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**A SELECTION OF PAPERS**

**Selected by Miloslav Ondrašovič**

**Košice — 2005**

# F O R E W O R D

*Ecological problems have become a serious issue on a worldwide scale. A systematic approach to these problems helps to increase the body of relevant knowledge and prevent negative phenomena that may adversely affect individual components of the environment. Each profession including the veterinary plays a more or less specific role in environmental protection.*

*With regard to this, we organised from June 16 to 17, 2005, the Vth International scientific conference „Ecology and Veterinary Medicine VI", which was dedicated to Associate Professors Vladimír Beňo, DVM, CSc. and Ľudovít Para, DVM, CSc., on the occasion of their anniversaries. Both of them with their scientific research results and teaching activities have contributed to the resolving of ecological problems and the successful organisation of previous conferences.*

*A specific objective of veterinary care on the environment in relation to human health is the prevention of zoonotic diseases and diseases that may develop as a result of the consumption of food containing residues of unwanted xenobiotics. The risk of pandemics spread through animals constitute an old but still persistent problem as indicated recently by avian influenza. Preventive measures taken against this disease stress the importance of the ecological dimension and the inevitability of the international collaboration of professionals involved in this area.*

*No less important are veterinary activities in the field of the monitoring of xenobiotic residues in live animals and products of animal origin that may eventually lead to the production of residuum-free foods and at the same time supply information needed for the taking of appropriate measures to ensure good quality animal products.*

*A topical task of the present is the calibration of Slovak legislation to EU standards. The ultimate target of all these efforts is an integrated prevention and pollution control concerning the environment as a whole integrating the steps and measures taken by individual countries and using the best available approaches. Within this process there is also space for veterinary care in the field of intensive animal rearing, food operations and processing of wastes from animal production.*

*Only a limited number of papers presented at our conference can be published in the Folia Veterinaria Supplement but they are all available in electronic form and can be obtained from the organisers of the conference. The issues discussed at preceding conferences and this one focused on environmental quality in relation to the health status and production of food. of animal origin. The conclusions point to the interest in these issues and a challenge to organisers to keep up with their work.*

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# RESULTS FROM THE CONTROL OF RESIDUES IN LIVE ANIMALS AND ANIMAL PRODUCTS PERFORMED BY VETERINARY ADMINISTRATION AUTHORITIES ACCORDING TO THE NATIONAL PLAN OF RESIDUE CONTROL

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## ABSTRACT

**The paper deals with results from the control of residues of foreign substances in live animals and animal products in Slovakia, the main and final aim of which is the control of the food chain in the interest of consumer health protection. It clarifies the principle and functioning of the monitoring system of foreign substance residues, the participation of veterinary administration authorities in the creation, implementation as well as the evaluation of this National Plan of Residue Control in Live Animals and Animal Products in the Slovak Republic. The paper explains the principle of the ordering of measures by veterinary administration authorities and subsequently the control of their implementation. It evaluates the results from the National Plan of Residue Control for the year 2004 in the Slovak Republic. In recent years considerable attention in the plans of control is paid to the control of live animals, in which the application and abuse of prohibited substances, which subsequently leave residues in animal products, are possible. A sufficient amount of high-quality raw materials and the use of Good Manufacturing Practice in obtaining hem guarantee the success in production of high-quality residue-free foodstuffs.**

**Key words: antibiotics; maximum residue limits; plan of control; prohibited substances; residues of inhibitory substances; suspect samples**

## INTRODUCTION

The State Veterinary and Food Administration of the Slovak Republic will elaborate the National Plan for Residue Control in Live Animals and Animal Products in the Slovak Republic yearly according to the Ordinance of the Government of the Slovak Republic on monitoring of certain substances and residues thereof in live animals and in animal products No. 320/2003 Coll. as amended when Council Directive 96/23/EC was transposed into the legal order of the Slovak Republic.

The State Veterinary and Food Administration of the Slovak Republic will evaluate the results of this monitoring program in the form of tables and reports, according to requirements of European Union experts and the results will also be sent yearly to the European Commission – Directorate General SANCO. Control of residues in live animals and products of animal origin leads in its final outcome to the production of residue-free foodstuffs and success in production of such foodstuffs depends to a great extent on the use of Good Practice in their reception. Monitoring and residue controls are performed at all levels of food production – through various monitoring programmes, plans, targeting controls and inspections performed.

## NATIONAL PLAN FOR RESIDUE CONTROL IN THE SLOVAK REPUBLIC

The aim of the control of foreign substance residues through the National Plan for Residue Control in Live Animals and

Animal Products is to obtain an overview on the current state of play of contamination of individual components of the food chain, performance of effective measures leading to the removal of identified deficiencies and prevention of entry of residues and their further occurrence. This shall be elaborated soon by the State Veterinary and Food Administration of the Slovak Republic in compliance with the valid legislation, taking into account the current situation in the Slovak Republic and also the results of the National plan in the previous year. The European Commission together with national experts of each Member State have started working on professional documents that should lead to a change of the monitoring system of foreign substance residue with regard to the fact that the legislation according to which the monitoring is performed, has already been in force for a ninth year in the European Union and, based on processed results from monitoring in the Member States, it is necessary to lay out new conditions and priorities of residue monitoring.

Prohibited substances and substances having anabolic effect belonging, under legislation, to Group A substances are monitored according to the plan. This Group is further divided into Groups A1 – A6. Stilbenes and stilbene derivatives belong in Group A1, thyreostatics into Group A2, steroids constitute Group A3, lactones of rezorcyl acid, including zeranole belong to Group A4, beta agonists into Group A5 and chloramphenicol, nitrofurane metabolites and nitroimidazol metabolites make up Group A6.

The division of these substances into these groups is the subject matter of discussions in working groups of the European Commission for proposals regarding the unification of division in compliance with Council Regulation setting maximum residue limits. Control of residues of prohibited substances is important to prevent abuse of hormones in meat production as well as to prevent abuse of substances for purposes other than they are intended, that is concerning cases of illegal treatment.

Hormones stimulate growth by increasing the efficiency of feedingstuffs and, in animals to which hormones are applied, promote feed conversion into muscle at the expense of fat more effectively. Use of hormones, such as 17-beta estradiole, progesterone, testosterone, zeranole, trenbolone and melengestrole acetate (MGA) for the purpose of the acceleration of growth in animal fattening develops risks for consumers. Unfavourable impacts include genetic, neurobiologic, genotoxic and carcinogenic effects. These effects can be either due to the original substance or its metabolites.

Moreover, veterinary drugs and substances that can be used for veterinary purposes and contaminants, which belong into Group B are monitored. Group B is divided into Groups B1, B2, B3. So called other veterinary drugs belong in Group B2 and they are more closely divided into sub-groups B2 a) – f). These subgroups a) – f) include antihelminthics, anticoccidics, carbamates and pyrethroides, sedatives, non-steroid antiflogistics and other pharmacologically active substances.

Contaminants in the environment are included in Group B3, that is divided into subgroups B3 a) – f), which contain organochlorinated compounds, including polychlorinated biphenyls, organophosphates, chemical elements, mycotoxines and colours.

The extent and frequency of official sampling for analyses of the above-mentioned substances within the National Plan for Residue Control are intended for cattle, sheep, goats and horses, broilers, spent hens, turkeys, other poultry, products of aquaculture, milk, eggs, rabbit meat and wild game meat and farmed game meat and for honey. The minimum number of samples to be taken in the said year for analyses of residues of Group A and B in live and slaughtered animals and animal products is determined by the number of slaughtered animals in the previous year at slaughterhouses in the Slovak Republic as well as by the number of produced raw materials of animal origin.

In compliance with the valid legislation the sampling will be performed by random selection, however in the case of known problems it will be preceded by targeting sampling, and the additional examination of purposes and performance of necessary veterinary measures.

The National Plan for Residue Control is modified yearly based on the situation in the Slovak Republic and also depending on results and positive findings in other Member States, for reason of the functioning of international trade and exchange not only within European Union countries. In the year 2004 the plan was amended and innovated mainly in the field of laboratory methods compared to the year 2003. More sensitive methods for the determination of residues of substances from the Group A1—A5 were introduced, control of substances from Group A6 was introduced, namely of chloramphenicol, nitroimidazoles and nitrofuranes in live animals and slaughtered animals, nitroimidazol metabolites were controlled in the year 2004 in raw cow's milk and raw sheep's milk. Control of a wider scale of antibiotics, antihelminthics and carbamates was introduced. New matrices were added in various animal species to determine mycotoxins and heavy metals.

The quality of the control of residues of prohibited substances, including hormonal substances depends on laboratory equipment and laboratory methods. The continued rapid development of new and more sensitive analytical methods leads to a decrease of detection limits and thus to recording a still lower amount of such substances. Laboratory methods are under constant development for the detection of a greater and greater amount of metabolites of these prohibited substances. The National Plan for Residue Control precisely specifies which analytical methods will be used for the analysis of official samples and which State Veterinary and Food Institute or other specialized workplace perform these analyses.

Analyses of official samples will be performed only by laboratories approved by the State Veterinary and Food Administration of the Slovak Republic and these laboratories will be accredited and they will take part in an internationally recognized scheme of quality control by participation in inter-laboratory tests. They will also demonstrate their competence by regular and successful participation in suitable schemes of eligibility tests organized by reference laboratories in the Slovak Republic and the reference laboratories of the European Community.

The quality of residue control also depends on the proper official sampling. The taking of official samples for analyses according to the plan and their wrapping are performed by a veterinary inspector of the District Veterinary and Food

Administration, who is responsible for the performance of sampling without prior notice and is obliged to ensure that animals from which the samples have been taken, must not leave the farm until all results from laboratory analyses are available. In the case of obtaining positive results from laboratory analyses, the official veterinarian will immediately order the performance of measures to prevent entry of residues into the food chain and the subsequent threat to human health.

In the case of the occurrence of a positive finding it is inevitable to detect the purpose of residue occurrence and to impose sanctions. Subsequently, the veterinarian is obliged to perform a repeated sampling; such samples taken from farm, flock, slaughterhouse, etc. as a result of the finding of a non-compliant result, are named suspect samples. Also samples taken by a veterinary inspector as a result of possession or presence of a prohibited substance at any point of their production, storage, distribution and sale in a production food or feed chain belong to suspect samples.

If a veterinarian takes samples in the case of suspicion or if he has evidence about illegal intervention, which means that an unauthorized substance came into use or could have come into use or a drug or the use of a substance or drug for other than its intended purpose, such samples are considered to be suspect samples. If a veterinarian has evidence or suspicion of non-compliance with the withdrawal period of authorized veterinary drugs, such officially taken samples are in the evaluation also included as suspect samples. Within the evaluation of results from the National Plan for Residue Control the results of targeting official samples and suspect samples are evaluated separately.

## RESULTS (IN 2004) AND DISCUSSION

Results from the National Plan for Residue Control in Live Animals and Animal Products are evaluated yearly by the State Veterinary and Food Administration of the Slovak Republic, and these results are also included in the Annual Report on Occurrence of Residues elaborated by the Food Research Institute in cooperation with the State Veterinary and Food Administration of the Slovak Republic. Within performed official samplings and controls of foreign substances in compliance with the National Plan for Residue Control a total of 4,584 samples were analysed in 2004, which were taken by veterinary inspectors of the District Veterinary and Food Administrations from live animals on farms and slaughtered animals at slaughterhouses of the Slovak Republic as well as from animal raw materials

Out of this total number (of 4,584) officially taken samples, residues of foreign substances were recorded in 14 samples, which means that 0.3 % of taken samples were not in compliance with the requirements of legislation. In 2004 a total of 327 hen egg samples for analyses of substances from Group A and B were taken. Out of 111 hen egg samples analysed for prohibited substances from Group A6, a total of 37 samples were analysed concretely for metronidazole. Out of these 37 samples, residues of metronidazole were recorded in one officially targeted hen egg sample, whereas subsequently 7 suspect samples were taken, from which 2 hen egg samples showed positive results of residues of metronidazole.

A further 2 suspect samples of the muscle, liver and kidney of respective hens were negative. 3 samples of drinking water showed positive findings of residues of nitroimidazole metabolites and one sample taken from a sewer in the vicinity of a water well was compliant. 2 suspect feedstuff samples and 1 suspect soil sample were compliant.

In the case of detection of metronidazole in hen's eggs the veterinary inspectors ordered their disposal in the establishment for the processing of high risk waste of Category 1. A ban on the sale and movement of laying hens and eggs was issued until a negative result from laboratory examination was available and veterinary inspectors ordered the ban on the use of source of drinking water from the water well, where the presence of the prohibited substance was confirmed. In the subsequent 12 months animal products and live animals from the farm in question are subject to strict controls for respective residues, which means an egg sample from each hall will be taken for examination for metronidazole once a month at the expense of the farm.

Totally 1,143 samples were taken from pigs, out of these 141 samples were taken from live pigs and 1,002 samples from animals slaughtered at the slaughterhouses. Residues of inhibitory substances were analysed in 247 samples taken from pigs and detected in 3 pig kidney samples. Subsequently suspect samples in numbering 15 were taken and out of these 6 samples were still positive and out of these 6 samples there were 4 positive findings in pig kidneys, one finding in a pig liver and in one case dihydrostreptomycine was recorded in a pig kidney, however its amount did not exceed the maximum residue limit. In each case of the occurrence of residues of inhibitory substances, examinations and controls were performed aimed at handling with veterinary drugs and record keeping.

