0.09	0.27	0.40*	0.47**	0.46**	1.00	
ML2	0.09	0.05	–0.02	0.04	0.03	0.15	0.00	0.07	0.49**	0.25	0.58**	0.44*	0.58**	1.00

P12, P19, P21, P28, P35, P40, P42 – milk progesterone content (ng/ml) on successive days after calving; P – mean milk progesterone concentration (ng/ml); R – number of days from calving to first estrus; M – mean milk urea concentration (mg/l); M1 – milk urea concentration (mg/l) at first test-day milking; M2 – milk urea concentration (mg/l) at second test-day milking; ML1 – daily milk yield (kg) at first test-day milking; ML2 – daily milk yield (kg) at second test-day milking; * coefficients significant at $P < 0.05$; ** coefficients significant at $P < 0.01$.

CONCLUSION

In summary, it should be mentioned that the milk progesterone content did not depend on the daily milk yield of the cows. Because of the high individual variation between the cows for milk progesterone concentration, it would be appropriate to determine postpartum progesterone profiles for each cow, which could contribute to efficient estrus detection. The level of progesterone in cow's milk had no significant effect on the day of first estrus after calving.

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Streszczenie: *Profile progesteronowe w okresie poporodowym u krów o różnej wydajności mlecznej*. Celem pracy było określenie kształtowania się profili progesteronowych u krów mlecznych w okresie poporodowym w zależności od ich wydajności. Analizie poddano również wpływ poziomu wydajności mlecznej i zawartości progesteronu w mleku na termin wystąpienia pierwszej rui u krów. Badania przeprowadzono w dwóch stadach krów mlecznych rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej. Objęto nimi 26 krów w stadzie wielkopolskim i 17 krów w stadzie małopolskim. Średnia wydajność mleczna krów utrzymywanych w tych stadach wynosiła odpowiednio 9921 i 7640 kg mleka za laktację pełną. Krowy były utrzymywane w systemie wolnostanowiskowym i żywione z wykorzystaniem systemu TMR (ang. *total mixed ration*). Do oznaczenia zawartości progesteronu w mleku wykorzystano aparat FT Multilyser. Próbki mleka pobierano rano i wieczorem w 12. 19. 21. 28. 35. 40. i 42. dniu po wycieleniu. Z raportów wynikowych RW-2 odnotowano wydajność mleka oraz zawartość mocznika w mleku krów w pierwszym i drugim dniu próbnego udoju. Stwierdzono statystycznie istotny wpływ stada na poziom progesteronu w mleku krów, który wynosił średnio (ze wszystkich badanych dni) 15,5 mg/l w stadzie małopolskim i 24,5 mg/l w stadzie wielkopolskim. Nie stwierdzono statystycznie istotnego wpływu poziomu dziennej wydajności mlecznej krów na zawartość progesteronu w mleku. Wykazano duże zróżnicowanie osobnicze między krowami odnośnie zawartości progesteronu w mleku. Zawartość progesteronu w mleku była ujemnie skorelowana z liczbą dni od wycielenia do wystąpienia pierwszej rui. Stwierdzono istotny wpływ poziomu dziennej wydajności mlecznej na termin wystąpienia pierwszej rui po porodzie.

Słowa kluczowe: krowy mleczne, profile progesteronowe, wydajność mleczna

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Authors' address:
Zygmunt Gil
Zakład Hodowli Bydła
Wydział Hodowli i Biologii Zwierząt
Uniwersytet Rolniczy im. Hugona Kołłątaja
w Krakowie
al. Mickiewicza 24/28, 30-059 Kraków
Poland
e-mail: rzgil@cyfronet.pl

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Compatybility of entomopathogenic nematodes with active substances of popular biocidal products, in controlling of the lesser mealworm beetle – *Alphitobius diaperinus* (Panzer, 1797)

KORNELIA KUCHARSKA, BARBARA ZAJDEL, ELŻBIETA PEZOWICZ, JOANNA JARMUŁ-PIETRASZCZYK
Faculty of Animal Sciences, Warsaw University of Life Sciences – SGGW

Abstract: *Compatybility of entomopathogenic nematodes with active substances of popular biocidal products, in controlling of the lesser mealworm beetle* – Alphitobius diaperinus *(Panzer, 1797)*. Different species of entomopathogenic nematodes (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae and S. carpocapsae* DD-136) and insecticides (chitin synthesis inhibitors – Baycidal WP 25 and pyrethroid – Solfac 10 WP) were used against larvae of *Alphitobius diaperinus* (Panzer, 1797) in a series of laboratory experiments. Four week larvae of the lesser mealworm were exposed to various species of EPNs (entomopathogenic nematodes), which survived contact with insecticides and to the residues of chemical substances. Mortality of *A. diaperinus* and the extensity of infection was assessed weekly. This study indicates that entomopathogenic nematodes and insecticides could serve as a more environmentally friendly integrated pest management method against harmful pests like *A. diaperinus* compared with conventional methods. The results of this study can be used as a basic information for the application of IPM (integrated pest management) in broiler houses.

Key words: IPM, integrated pest management, *Alphitobius diaperinus*, EPNs, entomopathogenic nematodes, insecticides

INTRODUCTION

The needs of modern farming and livestock production make environmental protection and the production of organic food the priority tasks. Chemical methods to combat harmful organisms increasingly give way to biological and integrated pest control methods (known as integrated pest management – IPM).

Alphitobius diaperinus (Panzer, 1797) (Coleoptera: Tenebrionidae) is an economically important pest in broiler houses and other livestock housing, warehouses and storages of food products and in the cellars. Increased mortality of birds is noted during the mass occurrence of pests in broiler houses. *Alphitobius diaperinus* is a vector of many pathogens like viruses (causing Mareka, Gumboro and Newcastle diseases, bird flu and enteritis), fungi (*Aspergillus* sp., *Fusarium roseum*), bacteria (*Escherichia* sp., *Salmonella* sp., *Bacillus* sp., *Streptococcus* sp.), protozoans (*Eimeria* sp.) and tapeworm larvae (*Raillietina* sp., *Choanotaenia* sp.) (De la Casas et al. 1976, Chernaki-Leffer et al. 2010). In addition, the larvae of lesser mealworm beetle feed on the eggs and larvae of advantageous beetle (*Carcinops pumilio*), which significantly reduces the population of *Musca domestica* in broiler houses (Watson et al. 2001). The larvae of *A. diaperinus* burrow tunnels in the insulating material of buildings and destroy

wood and foamed polystyrene elements reducing thermal insulation properties of buildings by up to 30% (Chernaki-Leffer et al. 2010).

Currently, organophosphorus insecticides, synthetic pyrethroids and insect growth regulators are mainly used to control *A. diaperinus*. However, the insecticides do not totally eliminate insect populations because they do not reach into all the gaps, in which the pests can hide. Moreover, individuals resistant to chemicals appear in isolated populations (Lambkin 2005). Therefore, the development and demonstration of alternative integrated pest management methods are needed. Several non-chemical control methods have been developed against lesser mealworm beetle, but all of them have limitations (Pezowicz 2005).

Entomopathogenic nematodes of the Steinernematidae and Heterorhabditidae (Rhabditida) families are lethal parasites associated with symbiotic bacteria in the *Enterobacteriacae*. Steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids with *Photorhabdus* spp. They are characterized by a high tolerance for insecticides and other chemicals. Studies confirmed the possibility of parallel application of biological and chemical means, which increases the severity of disease processes and accelerates the death of pests. Thus, entomopathogenic nematodes offer an environmentally safe and IPM compatible alternative. Selection based on two factors also prevent the development of resistance in the population of insects treated with IPM methods (Koppenhöfer et al. 2000b).

In the simultaneous infection of *A. diaperinus* by nematodes *Steinernema feltiae* (Owinema) (Filipjev) and entomopathogenic fungi *Beauveria bassiana* (Naturalis – L) (Vuill.) or *Metarhizium anisopliae* (Metsch.) it was found that the mortality of beetles depended on various factors (the species of entomopathogenic organism, dose of bioinsecticides, developmental stage of an insect, the type of substrate). The mortality of larvae on straw after the simultaneous contact with nematodes and fungi *B. bassiana* or *M. anisopliae* was about 70 or 20%, but in the case of imagines it was 50 or 48%, respectively (Pezowicz 2005).

In this research, we studied the combined effect of five species of entomopathogenic nematodes *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae* and *S. carpocapsae* DD-136) with three doses of two insecticides (chitin synthesis inhibitors – Baycidal WP 25, and pyrethroid – Solfac 10 WP). The hypothesis was that EPNs (entomopathogenic nematodes which survived contact with insecticides) and the residues of insecticides act as a stressors and cause high mortality of larvae of *A. diaperinus*. A stressor is defined as any stimulus that disrupts the homeostasis of an organism. Our objectives were: to determine the susceptibility of IJs (infective juveniles) to different doses of insecticides (the recommended dose, dose 10 times higher than recommended and dose 10 times lower than recommended); to determine the susceptibility of larvae of *A. diaperinus* to strains of entomopathogenic nematodes and the residues of insecticides.

MATERIAL AND METHODS

Insect and nematodes

Alphitobius diaperinus originated from a broiler farm located near Warsaw, Poland. Lesser mealworm beetles were cultured in 10 five-litre plastic boxes that contained 1 kg of artificial medium (Sante wheat bran, Dr Oetker yeast and apple halves). Wheat mediums were the most common culture diets in published lesser mealworm rearing systems, particularly wheat enriched with yeasts. Each box contained pupation substrate (cotton wool), in which the final instar larvae could burrow and pupate. The boxes had a plastic lid with a 10 × 5 mm vent and organdy cloth sealed between the lid and box, which closed the vent while allowing passage of air. The insects were stored under laboratory conditions at temperature 30°C, humidity 70%. The optimum temperature and humidity for lesser mealworm is 32°C and 70% RH, respectively (Steinkraus et al. 1991). Four-week larvae of the lesser mealworm beetle were used in experiments.

Heterorhabditis bacteriophora (origin: Warsaw University of Life Sciences – SGGW), *H. megidis* (origin: Warsaw University of Life Sciences – SGGW), *Steinernema affine* (origin: University of Kiel), *S. carpocapsae* (origin: Warsaw University of Life Sciences – SGGW) and *S. carpocapsae* strain DD-136 (origin: University of Kiel) were cultured at 25°C in last – instar larvae of the greater wax moth *Galleria mellonella* (L.) according to procedures described by Kaya and Stock (1997). Infective juveniles emerging within the first 2–6 days were collected from White traps and stored at 4°C for 2 weeks. Before use in the laboratory experiments, nematodes were acclimatized to room temperature for at least 2 h.

Insecticides

Triflumuron, 2-chloro-N-[[4 (trifluoro methoxy)phenyl]carbamoyl]benzamide (chitin synthesis inhibitors) was obtained as a wettable powder with 25% active ingredient (Baycidal WP 25) and 75% inert ingredients.

Cyfluthrin, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate--α-cyano-4-fluoro-3-phenoxybenzyl (pyrethroid) was obtained as a wettable powder with 10% active ingredient (Solfac 10 WP) and 90% inert ingredients.

Laboratory experiments

The effect of Baycidal WP 25 in three doses (0.013 g – recommended one, 0.13 g and 0.0013 g) dissolved in 10 ml of distilled water and the effect of Solfac 10 WP in three doses (0.0013 g – recommended dose, 0.013 g and 0.00013 g) on mortality of entomopathogenic nematodes (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae and S. carpocapsae* strain DD--136) were studied in experimental conditions at a temperature of 20 ±1°C. Each Petri dish, 9 cm in diameter (surface area: 63.6 cm^2) lined with filter paper contained about 1,600 IJs and appropriate dose of insecticide. During 7 days the number of dead and alive nematodes in 1 ml of suspension was determined daily. Tests were made in five repetitions. Infective juveniles from control group were kept in distilled water. Percentage IJs mortality was calculated on every Petri dish. Means and standard deviations were calculated for

each five repetitions of every treatment. After 7 days the nematodes that survived contact with insecticides were separated by sedimentation. The sedimentation did not allow for complete removal of chemical compounds from the sample. Thus, four-week larvae of *A. diaperinus* were exposed to the residues of chemical substances and to entomopathogenic nematodes that survived seven-day contact with these substances.

Next experiments were initiated by transferring 10 larvae of *A. diaperinus* to Petri dishes (9 cm in diameter) lined with filter paper. For treatments with entomopathogenic nematodes, the filter paper was moistened with the suspension of one species of IJs (500 IJs per Petri dish) and the residues of chemical insecticides (Baycidal WP 25 or Solfac 10 WP). In all experiments, each treatment was replicated six times with 10 larvae of *A. diaperinus* per replicate. Insects from control group were infected by IJs deprived of the contact with insecticides. Experiments were conducted at 25 ±1°C and 85–90% relative moisture of the substratum. The mortality and the extensiveness of infection of insects were assessed daily through 7 days. Dead insects were transferred to empty dishes and placed in the incubation chamber for 48 h. Later, the larvae were sectioned to check whether nematodes and associated mutualistic bacteria were the cause of their death. Percentage larvae mortality and extensiveness of infection was calculated for every Petri dish. Means and standard deviations were calculated for six repetitions of each treatment.

Statistics

Data were subjected to analysis with SPSS 15.0 and SAS 9.2 software. The differences between groups, the percentage EPNs mortality for each treatment, insects mortality, their extensiveness and intensity of infection were analyzed using one-way ANOVA. For each nematode species differences between concentrations of the active substance were analyzed. When ANOVA showed the significance of difference, means were compared by using Tukey's post hoc test. Results of this test are marked with letters (a, b, c, d) in tables. Different letters indicate significant differences at $P < 0.05$.

RESULTS AND DISCUSSION

Many scientists studying the effect of various insecticides on nematodes focuses on the toxicity of these compounds to IJs. The harmfulness of insecticides manifests itself in, for example, disturbances in movement and inability or limited ability to kill the host (Li et al. 2012). Different species of the same genus (*Steinernema* and *Heterorhabditis*) are characterized by diverse sensitivity to biocidal products and their different concentrations. For example, beta-cyfluthrin (Turbo biocidal product) and triflumuron (Certero biocidal product) are more toxic to *S. carpocapsae* (nematode mortality was respectively 50 and 25%) but less toxic to *S. glaseri* (nematode mortality was respectively 18.8 and 7.8%) (Negrisoli et al. 2010b). Solfac 10 WP of the group of pyrethroids caused higher mortality in *H. bacteriophora* and *H. megidis* (all doses) than Baycidal WP 25 (Table 1).

Cuticle of EPNs is composed of several layers: the cortex, elementary and fibre, which are a barrier against adverse

TABLE 1. Effect of various doses of Baycidal WP 25 and Solfac 10 WP on mortality of invasive larvae (IJs), various species and strains of entomopathogenic nematodes, after 7 days of contact with insecticides

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with insecticides				
			Invasive larvae of entomopathogenic nematodes				
			Mortality (%) ±*SD*				
			K	A	B	C	ANOVA
Baycidal WP 25	Triflumuron	*S. carpocapsae*	22^{b} ±6	3^{c} ±1	10^{c} ±2	57^{a} ±4	$P < 0.0001$
		S. carpocapsae (DD-136)	20^{b} ±3	5^{c} ±3	9^{c} ±2	44^{a} ±3	$P < 0.0001$
		S. affine	17^{b} ±4	2^{c} ±1	8^{c} ±3	63^{a} ±4	$P < 0.0001$
		H. bacteriophora	1^{b} ±1	1^{b} ±0	1^{b} ±0	3^{a} ±1	$P < 0.0005$
		H. megidis	1^{b} ±1	4^{a} ±1	4^{a} ±1	4^{a} ±1	$P < 0.0001$
Solfac 10 WP	Cyfluthrin	*S. carpocapsae*	22^{a} ±6	2^{c} ±1	3^{c} ±1	10^{b} ±1	$P < 0.0001$
		S. carpocapsae (DD-136)	20^{a} ±3	7^{b} ±2	11^{b} ±3	9^{b} ±2	$P < 0.0001$
		S. affine	17^{a} ±4	$0{,}3^{b}$ ±0	2^{b} ±2	2^{b} ±1	$P < 0.0001$
		H. bacteriophora	1^{d} ±1	11^{c} ±1	14^{b} ±2	18^{a} ±2	$P < 0.0001$
		H. megidis	1^{d} ±1	10^{c} ±2	14^{b} ±2	18^{a} ±2	$P < 0.0001$

SD – standard deviation, different small letters denote significant differences at $P < 0.05$ within each nematode species and at various concentrations of insecticides – Tukey test. K – control (nematodes from distilled water without insecticides). Concentrations of insecticides: A – 0.0013 g Baycidal WP 25 or 0.00013 g Solfac 10 WP, B – 0.013 g Baycidal WP 25 or 0.0013 g Solfac 10 WP, C – 0.13 g Baycidal WP 25 or 0.013 g Solfac 10 WP.

external factors. Infective juveniles have also obstructed intestinal tract, and in addition, they are covered by moult of earlier developmental stage L2. Their contact with the external environment is significantly limited, which largely determines the survival in many unfavourable conditions, and total or partial preservation of the ability to infect hosts. In all EPN species tested after treatment with insecticides Baycidal WP 25 and Solfac 10 WP, not 100% mortality was observed (Table 1). Baycidal WP 25 containing triflumuron, probably did not cause microdamages in the cuticle of IJs, because their cuticle does not contain chitin. Negrisoli et al. (2010a) also reported such observations. Mortality of *S. carpocapsae*, which had contacted the insecticide Certero (triflumuron) was 25%, and those that survived caused 70.2% mortality of *G. mellonella*. In turn, mortality of *Spodoptera frugiperda* larvae 2 and 4 days after application of the pesticide Certero and nematodes amounted to 26 and 72%, respectively (Negrisoli et al. 2010b). This shows that entomopathogenic nematodes are able to adapt to changing environmental conditions (Gondek and Ropek 2007). Infective juveniles after contact with different chemicals preserved the pathogenic properties against larvae of *A. diaperinus*. Many chemicals are not toxic to nematodes or their toxicity is minimal (pyrethroids, chlorinated hydrocarbons, preparations uracil and triazine). For example, triazine compounds (metribuzin, prometryn) caused a mortality of *S. feltiae* not exceeding 30% (Kamionek 1992). According to Kamionek (1992), the high concentrations of herbicides, even 10 times higher than those used in field treatments, also had no significant impact on the mortality of *S. carpocapsae*. Only a limited reproduction of EPNs in the host's body was observed. In other cases, the active substances, e.g. oxamyl (nematicide) and other pesticides stimulate nematode movement (sinusoidal movement, vibration, and turns). Such stimulation increased the efficiency of infection of insects by nematodes (Fedorko et al. 1977). The mortality of *S. affine* after exposure to insecticides Baycidal WP 25 and Solfac 10 WP (10 times lower and recommended doses), were lower than in controls. In all of these variants the differences were statistically significant in relation to the control groups (Table 1). This species was also characterized by usually higher than in the control pathogenicity (mortality, extensiveness of infection) with respect to larvae (Table 2).