Totally 1,488 samples were taken and subsequently analysed from cattle, out of these totally 259 samples were taken from live animals and 1,229 samples were taken from animals slaughtered at slaughterhouses.

The total number of samples taken from cattle for analyses of residues of inhibitory substances was 313, out of these 5 samples were positive. Residues of inhibitory substances were detected in 2 cases, in the liver and muscle of bovine animals, whereas by additional examination, aimed at specific antibiotics, performed by the State Veterinary and Food Institute in Dolný Kubín the result was negative, which means that positive residues of inhibitory substances could be caused by non-specific antibiotics or antibiotics that are not determined in the State Veterinary and Food Institute Dolný Kubín. Subsequent suspect samples showed a negative result.

In one liver sample and one kidney sample, positive for residues of inhibitory substances taken from the calf, by additional examination the maximum residue limit of dihydrostreptomycine was found to be in excess. The subsequent 3 suspect samples taken from cattle and analysed for residues of inhibitory substances showed a negative result. In one case a finding of tylosine was detected in cattle muscle, the amount of which did not exceed the maximum residue limit. The total number of samples taken from cattle and analyzed for residues of tylosine was 30. In the case of a result compliant with the regulation for residues of inhibitory substances the raw materials were released into food chain.

In 2004, in the National Plan for Residue Control, the positive finding of tylosine was recorded twice in honey, the suspect sample without a finding of residues. In the case of a compliant result raw materials were released into the food chain. In this monitoring plan totally 20 honey samples were analysed for tylosine and a total of 186 honey samples were taken and analysed. In the case of the finding of residues of tylosine in honey, a ban on placing the honey on the market for human consumption was issued.

A total of 444 milk samples were taken in 2004. In the targeted samplings for Group of B3a) substances one positive finding of DDT in raw sheep's milk was recorded, whereas repeated sampling was with negative result. A total of 15 samples of raw sheep's milk and 49 samples of raw cow's milk were analysed for specific parameters of DDT. In the case of the occurrence of DDT in raw sheep's milk, the veterinary inspectors issued a decision on a ban to place sheep's cheese into circulation. The decision was cancelled after receiving negative results from repeated samplings from the laboratory.

In the muscle of roe deer the maximum residue limit for lead was exceeded in 2 cases, and suspect samples will be taken on further shooting of roe-deer in the respective region. A total of 125 samples were taken and analysed for Group A and B substances. In each case of the finding of foreign substance residues, the veterinary inspectors of District Veterinary and Food Administrations ordered the immediate performance of measures without any delay in compliance with the valid legislation, they carried out an investigation of reasons for finding the residue and also ordered the execution of measures to prevent he entry of residues into the food chain.

## CONCLUSION

Each Member State is obliged, in compliance with the legislation, to submit to the European Commission the results from the National Plan for Residue Control by 31 March of each calendar year. These results are subsequently evaluated and summarized by the European Commission in the Annual Report and now already results from 25 Member States are included. The European Commission in cooperation with national experts of each Member State is elaborating the working documents analysing performed monitorings, plans as well as results thereof and they lead to proposals for new legislation or to changes of already valid legal rules and in final version they should lead to a still more complete functioning of European trade.

In future, new documents for a change in the system of residue monitoring in live animals, animal products and also in imported products will be prepared. A yearly elaborated National Plan for Residue Control in Live Animals and Animal Products, according to the legislation of the European Union will be divided into several parts; one of these parts will probably be also import control. A lot of questions exactly concerning this new system of residue monitoring will be solved in working groups of Member State experts, led by the European Commission, where the Slovak Republic also has its representatives and the prepared documents will be, following approval and legal incorporation into legislation, implemented in Member States of the European Union.

## REFERENCES

**1. Ordinance of the Government of the Slovak Republic on monitoring of certain substances and residues thereof in live animals and in animal products No. 320/2003 Coll. as amended.**

**2. The National Plan for Residue Control in Live Animals and Animal Products in the Slovak Republic.**

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# CRITERIA FOR THE APPLICATION OF THE RULES OF INTEGRATED PREVENTION AND ENVIRONMENTAL CONTROL FROM THE VIEWPOINT OF VETERINARY CARE IN THE SLOVAK REPUBLIC

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## ABSTRACT

**Integrated prevention and control of environmental pollution involves large industrial operations and in the Slovak Republic it is a part of Act No. 245/2003 of this Civil Code. Supplement No. 1 of this Act defines the categories of industrial activities that are subject to this Act and, in relation to productive activities, it also includes slaughter houses, plants for the treatment and processing of food and feed, rendering plants and farms for the intensive production of poultry or pigs. The second part of the Act deals with integrated permits, which define the conditions for operations and approve new operations with the aim of ensuring the integrated prevention of the environment and its components. This part also deals with issues of the veterinary protection of the state territory according to Act No. 488/2002 of the Slovak Civil Code on veterinary care.**

**Key words: industrial operations; integrated permits; pollution prevention; veterinary care**

## INTRODUCTION

Recently, increased attention has been paid to environmental issues on both theoretical and practical levels. The intensification of industrial and agricultural production has disturbed the relationship between living organisms and their surroundings to such a degree that natural autoregulation processes can no longer maintain the natural balance. The man himself is a part of the biosphere and depends on its ecological relationships but, by his activities, interferes with them constantly and contributes to the pollution of air and soil and subsequently also surface and ground water.

Integrated pollution prevention and control (IPPC) represents a practical approach to resolving these problems. The up-to-date partial efforts of individual countries have been integrated within the European Union, which has defined its common target and procedures for environmental protection. The basic aims and requirements concerning the prevention of environmental pollution by selected large industrial operation are defined in European Directive 96/61 ES. The forms and means for its implementation in national legislation and implementation in practice are determined individually by the respective EU Member states. Its implementation in the Slovak Republic is ensured by Act No. 245/2003 of the Civil Code and Decree of the Ministry of Environment SR No. 391/2003 of the Civil Code.

## ACT No. 245/2003 OF THE SLOVAK CIVIL CODE OF JUNE 19, 2003, ON INTEGRATED POLLUTION PREVENTION AND CONTROL AND AMENDMENTS AND SUPPLEMENTATION OF SOME ACTS

The Act is composed of five parts and is effective from July 31, 2003. In its first part, the Act defines operations from the viewpoint of IPPC and characterises them as either existing operations, i.e. those, which, according to this Act, had to apply for building permit before October 30, 1999 and started operation before October 30, 2000, or as new operations, which applied for a building permit and started operation after the specified dates.

The following measures have been taken to implement this Act:

a) integrated granting permissions for operations and changes in operations,

b) inspection of raw materials, substances and energy used in or produced by these operations,

c) determination of emission limits and conditions for running the operations,

d) monitoring of emission sources,

e) determination of best available techniques,

f) control of operations, sampling, measurement of emissions and their collection, evaluation and reporting of data and information,

g) reporting duties of operators,

h) control of manipulation with wastes,

i) control and survey of conditions for integrated permits.

In the field of granting integrated permits for operations or changes in operations the aims of proceedings involve also veterinary protection of the territory according to § 6, section 2 of letter i), item four, and § 39, section 1, letter a), and section 2, letters a) and b), and Act No. 488/2002 of the Civil Code on veterinary care, which, according to section g) requires the following:

1. issuing obligatory expert opinion on construction and approval actions involving buildings intended for animal rearing, production, processing, treatment or storage of animal products and feed, preparation, production, storage or distribution of medicated feed, and storage, processing or harmless disposal of wastes of animal origin,

2. issuing obligatory expert opinion on proposals of new technological or working procedures used in animal rearing or animal transport, production, processing, treatment or transport of animal products, preparation of medicated feed or disposal and treatment of wastes of animal origin,

3. proceedings concerning the process of granting permission for operations intended for processing wastes of animal origin and manipulation with such wastes, introducing them to the market and exchanges with EU Member states and countries of European economic space with exception of operations intended for production of feed for companion animals and production of pharmaceutical and technical products from low-risk wastes of animal origin.

In the case of integrated permission-granting that includes permits for the construction of new building or reconstruction of existing building, construction proceedings are also involved.

The concluding enactment of this Act stipulates that the operator of an existing operation, who intends to run the respective operation after October 30, 2007, is obliged to submit an application before the date stipulated by a respective administrative body, or before December 31, 2005, at the latest.

For some selected operations, upon agreement of the European Commission, the administrative body may issue a permit allowing them to fulfil individual permit conditions over a certain period and complete the process by a later date. New operations are required to submit an application before the date determined by the respective administrative body or before October 31, 2003, at the latest. According to this Act, permits were not required up to April 30, 2004, for new operations and will not be required up to October 30, 2007, for existing operations.

## SUPPLEMENT No. 1 TO THE ACT No. 245/2003 OF THE CIVIL CODE ON IEPPC

Supplement No. 1 to this Act divides industrial activities into the following six categories:

1. power production,
2. production and processing of metals,
3. processing of minerals,
4. chemical industry,
5. manipulation with wastes,
6. other operations.

The last category comprises 8 sub-categories. From the veterinary point of view, we consider important the sub-categories 6.4, 6.5 and 6.6, involving the following activities:

**6.4.** a) slaughterhouses of capacity higher than 50 tonnes per day,

b) treatment and processing operations intended for production of food or feed

1. from raw materials of animal origin (excluding milk) with production capacity exceeding 75 tonnes of final products per day,

2. from raw materials of plant origin with production capacity exceeding 300 tonnes of final products per day (3-month mean),

c) operations for treatment and processing of milk collecting more than 200 tonnes per day (annual mean).

**6.5.** Operations intended for disposal or processing and reuse of animal carcasses and animal wastes of processing capacity higher than 10 tonnes per day.

**6.6.** Operations for intensive rearing of poultry or pigs, capable of housing more than

a) 40,000 poultry,

b) 2,000 pigs (b.w. higher than 30 kg) or

c) 750 sows.

## DISCUSSION

Implementation of the Act No. 245/2003 of the Civil Code on IEPPC in agricultural production or food production, which is time-limited, will raise many problems but it is very urgent with regard to environmental management and protection. As an example we can cite the contamination of ground water with nitrogen substances. This discussion will deal only with the problems of environmental pollution concerning organic matter, specifically N-substances, as animal production is one of the principal sources of such contamination.

Results of investigations, carried out at the Department of the Environment of UVM Košice, focused on the examination of drinking water, serve as an example. Although localisation and protection of the investigated source of drinking water corresponded to requirements, microbial findings, N-substances and chlorides exceeded the limits for drinking water. Examination of other water sources in the respective area indicated that the main cause of such situation was contaminated ground water (2).

Decree No. 392/2004 presents the Programme of agricultural activities in announced vulnerable regions as specified by government Regulation SR No. 249/2003 of the Civil Code. The aim of the relevant legislative standards is to prevent the pollution of ground water by nitrates originating from mineral fertilisers and solid or liquid animal excrements. It focuses specifically on the handling and disposal of manure that differs in sensitive and vulnerable regions with regard to quantity, configuration of terrain, season, weather, etc.

In relation to this, considerable attention has been paid to manure storage tanks, particularly to their construction, capacity requirements and necessary safety mechanisms. The related problems are urgent with regard to the Act. No. 488/2002 of the Civil Code on veterinary care in the field of issuing obligatory expert opinions for construction procedure, proposals of new technologies or working procedures or granting permits for operation of facilities processing wastes of animal origin.

It could be stated in general that the capacity of storage tanks should exceed the volume of slurry produced by farm animals during the period when it cannot be applied to agricultural soil or, more accurately, should correspond to 6-month production.

The size of storage tanks can be calculated on the basis of the number of animals and respective animal species which is a part of Decree No. 392/2004.

With regard to its manuring value, poultry droppings are especially valuable because of their high content of nutrients, such as nitrogen, phosphorus, calcium and other. According to Hlouška *et al.* (3), hens produce between 14 and 16 kg of droppings per year. Their optimum use, which is one of the aims of IPPC, requires construction storage tanks for slurry from the cage system of capacity 0.030 $m^3$ per head over 6 months at a consumption of feed at 0.180 kg per day. When calculating the volume, 10 % loss due to storage and transportation should be considered. The storage capacity for dried poultry manure from the cage system should correspond to 0.015 $m^3$ per head over 6 months. The term dried manure is used for manure dried to 40 % dry matter.

Daily production of faeces and urine by pigs is approximately 4.5 kg (1). The capacity of storage tanks per head over 6 months ranges between 0.81 and 1.07 $m^3$ depending on body weight of pigs (Decree 392/2004 of the Civil Code). Mating and gravid pigs produce about 8.8 kg faeces and urine per day, therefore 2.77 $m^3$ of storage tank capacity (6 months) must be provided per head and day. Sows in high stage of gravidity and suckling sows produce 14.4 kg of faeces and urine per day and the respective storage capacity requirement is 3.2 $m^3$.

To complete information on the required capacity of slurry storage tanks one should mention that 0.92 $m^3$ of waste water is discharged from a milking parlour per one dairy cow over 6 months.

## CONCLUSION

Act No. 245/2003 of the Slovak Civil Code is the legal basis for integrated pollution prevention and control concerning large industrial operations. Supplement No.1 of this Act defines categories of industrial activities that are subject to this Act. It also determines the tasks in the field of veterinary care concerning the intensive rearing of poultry and pigs, food operations and processing of wastes from animal production. It is necessary to ensure appropriate education of professionals involved in the respective activities at all levels of veterinary care. At the same time, according to government Decree of the SR No. 391/2003 it is possible to obtain a certificate on the professional expertise of veterinarians and their capability to provide professional consultations in this area. This could help considerably in coping with the topical issues of environmental protection.

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# ANIMAL HYGIENE, WELFARE AND ENVIRONMENTAL PROTECTION IN RELATION TO IMPLEMENTATION OF EU LEGISLATION IN ANIMAL PRODUCTION

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## ABSTRACT

**Environmental conditions have an important influence on the health and welfare of farm animals. Especially with regard to the increasing industrial character of animal production the housing environment, particularly the microclimate, becomes an important health, production and, last but not least, an economic factor.**

**Key words: environmental protection; legislation; welfare**

## INTRODUCTION

The immediate housing environment may influence animal well-being either positively, when most of the important requirements are respected, or negatively, when animals are exposed to various stress factors that contribute to animal diseases. The immediate environment includes physical, chemical and biotic factors, housing technology, feeding and watering strategy and social aspects especially in terms of ensuring individual treatment and health care (9).

Air temperature is the key microclimatic parameter, which is affected by other physical factors (air flow, air humidity). A comfortable temperature range depends on animal species, age and total metabolic level (12). Light influences the organism through photoperiod or duration, intensity illumination, wavelength and affects growth, development, reproduction and production (3). Noise becomes a stress factor when a certain maximal threshold is exceeded, but its frequency and other characteristics are also important (6).

The metabolic processes of animals change the chemical composition of the air. Carbon dioxide, ammonia, sulphide and methane are the main gasses detected in increased levels in animal housings. This is important with regard to the emission of noxious gases by animal farms and must be measured when the capacity of cattle exceeds 180 head (Air protection Act No. 86/2002 of the Civil Code–CzR ). Airborne dust and micro-organisms are also an important factor (8).

The technology of livestock housing and management affect not only the physical and psychical wellbeing of animals but may pose a danger to the health and life of animals (11). Design parameters and quality of technological elements have considerable impact on animal welfare. Minimum housing and management standards are an important part of legal regulations.

Intensification of agriculture and associated minimisation of individual care results in changes in the structure of diseases, predomination of diseases of multi-factorial origin and increased demands on bio-security measures.

## MATERIAL AND METHODS

Animal husbandry and environmental protection have many contact points. Environmental conditions affect animal health and welfare considerably and the vital processes of animals and the animals influence the environment round the farm

negatively. The principal objectives of animal husbandry include the keeping of animals in a suitable living environment in terms of health and welfare, but also the protection of the outdoor environment against an excessive load of pollutants. Both objectives must be integrated in the respective legislation.

## RESULTS AND DISCUSSION

Many welfare problems are the result of animals not being fully adapted to the production system. The consequences of poor welfare include those of disease, injury, starvation, beneficial stimulation, social interaction, housing conditions, deliberate ill treatment, human handling, transport, laboratory procedures, various mutilations, veterinary treatment or genetic change by conventional breeding or by genetic engineering (2).The rapidly changing conditions prevent animals to adjust and cope with the changes (7).

The extent to which husbandry systems take account of the needs of livestock can be established with the help of various complementary parameters:

– **Production indicators**

Performance, yield. However, high productivity does not necessarily imply a good welfare state.

– **Veterinary approach**

Examples relevant to the assessment of well-being include studies linking injury to housing conditions, respiratory tract lesions to air quality, and gastrointestinal lesions to management and nutritional factors.

– **Physiological approach**

Classical stressors affect not only the adrenal cortex but practically all major endocrine systems of the body together with other widespread effects on reproduction, immunity, and metabolism. The use of "pre-pathological states" as a more useful index of well-being has been suggested. This state is connected with the reduction of immune competence, suppression of an endocrine response fundamental to reproduction, or display of increased social aggression. A second important application of physiological measures involves short-term indices of emotional upset.

– **Behavioural approach**

Behaviour provides a more direct window on the animal's own perceptions of events. Abnormal activities, such as self-mutilation, extreme aggression, or behaviour that appears to denote depression, are among the most persuasive indicators of impaired well-being.

– **Environmental preference testing**

Also preference testing has a number of limitations. Both the preferred and non-preferred options may be perfectly acceptable, or both may be seriously deficient. Tests may be influenced by ambient temperature or diurnal patterns too (5).

The motivation for EU directives on animal welfare is to facilitate the common market's function by introducing obligatory minimum animal welfare standards. These are intended to prevent competition between member countries caused by differences in national animal welfare laws, and, should the occasion arise, they remove technical hindrances for trade of live animals while at the same time trying to attain a satisfactory level of protection for the animals involved.

In the Czech Republic the animal welfare activities are implemented pursuant to Act No. 246/1992 of the Civil Code on protection of animals against cruelty (Welfare Act). The purpose of Act No. 246/1992, amended in 2004 by Act No. 77/2004 of the Code, is to protect animals, which as living and sentient creatures can feel pain and suffering, against cruelty, damage to their health or killing without reason by man, even if caused by negligence.

Additional direct and indirect protection of animals is covered by respective national and EU regulations and European conventions and treaties (4).

Protection of the Environment is ensured by Act No. 17/1992 of the Code on the environment, as amended Act No. 100/2001 of the Code on the environmental impact and assessment (Environmental Impact Assessment Act) and Act No. 86/2002 of the Code on air protection.

According to the current legal regulations the animals can still be kept under conditions that are unsatisfactory in terms of animal welfare. For example stanchion housing for cattle is not outlawed and freedom of movement is involved only in a minimal area (possibility of stand up, lie down, turn round, other natural movements). However trends in newly edited legal regulations are leading to the outlawing stanchion housing for cattle except for inferable exceptions. Council Directive 97/2/EC, concerning the protection of calves, determines that no calf shall be confined in an individual pen after the age of eight weeks and individual stabled calves (younger age) must have visual and tactual contact.

Welfare requirements may be in some cases in defiance of demands for environmental protection. For example, a larger area for animal locomotion and staying in egress may be associated with increased emissions and runoff. On the other hand regular removal of manure from stables improves animal welfare and decreases emissions and contamination of ambient water and soil.

In general, animal husbandry has negative environmental influences. Modern intensive livestock farms are point sources of environmental contaminants (1, 10). However, some measures can decrease atmospheric pollution, e.g. bio-technological agents for reduction of ammonia emissions (Bio-Algeen, Sannisty). These agents are classified as Best Available Techniques (or BAT) within the context of the enforcement of Act No. 76/2002 of the Code on Integrated Pollution Prevention and Control (IPPC). Other measures include the use of feed additives or acid, salts and natural substances added to slurry (vegetable oil, herbal extracts, essential oil, paraffin), and various absorbents with affinity to ammonia ions (zeolites, bentonites).

High-density livestock farms create the greatest problems for the environment. In the Czech Republic

there are more than 400 farms with high numbers of cattle and more than 200 farms with a high density of pigs. The negative effects of these farms are many and are augmented by unsuitable locations with regard to human dwellings and objects of hygienic protection.

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# RESOLVING THE PROBLEMS OF ENVIRONMENTAL PROTECTION IN RELATION TO THE STATUS OF HEALTH AND PRODUCTION OF FOOD

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## ABSTRACT

**In recent years, due to complex climatic and anthropogenic factors with synergistic action, considerable negative effects on the environment have been recorded. The toll which humankind has to pay for a high degree of civilisation and industrialisation has been increasing constantly. Animal production belongs among the important sources of environmental pollution. Modern animal production produces solid, liquid and gaseous pollutants, which affect unfavourably all components of the environment by both chemical compounds and microbiological agents and contribute to local, regional and global environmental changes. Specific problems are associated with micro-organisms, which by their diversity and tenacity can result in outbreaks of diseases including zoonoses. All up-to-date environmental problems, such as eutrophication, contamination with heavy metals, residues of pesticides and antibiotics, massive death of fish, acid rains, damage to ozone layer, genetic defects, waste dumps, new mutation of viruses and others, result from human activities and disturb the balance or even the integrity of the biosphere.**

**Key words: animal production; environmental protection; health; pollutants**

## INTRODUCTION

In recent years, complex climatic and anthropogenic factors with a synergistic action have negatively affected our environment. The toll which humankind has to pay for high degree of civilisation and industrialisation has been increasing constantly. The negative influences have local, regional and global consequences.

Modern animal production is one of the important sources of environmental pollution. It produces solid and liquid wastes and gaseous emissions that contribute to microbiological and chemical contamination of all components of the environment.

Changes in the chemical composition of air resulting from anthropogenic activities are associated with unforeseen climatic changes. The most important aerial pollutants produced by animal farms include odour, gases, dust, micro-organisms and endotoxins. More than 130 various gases have been identified in animal houses. Animal production is associated with emissions of ammonia, hydrogen sulphide, carbon dioxide and methane which participate in local, regional and global environmental changes. Ammonia from intensive animal farms contributes to soil acidification. Specific problems are associated with micro-organisms which by their diversity and tenacity can cause outbreaks of diseases including zoonoses (1).

Bacteria released from animal houses may spread to distances of between 400 and 500 metres while transfer of viruses over distances greater than 50 kilometres has been reported. Aerial pollutants in animal houses cause respiratory diseases and allergies not only in animals but also in human personnel. *Post mortem* examination of animals from houses with decreased hygiene of air has indicated an increase in pneumonia, pleuritis and similar. Lung lesions associated with the quality of air have been reported in poultry.

Water is essential for mankind and animals and contributes to all life processes. Wastes from animal production represent

an important point and diffuse source of contamination of surface and ground water (2). Increasing requirements in the capacity and quality of water sources require appropriate protection measures. The aim of the protection of water is to prevent such interventions and activities that could affect negatively the quality and quantity of water in the its natural water cycle. According to WHO, about 39 % of world population is fully supplied with drinking water, 35 % has no reliable and steady supply and the remaining 26 % suffer from shortage of good-quality drinking water.

Still more serious is the fact that the availability of freshwater sources has been decreasing. According to FAO, in the period from 1950 until 2000 drinking water supplies in Europe decreased by one third. The disproportion between water supplies and demands increases. Agriculture is one of the branches that has high requirements of water. Intensification in production, exploitation of agricultural soil, deforestation, application of organic fertilisers, pesticides and other chemicals pose considerable risk to quality of water sources. A small source of pollution can contaminate millions of cubic meters of ground and surface water.

## MECHANISMS OF ENVIRONMENTAL POLLUTION

Many organic substances and a considerable proportion of dissolved substances from animal production enter the soil and eventually also ground water and decrease its quality. Farmyard manure, slurry and wastewater contain nitrogen and other important plant nutrients but, after their application to soil some of these nutrients, such as nitrates and phosphates, together with micro-organisms, they pollute ground and surface water. One should also consider the potential contamination of soil with heavy metals, such as zinc and copper, that are used as stimulators in feed and part of them passes into excrement, particularly to pig slurry. An additional potential risk is associated with residues of antibiotics that may persist in animals after medical treatment and pass to the environment with animal manure or during grazing and may contribute to development of antibacterial resistance. Similar risk develops when sludges and effluents from treatment of municipal wastewaters contain antibiotic residues resulting from treatment of humans and are used for irrigation or disposed to the soil.

The supply of population with high quality drinking water is one of the most important measures of protection of health and its level characterises the quality of life in the respective country. Drinking water must comply with the health and hygienic requirements set by national legislation. It must not contain such organisms or concentrations of substances that can unfavourably affect the health of humans and animals, which consume this water over a prolonged time (4). Water supplied to animals must be of the same quality as water intended for human use.

An increased requirement in bacteriological quality applies to water used in the food industry. Bacteriological examination provides information about the immediate quality of water. It is the most sensitive indicator of direct and indirect faecal contamination. According to WHO diseases spread by microbiologically contaminated water endanger more than 500 million people and more than 10 million people die every year of diseases transmissible by water. Many infectious diseases can be spread by water. Waterborne infections include 80 % of all worldwide occurring infectious diseases and 90 % of all infectious diseases in the developing countries. Insufficient sanitation of water is associated with approximately 200 million human infections, particularly gastroenteritis and with approximately 4 million deaths annually, particularly in children. Protozoan diseases, giardioses and cryptosporidioses can be spread by drinking water. U.S.H.O. has reported 900,000 cases every year and out of that 900 cases are fatal (2).