Some compounds may, however, reduce the pathogenic properties of organisms, which in turn affects the development of the next generations of nematodes. Pesticides, belonging to carbamates, urea compounds and organophosphates limited pathogenicity and proper development of nematodes, causing paralysis and death. For example, carbamates and preparations of urea completely killed all *S. feltiae* nematodes after 72 h (Hara and Kaya 1983, Kamionek 1992). Witkowski (1979) and Hara and Kaya (1983) found that chemical substances (carbofuran, DDT, lindane and oxamyl) negatively affected entomopathogenic nematodes. Their numbers, after using these substances, decreased by about 50%. Jaworska et al. (2002) also found the high sensitivity of *Xenorhabdus* bacteria colonizing

TABLE 2. Pathogenic properties of entomopathogenic nematodes (percentage mortality, extensity and intensity of infection of *A. diaperinus* larvae) which contacted insecticides of Baycidal WP 25 and Solfac 10 WP in various concentrations (n = 60 in every variant of experiment)

Trade name	Active substance	Nematode species	Permanent contact of entomopathogenic nematodes with insecticides														
			A. diaperinus larvae														
			Mortality (%) ±*SD*					Extensity of infection (%) ±*SD*					Intensity of infection (nematodes per host) ±*SD*				
			K	A	B	C	ANOVA	K	A	B	C	ANOVA	K	A	B	C	ANOVA
Baycidal WP 25	Triflumuron	*S. carpocapsae*	100 ±0	90 ±10	87 ±15	100 ±0	$P = 0.03$	98^{a} ±4	87^{a} ±15	83^{b} ±12	100^{a} ±0	$P = 0.017$	6.9^{b} ±4.1	12.7^{a} ±12.3	12.8^{a} ±16.1	12.7^{a} ±7.4	$P = 0.002$
		S. carpocapsae (DD-136)	100 ±0	100 ±0	100 ±0	97 ±5	$P = 0.24$	97 ±5	90 ±10	93 ±6	87 ±8	$P = 0.16$	8.4^{b} ±5.2	35.6^{a} ±23	36.1^{a} ±26.2	7.4^{b} ±6.8	$P < 0.0001$
		S. affine	78 ±16	100 ±0	90 ±10	85 ±10	$P = 0.12$	35^{b} ±14	87^{a} ±6	67^{a} ±6	0^{c} ±0	$P < 0.0001$	1.7^{c} ±3.3	22.4^{a} ±19.6	10.2^{b} ±14.3	0^{c} ±0	$P < 0.0001$
		H. bacteriophora	93^{A} ±12	60^{B} ±10	83^{AB} ±12	100^{A} ±0	$P = 0.004$	70 ±20	50 ±17	57 ±6	67 ±23	$P = 0.53$	7.2^{a} ±11.3	1.5^{b} ±2.3	1.6^{b} ±2	3^{b} ±3.2	$P = 0.001$
		H. megidis	53 ±23	80 ±17	93 ±12	80 ±26	$P = 0.18$	7^{b} ±12	53^{a} ±29	30^{ab} ±10	7^{b} ±6	$P = 0.025$	0.2^{b} ±0.8	3.8^{a} ±5.6	3.7^{a} ±8.2	0.6^{ab} ±2.3	$P = 0.006$
Solfac 10 WP	Cyfluthrin	*S. carpocapsae*	100 ±0	100 ±0	100 ±0	100 ±0	–	98^{a} ±4	93^{a} ±6	93^{a} ±6	73^{b} ±15	$P = 0.007$	6.9^{b} ±4.1	15.7^{a} ±13.6	19.4^{a} ±18.5	13^{ab} ±14.3	$P < 0.0001$
		S. carpocapsae (DD-136)	100 ±0	100 ±0	100 ±0	100 ±0	–	97 ±5	90 ±10	100 ±0	100 ±0	$P = 0.15$	8.4^{b} ±5.2	24.3^{a} ±15	25.2^{a} ±21.9	33.8^{a} ±18.1	$P < 0.0001$
		S. affine	78 ±16	100 ±0	100 ±0	100 ±0	$P = 0.022$	35^{b} ±14	100^{a} ±0	97^{a} ±6	97^{a} ±6	$P < 0.0001$	1.7^{c} ±3.3	15.2^{b} ±9.8	16.4^{ab} ±11.5	21.8^{a} ±13.4	$P < 0.0001$
		H. bacteriophora	93 ±12	100 ±0	100 ±0	100 ±0	$P = 0.44$	70^{a} ±20	37^{ab} ±12	27^{b} ±12	53^{ab} ±12	$P = 0.025$	7.2^{a} ±11.3	1.8^{b} ±3.3	1.4^{b} ±3.5	2.8^{ab} ±3.8	$P = 0.003$
		H. megidis	53^{B} ±23	100^{A} ±0	100^{A} ±0	100^{A} ±0	$P = 0.002$	7^{b} ±12	77^{a} ±6	67^{a} ±23	70^{a} ±20	$P = 0.003$	0.2^{b} ±0.8	9.8^{a} ±16	5.6^{ab} ±8	5.7^{ab} ±7.1	$P = 0.003$

SD – standard deviation, different small letters denote significant differences at $P < 0.05$ within each nematode species and at various concentrations of insecticides – Tukey test. K – control (nematodes from distilled water without insecticides). Concentrations of insecticides: A – 0.0013 g Baycidal WP 25 or 0.00013 g Solfac 10 WP, B – 0.013 g Baycidal WP 25 or 0.0013 g Solfac 10 WP, C – 0.13 g Baycidal WP 25 or 0.013 g Solfac 10 WP.

the digestive tract of Steinernematidae to heavy metal ions (Cu, Mn, Ni). Heavy metals negatively affected the growth rate of bacterial colonies. High mortality of *S. carpocapsae* strain DD-136 (44%), *S. carpocapsae* (57%) and *S. affine* (63%) was caused by the highest dose of Baycidal WP 25 (Table 1). In relation to the controls all the differences were statistically significant. In most cases, these nematodes did not lose pathogenicity and contributed to the relatively high mortality and extensiveness of infection of *A. diaperinus* (Table 2). Baycidal WP 25 and Solfac 10 WP are soluble in water, and not long overdue in the soil. However, the use of excess doses of these agents in relation to the recommended doses could have a negative impact on beneficial organisms, for example entomopathogenic nematodes.

In experiments with invasive larvae contacting insecticides, Solfac 10 WP caused no more than 18% mortality (*H. bacteriophora* and *H. megidis* at the highest dose), whereas the contact of IJs with Baycidal WP 25 caused of up to 63% mortality in *S. affine* at a dose 10 times higher than recommended (Table 1). In all cases, experimental results significantly differed from the control. The diverse impact of chemicals on the IJs could be affected by various defensive and/or compensative mechanisms induced by these substances in the body. In the natural environment such direct and continuous contact of these animals with insecticides is not possible and results in limited mortality of entomopathogenic nematodes.

In studies concerning the occurrence of nematodes in forests contaminated in different degree by industrial pollutants Kamionek et al. (1995) found that invasive larvae are often resistant to a variety of chemicals, which results in their low mortality. The highest number of *S. carpocapsae* and *S. feltiae* (32,378 individuals per m^2) was found in areas with the highest concentration of industrial pollution (southern Poland). In areas with low and medium contamination, much smaller numbers of nematodes were noted. Measured abundances were, respectively, 9,548 individuals per m^2 and 7,145 individuals per m^2. The presence of heavy metals in the environment also positively influenced the pathogenicity of *H. megidis*. Reproduction of this species was possible even in very contaminated area (Jarmuł and Kamionek 2004). Pezowicz (2002) noted the presence of invasive larvae of entomopathogenic nematodes in soil with a high content of lead (200–300 mg Pb/kg), their density was 714 individuals per m^2. In the present study, mortality of IJs was low after treatment with high doses of insecticides. For most species and strains of entomopathogenic nematodes, their mortality did not exceed 18% for the highest dose of Baycidal WP 25 or Solfac 10 WP. The exception was the nematodes *S. carpocapsae*, *S. carpocapsae* DD-136 and *S. affine*. However, they also retained the pathogenic properties. The lowest doses of chemicals, to which IJs were exposed caused lower mortality, usually not exceeding 10% (Table 1). This shows that the entomopathogenic nematodes can be used in integrated control of *A. diaperinus*.

Most insecticides successfully controlled larvae of *A. diaperinus*. However, long term use of chemicals resulted in the emergence of biotypes of

A. diaperinus resistant to pesticides. Their number has increased in recent years (Lambkin 2005). Control of *A. diaperinus* through the use of the active substance – cyfluthrin and triflumuron was effective. The observed decline in the number of *A. diaperinus* in broiler houses reached 86%. The insecticides, however, did not penetrate all the places where pests could hide. Some individuals survived aerial spraying and after 2 months the pests appeared in the poultry house again. Insect resistance was also associated with their lowered mobility. Occasionally, immobile stages (pupae) appeared. To prevent the occurrence of insect resistance to insecticides, rotation of pesticides and methods of integrated pest management should be used. Integrated pest management counteract the formation of immune insects and can increase the effectiveness of infection by nematodes. Friedel et al. (1988) found that cyromazine (chitin biosynthesis inhibitor) damages the cuticle and the gut of insects and causes muscular dystrophy. Cracked cuticle helps EPNs to penetrate the host, which in turn improves the effectiveness of the biological agent. Reduced doses of chemicals, in combination with biological agents are very effective. Contact with a toxic substance decreases the immunity of beetles. Furthermore, the chemicals, such as fertilizers (NPK, Mikrovit) and calcium, have also impact on the average time required to kill the hosts. Gondek and Ropek (2007) noted slightly shorter time (2.25 days compared to the control of 2.52 days) required to kill wax moth caterpillars by *S. feltiae*, which were exposed to the fertilized soil. Also, the percentage of infected insects by EPNs was the highest in the experiment with mineral fertilizers and liming. It reached the value of 95%. Synergism was also observed in the case of extensity of infection of *A. diaperinus* by nematodes. The extensity of larvae infected by EPNs, which were in contact with Baycidal WP 25 (the dose 10 times lower than recommended) reached a value of 87% for *S. affine*, 53% for *H. megidis*. An analogous phenomenon in variants with Solfac 10 WP (the dose 10 times lower than recommended) was noted for the extensity of larvae (100% for *S. affine* and 77% for *H. megidis*) – Table 2. All the differences between experimental groups and controls were statistically significant. Koppenhöfer and Fuzy (2008) studied the effect of integrated pest management on beetle grubs (Scarabaeidae) using an insecticide containing chlorantraniliprole and *H. bacteriophora*. In the greenhouse and field experiments, synergistic effect in the control of *Popillia japonica*, *Cyclocephala borealis* and *Anomala orientalis* was observed in 64% of combinations. For example, mortality of *C. borealis* after application of invasive larvae (109 IJs/ha) and insecticide (0.3 kg ai/ha) approached 80%, while after the contact with nematodes or pesticide alone, the mortality did not exceed 20%. Also *S. glaseri* or *H. bacteriophora* in combination with an insecticide (imidacloprid) showed a synergistic effect in the control of grubs. Beetle larvae mortality was significantly higher after the use of combination of the insecticide and *H. bacteriophora* nematodes (63–90%) than after the application of imidacloprid alone (1–20%) or just *H. bacteriophora* (30%) (Koppenhöfer et al. 2000a, b). The increased infection of

insect larvae was determined by reduction of the preening intensity (Koppenhöfer et al. 2000a, b). Combined control of *Hoplia philanthus* larvae by spores of *M. anisopliae* fungus with the nematodes *H. megidis* or *S. glaseri* increased insect mortality. *Metarhizium anisopliae* was a stressor, which increased the susceptibility of insects to infection with EPNs. The high value of synergistic effect was observed when *H. philanthus* larvae were infected with EPNs after at least 3 or 4 weeks earlier exposition to the fungus spores (Ansari et al. 2004). Irigaray et al. (2003) concluded that biopreparation Naturalis – L containing the spores of *B. bassiana* can be used in the integrated pest management of *Tetranychus urticae* in combination with the active substance – triflumuron. Chitin synthesis inhibitor, did not inhibit the germination of fungal spores but inhibited the proper conduct of melanization and damaged cuticle of spider mites. Such activity resulted in an increase in the susceptibility of *T. urticae* to *B. bassiana* (mortality was 38%). In other studies, *H. megidis* (density of 1,035 mg/ml), that survived contact with the colloidal silica solution, showed high pathogenicity against larvae of *T. molitor* (Wilson and Ivanova 2004).

Pezowicz (2005) also observed that the mortality of *A. diaperinus* larvae in one week old litter with a mixed infection was lower (30%) from the mortality of insect infected just by *S. feltiae* nematodes (45%). In our experiments, similar effect was also observed when comparing the use of nematodes and chemicals with the effect of *H. bacteriophora* alone. The mortality of *A. diaperinus* larvae (60%) was lower after the application of nematodes and residues of Baycidal WP 25 (the lowest dose), than after the application of nematodes, which have had no contact with the insecticide (93%) – Table 2. Statistically significant differences from controls were noted.

Mortality of insects, including *A. diaperinus*, is influenced by the nematode species/strain, by contact or a lack of contact with chemicals, by virulence of symbiotic bacteria and stage of insect development.

Contact of IJs with different doses of insecticides did not cause the loss of their invasive abilities. This allows to use them in IPM methods in broiler houses. Geden et al. (1987) also confirmed the possibility of using EPNs indoor in farms in the system of biological control methods. Entomopathogenic nematodes caused up to 87% mortality of *A. diaperinus*. Extensiveness of infection of beetles was lower than or equal to mortality. Only in a few cases the extensiveness of infection equal 0% was found (*S. affine* against larvae, Baycidal WP 25, the highest dose – Table 2). Symbiotic bacteria that colonize the digestive tract of nematodes, after the release, can kill the host and inhibit the development of nematodes. This case makes determining the presence of EPNs during the autopsy of insects impossible (Pezowicz 2005).

All entomopathogenic nematodes used in these experiments have the ability to infect *A. diaperinus*. An interesting observation was that *H. megidis* IJs which survived contact with the highest dose of Solfac 10 WP (mortality rate of 18%) (Table 1) contributed to the higher mortality (100%) and the extensiveness of infection (70%) of *A. diaperinus* larvae, compared to the control (Table 2).

Chemical insecticides can stimulate EPNs to increased activity associated

with finding new host, which results in an increased mortality and the extensiveness of infection of pests. The literature data confirm that the use of neonicotinoid substances results in an increased number of IJs attached to grubs (Koppenhöfer et al. 2000a, b).

Many studies have shown that the effectiveness of Heterorhabditidae is lower than that of Steinernematidae. Sandner and Pezowicz (1986) and Renn et al. (1985) found above-mentioned relationship by examining the impact of nematodes from both families on larvae of *Barathra brassicae* and *Musca domestica*. A similar relationship was observed for the extensiveness of infection of *A. diaperinus* larvae by *H. bacteriophora* and *H. megidis* (Baycidal WP 25 and Solfac 10 WP, all doses). In comparison with the majority of nematodes of the genus *Steinernema*, the value of extensiveness of infection caused by the *Heterorhabditis* EPNs were from tens to dozen percent lower (Table 2). Differentiated pathogenicity also characterized two *Heterorhabditis* species. Values of extensiveness of infection in larvae after application of *H. megidis* at all Solfac 10 WP doses, were typically a several dozen percent higher than after the application of *H. bacteriophora* (Solfac 10 WP). In the control groups the extensiveness was 7% for *H. megidis* and 70% for *H. bacteriophora* (Table 2). Perhaps increased pathogenicity of *H. megidis* nematodes was caused by contact with the insecticide. Studies of Kaya and Stock (1997) confirmed that some biologically active compounds can stimulate the movement of parasites, which increases the efficiency of finding and infecting the host. This may also be caused by diverse degree of penetration of the host's body.

Increased demand for livestock production requires the use of chemicals to control pest and disease – causing pathogens. Therefore, more or less negative impact of the environment (including the organisms inhabiting the soil) cannot be avoided. As many as 99% of soil mesofauna are nematodes from different trophic groups, including species associated with bacteria. Effect of different chemicals may reduce populations of beneficial EPNs and affect their pathogenicity to insects. It is therefore necessary to limit the use of chemicals that are toxic to the environment. It is proposed to apply integrated pest control methods (for example EPNs and reduced doses of insecticides). From 1 January 2014 the introduction of protection by IPM is obligatory in the European Union (Article 14 of Directive 2009/128/EC and Regulation EC 1107/2009). It is necessary to: use biopreparations based on different living organisms or their spores, use chemicals least harmful for the beneficial organisms and the environment, reduce insecticide doses and use chemicals from a variety of groups and with different mechanisms of action (Olejarski and Ignatowicz 2011).

CONCLUSIONS

Use of insecticides in agriculture does not affect negatively EPNs pathogenicity under laboratory conditions. Instead of that, addition of triflumuron and cyfluthrin may slightly increase the efficacy of nematodes. Further experiments are needed in field trials to verify the observed effects.

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Streszczenie: *Możliwości stosowania nicieni entomopatogenicznych i substancji czynnych zawartych w popularnych produktach biobójczych, do ograniczania liczebności pleśniakowca lśniącego* Alphitobius diaperinus *(Panzer, 1797).* Larwy pleśniakowca lśniącego zostały zarażone pięcioma gatunkami i szczepami nicieni entomopatogenicznych: *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema affine*, *S. carpocapsae* i *S. carpocapsae* DD-136. Wcześniej nicienie miały kontakt z insektycydami Baycidal WP 25 oraz Solfac 10 WP. Przez 7 dni badano śmiertelność, ekstensywność i intensywność zarażenia larw. Prawie wszystkie badane EPN i pozostałości insektycydów powodowały dużą śmiertelność i znaczną ekstensywność zarażenia larw gospodarza, odpowiednio od 60 do 100% oraz od 0 do 100%. Zaobserwowano negatywny wpływ nicieni *S. affine* i Baycidal WP 25 na ekstensywność zarażenia larw. W wielu przypadkach kontakt nicieni z insektycydami nie hamował działania biopreparatów. Zmniejszone dawki insektycydów mogą być z powodzeniem stosowane w integrowanym zwalczaniu szkodników.