To prevent the spread of diseases transmissible by water, the water intended for drinking must be disinfected. The disinfectant most frequently used for this purpose is gaseous chlorine or chlorine preparations which, at an incorrect dose, may cause serious diseases. The most important of them include anaemia, hypertension, allergies and cancer of the stomach and large intestine. According to US Council (3) the risk of carcinogenic diseases in humans consuming water with a higher concentration of active chlorine is 93 % higher compared to those who have consumed water free of chlorine. Active chlorine reacts with other substances present in water and produces carcinogenic trihalomethanes. However, according to some institutions engaged in the investigation of water disinfectants, some viral and parasitic disease agents are resistant to chlorine and may survive in water for a relatively long time.

An important environmental component affected considerably by animal production is soil. Soil becomes contaminated by a wide spectrum of chemicals that enter soil due to erosion, during manipulation with manure and its storage and as a result of the use of various plant and insect pesticides. Survival of micro-organisms in the soil and the spread of various bacterial, viral and parasitic diseases depends on the particular conditions. These micro-organisms affect the plans, grazing animals and eventually also people. Children frequently suffer from diseases caused by helminths. The most important parasitic diseases include diseases caused by *Strongyloides stercoralis* and *Ascaris lumbricoides*.

All up-to-date environmental problems, such as eutrophication, contamination with heavy metals, residues of pesticides and antibiotics, massive death of fish, acid rain, damage to the ozone layer, genetic defects, waste dumps, new mutation of viruses and others, result from human activities and disturb the balance or even the integrity of the biosphere.

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# THE EFFECT OF THE APPLICATION OF PIG SLURRY ON THE HYGIENIC SAFETY OF DRINKING WATER

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## ABSTRACT

**The aim of the study was to assess the effect of the soil application of pig slurry on the hygienic quality and health safety of water from three sources; natural spring, well, and artesian well. The swine farm investigated is located in west Slavonia, Croatia, and produces about 25,000 fattened hogs per year. Pig slurry is first discharged into lagoons and then applied to surrounding fields sown with cereals. Analysis of water samples from the three investigated sources showed that pig slurry used for fertilization was not the only source of water pollution with nitrogen compounds. The unfavorable microbiological water findings may have been due also to other causes. The conclusion is that appropriate use of slurry for soil fertilization according to agrotechnical terms has no impact on the safety of drinking water sources.**

**Key words: pig slurry; swine farm; water safety**

## INTRODUCTION

The large amounts of slurry discharged by intensive pig breeding farms pose a great environmental risk and are occasionally a limiting factor in this industry. The quantity of slurry daily produced by animals may vary considerably depending on the animal category and size, type and quality of feed, type of feeding and watering system, physiological conditions, housing, microclimate and other factors. The total faeces and urine discharged by pigs account for approximately 5 to 7 % of their body mass (5). Pig faeces contains 65 to 85 % of water, 10 to 20 % of organic matter and some 10 % of inorganic matter. As manure is mostly used as a field fertilizer, the content of nitrogen, potassium, calcium and phosphorus available to plants is of utmost importance. The COD of pig slurry ranges between 22,000 and 35,000 $mg.l^{-1}$ (6). Overuse of liquid manure on sown fields may affect soil structure and texture considerably. Various chemical compounds from slurry, e.g., nitrogen and phosphates, may penetrate to deeper strata and contaminate ground waters.

## MATERIAL AND METHODS

Water samples were collected during 2000 at monthly intervals in west Slavonia, Croatia, from a natural spring at a 4 kilometer distance from the pig farm, three wells at a 2 to 3 kilometer distance and from two artesian wells on pig farm. The depth of wells was 6—10 metres and of the artesian wells 60—70 metres.

The capacity of the farm was 25,000 fattened hogs per year. The slurry was transferred through a hose system into lagoons and then applied to surrounding fields sown with cereals.

Water samples were tested in a laboratory by standard photometric methods (1, 2) using HACH/DREL/400 appartus. The results were evaluated according to regulations on the safety of drinking water.

## RESULTS AND DISCUSSION

The results are presented as means of values determined in the analysed water samples over a one-year period (Table 1).

**Table 1. Mean values of hygienic parameters in drinking water from natural spring, well and artesian well**

| Parameter | Natural spring | Well | Artesian well |
|---|---|---|---|
| Colour ($mg.l^{-1}$ Pt/Co scale) | 17 | 23 | 14 |
| Turbidity (NTU) | 2 | 5 | 3 |
| pH | 7.1 | 7.2 | 7.1 |
| Electric conductivity ($\mu S.cm^{-1}$) | 772 | 765 | 731 |
| COD $KmnO_4$ ($mg.l^{-1}$) | 2.81 | 3.91 | 5.07 |
| Chlorides ($mg.l^{-1}$) | 26 | 33 | 58 |
| Ammonium ($mg.l^{-1}$) | 0.33 | 0.40 | 0.14 |
| Nitrite ($mg.l^{-1}$) | 0.007 | 0.195 | 0.011 |
| Nitrate ($mg.l^{-1}$) | 6.3 | 5.0 | 3.6 |
| CFU.1 $ml^{-1}$ (22 °C) | 112 | 80 | 300 |
| CFU.1 $ml^{-1}$ (37 °C) | 42 | 75 | 29 |
| Coliform bacteria CFU.100 $ml^{-1}$ | 240 | 240 | 38 |

A considerable quantity of slurry produced by the swine farm is stored in lagoons for a certain period of time and then is applied to the surrounding fields. It is a valuable fertilizer if properly used in terms of amount, timing, and plant growth. However, uncontrolled application may lead to the penetration of organic and inorganic substances, especially nitrogen compounds, e.g., ammonium, nitrites, nitrates and phosphates, into deep strata, thereby contaminating ground waters (9). In this way various micro-organisms may also reach the deepest water-bearing strata (7).

The parameters analyzed in water samples from the three sources (spring, wells, and artesian wells) generally showed satisfactory levels according to the national provisions on drinking water safety (8). Water from the natural spring showed an increase in total bacterial count and coliform bacterial count. Water from the wells failed to meet the national regulations concerning the maximum permitted values for colour, turbidity, COD, nitrates, and total bacterial count and coliform bacterial count. Water from the artesian wells showed increased COD as well as total bacterial count and coliform bacterial count. Consistent results for well water from similar sites have been reported by Hadžiosmanović *et al.* (3, 4).

## CONCLUSION

The results of the analysis of water samples from the three water sources indicated that slurry used for fertilization of the surrounding fields did not cause water source pollution with nitric compounds. The unfavorable microbiological picture of water may have been due to fertilization with slurry as well as some other sources of contamination. The conclusion is that appropriate use of slurry for soil fertilization does not affect drinking water quality if applied with due consideration of the agrotechnical terms.

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# ECOLOGICAL AGRICULTURE AND THE ENVIRONMENT

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## ABSTRACT

**Agriculture is considered a strategic economic sector as it guarantees the nutrition of population and a direct relationship to the soil. Animal production as one branch of agriculture plays an important role in this direction. On the one hand, it is a source of wholesome food and on the other hand it produces precious organic wastes. There is no doubt that with regard to the ever-increasing human population (about a 77 million annual increase) intensive animal production will be irreplaceable despite some adverse impact on the environment and animals.**

**Key words: agriculture; ecology; environment; organic farm; residues**

## INTRODUCTION

The intensification of agriculture, as a result of efforts to increase the effectiveness of agricultural production, has caused serious environmental problems associated with the use of excessive doses of industrial fertilisers, pesticides, nutrition supplements for cattle, excessive exploitation of pasture, soil and similar (4).

Due to agricultural use soil is exposed to physical, chemical and biological degradation.

Physical degradation is caused by erosion, drying, soil drenching and compacting.

Chemical degradation occurs due to acidification, salinisation, contamination with heavy metals and other micro-contaminants.

Biological degradation includes the mineralization of humus and changes in biodiversity.

In relation to this, a new area opens up in agricultural production for the development of alternative forms of farming that allow farmers to reduce or even eliminate undesirable xenobiotics. These alternative forms are generally known as ecological or organic agriculture and may include several forms.

Ecological agriculture is also one of the sustainable ways of the utilisation of the landscape and its agrosystem, which uses predominantly local and renewable resources, ecologically friendly technologies and procedures which minimise damage to the environment. Sustainable utilisation of natural resources is based on their appropriate use, which causes no damage resulting in irreversible changes or even extinction. Sustainability also means that the limits of ecological, social and economic stability are not exceeded (5).

Sustainable agriculture is defined as an optimum desirable relationship between agriculture and the environment (3).

Ecological agriculture represents an interconnection of traditional agrotechnical methods with recent scientific knowledge, which allows farmers not only to produce bio-products and biofoods, but also resolves environmental problems.

F o s s l e r *et al.* (1) have conducted a study on 110 organic and conventional farms in the USA focused on the monitoring of the occurrence of salmonellae between October 2001 and August 2004. They collected altogether 22,417 samples of cattle faeces and 4,570 samples from different parts of farm

premises (water, milk, storage tanks, feed, cubicle floors). They found that on 92.7 % of farms at least one sample was salmonella positive and 25 % of farms showed that they were higher than 75 % salmonella positive. According to the authors, this information indicates a challenge for taking the appropriate measures to decrease the occurrence of salmonella in dairy cow herds.

H a m s c h e r and N a u (2) have conducted a study, which focused on the comparison of residues and contaminants in products of animal origin produced by ecological and conventional farms. The differences were much smaller than expected and the levels of residues and contaminants were in the majority of cases lower than the maximum permitted values. A remarkable observation was that milk produced by conventional farms was much more contaminated with *aflatoxin M* than milk from ecological farms.

Examination of eggs produced by layers kept in various systems showed that the highest concentrations of *dioxins* were determined in eggs of free-range layers on organic farms. In contrast, higher levels of *propoxur,* an insecticide used against skin mites (*Dermanyssus gallinae*), were determined in eggs of hens kept in battery cages compared with those produced by layers housed in enriched cages in alternative halls. Additional research in this area appears very promising, but the data obtained at national and international level should be more sophisticated.

Because agriculture and environmental protection are based on contrasting activities, but are inevitably part of everyday real life, it is necessary to rationalise those systems of agricultural production, which can improve the quality of the environment and food. Ecological agriculture has great potential for the successful implementation of these requirements in practice.

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# HYGIENIC AND ECOLOGICAL ASPECTS ASSOCIATED WITH THE UTILISATION OF BIOSOLIDS AND EXCREMENT IN AGRICULTURE

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## ABSTRACT

**The effective sanitation of the environment, particularly of its special parts, which can be a source for spreading infections, plays an important role in the prevention of infectious diseases. In this respect special attention should be paid to the sanitation of the excrement of farm animals to ensure the effective devitalisation of infectious agents and comply with the need to preserve the nutrients in manure. Agricultural reuse of sludge and manure is acceptable only if their sanitary quality is guaranteed sufficiently and the issues of public concern are handled in the proper way.**

**Key words: biosolids; excrements; pathogens; wastewater treatment plant**

## INTRODUCTION

Biosolids are primarily organic, accumulated solids separated from wastewater that has been stabilised by treatment. Their recycling depends on their composition and treatment. By definition, biosolids are the solid by-product of wastewater treatment. Beneficial use of biosolids is defined as the use of biosolids in circumstances and situations where "risks to human health, animal health, the environment and quality of agricultural products are within acceptable limits".

Land application of manure provides agricultural benefits and presents the optimum use of animal excrement if treated and handled properly. The principal health concerns associated with the agricultural use of biosolids and animal manure include pathogen transmission, contamination of ground or surface water with faecal material from field run-off, and build-up of heavy metals or organic contaminants.

## PATHOGENS IN BIOSOLIDS AND MANURES

The bacterial pathogens most important with regard to human health include, for example, *Salmonella* sp., *E. coli* O157 : H7, *Campylobacter jejuni, Yersinia enterocolitica and C. perfringens*. *Salmonella* are Enterobacteriaceae, which are widely distributed in the environment and include more than 2,000 serotypes. They are one of the most predominant pathogenic bacteria in wastewater and include serotypes that cause typhoid, paratyphoid fever and gastroenteritis. This pathogen produces endotoxins that cause fever, nausea and diarrhoea and may be fatal if not properly treated by antibiotics (1). Serotypes implicated in food contamination are *S. paratyphi* and *S. typhimurium*. These species can grow readily in contaminated foods and cause food poisoning (2). Shiga-like-toxin-producing *Escherichia coli* strains are major food-borne bacterial pathogens that have been implicated in diarrhoea, haemorrhagic colitis, and the haemolytic-uremic syndrome. One serotype, O157:H7, is the dominant serotype associated with diseases worldwide.

Manure pathogen levels depend on the animal source, animal health, method of manure storage and its treatment before use. Special attention should be paid to pig slurry solids

collected in the first stage of treatment as they are used almost exclusively for agriculture purposes and require proper sanitation (8).

Infected animals shed more than 30 causative agents of public health importance. The use of excreta (manuring) and wastewater (irrigation) in agriculture may result in a public health risk only if all of the following prerequisites concur:

(a) if an infective dose of an excreted pathogen reaches a pond or a natural water body;

(b) if this infective dose reaches a human host;

(c) if this host becomes infected;

(d) if this infection causes disease or further transmission.

At present, helminth pathogens play a minor role in industrialised countries. Many helminths are, however, endemic in the majority of tropical, less industrialised countries, and have various potential transmission patterns. *Ascaris* (roundworms), the most common helminth pathogen in developing countries, may be carried passively by fish and other water fauna although its major transmission foci are soils and waste-fertilised crops.

More than 140 enteric viruses can be transmitted by biosolids. The caliciviruses, adenoviruses, hepatitis A and E viruses, astroviruses, and rotaviruses are of particular concern. It is notable, however, that zoonotic viruses of humans continue to be discovered or appear to re-emerge as important human pathogens.

## TECHNIQUES FOR REDUCING PATHOGENS

Effective biological processes include digestion, composting, and storage of biosolids.

Aerobic stabilisation of sewage sludges makes use of the anabolic and catabolic activity of aerobic micro-organisms capable of producing heat. When all the conditions are met, the temperature can rise to the thermophilic range (45—70 °C) in which pathogens are killed and the sludge becomes stabilised, odour free and suitable for agricultural use as a high-quality organic fertiliser (3, 9).

Composting is one of several methods for treating biosolids to create a marketable end product that is easy to handle, store, and use. Pig slurry solids obtained during waste-water treatment of pig slurry are valuable material, which also presents some risk if not treated properly. A number of authors (4, 5, 6) have reported that composting results in significant decrease in pathogenic bacteria, fungi and helminth eggs and high quality organic manure with a considerable portion of humic substances. Two of the physical factors important in the reduction of pathogens are the heating and structure of the substrate. Inactivation is affected by many factors including self-heating, structure and the water content of the substrate, pH, organic constituents, and others.

In order to eliminate hygiene risk factors associated with different technologies of treatment, liming of sludge ($Ca(OH)_2$ or CaO) has been investigated in detail (5, 6). Besides lime, the effect of some natural materials, such as zeolite, alone or in combination with lime, has been investigated during the storage of biosolids and pig slurry solids (7, 10, 11).

The environmental benefits of better manure management will only be fully achieved if there is a broad acceptance of optimum techniques in all key areas. It is possible that larger livestock units could accommodate such technologies with relatively little outside support, but for smaller farms such practice is expensive and unlikely, therefore the building of centralised facilities may need to be considered.

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# THE PERFORMANCE OF NATURAL ZEOLITE AS A FEED ADDITIVE IN REDUCING AERIAL AMMONIA AND SLURRY AMMONIUM ION CONCENTRATION IN THE PIG FARM NURSERY

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## ABSTRACT

**The investigations were carried out in a pig nursery on 985 piglets (554 experimental and 431 control piglets) for 50 days. The effect of natural zeolite (55 % clinoptilolite) used as a feed additive on the concentration of ammonia in the air was investigated as an important factor affecting the health and welfare of piglets. Our investigations included the determination of microclimatic parameters, aerial ammonia and physicochemical parameters in the slurry. Results of the study proved that the respective feed additive decreased ammonia emissions in the experimental unit. The addition of 2 % of zeolite to mixed feed resulted in a decrease in ammonia emission by about 33 % and a decrease in ammonium concentration in the slurry by 25 %.**

**Key words: aerial ammonia; ammonium ion; clinoptilolite; pig slurry; zeolite**

## INTRODUCTION

Intensive livestock production is increasingly regarded as an important source of gaseous and malodorous emissions due to the production of large quantities of animal excreta and their composition. One of the important air pollutants is ammonia. Other gases released from animal housings are methane, carbon dioxide, hydrogen sulphide, nitrous oxide, and additional more than 130 trace gases (7).

The presence of ammonia in animal housings affects the health of housed animals. Ammonia causes direct irritation of the retinal and respiratory mucosa resulting in conjunctivitis, coughing, and reduced lung capacity (9, 14). The possibility of intoxication increases with the duration of stay in an ammonia-rich atmosphere. The maximum allowed concentration of ammonia is 25 ppm.

Efforts aimed at reducing aerial ammonia in animal houses are based on various feed and manure additives, for example zeolites (10, 13), glycocomponents from the *Yucca shidigera* plant (1, 15), and a preparation containing sodium alginate obtained from brown seaweed *Ascophillum nodosum* (12).

Zeolites are naturally occurring three-dimensional, microporous, hydrated aluminosilicate minerals characterized by high internal surface area and high cation exchange capacities. Zeolites remove ammonia from slurry by trapping and exchanging it in its crystalline structure. There are more than 50 different types of natural zeolites differing in their selectivity towards various cations. The zeolite clinoptilolite has a specific affinity for ammonium ions and ammonia (11). Moreover, zeolites are non-toxic, non-hazardous and valuable soil conditioners when added to the soil with manure (2).

In the present study, the effect of a commercial preparation "Pigozen", added to piglet feed, was assessed in terms of aerial ammonia and physicochemical composition (ammonium ion) of the slurry.

## MATERIALS AND METHODS

The study was conducted on Dubravica Pig-breeding farm in Hrvatsko Zagorje, Croatia. Measurements were taken in nursery units during the winter period. Piglets were housed in two equal nurseries, in boxes with 30 animals in each, on a partially slatted floor, and were supplied feed and water *ad libitum.*

There were 431 piglets in the control unit and 554 in the experimental. The experiment lasted 50 days.

The commercial preparation "Pigozen", a natural zeolite containing feed additive, was added to the mixed feed in the experimental group at an amount of 2 % by weight.

The microclimatic parameters (temperature, relative humidity and air velocity) were determined (n = 7) every week using a Testo 625 thermohygrometer and Testo 415 anemometer. $CO_2$ and $NH_3$ concentrations were determined by a Dräger-Acuro gas detector pump with respective detector tubes.

Untreated samples of pig slurry were used as a substrate in the study. Grab-bottle, dark green-brown, malodorous specimens were sampled from the channel under the slatted floor once a week (n = 7).

Standard physicochemical parameters in the slurry (dry matter — DM, inorganic matter — IM, pH, biochemical oxygen demand — $BOD_5$, and ammonium, nitrite and nitrate ions) were determined in accordance with standard methods (3, 4) using titration and photometric procedures and a HACH DREL/ 4000 chemistry/apparatus module.

## RESULTS AND DISCUSION

The results obtained by the measurement of microclimate parameters in the control and experimental nursery units are presented as means in Table 1.

**Table 1. Microclimate parameters in control and experimental nursery units**

| Parameter | Control unit | Experimental unit |
|---|---|---|
| Temperature (°C) | 24.6 | 24.2 |
| Relative humidity (%) | 70.1 | 66.2 |
| Air velocity ($m.s^{-1}$) | 0.09 | 0.08 |

The piglets' health and productivity are greatly influenced by microclimate parameters. Their values measured met the criteria set for the respective animal species and category and were comparable between the control and experimental units. The mean temperature of about 24 °C corresponded to the requirements for this animal category (20—28 °C). Humidity is a factor closely related to air temperature. The relative humidity ranged from 50 % to 70 % in both control and experimental units. Air velocity ranging from 0.05 to 0.2 $m.s^{-1}$ corresponded to the requirements for this animal category (5, 8). Although natural zeolites have great affinity for small polar molecules like $H_2O$, $SO_2$ and $NH_3$, lower relative humidity in experimental unit could not be caused by 2 % addition of zeolites (6).

The effect of "Pigozen" was reflected in a considerable difference in the mean aerial gaseous contamination, especially ammonia, between the control and experimental nursery units (Table 2). According to the manufacturer's instructions, this feed additive greatly inhibits the emission of ammonia from animal excreta, thus improving the housing microclimate, which in turn results in reduced mortality and better health in piglets.

**Table 2. Mean level of air pollutants in control and experimental nursery units**

| Parameter | Control unit | Experimental unit |
|---|---|---|
| Carbon dioxide $CO_2$ (% by vol.) | 0.16 | 0.14 |
| Ammonia $NH_3$ (ppm) | 4.04 | 2.68 |
| Reduction of $NH_3$ in comparison with control (%) | | 33.67 |

During the study period, the concentration of ammonia in the experimental unit was reduced by some 33 % on average, which could be attributed to the effect of clinoptilolite. The concentration of carbon dioxide as an indicator of ventilation showed a negligible decrease in the experimental unit.

The influence of zeolite on physicochemical parameters in the slurry differed according to individual parameters (Table 3 and 4). The pH of slurry showed a very small difference and DM content and IM was about 1 % higher in the experimental group, which is related to zeolite addition. On the other hand, the mean value of $BOD_5$ was lower in the experimental group.

**Table 3. Physicochemical parameters in the slurry from control and experimental nursery unit**

| Parameter | Control unit | Experimental unit |
|---|---|---|
| pH | 7.1 | 7.0 |
| Dry matter (%) | 7.1 | 8.1 |
| Inorganic dry matter (%) | 24.1 | 25.8 |
| Biochemical oxygen demand ($mg.l^{-1}$) | 6764 | 6280 |

The addition of zeolite-clinoptilolite to mixed feed reduced the concentration of nitrogen compounds — ammonium and nitrate ions by about 26 % and nitrite ions by 12 % (Table 4).

In conclusion, a positive effect of "Pigozen" was observed on the quality of air in terms of aerial ammonia,

**Table 4. Level of nitrogen compounds determined in the slurry from control and experimental nursery unit**

| Parameter | Control unit | Experimental unit |
|---|---|---|
| Ammonium $NH_4^+$-N ($mg.l^{-1}$) | 1880 | 1378 |
| Reduction in $NH_4^+$-N in comparison with control (%) | | 26.7 |
| Nitrite $NO_2^-$-N ($mg.l^{-1}$) | 6.4 | 5.6 |
| Reduction of $NO_2^-$-N in comparison with control (%) | | 12.2 |
| Nitrate $NO_3^-$-N ($mg.l^{-1}$) | 2385 | 1771 |
| Reduction of $NO_3^-$-N in comparison with control (%) | | 25.8 |

which was decreased by 33 % in the experimental unit. The concentration of ammonium ions and other investigated nitrogen substances in the slurry was also decreased by between 12 and 26 %.

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# THE INFLUENCE OF THE AMENDMENT OF SEWAGE SLUDGE WITH ZEOLITE AND LIME FROM THE MICROBIOLOGICAL POINT OF VIEW

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## ABSTRACT

**Sewage sludge amended with zeolite and lime was stored for 42 days and investigated for plate counts of coliform faecal coliform bacteria and faecal streptococci. A decrease in plate counts of the observed micro-organisms in experimental substrates was recorded compared to the control sludge, related to changes in pH. The devitalisation effect of lime persisted only for the period of increased pH. The following decrease in pH level resulted in revitalisation of the respective bacteria even to levels exceeding the original.**

**Key words: coliforms; faecal coliforms; liming; sewage sludge; streptococci; zeolite**

## INTRODUCTION

Successful removal of potential pathogens from sewage sludge may be achieved by physical, chemical and biological methods (4). The use of natural materials for this purpose eliminates the risk of negative influences on the structure and properties of soil and soil micro-organisms.

Zeolites are minerals with a unique, three-dimensional structure and a large outer and internal surface available for ion exchange.

Lime is used in the form of oxide or hydroxide. It is known for its bactericidal influence on vegetative bacteria in animal slurry, leaves no residues and has no phytotoxic effects. From the viewpoint of devitalisation effects, one should stress the influence of lime on pH that may increase to a level between 10 and 12 (5, 7).

## MATERIAL AND METHODS

Treated sewage sludge, obtained from municipal waste-water treatment plant in Kokšov-Bakša, was mixed with one of the following (3 % by weight additions): powdered burnt lime S + L), natural powder zeolite (S + Z) and mixture of zeolite and lime (S + ZL). The substrates obtained together with the control were stored for 42 days at laboratory temperature (14.5 °C—17.9 °C). During this time samples were taken at 5-day intervals for determination of plate counts of coliform and faecal coliform bacteria and faecal streptococci according to respective Slovak standards. The samples were also examined chemically.

## RESULTS AND DISCUSSION

Results of microbiological examination indicate that the survival of coliforms and faecal coliforms in experimental substrates and the control differed from that of faecal streptococci. Fig. 1 shows that lime alone or in combination with zeolite considerably affected plate counts of coliform bacteria. However, this effect persisted for between only 2 to 4 weeks and we assume that it was related to pH in the respective substrates (11.8 in S + L and 11.9 in S + ZL). After this time (14 to 28 days), coliform counts increased considerably and in some cases reached levels higher than the original ones. This increase was related to a decrease in pH on day 28 of storage down to 9.55 in S + L and to 10.86 in S + ZL. On day 42, the pH of all substrates was below 9. No marked decrease in plate counts of coliforms was observed in S + Z compared to the control (Fig. 1).