Słowa kluczowe: IPM, integrowane metody zwalczania szkodników, *Alphitobius diaperinus*, EPN, nicienie entomopatogeniczne, insektycydy

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Authors' address:
Kornelia Kucharska
Zakład Zoologii
Katedra Biologii Środowiska Zwierząt
Wydział Nauk o Zwierzetach
Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
ul. Ciszewskiego 8, 02-787 Warszawa
Poland
e-mail: kornelia.kucharska@op.pl

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Small mammals of xerothermic grasslands of south-eastern Poland

RAFAŁ ŁOPUCKI[1], IWONA MRÓZ[2], DANIEL KLICH[3], IGNACY KITOWSKI[4]
[1]Centre for Interdisciplinary Research, The John Paul II Catholic University of Lublin
[2]The John Paul II Catholic University of Lublin
[3]Faculty of Animal Sciences, Warsaw University of Life Sciences – SGGW
[4]Institute of Agricultural Sciences, State School of Higher Education in Chełm

Abstract: *Small mammals of xerothermic grasslands of south-eastern Poland*. Xerothermic grasslands are open habitats with rich and diverse grassy and herbaceous flora. The abundance of fauna is also related to the richness and uniqueness of flora, especially stenotopic species of invertebrates. Much less is known about the vertebrate fauna of these habitats. The vertebrates which can potentially form characteristic assemblages on xerothermic grasslands are small mammals. The aim of this paper is to study the quantitative and qualitative structure of small-mammal communities that inhabit protected xerothermic grasslands in south-east of Poland at the interface of three geographical macroregions: Lublin Upland, Volhynian Upland and Western Bug Basin. 488 small mammals of 13 species were captured over the 1,558 days of live-trapping. It was found that xerothermic grasslands are quite rich habitats in terms of small mammal fauna: species richness from 8 to 11; species diversity index from 1.7 to 2.0; number of mammals captured per 100 trap-days from 24.7 to 50.9. The most frequent species were *Microtus arvalis* (Pallas, 1778), *Apodemus agrarius* (Pallas, 1771) and *Sorex araneus* (Linnaeus, 1758), i.e. mammals that are also typical of agricultural areas. About 20% of captured mammals were species typical of other (e.g. wet or wooded) habitats neighbouring xerothermic grasslands. During our study, we did not catch any the southern birch mouse *Sicista subtilis* (Pallas, 1773); this indicates that this mammal, if still present in this area, is an extremely rare species. The data presented in this paper may be used to create or update protection plans for the surveyed nature reserves or Natura 2000 sites.

Key words: xerothermic grasslands, small mammals, nature reserves, Natura 2000

INTRODUCTION

Xerothermic grasslands are thermophilic steppe-type grass communities whose occurrence is conditioned by climate, soil, and landform. In Poland these habitats occur outside the climatic zone of the steppes, mainly in the uplands of southern Poland, and the Vistula, Odra, and Warta valleys (Mróz and Bąba 2010, Barańska et al. 2013). Xerothermic grasslands are open (usually treeless) habitats with rich and diverse grassy and herbaceous flora, often with relict and rare species (Barańska et al. 2013). They are dominated by plant and animal species from the southern Palearctic areas, representing Pontic-Pannonian, Mediterranean and Irano-Turanian elements (Mazur and Kubisz 2000). From the phytosociological point of view, these communities belong mainly to the *Festuco-Brometea* class; however, they are strongly diversified in terms of species composition, the genetics of these species, soil type, and historical transformations and land use (Barańska et al. 2013).

The flora of xerothermic grasslands in Poland is well known and, due to its uniqueness, diversity, and richness, is the object of both scientific research and various conservation measures (Bąba 2003, 2004, Czarnecka 2009, Denisow and Wrzesień 2015). These activities most often relate to strengthening the populations of the rarest species and maintaining optimal habitat features, mainly by restraining ecological succession by removing trees and shrubs, mowing or grazing, raking out needle cover, etc. (Barańska et al. 2013). The abundance and diversity of fauna are also related to the richness and uniqueness of the flora of xerothermic grasslands. In particular, invertebrates are commonly studied (Mazur and Wanat 1994, Mazur and Kubisz 2000, Weiss et al. 2013) because in these systematic groups there are many stenotopic species that are closely related to xerothermic habitats (Mazur and Kubisz 2000). On the example of the Przęślin steppe reserve near Wiślica, Mazur and Wanat (1994) showed how rich this stenotopic fauna can be: in an area of less than 1 ha, about 150 species of beetle from the family Curculionidae live, of which nearly 30% of the species are characteristic to this environment.

Much less is known about the vertebrate fauna of xerothermic grasslands. Larger vertebrates usually do not create the characteristic multi-species communities that are typical of invertebrates in these habitats, mainly because xerothermic grasslands are usually small area habitats (several, a dozen, or sometimes several dozen hectares). They use these habitats only as a part of their home range, e.g. as feeding grounds or places for nesting. Such species in xerothermic grasslands are often an important element of their biodiversity, but they do not usually constitute indicator species for these habitats.

The vertebrates which can potentially form characteristic communities on xerothermic grasslands are small mammals. The open nature of these habitats, the specific thermal and hydrological conditions, the low vegetation, and the large number of invertebrates (as a potential source of food) may result in the formation of a relatively specific assemblage of small mammals in these areas. In addition, there is also a species that could be considered as a characteristic species of these steppe (xerothermic) habitats: the southern birch mouse *Sicista subtilis* (Pallas, 1773) (Cserkész et al. 2009), which has been caught in Poland in the xerothermic habitat of Machnowska Góra reserve (Baraniak et al. 1998), however, the exact habitat of this species and the current composition of small mammal fauna throughout the south-eastern part of the Lublin region are both relatively poorly understood. Only very little data is available in atlas publications (Pucek and Raczyński 1983, Okarma et al. 2016) and a few reliable faunistic works based on trapping data (Ziomek 1998).

The aim of this paper is to study the quantitative and qualitative structure of the small-mammal communities inhabiting various xerothermic grassland habitats in the south-eastern of Poland at the junction of three geographical macroregions: Lublin Upland, Volhynian Upland and Western Bug Basin (Kondracki 2002). Previously the structure of the small-mammal communities inhabiting these specific habitats has not been analysed in detail. One of the few excep-

tions is the work of Ziomek (1998) on the small mammals of Central Roztocze Upland, in which some typical xerothermic habitats were studied. In our study, only protected xerothermic habitats were chosen (the most valuable and best-preserved habitats); allowing to use our dataset for conservation, or management plans of these protected areas.

MATERIAL AND METHODS

Study area

The study was carried out in Poland (Europe), in the south east part of Lubelskie Voivodeship in three macroregions: Lublin Upland (Mesoregion Grabowiec Interfluves), Volhynian Upland (Mesoregions Hrubieszów Basin and Sokal Ridge) and Western Bug Basin (Mesoregion Belz Plain) (Kondracki 2002). The studied region is agricultural with a low proportion of wooded areas (below 20%) and a low level of urbanization. It is characterized by hot summers (mean above 17°C), cold winters (mean below –3°C), and low annual precipitation (mean below 550 mm).

Five protected area consisting the most valuable and best-preserved xerothermic grassland habitats were selected: Rogów, Machnowska Góra, Gliniska, Skarpa Dobużańska and Zachodniowołyńska Dolina Bugu. In case of Rogów, Machnowska Góra and Skarpa Dobużańska, the study was conducted on the whole area of reserves, whereas in the case of Zachodniowołyńska Dolina Bugu, the study was conducted only in the xerothermic part of the grasslands on loess slopes in the Bug valley between the villages of Czumów and Gródek. In Gliniska the study was carried out with the exception of places where regular mowing was carried out and the vegetation was very short (part of the reserve was prepared as a place for the reintroduction of *Spermophilus suslicus*). Detailed data on all study areas are presented in Table 1.

Trapping procedure

Small mammals were captured in live traps, marked, and then released at the trapping site. Three types of live traps were simultaneously used: wooden box traps (90 × 80 × 200 mm), metal multicapture Ugglan traps (60 × 90 × 240 mm) and pitfall traps (plastic buckets with a diameter of 30 cm and a depth of 50 cm, buried even with the surface of the ground). The proportion of traps used for each study site was as follows: wooden traps 50%, Ugglan traps 25%, pitfall traps 25%. Traps were loaded with food bait (a mixture of oats, pumpkin seeds, peanuts, and some frozen crickets) and a piece of apple. Traps were set along a transect, usually consisting of 20–30 traps spaced about 20 m from one another (traps of different types were set in random order). Each trapping session lasted usually four days. Captured animals were described in terms of species, sex (if possible – the sex of shrews was not determined), reproductive activity and body mass. Individuals captured for the first time were marked by fur clipping. Trapping sessions were carried out in July and August for three years for each study site. Detailed information on study years, trapping effort and number of trapped animals is presented in Table 2. During 1,558 trap-days, 488 individuals of 13 species were captured. The study was conducted according to the permis-

TABLE 1. Characteristics of the study areas: their size, protection status and location.

Name of the study area	Protection status	The objectives of protection*	Area (ha)*	Geographical coordinates*	Physico-geographical location **
Machnowska Góra	Nature reserve (also part of Natura 2000 site PLH060018)	preservation, for scientific and didactic reasons, of xerothermic communities with numerous protected species of flora and fauna	25.30	23.58105 50.37171	Macroregion: Western Bug Basin Mesoregion: Belz Plain
Skarpa Dobużańska	Nature reserve (also part of Natura 2000 site PLH060039)	preservation of xerothermic communities with rare species of steppe plants	5.07	23.71486 50.58048	Macroregion: Volhynian Upland Mesoregions Sokal Ridge
Rogów	Nature reserve (also part of Natura 2000 site PLH060062)	preservation, for scientific and didactic reasons, of the places of occurrence of rare steppe plants and xerothermic plants with a relict population of *Carlina onopordifolia*	0.95	23.52335 50.79794	Macroregion: Lublin Upland Mesoregion: Grabowiec Interfluves
Zachodnio-wołyńska Dolina Bugu	Natura 2000 site PLH060035	inter alia: conservation of xerothermic grasslands in a favourable condition	77.81(total area 1556)	24.0612 50.6786	Macroregion: Volhynian Upland Mesoregion: Hrubieszów Basin
Gliniska	Nature Reserve (also part of the Natura 2000 PLH060029)	preservation of the place of occurrence of *Spermophilus suslicus*	34.00	23.63278 50.85997	Macroregion: Lublin Upland Mezoregion: Grabowiec Interfluves

* Source of information: http://crfop.gdos.gov.pl; ** according to regionalization of Poland (Kondracki 2002).

sion of the Regional Director for Environmental Protection in Lublin.

Data analysis

Data were analysed on the basis of the following parameters and methods:

– Community composition, characterized based on the relative abundance of each species, calculated as the number of individuals of a given species divided by the total number of individuals of all species and expressed as a percentage.
– Species richness, defined as the number of species recorded within a given study site.
– Species diversity, calculated by the Shannon–Wiener index (H') for each site using a natural logarithm.
– Relative abundance of the small mammal, defined as the number of

TABLE 2. Species composition and contribution of small mammals, species richness, and species diversity index in particular studied sites. Characteristics of the study years and trapping effort for the particular study sites

Specification	Zachodnio--wołyńska Dolina Bugu	Gliniska	Skarpa Dobużańska	Rogów	Machnowska Góra
Apodemus agrarius	6.25	9.86	17.35	29.46	38.74
Apodemus flavicollis	0	11.27	6.12	7.14	0
Apodemus sylvaticus	3.13	2.82	0	0	11.71
Micromys minutus	0	1.41	0	1.79	3.60
Mus musculus	1.04	5.63	0	0	0
Microtus arvalis	11.46	46.48	15.31	27.68	24.32
Microtus oeconomus	20.83	2.82	15.31	4.46	5.41
Microtus subterraneus	15.63	1.41	5.10	0	0.90
Myodes glareolus	1.04	2.82	6.12	15.18	0
Sorex araneus	28.13	11.27	27.55	12.50	8.11
Sorex minutus	8.33	4.23	5.10	1.79	5.41
Crocidura leucodon	2.08	0	2.04	0	1.80
Neomys fodiens	2.08	0	0	0	0
Number of individuals	96	71	98	112	111
Trapping effort (number of trap-days)	388	260	290	220	400
Number of individuals per 100 trap-days	24.74	27.31	33.79	50.91	27.75
Study years	2012; 2013; 2014	2012; 2013; 2014	2011; 2012; 2013	2010; 2011; 2012	2011; 2012; 2014

individuals (all species) captured per 100 trap-days.

– The dissimilarity in the composition of small-mammal communities among study sites was evaluated by means of agglomerative hierarchical clustering analysis (AHC) and was expressed as a dendrogram. We used Euclidean distance to present dissimilarity between sites and Ward's method to agglomerate the analysed sites into clusters (classes). Statistical tests were performed using XLSTAT statistical software.

RESULTS

In total, we captured 488 small mammals, representing 13 species:

– Five species from family Muridae: striped field mouse *Apodemus agrarius* (Pallas, 1771); yellow-necked mouse *Apodemus flavicollis* (Melchior, 1834); wood mouse *Apodemus sylvaticus* (Linnaeus, 1758); Eurasian harvest mouse *Micromys minutus* (Pallas, 1771) and house mouse *Mus musculus* (Linnaeus, 1758).

– Four species from subfamily Arvicolinae (family Cricetidae): common vole *Microtus arvalis* (Pallas, 1778); root vole *Microtus oeconomus* (Pallas, 1776); field vole *Microtus subterraneus* (de Selys-Longchamps, 1836); bank vole *Myodes glareolus* (Schreber, 1780).
– Four species from family Soricidae: common shrew *Sorex araneus* (Linnaeus, 1758); pygmy shrew *Sorex minutus* (Linnaeus, 1766); Eurasian water shrew *Neomys fodiens* (Pennant, 1771) and bicoloured white-toothed shrew *Crocidura leucodon* (Hermann, 1780).

The studied habitats had the following species richness: range 8–11, mean 9.6; standard deviation 1.34. The most frequent species captured on all sites were *M. arvalis*, *A. agrarius* and *S. araneus* (Fig. 1). The total share of these three species in the small mammals' community was 63%. The other species can be divided into two groups. The first group (total share 14.35%) includes species whose habitat preferences may also include open, dry areas (*C. leucodon*, *S. minutus*, *M. subterraneus*, *A. sylvaticus*), whereas the second group (about 22% of the animals caught) incudes species typical for wet (*M. oeconomus*, *N. fodiens*) and wooded habitats (*A. flavicollis*, *M. glareolus*), or synanthropic species (*M. musculus*).

The studied xerothermic habitats differed from each other in species composition and proportion of particular species (Table 2). Agglomerative hierarchical clustering (Fig. 2) showed three groups (classes) of the studied sites. The first class includes the Zachodniowołyńska Dolina Bugu and Skarpa Dobużańska sites, mainly due to the large propor-

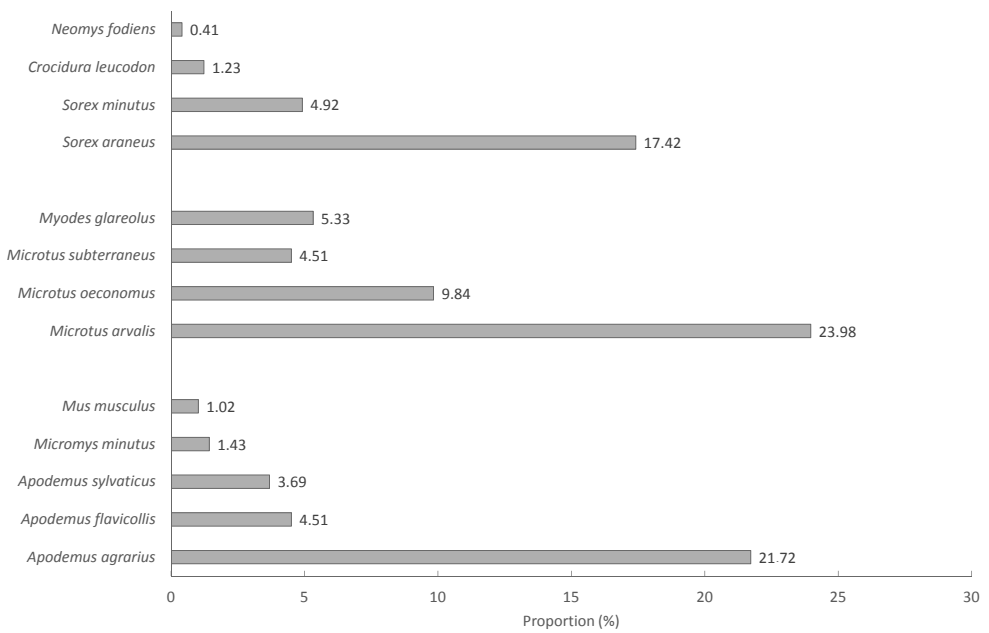

FIGURE 1. Species composition and proportion of species in all xerothermic habitats studied

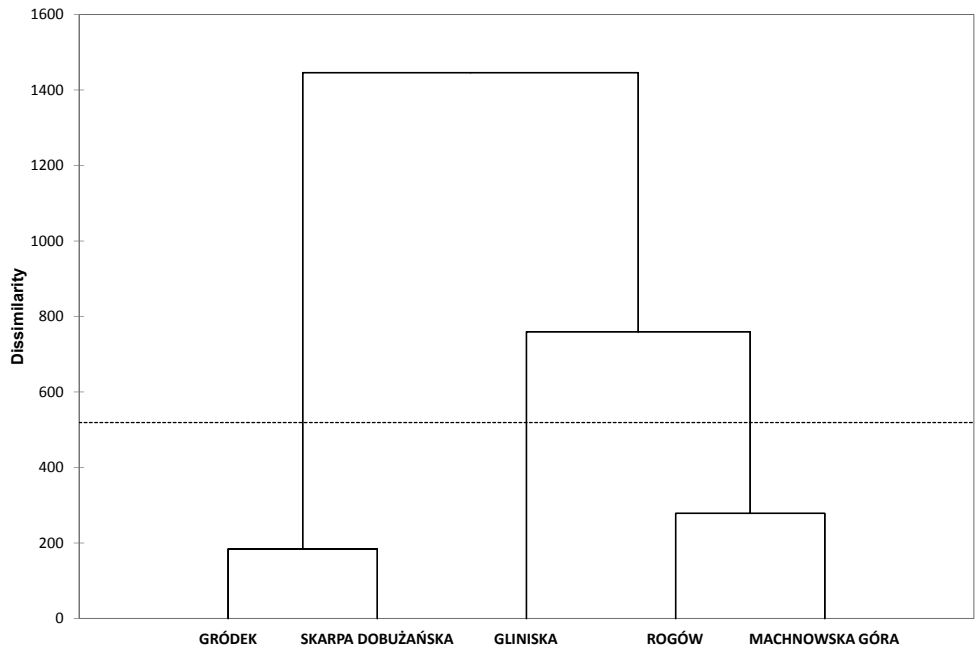

FIGURE 2. Dendrogram of dissimilarity in composition of small mammals' communities in the studied sites (agglomerative hierarchical clustering; dissimilarity – Euclidean distance; agglomeration method – Ward's method)

tion in the community of *S. araneus* and *M. oeconomus*. The second class includes only the Gliniska site, mainly due to the large proportion of *M. arvalis*. The third class includes the Rogów and Machnowska Góra sites, mainly due to the large proportion of *A. agrarius* and *M. arvalis*.

The species richness and diversity index for the surveyed small mammal communities in xerothermic habitats were relatively high: 8 to 11 species were found on each sites, which gave high species diversity index values, from 1.7 to 2.0 (Fig. 3).