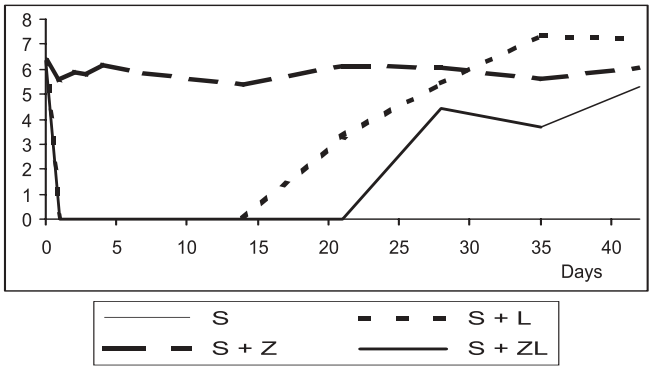


**Fig. 1. Plate counts of coliform bactera ($log_{10}$CFU.ml$^{-1}$) during 42-day storage**

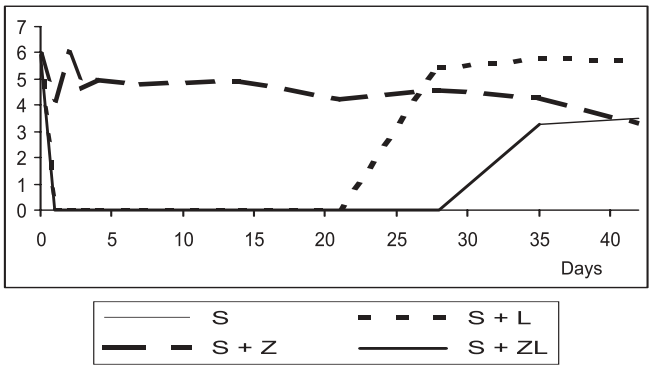


**Fig. 2. Plate counts of faecal coliforms ($log_{10}$CFU.ml$^{-1}$) during 42-day storage**

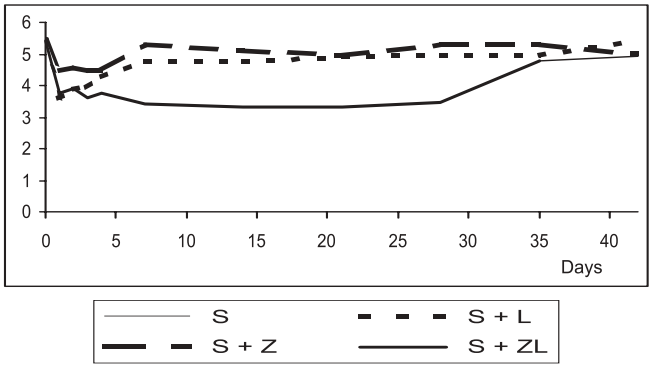


**Fig. 3. Plate counts of faecal streptococci ($log_{10}$CFU.ml$^{-1}$) during 42-day storage**

The final counts of faecal coliforms were lower by 2 orders compared to those at the start in S + Z ($3.3.10^3$ CFU.ml$^{-1}$) and S + ZL ($5.3.10^3$ CFU.ml$^{-1}$) substrates and only by one order in the control (Fig. 2).

The highest counts of faecal streptococci were found in the control and the lowest in the substrate S + ZL. However, by the end of the experiment the counts differed only little. A decrease observed between days 1 and 28 did not exceed two orders of magnitude (Fig. 3).

The results obtained indicate that increase in pH to highly alkaline was evidently the main devitalisation factor as the temperatures remained at the laboratory level and fluctuated very little.

The devitalisation effects of lime related to increased pH and release of ammonia have been reported by a number of authors. B i t t o n *et al.* (1) have reported reduction in micro-organisms in sludge at pH above 9 after liming. J e p s e n *et al.* (3) have observed reduction of faecal streptococci by three orders at pH 12.3. Liming of sludge resulted in elimination of *Salmonella* spp. within 24 h at pH 10 (2). The storage of sludge itself decreased counts of faecal coliforms and *Salmonella* spp. below detection limit.

An important conclusion of our study is that the effect of zeolite and lime in relation to devitalisation of indicator micro-organisms was higher than of the lime itself. It can be related to the affinity of zeolite for ammonia and its subsequent release as $NH_4OH$ also exhibits devitalisation effects. In a study by P a v o l o v á *et al.* (6) sanitation of sludge occurred at a ratio of 90 : 5 : 5 (raw sludge : CaO : zeolite).

The majority of authors have investigated the effects of lime immediately after its application but long-term investigations are not available. Liming decreases the counts of pathogenic organisms in sludge but fails to eliminate them completely. Therefore additional processing of sludge is required, e.g. chemical disinfection, composting or at least long-term storage on sludge fields.

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# HEAVY METALS IN SEWAGE SLUDGE AND PIG SLURRY SOLIDS AND THE HEALTH AND ENVIRONMENTAL RISK ASSOCIATED WITH THEIR APPLICATION TO AGRICULTURAL SOIL

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## ABSTRACT

**The examination of heavy metals (Cd, Pb, Zn and Cu) in influent, effluent, solid fraction or sewage sludge was carried out in two biological wastewater treatment plants treating pig slurry (WWTP-1) and municipal sewage (WWTP-2). Our results showed that higher than 80 % efficiency of metal removal was reached in both WWTPs. The levels of metals in the influent were evaluated with regard to their effect on the aquatic ecosystem in the recipient. The levels of Pb, Cu and Zn in pig slurry solids corresponded to national regulations but the levels of Cd exceeded the maximum acceptable concentration (10 mg.kg$^{-1}$ dry matter) in three samples of pig slurry solids and in two sludges.**

**Key words: heavy metals; hygiene risk; pig slurry solids; sewage sludge**

## INTRODUCTION

The constant growth of human population and increased demands on production of food are associated with marked changes in agriculture including animal production. Intensive animal production methods change the character of excrements, prevent their direct application to the soil and require additional treatment. An increasing proportion of the human population is concentrated in towns, which is associated with the production of large volumes of waste-water that must be treated before discharge into a recipient. Heavy metals from waste-waters are partitioned into sewage sludge and the effluent and are returned to the environment where they may contaminate soil, water and eventually also the food chain. While some metal compounds are essential to animals and humans, others are known to be toxic and the environmental impact of many of them has to be elucidated.

Our study investigated the potential risk arising from the presence of selected heavy metals in the effluent from waste-water treatment and in the solid by-products of the treatment of pig slurry and urban waste-waters.

## MATERIAL AND METHODS

The efficiency of waste-water treatment with regard to the removal of Cd, Pb, Zn and Cu was investigated in two aerobic waste-water treatment plants, one treating slurry produced by 20,000 fattening pigs (WWTP-1) and the second treating urban waste-waters from a conglomeration of approximately 100,000 inhabitants (WWTP-2). The solid fraction from WWTP-1 is stored and applied to agricultural soil and the sewage sludge from WWTP-2 is treated anaerobically and used in horticulture or for other agricultural purposes.

The level of Cd, Pb, Zn and Cu in the influent, effluent, pig slurry solids and sewage sludge was determined once a month for six months by AAS using the method by K o c o u r e k (1). The results are presented as minimum, maximum, mean ± SD in the influent, effluent, sludge and the solid fraction and efficiency of removal (RE). The results for solid fraction

and sludge were obtained on a wet basis and recalculated per dry matter (DM). The results were evaluated statistically with the software Microsoft Excel 7.0.

## RESULTS AND DISCUSSION

Metals are a unique group of toxic substances. They occur and persist in nature and their toxicity depends on their form (4). Inputs of metals to urban waste-water occur from theree generic sources: domestic, industrial and urban runoff. Human faecal matter typically contains 250 mg $Zn.kg^{-1}$, 70 mg $Cu.kg^{-1}$, 5 mg $Ni.kg^{-1}$, 2 mg $Cd.kg^{-1}$ and 10 mg $Pb.kg^{-1}$. The content of metals in animal excrements depends mostly on their species, category, age and feed rations. Approximately 70—75 % Zn, Cu, Cd, Cr, Hg and other metals in raw sewage is removed and transformed to sludge and the concentration of these elements in the final effluent would be expected to decrease by the same amount (5).

Cadmium as highly toxic metal ranks among the most hazardous metal pollutants. Cd has accumulated in some plants to levels that may be hazardous to humans. Its concentration in vegetable grown on cadmium polluted soil was higher. K o r é n e k o v á *et al.* (2) have analysed samples from 35 cows from an area polluted by industrial emissions and observed that Cd levels close to acceptable limits were detected in 7 out of 35 liver samples and in 2 out of 35 muscle samples.

The concentration of lead in biological tissues corresponds to environmental pollution and varies significantly with geographical area and demographic factors. It has no known essential role in an organism and its accumulation in tissues may cause a number of health hazards including neurotoxicity, haematotoxicity. Samples from cattle grazing on pasture contaminated with lead contained 40-times higher levels of this element (7).

Copper is an essential trace metal. The human population may be exposed to increased levels of CU in drinking water. Liming of sludge to pH 12 increases the mobility of Cu and Ni and therefore also their leaching (6).

Zinc is a cofactor in scores of metalloenzymes and is therefore an essential element but mey be toxic at high levels of exposure. Clinical signs include elevated liver enzymes (LOH, AST, SDH). A study by K o t t f e r o v á *et al.* (3) has shown that the liver is the main organ of accumulation of zinc, followed by the heart, kidney and leg muscles.

The results obtained in our study are summarised in Tabs. 1 and 2:

Our results show that a relatively high removal of metals (80.3—98.9 %) of metals was ensured in both WWTPs. The higher level of metals in the influent to WWTP-1 was reflected in the quality of the effluent. With the exception of Cu, metal levels in the effluent from WWTP-2 complied with acceptable limits for surface water. Cu and Zn levels in effluent from WWTP-2 complied with the limits for water for irrigation but the levels of Pb and Cd were higher. However, with regard to the considerable dilution of effluent in the recipient no serious risk to water fauna could be expected.

Sewage sludge contains many more heavy metals and other trace elements than artificial fertilisers. Its frequent application to soil may result in the accumulation of metals in soil and their subsequent release, for example as a result of acid rains, to soil solution and ground water. Act No. 23/2003 of the Code limits for the appli-

**Tab. 1. Content of selected heavy metals in WWTP-1**

| Metal | Influent ($mg.l^{-1}$) Min–max | Mean ± SD | Effluent ($mg.l^{-1}$) Min–max | Mean ± SD | RE (%) | Solid fraction ($mg.kg^{-1}$ dm) Min–max | Mean ± SD |
|---|---|---|---|---|---|---|---|
| Cd | 0.01–0.80 | 0.29 ± 0.31 | 0.006–0.01 | 0.008 ± 0.002 | 97.3 | 0.6–15.2 | 7.45 ± 5.90 |
| Pb | 0.02–1.70 | 0.66 ± 0.71 | 0.02–0.23 | 0.125 ± 0.082 | 80.3 | 3.8–45.2 | 35.38 ± 15.68 |
| Cu | 0.50–2.10 | 1.22 ± 0.64 | 0.03–0.10 | 0.067 ± 0.035 | 94.5 | 37.5–45.2 | 41.40 ± 2.,42 |
| Zn | 0.32–14.5 | 7.15 ± 5.86 | 0.12–0.44 | 0.27 ± 0.12 | 96.2 | 201.2–310.2 | 252.40 ± 34.96 |

**Tab. 2. Content of selected heavy metals in WWTP-2**

| Metal | Influent ($mg.l^{-1}$) Min–max | Mean ± SD | Effluent ($mg.l^{-1}$) Min–max | Mean ± SD | RE (%) | Solid fraction ($mg.kg^{-1}$ dm) Min–max | Mean± SD |
|---|---|---|---|---|---|---|---|
| Cd | 0.002–0.009 | 0.006 ± 0.002 | < 0.001 | | 83.3 | 2.60–12.4 | 8.00 ± 3.74 |
| Pb | 0.12–0.19 | 0.16 ± 0.03 | 0.001–0.005 | 0.003 ± 0.001 | 98.1 | 62.4–195.5 | 151.65 ± 41.99 |
| Cu | 1.02–1.08 | 0.72 ± 0.50 | 0.038–0.046 | 0.043 ± 0.003 | 94.0 | 172.5–299.2 | 258.97 ± 45.95 |
| Zn | 0.80–1.91 | 1.23 ± 0.43 | 0.012–0.018 | 0.014 ± 0.002 | 98.9 | 1110.4-790.3 | 1464.9 ± 248.32 |

cation of sewage sludge to agricultural soil are as follows: 10 $mg.g^{-1}$ DM for Cd, 750 $mg.g^{-1}$ DM for Pb, 1000 $mg.g^{-1}$ DM for Cu and 2 500 $mg.g^{-1}$ DM for Zn. The levels of investigated metals were below these limits as in sewage sludge as in pig slurry solids except for cadmium which exceeded the limit in three samples from WWTP-1 and two from WWTP-2. The risk of contamination of soil with Cd resulting from application of sludges and pig slurry solids may be further increased in industrially polluted areas where plants and animals may become affected and cause contamination of the entire food chain

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# THE EFFECT OF CADMIUM AND LEAD POLLUTION ON HUMAN AND ANIMAL HEALTH

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## ABSTRACT

**Our environment is affected by a great variety of pollutants. What they all have in common is that only their absence can eliminate the respective health risk. Therefore it is very important to recognise the problems and take appropriate measures early. The health risk arising from environmental contaminants depends on many factors including absorption and toxicity of the substance, its level in food, the quantity of contaminated food consumed and duration of exposure. Another complicating factor concerning chemical contaminants is that the toxicity of contaminants must be frequently extrapolated from animal studies. Whether the substances exert the same effect in humans is not always known with absolute certainty. Food products account for most of the human exposure to cadmium except for areas in the vicinity of cadmium-emitting industries. Cadmium is taken up by roots of plants and passes to edible leaves, fruits and seeds. It will also accumulate in animal milk and fatty tissues. Therefore people are exposed to cadmium upon the consumption of cadmium containing plants or animals. All food of animal origin with the exception of milk contains lead in higher concentration than in that of plant origin. The amount of lead in food has been reduced since 1990 due to the decline in production of some goods, the use of unleaded fuel, and other environmental protection measures. The mean weekly lead intake is less than 70 % of the tolerated value (PTWI) calculated on the basis of food consumption and the relevant levels of lead in food.**

**Key words: cadmium; environmental pollution; food chain; lead**

## INTRODUCTION

One of the most important aspects of environmental pollution is that people take up many pollutants through food. Animals are a rich source of food but also of various contaminants. In order to prevent danger to humans it is necessary to eliminate or at least minimise intentionally and systematically the penetration of extraneous matter into feed and the human food chain. This means that it is necessary to ensure that a minimum quantity of extraneous substances can pass to human beings so their levels cannot affect their health or the health of their offspring. One of the promising ways towards reaching this goal is continuous qualitative and quantitative determination of residues of extraneous substances in individual components of the environment and their subsequent monitoring in basic food commodities. It is also important to carry out complex studies dealing with potential elimination of these contaminants and their negative influence on live organisms.