DISCUSSION

Xerothermic grasslands are considered as one of the richest plant communities, consisting many protected and rare, often relict species of plants and animals (especially invertebrates). Important group of these habitats is the small mammals, which occur there quite often compared to other habitats studied in the Lublin Region. For the habitats studied in this work, the average capture rate per 100 trap-days was in the range 24.7–50.9 individuals, while for other habitats in nearby regions described in the literature, the capacity was similar or even lower. For example, in the agricultural landscapes of Lublin Upland the number of mammals captured per 100 trap-days was 15–54 (Łopucki et al. 2013). In Roztocze Region, Ziomek (1998) trapped 16.1–31.8 small mammals per 100 trap-days in forest habitats and 10.7–33.3 individuals in open habitats. Of course, these comparisons should be treated cau-

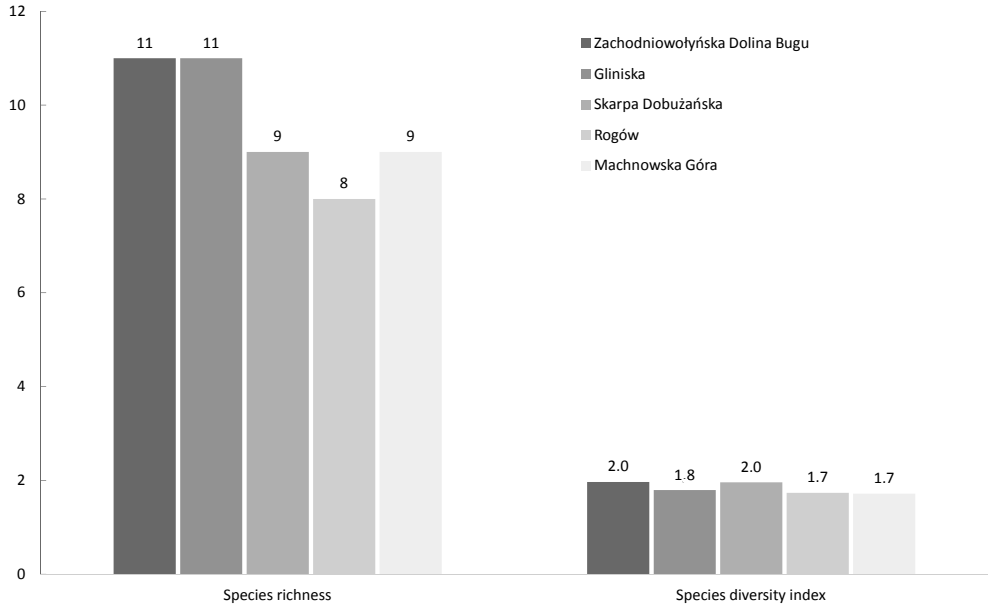

FIGURE 3. Species richness and diversity index of the studied xerothermic habitats

tiously because data presented in different works could have been obtained by various methods and in different seasons. Nevertheless, the presented comparison shows that diverse and abundant small mammal fauna lives in the studied xerothermic habitats. The high species richness (8–11) and species diversity (1.7–2.0) indexes recorded on the studied sites are probably related to the fact that these xerothermic habitats are relatively small in size and most often have an elongated shape, thus the impact of neighbouring habitats is high. For the xerothermic habitats of Roztocze Region, also were found a similar species richness index of 11 and captured species typical for other (mainly forest) habitats (Ziomek 1998).

In our study, the most frequent species captured on all sites were *M. arvalis*, *A. agrarius* and *S. araneus*; these species are typical also in agricultural areas (Gliwicz and Kryštufek 1999, Zima 1999). The proportion of these three species in the small mammal community was 63%. These results partly correspond to the results obtained by Ziomek (1998) for the xerothermic habitats of Roztocze Region, where *M. arvalis* was the dominant rodent species and *S. araneus* was the dominant shrew species; however, the proportion of *A. agrarius* was very low and *M. glareolus* and *A. sylvaticus* were the subdominant species. In our study the smaller proportion of species associated with trees and shrubs, as well as the increased proportion of species typical for open habitats (including arable lands), may be a result of the conservation activity on the studied reserves, including removal of shrubs to maintain their open character (Barańska et al. 2013). Other

species we captured include those whose habitat preferences may also cover the dry open habitats (*C. leucodon*, *S. minutus*, *M. subterraneus* and *A. sylvaticus*), as well as species typical of other, e.g. wet (*M. oeconomus*, *N. fodiens*) or forests habitats (*A. flavicollis*, *M. glareolus*), or synanthropic species (*M. musculus*). The presence of species of humid or forest habitats in xerothermic grassland is caused by the fact that these species are common in the neighbouring habitats (they probably used xerothermic habitats despite other habitat preferences or migrating animals were captured). Such a phenomenon of increased presence of untypical species for xerothermic habitats is particularly visible for the study sites located near river valleys (Skarpa Dobużańska, Zachodniowołyńska Dolina Bugu) of near forests (Rogów).

During our study, we did not catch any southern birch mouse *S. subtilis*, despite the fact that we conducted research in theoretically suitable habitats for it at the right time of the year, and with the recommended trapping methods (Cserkész and Gubányi 2008, Cserkész et al. 2009). This may mean that *S. subtilis*, if it is still present in this area, is an extremely rare species (as was also suggested by Cserkész et al. 2009). The presence of *S. subtilis* in Poland is still waiting for confirmation on the basis of intentionally conducted trapping studies. All current knowledge about this species is based on one accidental capture in 1994 (Baraniak et al. 1998) and bone material from owls' pellets (Cserkész et al. 2009), so it was even impossible to establish the taxonomical position of the Polish population (Cserkész et al. 2016).

CONCLUSIONS

The data presented in this paper are potentially useful for create or update protection plans of the surveyed nature reserves or Natura 2000 sites. Our results contain qualitative and quantitative data on a group of animals that have not been studied before in detail, therefore they expanded the knowledge about the natural value of these reserves. In addition, our data can be used in future research on *S. subtilis*. The trapping effort presented in this paper and lack of success in capturing *S. subtilis* could be an indication that when planning monitoring of this species in the future, much more intense study is needed (many more trapping days should be planned).

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Streszczenie: *Drobne ssaki muraw kserotermicznych w południowo-wschodniej Polsce.* Murawy kserotermiczne są otwartymi siedliskami z bogatą i różnorodną roślinnością trawiastą oraz zielną. Z unikalną roślinnością tych siedlisk powiązane jest także bogactwo faunistyczne, w szczególności dotyczy to stenotopowych bezkręgowców. Fauna kręgowców tych siedlisk jest słabo zbadana. Kręgowcami, które mogą tworzyć specyficzne zespoły gatunków muraw kserotermicznych, są drobne ssaki. Celem pracy było poznanie jakościowej i ilościowej struktury zespołów drobnych ssaków zasiedlających najlepiej zachowane i chronione fragmenty muraw kserotermicznych w południowo-wschodniej Polsce na styku trzech makrore-

gionów geograficznych: Wyżyny Lubelskiej, Wyżyny Wołyńskiej oraz Kotliny Pobuża. Podczas 1558 pułapkodób schwytano 488 drobnych ssaków reprezentujących 13 gatunków. Wykazano, że murawy kserotermiczne mają względnie bogatą faunę drobnych ssaków: bogactwo gatunkowe wyniosło od 8 do 11; wskaźnik różnorodności gatunkowej wyniósł od 1,7 do 2,0, liczba osobników schwytana na 100 pułapkodób wyniosła od 24,7 do 50,9. Najczęściej spotykanymi gatunkami były *Microtus arvalis*, *Apodemus agrarius* i *Sorex araneus*, tj. ssaki typowe również dla obszarów rolniczych. Około 20% schwytanych ssaków reprezentowało gatunki typowe dla innych siedlisk (np. wilgotnych lub leśnych), które sąsiadowały z płatami muraw. Podczas badań nie schwytano smużki stepowej *Sicista subtilis*, co świadczy o tym, że ten ssak o ile jest wciąż obecny w tym rejonie, o tyle jest niezwykle rzadkim gatunkiem. Dane zaprezentowane w niniejszej pracy mogą być użyte do tworzenia i aktualizacji planów ochrony badanych rezerwatów lub obszarów Natura 2000.

Słowa kluczowe: murawy kserotermiczne, drobne ssaki, rezerwaty, Natura 2000

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Authors' address:
Rafał Łopucki
Interdyscyplinarne Centrum Badań Naukowych
Katolicki Uniwersytet Lubelski Jana Pawła II
ul. Konstantynów 1 H, 20-708 Lublin
Poland
e-mail: lopucki@kul.pl

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The effect of oil type in diet in broiler chicken on pro-health and organoleptic properties of meat

ANNA MILCZAREK, MARIA OSEK, TERESA BANASZKIEWICZ
Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities

Abstract: *The effect of oil type in diet in broiler chicken on pro-health and organoleptic properties of meat.* The studies covered the analysis of the pro-health and organoleptic properties of the meat of chickens for fattening fed with mixtures oiled with three different oils. The research material consisted of 36 samples of pectoral and legs muscles. Samples were collected from 42-day old birds from three feeding groups. Group I chickens received mixtures oiled with soybean oil, while groups II and III chickens were fed with mixtures oiled with rapeseed oil and linseed oil, respectively. The use of rapeseed or linseed oil in mixtures for chickens for fattening does not affect the content of basic ingredients in muscles, but significantly reduced the proportion of crude fat in pectoral muscle. The differentiation of the fatty acids content in oils was reflected in muscle lipids. The obtained results provide grounds to recommend oiling of compound mixtures primarily with linseed oil, as it increases ($P < 0.01$) the content of PUFA n-3. However, it should be taken into account that taste qualities will thus deteriorate.

Key words: broiler chickens, muscles, fatty acids, basal nutrients

INTRODUCTION

Healthy nutrition is needed in the development and preservation of human health. It means that it is necessary to cover the body's needs for energy and nutrients, including fat (EFSA 2010). Currently, consumption of the above mentioned ingredient, with regard to quantity and quality as well, is one of the most common dietary mistakes. The high proportion of fat in a diet is commonly associated with the risks of the so-called civilization diseases (obesity, cardiovascular disease, tumours, etc.). However, it is indispensable in the human diet, as it is a carrier of polyunsaturated fatty acids belonging to the families of omega-6 and omega-3, that are particularly important to health. They must be delivered with food in right proportions. According to Daley et al. (2010) and Gibbs et al. (2010), the superiority of omega-6 acids over omega-3 acids should not be greater than four times, while in the average diet it is 10–20 times. The deficiency of polyunsaturated fatty acids from the n-3 family in human nutrition can be supplemented by introducing the best of its sources to the diet, such as linseed oil, or the use of food products enriched with these acids in a direct or indirect way. Meat is such a product. It is a source of protein of high nutritional value and fat, but also of a number of pro-health ingredients (Marco et al. 2013, Puvača et al. 2014, Valavan et al. 2016). Due to the fact that the largest controversy concern meat lipids, the recent years studies focused on improvement of the profile of fatty acids of intramuscular fat

in the pro-health direction. It is based on feeding the animals with properly selected fats. Studies by various authors (Schneiderová et al. 2007, Konieczka et al. 2017, Li et al. 2017) indicate the physiological ability of animals to incorporate fatty acids from diet into abdominal and intramuscular fat. Introduction of feed biocomponents rich in polyunsaturated fatty acids (PUFA) to the diet of chickens allows for their increased content in the tissues of birds. However, the experiments carried out have proved that the effectiveness of this treatment depends mainly on the type and quantity of feed material used in mixtures for chickens. What is more, the quantity and quality of fat affect the quality of meat related to organoleptic values, such as the smell in particular (Wood and Enser 1997, Betti et al. 2009, Jankowski et al. 2012). Marco et al. (2013) and Valavan et al. (2016) state that inclusion of n-3 lipid sources in broiler ration had no adverse effect on meat quality in terms of organoleptic assessment such as appearance, juiciness, flavour, tenderness and overall acceptability scores.

The purpose of the studies was to assess the pro-health and organoleptic properties of meat of chickens fed with mixtures oiled with three different vegetable oils.

MATERIAL AND METHODS

The research material consisted of 36 samples of muscles of pectoral muscles

TABLE 1. Feed ingredients and nutritive value of the mixtures

Item	Mixtures	
	starter	grower
Feed ingredients ($g \cdot kg^{-1}$)		
Maize	503.2	539.5
Soybean meal	410.0	364.5
Oil*	45.00	55.00
L-lysine, DL-methionine, limestone, monocalcium phosphate, salt	36.80	36.00
Premix**	5.00	5.00
Nutritive value per 1 kg of mixtures		
ME-EM (MJ)	12.53	12.77
Crude protein (g)	217	199
Crude fibre (g)	25.9	25.2
Lysine (g)	11.7	9.7
Methionine + cysteine (g)	8.9	8.4
Calcium (g)	9.1	8.9
Phosphorus available (g)	4.2	4.1
Sodium (g)	1.6	1.7

* Soybean oil – group I, rapeseed oil – group II, linseed oil – group III.
** Premix starter in starter mixtures, premix grower in grower mixtures.

and muscles from legs of Ross 308 chickens for fattening. Samples were collected from 42-day old chickens from three feeding groups (I, II, III). All mixtures were prepared in-house based on maize meal, post-extraction soybean meal. The experimental factor were oil: soybean (group I), rapeseed (group II) or linseed (group III) introduced into the starter and grower mixtures (Table 1).

Basal nutrients and fatty acid profile in muscles after stored by 2 weeks in frozen were analysed. Dry matter, ash, crude protein and ether extract contents in the carcass samples were determined according to AOAC International (2011) procedures No 934.01, 942.05, 984.13 and 920.39, respectively. The fatty acid profile in oils and in muscles was determined by gas chromatography (Folch et al. 1957). The fat extracted with light petroleum underwent the processes of alkaline hydrolysis using 0.5 n solution of NaOH in absolute methanol, then the released fatty acids were converted to methyl esters using 4% hydrogen chloride in methanol. The obtained esters were separated on a GC column and their total acid content was determined. A gas chromatograph was used for chromatographic separation. The fatty acid concentrations were expressed in g per 100 g of tissue because this nutritionally reflects possible changes in the fatty acid profile better than the percentage content as it takes the fat content in 100 g of tissue into account. Based on the percentage (% of the total) of fatty acids, we calculated the atherogenic (AI) and thrombogenic (TI) indexes, as well as the hypocholesterolemic-to-hypercholesterolemic fatty acids ratio (HH) according to Ulbricht and Southgate (1991) and Santos-Silva et al. (2002):

$$AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0) / / [\Sigma MUFA + \Sigma(n\text{-}6) + \Sigma(n\text{-}3)]$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0) /[0.5 \times \times \Sigma MUFA + 0.5 \times \Sigma(n\text{-}6) + 3 \times \Sigma(n\text{-}3) + + \Sigma(n\text{-}3) / \Sigma(n\text{-}6)]$$

$$HH = [(C18{:}1n\text{-}9 + C18{:}2n\text{-}6 + C20{:}4n\text{-}6 + C18{:}3n\text{-}3 + C20{:}5n\text{-}3 + C22{:}5n\text{-}3 + + C22{:}6\text{-}n) / (C14{:}0 + C16{:}0)].$$

Sensory evaluation of the muscles was performed after thermal treatment. Muscles (not frozen) were heated in 0.8% NaCl water solution (with a 1 : 2 meat-to--water proportion) until attaining 75°C in the geometric center of the sample. The assessment was carried out by a panel of six qualified subjects. The samples were evaluated for: flavour, tenderness, juiciness and palatability, according to a five--point scale: 1 (the lowest score) through 5 (the highest score) (Baryłko-Pikielna and Matuszewska 2014).

The results obtained were developed by one-way analysis of variance ($P < < 0.05$ and $P < 0.01$) and calculating the mean values for the groups and the (SD). The significance of differences between mean values of the analysed characteristics was determined by means of the Duncan test (post-hoc) with Statistica 12.5.

RESULTS AND DISCUSSION

The determined profile of fatty acids of oils (soybean, rapeseed, linseed) used to oil mixtures is shown in Table 2.

TABLE 2. Fatty acids profile (g·100 g^{-1}) of oils

Item	Oils		
	soybean	rapeseed	linseed
C 14:0	0.05	0.07	0.04
C 16:0	12.37	4.83	4.62
C 16:1	0.07	0.12	0.05
C 18:0	2.11	1.38	3.02
C 18:1	22.38	68.13	20.41
C 18:2 n-6	57.96	17.70	17.97
C 18:3 n-3	4.73	6.45	53.69
C 20:0	0.18	0.43	0.15
C 20:1	0.12	0.84	0.10
C 22:0	0.16	0.27	0.05
Others	0.08	0.16	0.06
Σ SFA	14.70	6.68	7.75
Σ UFA	85.22	93.16	92.19
Σ MUFA	22.53	69.01	20.55
Σ PUFA	62.69	24.15	71.64
PUFA n-6/n-3	12.63	2.78	0.34
AI	0.15	0.05	0.05
TI	0.27	0.09	0.04
HH	2.18	16.77	15.91

SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AI – atherogenic index; TI – thrombogenic index; HH – the ratios of hypocholesterolemic and hypercholesterolemic fatty acids.

It was proven that rapeseed and linseed oil contained more than three times less linoleic acid C 18:2 n-6, but more (linseed oil in particular – approx. 11 times) linolenic acid C 18:3 n-3 compared to soybean oil. The content of the above mentioned acids contributed to the narrowing of the total n-6 to n-3 PUFA ratio. In soybean oil, it was 12.63, in rapeseed oil – 2.78 and in linseed oil – 0.34. From the point of view of human nutrition, the most favourable proportion of n-6 to n-3 PUFA was found in rapeseed oil. The obtained own results are similar to data of Osek et al. (2004), Schneiderová et al. (2007) and Konieczka et al. (2017). The authors state that the fatty acid profile of oil depends on a number of factors, for example: the species and variety of seeds, method of pressing or conditions of product storage.

Significantly higher body weight of broilers from groups I and II at the age of 42 days in comparison with group III was proved (Table 3). Lower feed conversion ratio in broilers fed with mixtures containing soybean and rapeseed oils in comparison with birds of group III

TABLE 3. Rearing and post-slaughter results of broiler chickens

Item	Group						*P*
	I		II		III		
	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	
Body weight in day 42 (kg)	2.20b	0.03	2.21a	0.04	2.15ab	0.03	< 0.05
FCR (kg)	1.46b	0.02	1.54a	0.03	1.62ab	0.04	< 0.05
Dressing percentage (%)	76.10	1.26	76.40	1.48	75.50	1.28	0.921
Share in cold carcass (%)							
Muscles total including	44.4b	2.8	46.8ab	2.3	45.0a	1.9	< 0.05
breast	21.6	1.8	23.4	2.6	21.9	1.5	0.344
thigh	13.0	1.2	13.9	1.1	13.2	0.8	0.104
drumstick	9.8	1.2	9.5	1.2	9.9	1.1	0.125
Skin with subcutenous fat	12.9	1.9	13.0	1.5	13.1	1.7	0.103
Abdominal fat	2.2	0.8	2.2	0.6	2.3	0.4	0.090

a, a and b, b – in line the means marked with the same small letters differ statistically significantly at $P \leq 0.05$.

was also found. No influence of the oil type on dressing percentage and broiler fatness was observed, whereas the best shore of muscle ($P \leq 0.05$) in bird groups was found.

The introduction of soybean, rapeseed or linseed oils into mixtures for chickens for fattening did not affect the content of basic ingredients in muscles, except for crude fat (Table 4).

The smallest level of fat was found in the muscles of birds fed with mixtures oiled with rapeseed oil, and the statistically significant difference was confirmed against group I ($P < 0.01$) and group III ($P < 0.05$). The use of linseed oil (instead of soybean oil) in chicken diets significantly reduced the amount of lipids in the pectoral muscle only.

According to Zelenka et al. (2006), the addition (1, 3, 5 and 7%) of linseed oil to compound mixtures for chickens for fattening does not affect the crude fat content in both types of muscles, but its significant amount (7%) decreases ($P \leq 0.05$) the content of protein in pectoral muscles. Osek et al. (2004) indicate a significant influence of the type of oil used to oil mixtures for chickens on fat content in meat, as in pectoral muscles and in muscles of legs of chickens fed with mixtures oiled with linseed oil, smaller amount of this ingredient was found than in the muscles of birds fed with mixtures with rapeseed or soybean oil. Kanakri et al. (2017) state that the type of fat used in mixtures for chickens does not affect the content of crude fat in pectoral muscles and muscles of legs.

The fatty acid profile in muscle lipids (pectoral muscles and muscles of legs) was a reflection of the fatty acid profile of the oils used to oil mixtures for chickens (Table 5 and 6). Significantly ($P < 0.01$) more α-linolenic acid was found in both muscle of birds fed diets

TABLE 4. Basal nutrients (%) of muscles

Item	Groups						*P*
	I		II		III		
	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	
Pectoral muscles (*N* = 18)							
Dry matter	25.57	0.31	25.36	0.29	25.41	0.39	0.336
Crude ash	1.16	0.02	1.16	0.01	1.14	0.01	0.235
Crude protein	22.73	0.16	22.92	0.18	22.82	0.28	0.801
Crude fat	1.65 Aa	0.20	1.23 Aa	0.16	1.41 Ba	0.12	< 0.01
Leg muscles (*N* = 18)							
Dry matter	25.15	0.30	25.40	0.29	25.41	0.34	0.249
Crude ash	1.00	0.02	1.00	0.01	0.99	0.01	0.744
Crude protein	18.69	0.16	19.24	0.19	19.09	0.31	0.125
Crude fat	5.46 Aa	0.16	5.16 Aab	0.09	5.33 Bb	0.24	< 0.01

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A, A and B, B – in line the means marked with the same capital letters differ statistically significantly at $P \leq 0.01$.

with linseed or rapeseed oil compared to chickens receiving the mixtures with soybean oil. Such relations were also noted by Bou et al. (2005), Haug et al. (2007) and Marco et al. (2013) after the introduction of linseed oil to mixtures for chickens for fattening. Significantly lower level of saturated fatty acids, including hypercholesterolemic acids (OFA), and a higher proportion of PUFA in chicken muscles after introduction of rapeseed or linseed oils to mixtures is a confirmation of the results of Osek et al. (2004). Similarly, Kanakri et al. (2017) noted significantly less SFA and more PUFA in the muscles of chickens fed with mixtures containing linseed oil.

The calculated ratio of PUFA n-6 to n-3 in muscles clearly indicates that linseed oil most preferably modifies the fatty acid profile in the pro-health direction. Compared to the muscles of chickens fed with mixtures with rapeseed oil, and especially with soybean oil, this ratio was many times lower. Similarly, Nguyen et al. (2003) showed a narrowing of the n-6 to n-3 PUFA ratio in individual pieces of carcass after the introduction of rapeseed oil (approx. 2.5-fold) or linseed oil (approx. 10-fold) to the diet of chickens for fattening.

Considering the type of muscles of chickens for feeding, it has been shown that leg muscles are a better source of fatty acids from the omega-3 family, due to the higher content of fat, and thus fatty acids. Jarosz and Bułhak-Jachymczyk (2008) recommend to consume 2 g of α-linolenic acid and 200 mg of LC-PUFA n-3 per day in order to narrow the proportion of n-6 to n-3 acids in the human diet.

The fatty acid profiles also enabled the evaluation of the lipid fraction's nutritional quality index (INQ). The atherogenic-

TABLE 5. Fatty acids profile (g·100 g^{-1}) of pectoral muscles ($N = 18$)

Item	Group						*P*
	I		II		III		
	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	
C 14:0	0.004	0.001	0.004	0.001	0.004	0.001	0.234
C 16:0	0.366 A	0.001	0.399 AB	0.013	0.359 B	0.001	< 0.01
C 16:1	0.082 Aa	0.001	0.072 Ba	0.005	0.062 Aa	0.003	< 0.01
C 18:0	0.065 ab	0.002	0.072 b	0.001	0.074 a	0.006	< 0.05
C 18:1	0.588 ABa	0.009	0.893 Aa	0.189	0.639 Ba	0.007	< 0.01
C 18:2 n-6	0.120 A	0.001	0.176 A	0.001	0.171 A	0.002	< 0.01
C 18:3 n-3	0.003 A	0.001	0.016 A	0.001	0.086 A	0.001	< 0.01
C 20:1	0.002 A	0.001	0.004 AB	0.001	0.002 B	0.001	< 0.01
C 20:2	0.002 AB	0.001	0.001 A	0.0001	0.001 B	0.001	< 0.01
C 20:3	0.001	0.001	0.001	0.001	0.001	0.001	0.511
C 20:4	0.001 ABa	0.001	0.005 Aa	0.001	0.004 Ba	0.001	< 0.01
Others	0.004	0.001	0.005	0.001	0.005	0.001	0.238
Σ SFA	0.433 A	0.001	0.475 A	0.013	0.438 B	0.006	< 0.01
Σ UFA	0.792 A	0.001	1.170 A	0.014	0.968 A	0.007	< 0.01
Σ MUFA	0.666 A	0.002	0.970 A	0.014	0.704 A	0.010	< 0.01
Σ PUFA	0.126 A	0.001	0.200 A	0.001	0.264 A	0.004	< 0.01
PUFA n-6/n-3	39 A	1.147	11 A	0.799	2 A	0.006	< 0.01
AI	0.482 ABa	0.002	0.356 Ba	0.016	0.387 Aa	0.002	< 0.01
TI	1.013 A	0.001	0.665 A	0.033	0.368 A	0.006	< 0.01
HH	1.909 ABa	0.006	2.713 Aa	0.136	2.480 Ba	0.005	< 0.01

Explanations as in Table 2.
a, a and b, b – in line the means marked with the same small letters differ statistically significantly at $P \leq 0.05$.
A, A and B, B – in line the means marked with the same capital letters differ statistically significantly at $P \leq 0.01$.

ity (AI) and thrombogenicity (TI) indexes and the ratios of hypocholesterolemic and hypercholesterolemic fatty acids (HH) could therefore be determined. According to Turan et al. (2007), nutritional quality indexes can indicate a sample's potential for plaque aggregation. In other words, low AI and TI values indicate high quantities of anti-atherogenic fatty acids in oil or intramuscular fat. Ouraji et al. (2009) reported that AI and TI higher than 1 are harmful for human health. The values for the both indexes (AI and TI) showed that linseed and rapeseed oils had beneficial effects on pro-healthy properties of meat. The lower the AI and TI index values, the healthier the food. This is because these indexes report the relationship between fatty acids in food and their contribution to the prevention of coronary dis-

TABLE 6. Fatty acids profile (g·100 g^{-1}) of leg muscles ($N = 18$)

Item	Group						*P*
	I		II		III		
	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*	
C 14:0	0.017 AB	0.001	0.013 B	0.001	0.013 A	0.001	< 0.01
C 16:0	1.571 ABa	0.029	1.213 Ba	0.021	1.267 Aa	0.025	< 0.01
C 16:1	0.413 AB	0.022	0.284 A	0.014	0.277 B	0.012	< 0.01
C 18:0	0.254 a	0.013	0.199 ab	0.009	0.250 b	0.033	< 0.05
C 18:1	2.631 Ba	0.283	2.755 Aa	0.045	2.448 ABa	0.164	< 0.01
C 18:2 n-6	0.519 A	0.004	0.566 A	0.002	0.637 A	0.037	< 0.01
C 18:3 n-3	0.036 A	0.002	0.065 A	0.005	0.396 A	0.158	< 0.01
C 20:1	0.014 a	0.001	0.014 b	0.002	0.010 ab	0.002	< 0.05
C 20:2	0.003	0.001	0.003	0.001	0.003	0.001	0.590
C 20:3	0.003	0.001	0.003	0.001	0.003	0.001	0.630
C 20:4	0.012 A	0.001	0.010 B	0.001	0.008 A	0.001	< 0.05
Others	0.019	0.001	0.016	0.002	0.018	0.001	0.170
Σ SFA	1.813 A	0.013	1.425 A	0.031	1.530 A	0.054	< 0.01
Σ UFA	3.629 ABa	0.014	3.719 Ba	0.031	3.782 Aa	0.036	< 0.01
Σ MUFA	3.055 B	0.014	3.073 A	0.029	2.735 AB	0.173	< 0.01
Σ PUFA	0.574 Ba	0.001	0.647 Aa	0.001	1.047 ABa	0.194	< 0.01
PUFA n-6/n-3	14 A	0.0369	9 A	0.699	2 A	0.423	< 0.01
AI	0.446 AB	0.004	0.340 B	0.009	0.350 A	0.004	< 0.01
TI	0.916 A	0.012	0.669 A	0.026	0.439 A	0.108	< 0.01
HH	2.047 AB	0.023	2.789 A	0.089	2.726 B	0.030	< 0.01

Emplanations as in Table 2.
a, a and b, b – in line the means marked with the same small letters differ statistically significantly at $P \leq 0.05$.
A, A and B, B – in line the means marked with the same capital letters differ statistically significantly at $P \leq 0.01$.

eases (Turan et al. 2007, Cutrignelli et al. 2008).

Sensory evaluation of the muscles included: flavour, juiciness, tenderness and palatability (Figs. 1, 2).

Oiling mixtures with rapeseed or linseed oils worsened the flavour and palatability of pectoral muscles, and consequently the average for four rated traits ($P < 0.05$). Significant reduction of organoleptic qualities of muscles of chickens fed with mixtures containing 5% linseed oil was demonstrated by Osek et al. (2005), while Marco et al. (2013) did not note a decrease in the sensory qualities of meat of chickens fed with mixtures oiled with linseed oil. Meat from turkeys fed with mixtures oiled with rapeseed or linseed oils has significantly worse taste, while the muscles of birds having diets

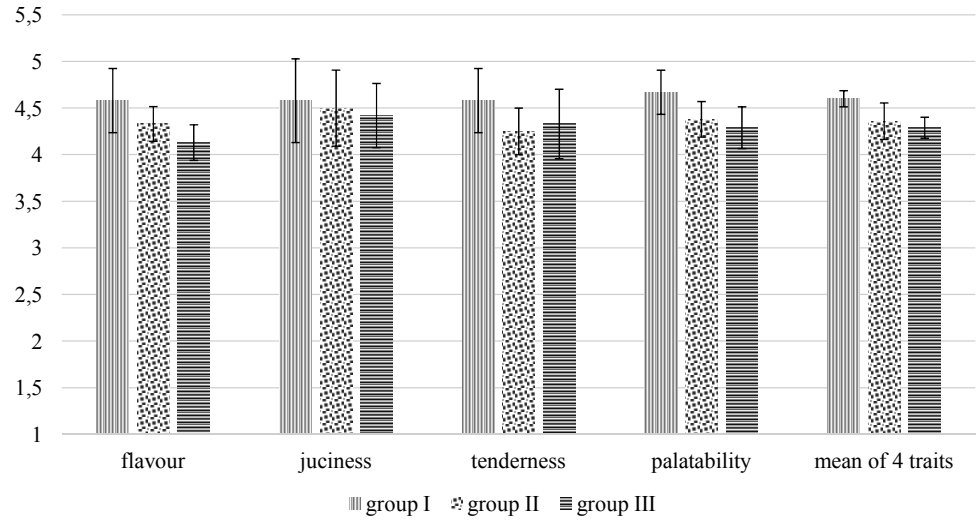

FIGURE 1. Sensory evaluation of pectoral muscles (point)

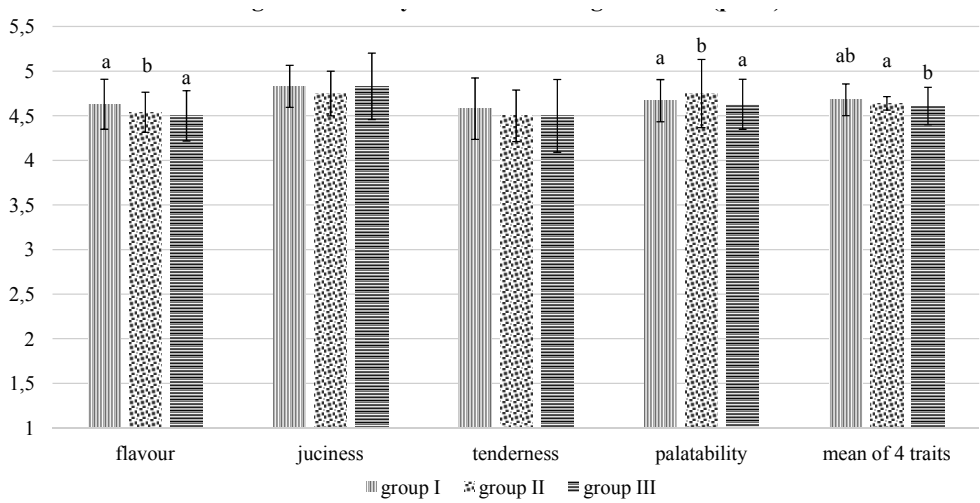

FIGURE 2. Sensory evaluation of thigh muscles (point)

with linseed oil received the lowest evaluation (Jankowski et al. 2012).

CONCLUSIONS

Oiling compound mixtures with rapeseed oil or linseed oil significantly reduced the content of crude fat in the pectoral muscles of chickens for fattening.

The contribution of fatty acids in oils has been reflected in muscle lipids. The most advantageous ratio of n-6 to n-3 PUFA was found in the muscles of chickens receiving mixtures with linseed oil.

The use of rapeseed or linseed oils in the mixtures worsened the smell and taste of pectoral muscles, and consequently the average for four rated traits ($P < 0.05$). Paying attention to the pro-health properties of the meat of chickens for fattening, the obtained results provide grounds to recommend oiling of compound mixtures primarily with linseed oil, as it increases ($P < 0.01$) the content of PUFA n-3. However, it should be taken into account that taste qualities will thus deteriorate.

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Streszczenie: *Wpływ rodzaju oleju w dietach kurcząt brojlerów na właściwości prozdrowotne i organoleptyczne mięsa.* W pracy dokonano analizy właściwości prozdrowotnych i organoleptycznych mięsa kurcząt rzeźnych żywionych mieszankami natłuszczanymi trzema różnymi olejami. Materiał badawczy stanowiło po 18 próbek mięśni piersiowych i nóg kurcząt Ross 308. Próbki pobrano od 42-dniowych ptaków z trzech grup żywieniowych. Kurczęta grupy I otrzymywały mieszanki natłuszczane olejem sojowym, a kurczęta II i III grupy mieszanki natłuszczone odpowiednio olejem rzepakowym i lnianym. Zastosowanie w mieszankach dla kurcząt brojlerów oleju rzepakowego lub lnianego nie wpłynęło na zawartość składników podstawowych w mięśniach, ale istotnie zmniejszyło w nich udział tłuszczu surowego (wyjątek mięśnie nóg ptaków żywionych mieszanką zawierającą olej lniany). Zróżnicowanie udziału kwasów tłuszczowych w olejach znalazło swoje odzwierciedlenie w lipidach mięśni. Mając na uwadze prozdrowotne walory mięsa kurcząt brojlerów, otrzymane wyniki upoważniają do zalecania natłuszczania mieszanek paszowych przede wszystkim olejem lnianym, bowiem zwiększa ($P \leq 0{,}01$) on udział PUFA n-3. Należy jednak liczyć się z pogorszeniem jego walorów smakowych.

Słowa kluczowe: kurczęta brojlery, mięśnie, kwasy tłuszczowe, składniki podstawowe

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Authors' address:
Anna Milczarek
Katedra Żywienia Zwierząt i Gospodarki Paszowej
Wydział Przyrodniczy
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach
ul. Prusa 14, 08-110 Siedlce
Poland
e-mail: anna.milczarek@uph.edu.pl

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Rearing performance of piglets from the aspect of mammary gland remodeling in sows of two genotypes

ANNA REKIEL, JUSTYNA WIĘCEK, MAGDALENA HOŁOWNIA, MARTYNA BATORSKA
Faculty of Animal Sciences, Warsaw University of Life Sciences – SGGW

Abstract: *Rearing performance of piglets from the aspect of mammary gland remodeling in sows of two genotypes*. The aim of the study was to determine rearing performance of piglets from the aspect of changes in some zoometric indicators of milk line during the reproductive cycle of Danhybrid and PIC crossbred sows. Data for analysis of mammary glands in the third and fourth reproductive cycles were collected from 50 multiparous sows, including 20 Danhybrid and 30 PIC. Number of teats, length of the milk line, base width of the mammary gland, and nipple length were determined. Measurements were made with a tape or caliper during three phases of the reproductive cycle: empty sow period (at mating), gestation (late pregnancy, 7 days before farrowing), and lactation (7th day of nursing). Data were collected for the number of total born piglets, number of live born and stillborn piglets, and number of piglets reared to the 21st day. The results were statistically analysed. Differences were found for base width of the mammary gland at mating ($P \leq 0.01$ in the third cycle and $P \leq 0.05$ in the fourth cycle) and length of the milk line during late pregnancy and lactation ($P \leq 0.05$ in the third cycle). During the reproductive cycle the magnitude of changes in zoometric indicators of the mammary gland was distinctly higher in PIC than Danhybrid sows, with a significantly lower number of total and live born piglets. The differences in the intensity of mammary gland remodeling in the two sow groups may be associated among others with the advancement of body development (slightly greater changes in the fourth compared to the third cycle), genetic dissimilarity (slightly greater changes in PIC compared to Danhybrid sows), better survival at birth and higher activity of piglets from the PIC compared to the Danhybrid group during the suckling period. Rearing performance of the piglets should be regarded as good, and losses were smaller in the PIC vs. Danhybrid sow group.

Key words: sows, Danhybrid, PIC, mammary gland, piglets born and reared

INTRODUCTION

One of the keys to success in pig production is the high reproductive efficiency of the sow, expressed as high prolificacy (Koketsu 2016, Mabry 2016). A prerequisite for a good result, i.e. at least 28 piglets reared per sow per year, is that the offspring make full use of sow's milk. A good growth rate of neonatal piglets and low mortality of suckling piglets are conditional on the sufficient number and quality of sow's teats and its high milk yield (Skok et al. 2007, Chalkias 2013, Rekiel et al. 2013, Sommavilla et al. 2015). Piglet growth and development as well as piglet losses also depend on anatomic location of the teats at which piglets suckle based on the social hierarchy (Skok et al. 2007). Sows of maternal breeds and lines should have at least seven pairs of active, morphologically normal mammary glands. The average heritability of the traits allows it to be improved, which is conducive to increasing

teat number and improving rearing performance of the offspring (Pumfrey et al. 1980, Borchers et al. 2002, Chalkias 2013, Ocepek et al. 2016).