## CADMIUM

Cadmium is a relatively volatile element not essential to plants, animals and humans. Its presence in live organisms is unwanted and harmful. An increased level of cadmium in the air, water and soil increases its uptake by live organisms. It

is taken up by plants and animals and through them also by humans. This leads to the cadmium cycle soil — plant — animal — man. P a v l í k *et al.* (8) have stated that up to 90 % Cd taken up by plants originates from soil and only 10 % from the atmosphere. Uptake of cadmium by plants occurs through roots and leaves. Plants take up cadmium from water only in the form of $Cd^{+}$ ion (after release from the sorption complex or from soil solution). Additional cadmium is transported to roots by diffusion and mass soil flow.

Green plants are the starting link of the food chain, which is the principal source of cadmium for animals and humans. Plants convert solar energy to chemical energy and store it in the form of organic compounds. They also accumulate unwanted substances including cadmium. Plants are consumed mostly by herbivores, which then become a source of food for carnivores. Omnivores consume plant products, herbivores and carnivores. The main port of entry of cadmium to the organism of animals is the digestive tract and alveolar absorption (2).

## LEAD

Lead is a microelement naturally present in trace amounts in all biological materials, i.e. in soil, water, plants and animals (9). It has no physiological function in the organism (7). According to C i b u l k a (1), the main sources of lead contamination are smelting works, application of wastewater treatment sludges to soil, transportation, rain, snow, hail and other. Approximately 98 % of lead in the atmosphere originates from human activities. N e u m a n n *et al.* (7) have extended the sources of lead pollution by paints, lead wastes, cell batteries, lead solders and forms.

Lead is absorbed by plants through roots where most of the lead is also accumulated. In the case of other than root uptake (along roads in urban regions) the content of lead decreases as follows: above ground part > roots > products (4). Intoxication of animals by lead occurs particularly after grazing on pasture contaminated with lead (10).

Lead enters the organism with food and air. In children it affects most the central nervous system. The toxic effects of lead in the prenatal stage cause a shortening of gravidity (higher rate of premature babies), decrease in birth weight and retardation of mental development.

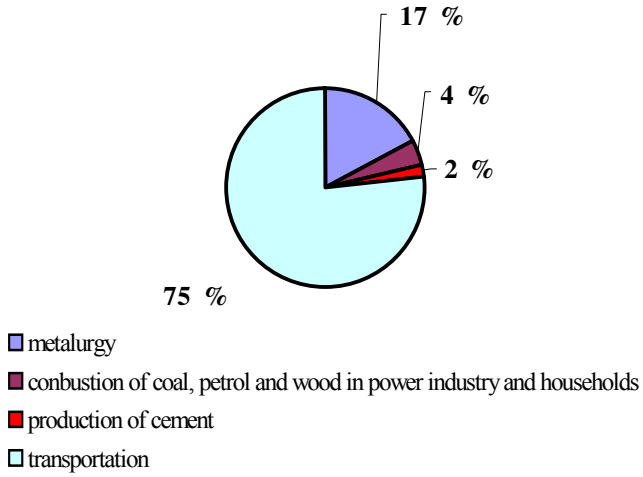


**Fig. 1. Percentage proportion of principal sources of environmental lead contamination (6)**

Lead intoxication is one of the most frequent diseases in farm animals, particularly in those grazing on pasture in the vicinity of metallurgic complexes but also close to busy roads. Species specific susceptibility to lead has been described in cattle, particularly the young one. Dairy cows eliminate Pb in milk. Intoxication with mercury results in inappetence, pathological changes in skin and hair and decreased milk yield in dairy cows. In animals cadmium causes softening of bones and decreased productivity. Cadmium is a typical example of a cumulative poison. Prolonged increased uptake of cadmium interferes with the function of the kidneys. Bone tissue is affected too as cadmium disturbs the metabolism of calcium (3).

## CONCLUSION

Chemical residues have raised serious problems and risks, which will persist also in the future. The reason for this pessimistic prognosis is the global terrestrial polycontamination of all ecosystems and components of the environment by human activities and local contamination of production areas with a range of chemical contaminants. There is an effort to decrease to a minimum or eliminate penetration of the most serious contaminants into the food chain and therefore also into the human body. One of the tools in this struggle is the target monitoring of relevant contaminants in raw materials and food of animal origin (5).

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# LYME BORRELIOSIS, THE PRESENT DIAGNOSTIC CRITERIA

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## ABSTRACT

**Recent clinical experience and literature sources have shown the co-incidence with multiple tick-borne pathogens resulting in Lyme disease, and ehrlichiosis and/or babesiosis. Diagnosis and differential diagnosis of these diseases require attention to the possibility of the presence of mixed infection. Lyme borreliosis risk prevalence, is common with other tick-borne disease and is closely relating to the ecology of an area. These phenomenon indicate a need to monitor cases with Lyme disease for all currently known tick borne pathogens.**

**Key words: babesiosis**; **dogs**; **ehrlichiosis**; **Lyme disease**; **ticks**

## INTRODUCTION

Lyme disease is a systemic diseases in man and animals, caused by *Borrelia burgdorferi* sensu lato, transfered by ticks from *Ixodes ricinus* in European conditions.

The environmental conditions and pathogenesis of Lyme borreliosis are not fully explained. It is possible to see the significant importance of variety of interrelations: of 11 genospecies of *Borrelia burgdorferi* sensu lato, their geographical distribution, ticks infestation, and so on. Tick saliva contains multiple pharmacologically active molecules with an immunossuppressive effect (8). The mechanisms of immunossuppression play an important role in the pathogenesis of arthritis. Lyme borreliosis (LB) can manifest in three stages. In a range of three to five days after a tick bite *erythema chronicum migrans* can develop which is an early stage of LB. In the next stage anorexia, weakness, vomitus, and so on can be present. The second stage, chronic borreliosis, can develop after one or two months post infection. Lyme arthritis is the most often occurring musculoskeletal symptom of LB. The third stage, chronic neuroborreliosis, is the final stage of LB.

Most of studies in humans as well as in animals showed the possibility of the coincidence of multiple tick borne diseases. It can be in a mixed infection developing at the same time, which could be presented with individual clinical signs, non-characteristic symptoms and with decreased sensitivity to standard diagnostic kits (3, 5).

## MATERIAL AND METHODS

A clinical and laboratory examination focusing Lyme borreliosis (LB), haematological, examination including blood smear and urine samples, CSF, and samples for aspiration cytology, were completed at the 1st Internal clinic of UVM, Košice and at the multiple private clinic in Czech Republic: Olomouc, Brno and Prague in dogs with a history data of tick infestation. From the differential diagnosis point view USG, RTG, ECG, endoscopy and so on were used. Serological examination for the detection of antiborrelia IgG antibodies were completed using the ELISA method (7) used for diagnosis in the Institute of Parasitology SAS, Košice and Faculty of Medicine UK, Prague.

## RESULTS AND DISCUSSION

From the 650 examined dogs included in the trial 38.6 % dogs showed the presence of antiborrelia IgG antibodies. Clinical signs did not correlate always with the presence of antiborrelial IgG antibodies. The common findings included locomotory system disorders with clinical manifestation of chronic arthritis and pododermatitis. In a case of 28 % of seropositive dogs arthritis was the most often and common form of borreliosis in dogs seen clinically with movement anomalies, reluctance to move, lethargy and so on. These signs have been shown also by other authors (1, 2). Clinical signs were recurrent and variable with a variety of complication in some of cases diagnosed months after the primary infection. LB showed significant leukocytosis. The presented work showed the importance of *Giardia* spp. and *Babesia* spp. in coincidence with immunossuppression in the aetiology of complicated cases.

For the **treatment** of LB penicillin and tetracyclin preparate are recommended. The later stages: cardiological, joint, neurological, and many other complications requires oral or intravenous application of antibiotics including cephalosporines.

**Prevention**: individual protection, protection of environment from ticks and effective immunization together with the usage of immunomodulators and other additives are discussed (2, 3, 5).

## CONCLUSION

At present, several cases of Lyme borreliosis have a complicated course. The possibility of coincidence of multiple pathogens transmitted by ticks such as *Giardia* spp. in seropositive cases of LB has shown the importance in the development of GIT complication the early detection of these cases requires selected diagnostic approaches (4, 6, 7).

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# APPLICATION OF THE DNA METHOD FOR THE DIAGNOSIS OF ANIMAL PATHOGENS

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## ABSTRACT

**The PCR conditions for rapid diagnosis of the strains *E. coli* (encoded F4 fimbriae) and *Salmonella* were tested. We determined the conditions for amplification of *E. coli* and *Salmonella* specific-DNA products from a bacterial culture which, besides the *E. coli* and *Salmonella*, also contained other bacteria.**

**Key words: DNA method; *E. coli* and *Salmonella* diagnosis; PCR reaction**

## INTRODUCTION

Diseases of humans and animals caused by bacterial pathogens present a serious heath and economical problem. Economic losses on animal farms associated with diagnostic costs, treatment of sick animals, laboratory diagnosis and costs of disinfection and disease prevention and control increase the total death losses considerably (11, 12). These economic losses can be decreased by limiting the incidence of diseases in animals and humans. One of the potential ways is the prevention of contact of humans and animals with infectious agents (4, 9).

Salmonelloses and diarrhoeal diseases caused by enteropathogenic *E.coli* strains (EPEC) are still a common problem. Therefore the need for rapid and reliable diagnostic methods involving these pathogens is very urgent (1, 2). Several methods have been developed for this purpose including bacteriological culturing, ELISA tests, DNA hybridisation tests, skin tests, radioimmunological methods and PCR (3, 5, 6, 8).

The study presents results of a PCR diagnosis of bacterial strains *Salmonella, Salmonella enteritidis* and ETEC *E. coli* strains expressing K88ab antigen.

## MATERIAL AND METHODS

**Bacterial strains and cultivation:** To optimise PCR conditions for the determination of the genus *Salmonella*, serovar *Salmonella enteritidis* and *E. coli* K88$^+$ we used bacterial strains *Salmonella enteritidis* 225 (a gift from Dr. Nagy, Hungary), a field isolate *Salmonella typhimurium* 4/5, *Salmonella typhimurium* TM 333, *E. coli* M1, *E. coli* 7301, *E. coli* G7, *E. coli* G205, *E. coli* 6012, *E. coli* 987, *Salmonella typhimurium* pYA, *E. coli* HB 101 and *Proteus vulgaris*. The strains were cultured in LB medium overnight at 37 °C with constant shaking.

**Isolation of DNA, PCR reaction:** according to Holoda *et al.* (7).

**Preparation of samples for PCR determination, Agarose gel electrophoresis:** according to V u – K h a c *et al.* (13).

## RESULTS AND DISCUSSION

Investigations were carried out to determine PCR conditions for the rapid diagnosis of bacterial strains expressing adherence of K88ab antigen. We tested the dependence of PCR reaction on temperature, time of individual steps, number of cycles and concentration of $MgCl_2$ in the reaction buffer. On the basis of the known sequence of the gene encoding K88ab antigen (10), a fragment of size 877 bp was expected in the case of strain *E. coli* M1. According to our expectations the best results were reached under reaction conditions summarised in Table 1.

To determine the conditions for the PCR diagnosis of the genus *Salmonella* and species *Salmonella enteritidis* under equal conditions (Table 2) we used DNA isolated from the bacterial strains *S. typhimurium*, *S. enteritidis* and *E. coli*.

To prove the specificity of the used primers we detected *Samonella* by PCR reaction under the conditions mentioned in a contaminated sample in the presence of *E. coli*, *S. typhimurium*, *S. enteritidis* and *Proteus vulgaris*. In none of the cases false negative or false positive results were obtained.