The mammary gland is subject over time to anatomical and functional changes, which begin during the period of fetal growth and development, and continue in the postnatal period. Their magnitude depends on the stage of body maturation, phase of the sow's reproductive cycle, and activity of suckling piglets (Motyl et al. 2001, Procak et al. 2004, Rząsa et al. 2005, Krzymowski and Przała 2015).

The aim of the study was to determine rearing performance of piglets from the aspect of changes in some zoometric indicators of milk line during the reproductive cycle of crossbred sows of two genotypes: Danhybrid and PIC.

MATERIAL AND METHODS

Data for analysis of mammary glands in two reproductive cycles (third and fourth) were collected from 50 multiparous sows, including 20 Danhybrid and 30 PIC. Number of teats (TN), length of the milk line (LML), base width of the mammary gland (BWMG), and nipple length (NL) were determined in the experimental sows. Zoometric measurements of the mammary gland were made with a tape and caliper to within 0.1 mm during three phases of the reproductive cycle: empty sow period (at mating), gestation (late pregnancy, 7 days before farrowing), and lactation (7th day of nursing). Data were collected for the number of total born piglets, number of live born and stillborn piglets, and number of piglets reared to the 21st day.

The results were statistically analysed. Differences between the groups were determined by the Mann–Whitney U-test (IBM SPSS Statistics 24). Tables bring together the results of zoometric measurements, arithmetic means, standard deviations, and the magnitude of changes in the analysed traits.

RESULTS AND DISCUSSION

There were no differences in the number of teats between Danhybrid and PIC sows ($P > 0.05$) (Table 1), but the number of piglets born and reared to 21st day was lower in PIC than in Danhybrid sows ($P \leq 0.01$). Differences were observed between the sow groups in the base width of the mammary gland during the empty sow period (third cycle, $P \leq 0.01$, fourth cycle, $P \leq 0.05$). There were significant differences in length of the milk line in the sows from the compared genetic groups during gestation and lactation ($P \leq 0.05$), which were observed in the third cycle, but not in the fourth cycle ($P > 0.05$) – Tables 1 and 2. During gestation and lactation, PIC compared to Danhybrid sows had longer milk line by 3.18 and 3.67 cm (third cycle) and 1.61 and 2.32 cm (fourth cycle), respectively. The extent of changes in the analysed zoometric indicators of the mammary gland during gestation compared to the empty sow period, and during lactation vs. gestation and the empty sow period was progressive (Tables 3 and 4), which is supported in the literature (Rząsa et al. 2005). Changes in PIC compared to Danhybrid sows, expressed in percentage points, were markedly higher for WMG and NL measurements during

TABLE 1. Mammary gland characteristics of the multiparous sows of two genotypes in the third reproductive cycle

Traits	Danhybrid, $n = 20$		PIC, $n = 30$		P
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	
TN	14.5	0.761	14.6	0.932	0.599
Empty sow period					
LML (cm)	66.30	4.707	66.27	6.871	0.655
BWMG (cm)	10.12	1.732	8.12	1.601	0.001
NL (cm)	1.97	0.385	1.78	0.380	0.135
Late pregnancy					
LML (cm)	70.55	4.049	73.73	6.368	0.039
BWMG (cm)	11.83	1.823	10.77	2.298	0.088
NL (cm)	2.20	0.287	2.31	0.289	0.172
Lactation period					
LML (cm)	76.63	3.594	80.30	6.733	0.020
BWMG (cm)	13.03	1.950	12.67	2.591	0.749
NL (cm)	2.63	0.299	2.73	0.315	0.252

TN – teat number; LML – length of milk line; BWMG – base width of the mammary gland; NL – nipple length.

TABLE 2. Mammary gland characteristics of the multiparous sows of two genotypes in the fourth reproductive cycle

Traits	Danhybrid, $n = 20$		PIC, $n = 30$		P
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	
Empty sow period					
LML (cm)	66.79	4.492	66.07	6.359	0.626
BWMG (cm)	9.46	1.763	8.16	1.382	0.012
NL (cm)	1.72	0.274	1.73	0.352	0.798
Late pregnancy					
LML (cm)	71.82	4.134	73.43	5.788	0.285
BWMG (cm)	11.52	1.705	10.50	2.077	0.058
NL (cm)	2.17	0.354	2.25	0.291	0.318
Lactation period					
LML (cm)	78.53	3.893	80.85	6.236	0.120
BWMG (cm)	13.51	1.425	12.60	2.603	0.350
NL (cm)	2.57	0.315	2.71	0.302	0.164

LML – length of milk line; BWMG – base width of the mammary gland; NL – nipple length.

TABLE 3. Changes in zoometric indicators of the mammary gland of sows in the third reproductive cycle

Period/traits	Danhybrid	PIC
Late pregnancy vs. empty sow period		
Change in LML (cm/%)	4.25 / 6.41	7.46 / 11.26
Change in WMG (cm/%)	1.71 / 16.90	2.65 / 32.64
Change in NL (cm/%)	0.23 / 11.68	0.53 / 29.78
Lactation period vs. late pregnancy		
Change in LML (cm/%)	6.08 / 8.62	6.57 / 8.91
Change in WMG (cm/%)	1.2 / 10.14	1.9 / 17.64
Change in NL (cm/%)	0.43 / 19.55	0.42 / 18.18
Lactation period vs. empty sow period		
Change in LML (cm/%)	10.33 / 15.58	14.03 / 21.17
Change in WMG (cm/%)	2.91 / 28.75	4.55 / 56.03
Change in NL (cm/%)	0.66 / 33.50	0.95 / 53.37

Explanation as in Table 2. WMG – width of the mammary gland.

TABLE 4. Changes in zoometric indicators of the mammary gland of sows in the fourth reproductive cycle

Period/traits	Danhybrid	PIC
Late pregnancy vs. empty sow period		
Change in LML (cm/%)	5.03 / 7.53	7.36 / 11.14
Change in WMG (cm/%)	2.06 / 21.78	2.34 / 28.68
Change in NL (cm/%)	0.45 / 26.16	0.52 / 30.06
Lactation period vs. late pregnancy		
Change in LML (cm/%)	6.71 / 9.34	7.42 / 10.10
Change in WMG (cm/%)	1.99 / 17.27	2.10 / 20.00
Change in NL (cm/%)	0.4 / 18.43	0.46 /20.44
Lactation period vs. empty sow period		
Change in LML (cm/%)	11.74 / 17.58	14.78 / 22.37
Change in WMG (cm/%)	4.05 / 42.81	4.44 / 54.41
Change in NL (cm/%)	0.85 / 49.42	0.98 / 56.65

Explanation as in Tables 2 and 3.

gestation and lactation compared to the empty sow period; this concerned the third and fourth cycles. A strong development of the mammary gland occurs during lactation and reflects the milk yield of individual glands, the enlargement of which is dependent on the activity of suckling piglets (Hurley 2001, Procak et al. 2004). Rząsa et al. (2005), who investigated the mammary gland in Polish Large White and Polish Landrace crosses, concluded that enlargement of the mammary gland was small during the first five days of lactation, and changed by 36% on 21st day of lactation. Suckled mammary glands compared to unsuckled teats expand more rapidly during the first and second lactation. The productivity of mammary glands can be influenced by their suckling in the previous lacta-

tion. According to Farmer et al. (2012), the glands that were not suckled in the previous lactation produce less milk than suckled ones. Procak et al. (2004), who analysed morphometric measurements of mammary glands, concluded that unsuckled glands in the first lactation, may reach their physiological capacity in the next lactation, on the first day after farrowing. However, their width on 1st and 21st days after farrowing was smaller than the width of the glands suckled in the previous lactation. The width of the mammary glands, which were not suckled by the piglets, decreased by 50% three weeks after farrowing, regardless of size of the litter reared by the sow. In our study, LML, BWMG and NL values in the three phases of the reproductive cycle (mating, gestation, lactation) were compared in the third and fourth cycle, in both genetic groups (Danhybrid and PIC). The stabilization of LML in multiparous third and fourth parity sows may indicate that the Danhybrid and PIC sows have completed their body development.

Danhybrid sows gave birth to 2.03 (parity 3) and 2.19 (parity 4) more piglets per litter compared to PIC sows (Tables 5 and 6). The number of piglets born alive by Danhybrid sows was also higher than in PIC sows, by 1.73 and 2.03 piglets in parity 3 and 4, respectively, with highly significant differences ($P \leq 0.01$). In relation to the average calculated for all the sows under analysis, the number of piglets reared to 21st day from Danhybrid sows was higher by an average of 10.6% (parity 3) and 10.5% (parity 4), and that from PIC sows lower by an average of 7.0% (parity 3) and 6.7% (parity 4), respectively. The number of piglets reared to 21st day per Danhybrid

TABLE 5. Number of piglets born and reared (head) (parity 3)

Number of piglets (head)	Danhybrid		PIC		*P*
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	
Total	15.00	2.176	12.97	2.008	0.002
Live born	14.20	2.397	12.47	2.129	0.007
Stillborn	0.80	1.196	0.50	0.682	0.442
Total losses	0.80	1.056	1.20	1.400	0.294
Reared to day 21	13.40	1.903	11.27	2.303	0.001

TABLE 6. Number of piglets born and reared (head) (parity 4)

Number of piglets (head)	Danhybrid		PIC		*P*
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	
Total	15.26	1.522	13.07	2.033	0.001
Live born	14.63	1.606	12.60	1.993	0.001
Stillborn	0.63	0.955	0.47	0.629	0.656
Total losses	0.84	0.958	1.03	1.066	0.450
Reared to day 21	13.74	1.327	11.60	1.868	0.001

sow per litter was higher by an average of 2.13 and 2.14 (parities 3 and 4) compared to PIC sows, and the differences were significant at $P \leq 0.01$ (Tables 5 and 6). Slightly better reproductive results were observed for sows of both genotypes in parity 4 compared to parity 3. This shows that the genetic potential for reproductive traits became more evident in older than in younger sows.

The sows of the two compared genotypes showed differences in their reproductive potential. This is supported in the literature on the subject, which shows that sow fertility depends on breed (Blicharski and Snopkiewicz 2016). The number of piglets born dead by the more fertile Danhybrid sows compared to PIC sows was higher in parities 3 and 4 by 0.3 and 0.17, respectively. This is consisted with the findings of Wallgren (2013), who reported that improvement of reproductive traits and consistent improvement of the basic reproductive indicator, i.e. number of piglets born per litter, is accompanied by an increased number of stillborn piglets. Comparison of piglet production results in Sweden (2013 vs. 2003) showed increases in prolificacy (by 2 piglets weaned per sow per year), number of total born piglets (by 1.72) and stillbirths (by 0.31) (Wallgren 2013). Stillbirths affect 48.3% of all litters and range from 5.2 to 7.5% (Pedersen et al. 2010). The improvements in sow fertility and the associated increase in stillbirths are influenced not only by breeding work and crossbreeding. Stillbirths of piglets are also affected by other factors including the sow's fat reserves, intrauterine growth restriction, birth order and the interval between each piglet being born, reproductive cycle, hypoxia, neonatal weight and sex (Vanderhaege et al. 2010, Baxter et al. 2012, Rutherford et al. 2013). In our study, piglet losses during the maternal rearing period were moderate and higher for PIC compared to Danhybrid sows (parity 3: 9.62 vs. 5.63%, parity 4: 7.94 vs. 6.08%). Data reported by Wallgren (2013) show that preweaning piglet mortality in Sweden was 14.3% in 2003 and 18.3% in 2013. These results are similar to the European Union average of 14–17% (Pedersen et al. 2010).

CONCLUSIONS

During the reproductive cycle, the magnitude of changes in zoometric indicators of the mammary gland was distinctly higher in PIC compared to Danhybrid sows, with a significantly lower number of total and live born piglets. The differences in the intensity of mammary gland remodeling in the two sow groups may be associated among others with the advancement of body development (slightly greater changes in the fourth compared to the third cycle), genetic dissimilarity (slightly greater changes in PIC compared to Danhybrid sows), better survival at birth and higher activity of piglets from the PIC compared to the Danhybrid group during the suckling period.

Rearing performance of the piglets should be regarded as good, and losses were smaller in the PIC vs. Danhybrid sow group.

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Streszczenie: *Wyniki odchowu prosiąt w aspekcie przebudowy gruczołu mlekowego loch dwóch genotypów*. Celem badań było określenie wyników odchowu prosiąt w aspekcie zmian wybranych wskaźników zoometrycznych listwy mlecznej w cyklu reprodukcyjnym u loch hybrydowych Danhybrid i PIC. Dane do analizy pozyskano od 50 loch wieloródek, w tym 20 Danhybrid i 30 PIC, wykonując ocenę gruczołu sutkowego w trzecim i czwartym cyklu reprodukcyjnym. Określono liczbę sutków (TN) oraz długość listwy mlecznej (LML), szerokość podstawy gruczołu mlekowego (BWMG) i długość brodawki sutka (NL). Pomiary wykonano taśmą lub suwmiarką w trzech fazach cyklu reprodukcyjnego: luźności (przy kryciu), prośności (ciąża wysoka, 7 dni przed porodem) oraz laktacji (7. dzień karmienia prosiąt). Zgromadzono dane dotyczące liczby prosiąt urodzonych ogółem, urodzonych żywo i martwo oraz odchowanych do 21. dnia. Wyniki opracowano statystycznie. Stwierdzono różnice w BWMG przy kryciu ($P \leq 0{,}01$ w cyklu 3 i $P \leq \leq 0{,}05$ w cyklu 4) oraz w LML w okresie wysokiej ciąży i w laktacji ($P \leq 0{,}05$ w cyklu 3). W cyklu reprodukcyjnym poziom zmian wskaźników zoometrycznych gruczołu mlekowego był wyraźnie większy u loch PIC w porównaniu do Danhybrid, przy istotnie mniejszej liczbie prosiąt urodzonych ogółem i urodzonych żywo. Zróżnicowana intensywność przebudowy gruczołu mlekowego dwóch grup loch może być związana m.in. z zaawansowaniem rozwoju somatycznego (zmiany nieco większe w cyklu 4 względem cyklu 3) oraz odmiennością genetyczną (nieco większe zmiany u loch PIC w porównaniu z Danhybrid), oraz z lepszą żywotnością noworodków i większą aktywnością prosiąt z grupy PIC w porównaniu z Danhybrid w okresie ssania. Wyniki odchowu prosiąt można uznać za dobre, straty były mniejsze w grupie loch PIC względem wyników Danhybrid.

Słowa kluczowe: lochy, Danhybrid, PIC, gruczoł mlekowy, prosięta urodzone i odchowane

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Authors' address:
Justyna Więcek
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach
Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: justyna_wiecek@sggw.pl

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Influence of transport on selected quality factors of rabbit meat

JOANNA SKŁADANOWSKA-BARYZA, AGNIESZKA LUDWICZAK, ŁUKASZ SIKORA, MARTA BYKOWSKA, MAREK STANISZ
Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences

Abstract: *Influence of transport on selected quality factors of rabbit meat.* The aim of this study was to examine the influence of transport on the quality characteristics of rabbit meat determining its technological usefulness. Meat from 20 hybrid rabbits (male crossbreds of two French hybrids: Martini and Hyla) aged 90 days was analysed in the study. The control group (10 rabbits) was transported after weaning (at the age of 50 days) and was fattened at the experimental farm until the age of 90 days. The experimental group (10 rabbits) was transported directly prior to slaughter. After 24 h and 48 h from the slaughter the pH in the experimental group was higher ($P \leq 0.01$) compared to pH of meat in the control group. The transport significantly affected L* measured 48 h post-slaughter. Rabbit meat from the experimental group was darker compared to meat from the control group ($P \leq 0.01$). The transport also caused a higher a* measured 45 min post-slaughter compared to the control ($P \leq 0.01$). The meat of rabbits fattened in the experimental farm characterised with significantly lower drip loss, free water and plasticity compared to the meat of rabbits transported directly prior to slaughter. On the basis of the research results one can conclude that the transport taking place directly before slaughter negatively affected the quality of rabbit meat, leading to abnormal quality conditions expressed with high pH and dark colour.

Key words: rabbit meat, transport, meat quality, transport, stress, DFD

INTRODUCTION

Nowadays a lot of attention is drawn to the origin of meat and meat quality. Due to increasing consumer awareness, dietary and health benefits of rabbit meat have been noticed (Petracci and Cavari 2013. The quality of rabbit meat is affected by the breed (Maj et al. 2012), nutrition (Gebler 2008), sex and age (Hernandez et al. 2004), body weight before slaughter and animal welfare (Zając et al. 1998). However, one of the major factors affecting the quality of meat is the pre-slaughter stress associated with transport and slaughter (Maj et al. 2012).

Studies on meat have shown that transport increases serum cortisol levels and has a negative effect on the quality of meat, which in turn classifies meat into PSE (pale, soft, exudative) meat quality or DFD (dark, firm, dry), as a consequence the pH value does not reach a level providing the microbiological stability of meat (Dal Bosco et al. 1997, Kowalska et al. 2016). The PSE abnormal condition is directly related to low water binding capacity. In spite of the basic effects, such as lowering the processing value, the meat is less often chosen by consumers, due to the worse organoleptic qualities (Kristensen and Purslow 2001). The DFD rabbit meat is dark, dry and highly viscous. The meat also characterizes with a reduced durability. The cause of this abnormal condition is too little glycogen in the muscle at the time of slaughter, as a consequence the

pH value does reach a level providing the microbiological stability of meat. One of the causes of this defect is the improper handling of the animal before slaughter (Rodríguez-Calleja et al. 2005).

Because the number of slaughter houses adapted to slaughter rabbits is limited, the commercial production of rabbits for meat is connected with a necessity of transport. Rabbits are known to be prone to stress, therefore all the research related to influence of transportation on rabbit meat quality provide valuable information on the proper pre-slaughter handling of this species. The aim of this study was to evaluate the effect of transport on quality characteristics of rabbit meat.

MATERIAL AND METHODS

The analysed material included 20 hybrid rabbits (crossbreds of two lines: Martini and Hyla, males) divided into two groups: the control group and the experimental group. Up to 50 days of age the animals were raised in the same rabbitry, and were fed the same diet. All rabbits were slaughtered at the age of 90 days at the experimental farm. The control group (10 rabbits aged 50 days) was transported in metal cages directly after weaning and fattened at the experimental farm until the age of 90 days. During the fattening period the animals were kept in the same groups as before weaning and during the transport. The rabbits (10 heads) from the experimental group were transported directly prior to slaughter. The time gap between the beginning of transport until the slaughter (including the loading of the rabbits and the transport time covering 120 km with an average speed of 50 km/h) was about 4 h. The rabbits were transported in metal cages, 4 rabbits per cage. The transport was carried out in the summer, early in the morning, at an environmental mean temperature of 16°C. Both groups were fasted 24 h prior to slaughter, with unlimited access to water. Prior to slaughter the animals were weighed. The slaughter included mechanical stunning (hit in the back of the head with a narrow rod) immediately followed by cutting the jugular veins (according to Council Regulation 1099/2009). The animals were hung by the hind limbs in order to allow bleeding out. After dressing, rabbit carcasses were kept at +2°C. Twenty-four hours post mortem the carcasses were weighed (cold carcass weight, kg) and the dressing percentage was calculated as a relation of the cold carcass weight to the pre-slaughter body weight of animals. Twenty-four hours after slaughter the right and left *m. longissimus thoracis et lumborum* (LTL) were cut from the carcasses in order to examine the quality of rabbit meat.

The pH was measured by inserting a calibrated combination glass calomel electrode (ERH-11X1,SCHOTT, Germany) connected to a portable pH meter (Handylab 2, SCHOTT, Germany) into the LTL. The pH was measured 45 min, 24 h and 48 h post mortem. The first measurement was made on the right LTL of carcasses, after dressing. The two following measurements were made after dissecting the LTL muscles from the carcasses.

The first colour measures were recorded 45 mins post mortem on the muscle surface, after removing the con-

nective tissues covering the lumbar part of the LTL. The colour was measured using a Minolta colour meter CR-200b (Konica Minolta, Netherlands) (illuminant D65, 2° observer with a eight-millimeter in diameter aperture size). The tristimulus CIE system which measures lightness (L*), redness (a*) and yellowness (b*) was used (CIE 1978). Colour measures were repeated 24 h and 48 h post mortem on the dissected muscles, in the previously defined point of measurement.

The drip loss, free water, cooking loss and plasticity were measured 24 h post mortem.

The drip loss (%) was measured after Honikel (1998). The three-centimeter thick, transverse slices of the LTL (25––30 g) were weighed, hung on hooks and placed in a container to reduce evaporation (+2°C). After 24 h, the samples were reweighed to calculate the change in the weight.

The free water (%) was measured using a filter-paper press method, after Grau and Hamm (1953) in modification of Pohja and Niinivaara (1957). Samples (0.3 g) of ground meat were placed on a filter paper between two glass tiles. A force of 2 kg was applied on each sample for 5 min. Then the samples were removed from the filter paper and reweighed straight after to calculate the change in the weight. The calculations were made using the following formula:

free water (%) = (*sample of ground meat* – *sample of meat after* 5 *min of* 2 *kg pressure*) · 100 / *sample of ground meat*)

Meat plasticity (cm^2) measurement was conducted according to Pohja and Niinivara (1957), simultaneously to the free water measurement and was expressed as the area of the compressed meat sample used for the measurement of free water.

The cooking loss (%) was measured after Honikel (2004). The three-centimeter thick, transverse slices of the LTL (25–30 g) were placed in thin polyethylene bags, with the bag's wall firmly adhered to the meat sample. The bags with meat were placed in a water bath at 75°C for 30 min, and then cooled to room temperature and reweighed after removing the excess of moisture with a paper towel. The change in the weight of the sample was calculated (%).

For the analysis of the chemical composition, muscle samples were minced in a food grinder. To determine dry matter content, 3 g samples of minced meat were dried in filter-paper bundles at 105°C to a constant weight (PN-ISO 1442:2000). For the protein content the Kjeldahl procedure was used (PN-A--04018:1975). The samples were boiled in concentrated H_2SO_2 for 30 min. The digest contained a catalyst (a mixture of $CuSO_4$ and K_2SO_4). The process ended with the acquisition of ammonium sulfate solution – $(NH_4)_2SO_4$. The distillation was performed by means of a B-324 Buchi distillation unit, using NaOH and H_3BO_3 solutions. The last phase was titration (Schott TitroLine, SCHOTT, Germany), which allows to quantify the amount of ammonia in the receiving solution (H_3BO_3) with the use of 1 M HCl. For the extracted fat content the Soxhlet method was used (PN-ISO 1444:2000). The samples used for determination of dry-matter content were placed in the Soxhlet extractor (MLL 147, AJL Electronics, Poland).The solvent used in the

procedure was petroleum ether. The effect of the group (control, experimental) on the body weight at slaughter, drip loss, free water, cooking loss, plasticity, dry matter, crude protein, extractable fat, water/crude protein ratio was calculated with the model given below.

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where:
μ – the overall mean of the analysed trait;
α_i – the fixed effect of the i-group ($i = 1, 2$);
e_{ij} – the random error.

The effect of the group (control, experimental), and time post mortem on the pH (pH_{45min}, pH_{24h} and pH_{48h}) and muscle colour L* (L^*_{45min}, L^*_{24h} and L^*_{48h}), a* (a^*_{45min}, a^*_{24h} and a^*_{48h}) and b* (b^*_{45min}, b^*_{24h} and b^*_{48h}) was calculated with the model given below.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{j(k)} + e_{ijk}$$

where:
μ – the overall mean of the analysed trait;
α_i – the fixed effect of the i-group ($i = 1, 2$),
β_j – the random effect of the j-animal;
$\gamma_{j(k)}$ – the effects of the k-time post mortem ($k = 1, 2, 3$) nested in the j-animal;
e_{ijk} – the random error.

There was no interaction between analysed effects, therefore they were not included in the model. All the statistical analysis were made with SAS (2011). Tukey–Kramer adjustment was implemented for multiple comparisons of LS-mean differences.

RESULTS AND DISCUSSION

The presented study revealed that the examined groups of rabbits did not differ with the body weight at slaughter (Table 1). In study conducted by Mazzone et al. (2010) animals treated gently during loading characterised with smaller weight loss (2.78 vs 3.0 %) and higher dressing percentage (61.0 vs 60.8%) compared to the rabbits treated roughly.

TABLE 1. Body weight of rabbits aged 90 days

Trait	Control group	Experimental group	Group effect
	LSM ±*SE*	*LSM* ±*SE*	
N	10	10	
Body weight (kg)	3.21 ±0.12	3.08 ±0.11	Ns

Ns – non-significant.

Presented results did not show the effect of the group on the pH measured in the rabbit meat 45 min after slaughter ($P > 0.05$). However, 24 h and 48 h post mortem the pH value in the experimental group was higher than in the control group ($P \leq 0.01$) (Table 2). In the control group a gradual decrease in the pH value was observed indicating a correct course of post mortem glycolysis. Dal Bosco et al. (2004) found that long distance transport caused higher initial (pHi) and final (pHu) pH value of meat compared to short transport (pHi: 6.35 vs 5.99; $P \leq 0.01$; pHu: 6.35 vs 5.99; $P \leq 0.05$). Opposite to the results of the Dal Bosco et al. (2004), Maria et al. (2006) observed that the transport duration did not affect

TABLE 2. The pH value of rabbit meat

Time	Control group	Experimental group	Group effect
	LSM ±*SE*	*LSM* ±*SE*	
45 min	6.66 ±0.05 AB	6.68 ±0.05 AB	Ns
24 h	5.81 ±0.05 A	6.22 ±0.05 A	**
48 h	5.78 ±0.05 B	6.20 ±0.05 B	**

The effect of the measurement time is indicated in the columns. Means within the same column with the same letters (A, B) are significantly different at $P \leq 0.01$; ** $P \leq 0{,}01$, Ns – non-significant.

the pH value in rabbit meat (1 h transport, pH 5.86 and 7 h, pH 5.83; $P > 0.05$). Similarly to Maria et al. (2006), Liste et al. (2008), also did not observed effect of the transport time on the pH value of rabbit meat. Study conducted by Lambertini et al. (2006) showed the effect of transport on the pH value measured 15 min and 24 h after slaughter, with lower acidification of the muscles characteristic for rabbits subjected to longer transport ($P \leq 0.05$).

In presented study transport had a significant impact on L* measured 48 h after slaughter. The meat of the experimental group was darker compared to the control ($P \leq 0.01$). The redness of the meat was affected by the time post mortem in both groups ($P \leq 0.01$). For both rabbit groups no changes of b* have been found in the analysed time periods ($P > 0.05$) – Table 3. Trocino et al. (2003) and Liste et al. (2008) reported that rabbits submitted to transport characterize with lower L* and higher a* compared to non-transported ones. Dal Bosco et al. (1987) observed darker, redder and yellower meat from rabbits exposed to long transport compared to the rabbits exposed to short transport (L* = 44.3 vs 61.4; a* = 24.2 vs 20.1; b* = 9.6 vs 3.1; $P \leq 0.01$).

In the presented experiment, the meat of rabbits transported directly after weaning characterised with lower drip loss, lower free water content and lower plasticity compared to meat of rabbits transported 24 h prior to slaughter ($P \leq 0.01$) – Table 4. The differences between analysed groups are related to the pH of rabbit meat. High pH value leads to increase capacity of muscle tissue to hold the residual water, expresses by lower drip loss and lower free water content. There were no differences between groups in the amount of cooking loss ($P > 0.05$). Trocino et al. (2003) also reported no effect of transport on the level of cooking loss in hybrid rabbits' meat ($P = 0.99$). Opposite to Trocino et al. (2003) and to the presented study, Dal Bosco et al. (1987) showed that meat of hybrid rabbits exposed to a long transport characterizes with a lower thermal drip (28.93 vs 31.98%; $P < 0.01$) and a higher drip loss (2.34 vs 1.69%; $P < 0.01$) compared to rabbits exposed to shorter transport time. Mazzone et al.

TABLE 3. The colour of rabbit meat

Trait	Time	Control group	Experimental group	Group effect
		LSM ±*SE*	*LSM* ±*SE*	
L*	45 min	48.58 ±1.01 A	48.22 ±1.01	Ns
	24 h	50.22 ±1.01 B	47.65 ±1.01	Ns
	48 h	55.56 ±1.01 AB	46.53 ±1.01	**
a*	45 min	0.79 ±0.66 A	3.93 ±0.66 A	**
	24 h	4.21 ±0.66 AB	4.22 ±0.66 B	Ns
	48 h	0.08 ±0.66 B	–0.28 ±0.66 AB	Ns
b*	45 min	5.98 ±0.65 a	6.02 ±0.65 a	Ns
	24 h	7.92 ±0.65 ab	6.05 ±0.65 b	Ns
	48 h	5.67 ±0.65 b	4.19 ±0.65 ab	Ns

The effect of the measurement time is indicated in the columns. Means within the same column with the same letters a, b (A, B) are significantly different at $P \leq 0.05$($P \leq 0.01$); ** $P \leq 0.01$, Ns – non-significant.

TABLE 4. The water fractions and capacity to hold inner water by rabbit meat

Trait	Control group *LSM* ±*SE*	Experimental group *LSM* ±*SE*	Group effect
Drip loss (%)	1.12 ±0.14	1.74 ±0.14	**
Free water (%)	31.4 ±0.8	36.6 ±0.8	**
Cooking loss (%)	20.90 ±0.42	21.65 ±0.42	Ns
Plasticity (cm^2)	4.22 ±0.11	4.53 ±0.11	*

* $P \leq 0{,}05$, ** $P \leq 0{,}01$,Ns – non-significant.

(2010) found a higher drip loss (3.21 vs 3.10%; $P < 0.05$) and lower thermal drip (17.92 vs 19.76%; $P < 0.05$) in the group of rabbits loaded in a gentle manner compared to rabbits treated roughly during the loading process. In the study conducted by Bianchi et al. (2010) hybrids had a drip loss of 1.02–1.17%. Opposite to our results, Apata et al. (2012) and Dal Bosco et al. (2004) obtained higher value of free water in meat of hybrid rabbit (50.20–62.37% vs 62.92–66.70%, respectively). The plasticity of meat is strongly related with its capacity to hold residual water. Hogh plasticity is a results of high fluidity of meat Grau and Hamm (1957). In presented study, the meat from the control group characterised with higher plasticity compared to the experimental group ($P \leq 0.05$).

The proximate chemical composition of the LTL of hybrid rabbits was not affected by the transport ($P > 0.05$) – Table 5. Similar results were obtained by

TABLE 5. The proximate chemical composition of rabbit meat

Trait	Control group	Experimental group	Group effect
	LSM ±*SE*	*LSM* ±*SE*	
Dry matter (%)	23.88 ±0.25	23.86 ±0.25	Ns
Crude protein (%)	21.59 ±0.41	21.86 ±0.41	Ns
Fat (%)	0.67 ±0.06	0.74 ±0.06	Ns
Water/Crude protein	3.54 ±0.07	3.48 ±0.07	Ns

Ns – non-significant.

Gasperlin et al. (2010), Metzger et al. (2006) (protein – 22.8%, fat – 0.49%) and Tumova et al. (2014) (dry matter – 25.3%, protein – 23.3%, fat 0.56%). Daszkiewicz et al. (2011) obtained similar results of water/crude protein ratio between *m. longissmus* and the leg muscle ($P \leq 0.01$).

CONCLUSIONS

On the basis of the presented results one can conclude that the transport taking place directly prior to slaughter negatively affected the quality of rabbit meat, causing the development of DFD-like abnormal quality. Due to limited shelf life and darker colour DFD meat is less useful for culinary and technological purposes and less attractive for consumers.

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Streszczenie: *Wpływ transportu na wybrane cechy jakościowe mięsa króliczego*. Celem doświadczenia była ocena wpływu transportu na cechy jakościowe mięsa królików decydujące o jego przydatności technologicznej. Badaniu poddano mięso pochodzące od 20 królików hybrydowych (samce, krzyżówka dwóch linii francuskich: Martini i Hyla) ubitych w wieku 90 dni. Grupa kontrolna (10 królików) została przetransportowana po odsadzeniu (wiek 50 dni) i utrzymywana na terenie gospodarstwa doświadczalnego do wieku 90 dni. Grupa eksperymentalna (10 królików) została przetransportowana tuż przed ubojem. Analizowane króliki brojlerowe nie różniły się istotnie masą ciała. Po 24 i 48 h pH w grupie było istotnie wyższe ($P \leq 0.01$) od tego w grupie kontrolnej. Transport miał znaczący wpływ na L* mierzone po 48 h od uboju. Mięso królików z grupy eksperymentalnej było ciemniejsze w porównaniu do królików z grupy kontrolnej ($P \leq 0.01$). Transport spowodował także większą wartość a* w grupie eksperymentalnej w stosunku do grupy kontrolnej ($P \leq 0.01$) 45 min po uboju. Mięso królików przewiezionych do gospodarstwa tuż po odsadzeniu charakteryzowało się znacznie mniejszym wyciekiem naturalnym, mniejszą zawartością wody wolnej oraz mniejszą plastycznością w porównaniu do mięsa królików przetransportowanych tuż przed ubojem ($P \leq 0.01$). Na podstawie wyników wnioskuje się, że transport mający miejsce bezpośrednio przed ubojem doprowadził do obniżenia jakości mięsa, powodując zbyt małe zakwaszenie poubojowe mięśni, a co za tym idzie ciemną barwę i dużą wodochłonność charakterystyczną dla tego typu surowca mięsnego.

Słowa kluczowe: mięso królicze, jakość mięsa, transport, stres, DFD

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Authors' address:
Joanna Składanowska-Baryza
Katedra Hodowli Zwierząt i Oceny Surowców
Wydział Medycyny Weterynaryjnej i Nauk
o Zwierzętach
Uniwersytet Przyrodniczy w Poznaniu
ul. Słoneczna 1, 62-002 Suchy Las, Złotniki
Poland
e-mail: jskladanowska92@gmail.com

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Management of hunting animals population as breeding work. Part II: Hunting and breeding work on red deer (*Cervus elaphus*) and elk (*Alces alces*) populations

KATARZYNA TAJCHMAN, LESZEK DROZD
Faculty of Biology, Animal Science and Bioeconomy, University of Life Sciences in Lublin

Abstract: *Management of hunting animals population as breeding work. Part II: Hunting and breeding work on red deer* (Cervus elaphus) *and elk* (Alces alces) *populations.* The paper presents the aim and rationale behind hunting and breeding practices in hunting districts. This part of the article series describes problems and errors in the management of game populations. The example of red deer and elk breeding shows that the difficulties faced by hunters in estimation of the game population size exert a huge impact on the implemented breeding procedures. Currently, there are no excellent methods for animal stocktaking; therefore, there are problems with determination of appropriate animal harvesting, which may result in overpopulation or a drastic decline in the population size. The study indicates that improvement of living conditions by management of habitats and maintenance of appropriate densities through translocation or periodic protection of species not only results in improvement of the ontogenic quality and fitness of animals but also offers the opportunity to restore populations.

Key words: breeding, game animals, stocktaking, translocation

INTRODUCTION

For several years, there has been a steady increase in the size of big game species populations. As described in the first part of the article, excess densities of animals have a disastrous effect both on the living habitat and on the game animals themselves. Presumably, the increase in the number of animals is caused by inappropriate methods for annual stocktaking, i.e. estimation of the size of game species population in hunting districts. In a majority of hunting districts, the stocktaking is reported to be based on year-round observations of animals. Since there are no methods for such observations, these may be only subjective estimates made by hunters, which sporadically reflect the population size of game animals. A result of such hunting management is the overpopulation of red deer, elk, and wild boars despite the apparently adequate culling schemes. Constant stress as well as a shortage of food and proper shelters lead to deteriorated immunity, increased prevalence of diseases, weakness, and mortality (in winter).

An attempt to improve the management of game populations was the establishment of hunting complexes by grouping hunting districts in 1997. Populations of game animals, especially larger or mobile ones, are not confined to single ecosystems but function within higher-order units, i.e. landscapes (Beszterda and Przybylski 2011). A principle was adopted that hunting complexes should take into account free migration of large mammals within larger forest complexes and limit errors in estimation of their

population size. Methods for estimation of the number of animals that disregard species biology and migrations yield inadequate schemes of animal harvesting. Overestimation and absence of proper selection practices have frequently led to a population decline, as in the case of elk or red deer population management, which resulted in translocation of *Cervus elaphus* by hunters and foresters or implementation of all-year species protection plans for *Alces alces*. Livestock breeders never encounter this type of difficulties. Therefore, game animals should be treated with particular care, taking into account the complexity of factors affecting their populations.

RED DEER POPULATION MANAGEMENT

Proper animal translocation can yield tangible benefits for nature conservation and hunting management. However, improper implementation thereof may contribute to destabilisation of local ecosystems. As specified in the definition proposed by the International Union for Conservation of Nature (IUCN), translocation is "premediated movement of living organisms from one area, with release in another" (IUCN 1987). These human-mediated practices have always been and still are carried out for three fundamental reasons: reintroduction, introduction, and improvement of the ontogenic quality of a local population. A good example of translocation targeted at reinforcement of local populations was the resettlement of the red deer throughout Europe, including Poland. These practices involved reinforcement of local (small-size) populations with additional individuals and, consequently, with a new genotype, although this aspect was not considered at that time (Borowski et al. 2013).

Humans have had a huge impact on the diversity of red deer populations since the beginning of settlement. In the Middle Ages, the species was one of the major game animal. Poaching was punished with the death penalty at that time; hence, a relatively large population size of deer was maintained. According to historical sources, King Władysław IV harvested 50 deer within 4 hunting days in Niepołomicka Primeval Forest in 1644 (Rokosz 1984 after Bobek et al. 1992).

In the Polish territories in the 19th century, red deer inhabited almost exclusively the land west of the Vistula river. In Białowieża Primeval Forest, red deer were extinct probably at the turn of the 17th and 18th centuries. In 1865, 18 red deer from Silesia were introduced and kept in a closed menagerie. Unfortunately, they had low body and antler weight probably due to low-quality nutrition. Only those animals that had managed to escape had stronger antlers. To "refresh the blood", deer from Spała, Silesia, the Carpathians, and Czech were introduced in Białowieża Primeval Forest in 1881; in total, 500 deer were introduced in the area in the second half of the 19th century. By 1914, the deer population in this region had increased to approx. 6,800 individuals. The red deer translocation brought the expected results and the population size of this species began to grow rapidly; however, the poaching practice, which was common during World War I, again reduced the population of the species in Poland. At the beginning of the 20th century, there was relatively strong

migration of deer from Białowieża Primeval Forest to Lithuanian forests, where they had been almost eradicated at the turn of the 19th and 20th centuries. At the same time, there were numerous red deer populations in Poznan Province, Pomerania, and the Carpathians (Sztolcman 1920 after Bobek et al. 1992).

Probably by the 17th century, the red deer had not been a permanent component of the Tatra fauna. The present population originates mainly from the animal menagerie maintained in 1850–1885 in Jaworzyna. The red deer in the menagerie originated from Czech, Slovakia, Ukraine, and England; there were also Asian and North American deer. After the fall of the estate, the red deer spread in the area, giving rise to the current population inhabiting the Tatra Mountains (Brągiel 1973 after Bobek et al. 1992).

Similarly, the red deer population size in Warmia and Mazury fluctuated substantially. Before the 17th century, the deer was regarded as a common species in this area. Intensive hunting and random exploitation of forests caused a rapid drop in the number of the animals. The problem was further aggravated and, in the 1930, only migrating red deer were observed in the surroundings of Mikołajki, whereas those from Piska Primeval Forest were extinct. As late as in the second half of the 19th century, the population size slowly increased and the 1897 stocktaking documentation reported approx. 1,600 deer. In 1900, restoration of red deer population was undertaken in Warmia and Mazury. To this end, red deer were brought from Hungary, surroundings of Potsdam and Berlin, and Romint. Thirthy eight years after the reintroduction, there were 2,000 individuals in this area. In 1938, 8,827 red deer were inventoried, and the culling rate in the 1936/1937 season was 3,179 animals, which was nearly 10-fold higher than in 1885/1886 (Dzięgielewski 1970).

After World War II, the population of this species declined again throughout the country. A particularly pronounced decrease in comparison with the prewar period was reported from Spała, Białowieża, and Bieszczady forests. In 1956, translocation of red deer mainly from western provinces to former Białystok, Łódź, Lublin, and Warsaw Provinces was initiated. The targeted breeding activities had resulted in relocation of 1,027 individuals by 1967 (Dzięgielewski 1970).

Until 1939, red deer were present only in three regions of the central-eastern Poland macroregion. They inhabited Solska Primeval Forest and Adampol forests in the Sobibór-Włodawa forest complex, where red deer were re-introduced in 1895, and there were few in Kozłówka forests. As indicated by data from 1928, the population of this species in the state forests of former Lublin Province comprised only 8 animals. During World War II, the red deer population in Lubelszczyzna was completely eradicated and the last individual died in 1948. Until the mid-1950s, red deer were temporarily present in the south of Lubelszczyzna as migrating animals. In 1958, the first attempts were made to restore the red deer population in this area, and 271 individuals were introduced from different parts of Poland. A majority of the red deer originated from Wielkopolska, Warmia and Mazury, Pomerania, and surroundings of Opole and Katowice. The reintroduction was carried out in nearly all

large forest complexes of Lubelszczyzna. The greatest number of individuals was brought to Józefów, Biłgoraj Janów Lubelski, and Lubartów Forest Districts. The intensive breeding work carried out by foresters and hunters resulted in a substantial increase in the deer population size. Currently, the red deer inhabits all Forest Districts in Lubelszczyzna. Numerous populations of this species are also present in Warmińsko-Mazurskie, Zachodniopomorskie, Dolnośląskie, Opolskie, and Podkarpackie Provinces (Bobek et al. 1992, Drozd et al. 2000).

At the end of the 20th and the beginning of the 21st centuries, the red deer population size in Poland increased. The clear rise persisted until 2013 when the population of this species in the country was 2.5-fold greater than in 1996. Deer harvesting increased as well and reached 70.1 thousand animals in the 2014/2015 season, which was two-fold higher than 10–15 years earlier. In recent years (2012/2013–2015/2016), the red deer population size stabilised at a level of 128 thousand individuals in leased hunting districts. The deer-harvesting rate per unit of forest area, which is an indicator of the density of the species, was higher in the west of Poland than in the other regions and especially in the centre of the country (Panek and Budny 2015).

Red deer individuals from different regions of the current occurrence range of the species differ significantly in their body size, weight, and antler shape, even in nearby hunting grounds. Animals inhabiting the Carpathians and Mazury regions are characterised by high body weight, compared with the individuals from the central and western part of the country (Bobek et al. 1992, Szczepański et al. 2006). This variability is confirmed by the genetic diversity of deer originating from the different habitats. It is inconsiderable, as shown by a country-scale analysis of the populations, whereas high local genetic variation has been revealed (Borowski et al. 2013). Such a pattern of spatial genetic variation is very likely to illustrate the impact of the historical translocations on the current population structure of this species. Besides the justifiable restoration practices described above, one of the basic targets of animal translocations was the improvement of the ontogenic quality of stags by upgrading the quality of individuals in the population. In this end, individuals from populations characterised by high body and antler weight were reintroduced. Despite all these attempts, the living environment (rather than the genotype) plays a major role in determination of the animal phenotype, as indicated in many studies mentioned in the first part of the article series (Borowski et al. 2013). This variability is probably caused by the food supplies with their varied nutritional value and the population density; all these factors are influenced by humans. As shown by Łabudzki (1993), stags living in the former Olsztyn Province weighed 79.26 kg in the second year of life. Investigations conducted in the central-eastern Poland macroregion have shown that the mean weight of stags at the same age was 85.32 kg (Krupka et al. 1986). In the analysis of material collected in three hunting grounds (former Olsztyn, Lublin, and Katowice Provinces). Dzięciołowski (1970) determined the mean the average weight of does in the following age groups: 1–3 years – 67 kg, 4–7 years – 82 kg, 8–11 years

– 89.3 kg, and older – 79.5 kg. The study presented by Łabudzki (1993) demonstrated that the mean carcass weight of 3,500 hinds from the former Olsztyn Province was 76.09 kg. In turn, female individuals from Wielkopolska Province weighed 70.19 kg on average. Comprehensive analyses of carcass weight in deer from Warmia and Mazury Province hunting grounds were carried out by Janiszewski and Szczepański (2004). The authors showed the following values of average carcass weight determined during 15 hunting seasons: stags – 114.5 kg, does – 76.6 kg, and calves – 43.5 kg. Changes in body weight (even by ca. 30 kg) may be influenced by the hunting season as well as various external factors such as weather conditions and anthropopressure or may be associated with the behaviour and physiological status of the animal (oestrus, pregnancy, lactation etc.). Every year, two factors indisputably induce reduction of body weight in stags: the rut period and the deteriorating feed supply in late autumn or in winter (Bobek et al. 1984). During the rut period, stags hardly feed, which results in loss of up to 25% of their weight over approximately 4 weeks (Krupka et al. 1986, Drozd et al. 2000). It has been shown that body weight in stags is accumulated earlier in rich habitats than in poor ones (Grudziński et al. 1972). Improvement of the habitat in terms of nutritional requirements of Cervidae is an important factor in the proper management of healthy populations of these animals. Nutrition is one of the three main determinants (besides age and genetics) of antler growth and body size in animals (Landete-Castillejos et al. 2013). Heritability in three populations analysed in the world has been found to vary in the range from 0.27 to 0.36. This indicates that the genetic component is responsible in 30% for inheriting the antler weight by male deer from parent animals (females transfer antler weight encoding genes as well). Other factors, such as the environment quality and climate, are responsible for the other 70% of the heritage probability (Borowski et al. 2013).

In 2003–2010, an analysis of the dependence of body weight on the population density in the white-tailed deer (*Odocoileus virginianus*) was carried out in the De Soto National Nature Refuge in eastern Nebraska. The density ranged from 36.5 to 50.5 individuals per km^2, whereas the cropland cover ranged from 14.9 to 23.2%. It was demonstrated that the deer body weight was inversely proportional to the density (21.4 kg per 5.5 deer per km^2) and proportional to the increasing crop area (21.3 kg to 3.1% of conversion of total land area to grassland). It was also shown that the estimated density of the white-tailed deer had to be reduced by 1.7 deer per km^2 per each 1% of conversion of total cropland area to grassland; this would ensure maintenance of appropriate body weight by the animals. In agricultural areas, female deer can consume more crops than stags, which indicates that hinds are more sensitive to changes in the agricultural land coverage. An inverse correlation between body weight and density was reported for both sexes and all age classes of deer (Hefley et al. 2013). Furthermore, another study of red deer demonstrated higher dependence of animal fitness and fertility on the density than on other factors, e.g. ambient temperature and precipitation. The effects of varying nutrition result-

ing from overpopulation during summer (the period of accumulation of supplies) had a significant effect on the fitness and reproduction. Fewer females conceived as the density increased, since their ontogenic quality declined. The percentage of two-year-old pregnant does was significantly lower in an area characterised by high density (20.1 individuals per km^2) than at lower density (4.1 individuals per km^2). However, larger differences were observed in middle-aged females (4–9 years) and the highest differences were noted in six-year-olds (the age of the highest productivity) (Stewart et al. 2005). The density of a Cervidae population is another determinant of animal health and body weight (Kie et al. 1983, Keyser et al. 2005), and this factor can be managed by hunters and foresters. As shown by the examples discussed above, hunting and breeding practices have an effect on not only animal condition but also female fertility and the health of offspring.

ELK POPULATION IN POLAND

The fate of the elk in Poland was slightly different. As demonstrated by historical records and archaeological excavations, under the pressure of a rapidly growing human population, the elk with its large population size across the forest zone of Europe, shared the fate of the aurochs, bison, and tarpan (Raczyński 2006). Poaching and increasing demand for skins led to eradication of elk in most western and central European countries. At the beginning of the 19th century, it was classified as a nearly extinct species in Sweden, Finland, Russia, or Poland (Rülcker and Stalfelt 1986, Schmölcke and Zachos 2005). In Poland, the degree of the elk population collapse was so high that the forests near Rajgród in the Biebrza river valley were the only westernmost refuge of this species (Brincken 1826). The distribution of elk in Poland did not change substantially until the interwar period when the species was present only in the eastern regions of the country (Raczyński 2006). A population consisting of several individuals inhabiting the Biebrza river valley was the only one to have survived World War II. This was possible thanks to the establishment of the Czerwone Bagno Reserve in 1925 with the aim of preservation of the occurrence of this rare species in Poland at that time (Lublinerówna 1935). The elk population from this area managed to survive World War II and gave rise to a group consisting of descendants of the native population, which was continued through elk restoration in this area in the 1950 (Dzięciołowski and Pielowski 1993, Raczyński 2006). The species was fully protected in Poland by virtue of the Regulation of the Minister of Forestry from 1952 until the publication of the Act of 17 June 1959 On breeding, protection of game, and hunting law. The Act defined elk as game animals with an all-year protection period, which in practice preserved the protection status of this species (Raczyński 2006). Besides the Biebrza population (developing since the late 1940s), a dynamic increase in the elk population size was also observed in the Kampinoski National Park. In contrast to the animals from the Biebrza river valley, this population was established by humans in 1951 by translocation of three young cows and

two bulls from Belarus (Dzięciołowski and Pielowski 1993). For about seven years, the animals and their offspring were kept in a specially prepared fenced area. The elk were released after a few years and they formed a new population (Dzięciołowski and Pielowski 1993). As shown by Gębczyńska and Raczyński (1999), the dynamic development of the Kampinos population led to colonisation of areas in western Poland by the elk and emergence of previously non-existent local populations.

After World War II, there was the so-called demographic increase of elk in the period from 1950 to 1970s. This was caused by the restoration of the species in Western Siberia, Kazakhstan, and the Baltic zone. The main breeding and hunting practices implemented by humans included introduction of rational population management principles, reduction of the abundance of predators threatening the elk population such as wolves, enlargement of the feed base by intensification of forest management, and establishment of large areas of forest crop cultivation and coppices (mainly pine).

As a large herbivore, the elk exerts a significant effect on the phytocoenoses and forest communities of its habitat (Hofmann 1985, Ratkiewicz 2011). As a result of the above-mentioned activities, the population size in the country in 1981 was 6,200 individuals, as indicated by the official hunting statistics (Dzięciołowski and Pielowski 1993, Gębczyńska and Raczyński 2001). As suggested by Raczyński (2006), an important role in the population restoration was played by the natural migration of elk from the territory of Belarus, Lithuania, Kaliningrad District, and Ukraine. Unfortunately, the excessive hunting exploitation in the 1980s and 1990s reduced their population size to 0,25 of the numbers recorded at the beginning of the 1980s. This reduced the occurrence range of the species in Poland. As in the case of other Cervidae, elk stocktaking carried out with the available tools and methods has serious limitations, which results in errors in the design of other breeding practices, e.g. reduction by culling. In 2001, the Minister of the Environment imposed a *moratorium* on elk harvesting, and 16 years of protection contributed to restoration of the population size. According to official statistics, the number of elk increased in 2013 to approximately 16 thousand individuals. Currently, there are three main elk refuges in Poland. The largest one, comprising ca. 70% of all living individuals, is the population in the northeast of Poland together with the Biebrza population. Another refuge is the population inhabiting the Kampinoski National Park and neighbouring forestry districts; it originates from individuals reintroduced from Belarus and from descendants of a Swedish stag introduced through secondary translocations from the Białowieża Primeval Forest (Karpiński 1951, Świsłocka 2014). The third group is formed by elks living in the Poleski National Park.

The annual rate of elk population growth within the last few years has decreased. It was approximately 20–25% at the beginning of the *moratorium* period; since 2008, it has dropped to 15% (Budny et al. 2010). This phenomenon is probably an effect of intrapopulation mechanisms triggered by an increase in population density (density dependence)

and primarily by the impoverishment of the feed base (Komenda 2001). Pullin (2004) has proved that the phenomenon can be observed when the species reaches half of the environmental capacity.

The elk population size in spring 2015 was estimated at 16.7 thousand animals. It was nearly 10-fold greater than at the turn of the 20th and 21st centuries, when the lowest population size over the last four decades was recorded (less than 2 thousand individuals) as a result of the decline in the 1990s. There was also an increase in the number of hunting districts inhabited by elk from ca. 400 at the turn of the centuries (8–9% of existing districts) to 1,500 in 2015 (32% of districts). The area of the occurrence of the species increased four times in this period. The highest elk density is reported from the northeast and east Poland, where 9–12 individuals per 1,000 ha of forests were noted in spring 2015 (Panek and Budny 2015).

As indicated by data on elk density in Estonia, the highest productivity of the species population (allowing maximum harvest) has been recorded at a density of ca. 5 animals per 1,000 ha of forest and wetland areas. In turn, the most optimal elk density in terms of species biology and ecology is 7–8 individuals per 1,000 ha, and the maximum capacity of forest and wetland habitats at which the population discontinues to grow is over 9 elks per 1,000 ha (Tõnisson and Randveer 2003). Assuming that similar parameter values can be adopted for the elk population within its permanent occurrence range in Poland, the population density of this species will range from 1–2 individuals per 1000 ha to ca. 10 individuals per 1,000 ha of forest and wetland areas (Ratkiewicz 2011).

Overpopulation and reduction of the feed base may be a stimulus for elks to wander over long distances in search of better refuges, which may result in expanding the occurrence range of the species (Dzięciołowski and Pielowski 1993). However, migrations of these animals are a cause of the increasing number of vehicle collisions, which unfortunately have very serious consequences. Maintenance of an appropriate density of the animals in a given area not only brings benefits to the game but also ensures safety to humans (Tajchman et al. 2017). The effect of multi-species and intense parasitic invasions on the condition of elk is yet unknown. Some of the recently recorded parasitoses may be a sign of the increased population density of these animals and environmental pressure (Filip et al. 2017).

The ranges of the elk population size presented above indicate that the density of this species in Poland has already been exceeded, and regulation of the population size by culling is an advisable hunting-breeding practice in this situation. It would prevent damage to forest complexes and reduce the danger associated with the frequent migrations (wildlife–vehicle collisions) as well as falls as an effect of epidemics or other pathologies.

CONCLUSIONS

Management of game populations may have not only positive effects, as in the case of roe deer, but also negative consequences related to errors resulting from

insufficient knowledge of animal biology and behaviour and, hence, inadequate application of stocktaking methods leading to overestimation of abundance and over-exploitation of game populations Therefore, a specific approach to each species, its requirements, and habitat preferences should be adopted. It should be borne in mind that populations of wild animals are subjected to the impact of external factors and environmental pressures. As proved in the case of red deer and elk, improvement of living conditions and maintenance of appropriate density values in habitats not only results in improvement of ontogenic quality of animals and health status but also offers a possibility to restore populations. An example of a hunting-breeding practice targeted at upgrading of the quality of local populations is the translocation of the red deer, which contributed to an increase in the population size and genetic variability of this species. Currently, research is being carried out on development of new reliable game counting methods, as further hunting and breeding procedures can be implemented only with sufficient knowledge of animal density and the quality of the habitat.

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Streszczenie: *Gospodarowanie populacjami zwierząt łownych jako hodowla. Część II: Prace łowiecko-hodowlane na przykładzie jelenia szlachetnego* (Cervus elaphus) *i łosia* (Alces alces). Praca przedstawia cel i zasadność prowadzenia zabiegów łowiecko-hodowlanych w obwodach łowieckich. W tej części cyklu artykułów przedstawiono problemy i błędy, którymi obarczone jest gospodarowanie populacjami zwierząt dzikich. Na przykładzie hodowli jelenia i łosia wykazano, że trudności, z jakimi spotykają się myśliwi przy szacowaniu liczebności gatunków łownych, mają ogromny wpływ na późniejsze zabiegi. Nie ma obecnie doskonałych metod inwentaryzacji zwierzyny, a w związku z tym pojawiają się problemy z określeniem odpowiedniego pozyskania, co może skutkować wystąpieniem przegęszczeń lub drastycznego spadku liczebności populacji. W pracy udowodniono, że poprawa warunków bytowania, poprzez kształtowanie środowiska życia oraz utrzymywanie odpowiednich zagęszczeń poprzez przesiedlanie lub okresową ochronę gatunku, prowadzi nie tylko do polepszenia kondycji osobniczej zwierząt i ich zdrowotności, ale też stwarza możliwość odbudowania populacji.

Słowa kluczowe: hodowla, zwierzęta łowne, inwentaryzacja, przesiedlenia

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Authors' address:
Katarzyna Tajchman,
Zakład Hodowli Zwierząt Dzikich
Katedra Etologii i Dobrostanu Zwierząt
Uniwersytet Przyrodniczy w Lublinie
ul. Akademicka 13, 20-950 Lublin
Poland
e-mail: katarzyna.tajchman@up.lublin.pl