**Table 1. PCR conditions for detection of K88 ab antigen**

| Temperature | Time (min) | Number of cycles |
|---|---|---|
| 95 °C | 4 | 1 — initial |
| 95 °C | 1.0 | |
| 52 °C | 1.0 | |
| 72 °C | 1.5 | 30 |
| 72 °C | 2.5 | 1 — final |
| Concentration of $MgCl_2$ 2.5 mM | | |

**Table 2. PCR conditions for detection of the genus *Salmonella* and species *Salmonella enteritidis***

| Temperature | Time (min) | Number of cycles |
|---|---|---|
| 95 °C | 2 | 1 — initial |
| 95 °C | 0.5 | |
| 54 °C | 0.5 | 30 |
| 72 °C | 0.6 | |
| 72 °C | 2.5 | 1 — final |
| Concentration of $MgCl_2$ 2.5 mM | | |


## ACKNOWLEDGEMENTS

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# MALASSEZIOSIS AND IMMUNITY

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## ABSTRACT

**Selected parameters of non-specific cell-mediated immunity were evaluated in seven dogs with malasseziosis (*Malassezia pachydermatis)* complicated with a secondary bacterial infection (*Staphylococcus intermedius, Staphylococcus haemolyticus*). Clinical disease persisted for two to three months. We observed a significant increase in total leukocyte count, phagocytic activity, ingestion ability of neutrophils and blastogenic response of lymphocytes and a non-significant decrease in metabolic activity of phagocytes.**

**Key words: blastogenic response of lymphocytes; dog; malasseziosis; phagocytosis**

## INTRODUCTION

Dermatomycoses in dogs are caused by dermatophytes belonging to the genera *Microsporum, Trichophyton, Malassezia* and *Candida.* The incidence of infections by *Candida* and *Malassezia* species has increased recently. An increasing use of antibiotics, immunosuppressants and cytostatics in small animal practice and insufficient care and hygiene contribute to the development of these diseases. Opportunistic fungi usually require a host that is immunosuppressed by metabolic problems, malnutrition, concurrent viral or bacterial infections or neoplasia. Generally, predisposing factors limiting development of dermatomycosis include any disease process that compromises the skin barrier and suppresses the immune response of a host (3).

Malasseziosis is caused by *Malassezia pachydermatis* that inhabits as a saprophyte the ears and skin and may become pathogenic. The main clinical signs are otitis externa, erythematous lesions, alopecia, hyperkeratosis. The condition is always accompanied by intense itching. In the case of mixed infections with Gram-positive bacteria as well as other types of yeasts, the clinical signs are pronounced and the course of disease is prolonged.

The aim of our study was to evaluate some parameters of non-specific cellular immunity in dogs with malasseziosis complicated with a secondary bacterial infection.

## MATERIAL AND METHODS

**Animals:** 7 dogs of average age 4.7 years, of different breed and sex with natural dermatomycosis confirmed by clinical and mycological examination. Dermatomycosis was complicated by a bacterial infection verified by BBL Crystal identification system (Becton Dickinson, USA). Twenty uninfected dogs of average age 3.1 years, of different breed and sex served as a control.

**The immunological parameters evaluated:**

***Blastogenic response of blood lymphocytes to mitogens:*** Lymphocytes were separated from venous blood on the Ficoll density gradient (Pharmacia Biotech AB, Sweden). The cultivation, mitogen stimulation and measurement of blastogenic response of lymphocytes by fluorescence method were performed according to Nakanishi *et al.* (4). The level of the blastogenic response of the lymphocytes was expressed as the stimulation index (SI).

**Table 1. Haematological and immunological parameters in dogs with malasseziosis compared with those in healthy dogs**

| Dog | Lc ($\times 10^9.l^{-1}$) % | Ne | PANe (%) | IPa Ne | IMA | SI |
|---|---|---|---|---|---|---|
| **1** | 12.6 | 59 | 81 | 18.1 | 2.250 | 1.71 |
| **2** | 10.6 | 53 | 84 | 16.7 | 2.103 | 1.14 |
| **3** | 12.2 | 71 | 87 | 25.7 | 2.169 | 1.84 |
| **4** | 9.2 | 51 | 88 | 21.8 | 2.477 | 1.25 |
| **5** | 18.8 | 60 | 80 | 16.4 | 2.413 | 1.75 |
| **6** | 21.2 | 58 | 81 | 19.5 | 1.546 | 1.35 |
| **7** | 16.8 | 58 | 79 | 15.4 | 1.813 | 1.50 |
| **Mean ± SD** | 14.5 ± 4.5 | 58 ± 6.4 | 82.5 ± 3.4 | 19.1 ± 3.6 | 2.110 ± 0.331 | 1.51 ± 0.3 |
| **Control** (n = 20) | 9.5 ± 3.5 | 65.6 ± 9.6 | 58.3 ± 5.7 | 7.2 ± 5.7 | 2.206 ± 1.057 | 2.90 ± 1.2 |
| *t*-test | $p < 0.005$ | NS | $p < 0.005$ | $p < 0.005$ | NS | $p < 0.01$ |

Lc — total leukocyte count, Ne — neutrophils, PANe — phagocytic activity of neutrophils
IPANe — index of phagocytic activity of neutrophils, IMA — index of metabolic activity, SI — stimulation index of lymphocytes

***The phagocytic activity of blood neutrophils*** was examined by the method of ingestion of MHSP particles (5). The phagocytic activity (PA) of neutrophils (Ne) was expressed as the percentage of the neutrophils phagocytizing 3 and more MSHP, and as the index of phagocytic activity (IPA) representing the ingestion ability of neutrophils (the ratio of the number of phagocytized MSHP and phagocytizing Ne).

***The metabolic activity of phagocytes*** was determined by modified INT (iodnitrotetrazolium) test and expressed as index of metabolic activity (IMA).

***Total leukocyte count and differential cell count*** were determined using common haematological methods.

Means and standard deviations were calculated from the respective results. The significance of differences was checked by Student's *t*-test.

## RESULTS AND DISCUSSION

Mycotic infection (*Malassezia pachydermatis*) and bacterial infection (in most cases *Staphylococcus intermedius*, in one dog *Staphylococcus haemolyticus*, in one dog *Enterococcus faecium* and *E. coli*) were identified in the examined dogs. Clinical signs persisted for two to three months. Alopetic and erythematous lesions, scaling and intense pruritus predominated. A typical feature of this condition was the progressive spreading to the whole body and the permanent formation of new lesions.

The results of immunological examination are shown in Table 1. Leukocytosis was found in all dogs examined. The percentage of phagocytizing neutrophils and index of phagocytic activity were significantly in contrast to those in healthy dogs. The metabolic activity of phagocytes was lower in affected dogs but without statistical significance. The proliferation of dog peripheral lymphocytes to nonspecific mitogen, concanavalin A was significantly lower when compared to the control group.

The monitoring of selected immunological parameters in dogs with malasseziosis revealed the most significant changes in the values of phagocytic activity and ingestion capacity of neutrophils. Similarly increase in the ingestion activity of phagocytes was observed in a study of trichophytosis in foxes (3) in the initial weeks of infection. When the disease process proceeded to the chronic stage, this value decreased significantly. In dogs clinical signs of the disease persisted for two to three weeks with the permanent formation of new lesions. The results were supported by haematological findings (leukocytosis).

Cell mediated immunity plays a decisive role in the protection of organisms against dermatophytosis. In our cases acute inflammatory process on the skin activated some immune functions, mainly a phagocytic activity of neutrophils. A decrease in some parameters of cellular immunity in human patients suffering from dermatophytosis was reported by Jautová *et al.* (2). De Moraes-Vasconcelos *et al.* (1) have presented an alteration of cellular immunity, especially of some functions of polymorphonuclear leukocytes and a proliferative response of lymphocytes in chronic dermatophytosis.

Alteration of any cellular immunity function worsens the infection and leads to secondary bacterial complications. Secondary bacterial infection, predominantly that caused by *S. intermedius*, developed in all the examined dogs. As the dogs were not examined repeatedly it is difficult to judge when the secondary pyoderma occurred or to determine the accurate cause of alteration of immunity. To provide appropriate explanation it is necessary to carry out additional observations in different stages of the disease or examine the affected dogs repeatedly.

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# ASANATION OF THE ENVIRONMENT CONTAMINATED WITH ENTERONEMATODE EGGS

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## ABSTRACT

The effect of the anaerobic composting of organic wastes from public spaces on the survival of helminth eggs *Toxocara canis* and *Ascaris suum* was observed in the Field Composting Plant in Brezno (SR). After 150-days of composting, most of *A. suum* eggs preserved their viability (63.47 ± 4.15 %) owing to the effect of anaerobic conditions, low temperature (40 °C), low C:N ratio (to 15:1) and changes in the physico-chemical properties of the composted organic wastes. In the control group, incubated under optimal aerobic conditions in distilled water, there were 84.48 ± 1.82 % vital eggs. In the process of composting the vitality of *T. canis* eggs was more influenced than the vitality of *A. suum* eggs. Following 150 days exposure in the composting pit on average there were only 20.11 ± 1.85 % vital eggs. The results indicate the risks of dissemination, survival and potential spread of developmental stages of endoparasites in the environment by means of organic wastes processed using cold composting.

Key words: anaerobic composting; *Ascaris suum* eggs; *Toxocara canis* eggs

## INTRODUCTION

Dogs and cats may be the reservoirs of many different zoonotic endoparasites including *Giardia* sp., *Balantidium* sp., *Toxoplasma* sp., *Ascaris* sp., *Toxocara* sp., *Toxascaris* sp., *Echinococcus* sp. and *Taenia* sp. Epidemiologically, toxocariasis, caused by nematodes *Toxocara canis* and *T. cati* is considered to be one of the most serious parasitic diseases of humans (1, 3). Therefore we should focus on the primary source of infection – dog and cat faeces. Excrements may be regarded as a type of municipal waste with no special requirements for its manipulation with regards to health protection. In order to reduce the occurrence of potential zoonotic pathogens in the environment, it is possible to use the composting of organic wastes from public areas. The aim of our work was to monitor the effect of anaerobic composting of organic wastes on the vitality and infectiousness of *T. canis* emryonated eggs and the viability of non-embryonated *A. suum* eggs resistant to the outer environment.

## MATERIAL AND METHODS

*A. suum* and *T. canis* eggs were isolated *via* dissection of uterine distal ends of female pig ascaris and *T. canis*. The eggs were inoculated into polyurethane carriers, prepared according to Plachý and Juriš (4) at a dose of 1,000 eggs per carrier and stored in a composting pile. The viability of exposed embryonated *T. canis* eggs was confirmed by a biological experiment on mice, ICR strain. The number of mice used in the experiment was limited by the number of viable eggs obtained after exposition in composting piles. The control group comprised five mice, infected with a dose of 500 eggs per mouse. The larvae were isolated from the brain and muscles on day 28 after infection using the modified digestive method (8). The viability of exposed unembryonated

*A. suum* eggs was determined by incubation in the embryonated stage in a thermostat at 26 °C over 21 days. The developmental ability of *A. suum* eggs was compared with the control, where the eggs were kept in distilled water in aerobic conditions.

The physical and chemical analysis of composting material was performed according to the standards for industrial composts (5). The C:N ratio was determined according to Navarro *et al.* (2).

The samples for analyses were collected on day 0, 35, 60, 110 and 150 of composting. The experiment was organised with three repeats.

## RESULTS AND DISSCUSION

The operating experiment was set up in a Field Composting Plant in Brezno, which processes the waste from public areas (composition: grass — 60 %, leaves — 25 %, tree branches— 5 %, soil — 10 %), parks and playgrounds (dogs excrements and other) along with animal manure mixed with soils and layered into the composting piles (2 metres high and 25 metres long). The surface of the piles is not covered with any material, resembling natural conditions. The temperature in the pile did not exceed 40 °C during the experiment.

In the composting organic substrate the following changes in physical and chemical properties were monitored — pH, dry matter, inorganic (IM) and organic (OM) matters, ammoniacal ions ($NH_4^+$), total nitrogen ($N_t$), total phosphorus ($P_t$) and C:N ratio (Table 1).

Due to the anaerobic conditions, low temperature, low C:N ratio after 150 days of composting, the number of *A. suum* eggs decreased by 21.0 % and *T. canis* eggs by 49.9 % (Table 2).Viable *T. canis* eggs were even after composting infectious.

Our results correspond with the results of Thevenot *et al.* (7). They found after 120 days of composting in 60 % of samples viable eggs of *Ascaris* sp., *Trichuris* sp., *Toxocara* sp. and *Hymenolepis* sp. Similarly Tharaldsen and Helle (6) have found that *Ascaris* sp. eggs were not devitalised even after three weeks of composting at 30 °C. The majority of *Ascaris* sp. eggs were devitalized at 37 °C in two weeks, 15 to 27 % of the eggs were able to develop further.

Even though during anaerobic composting in psychrophillic and mezophillic temperature zone the total devitalisation of model parasitic germs was not recorded, cold composting can be used in the processes of operated (regulated) composting. The problems relating to environmental sanitation and waste management is highly topical and it is a truth universally acknowledged that the majority of endoparasitic germs are able to cause infection after a year or two in animals as well as in humans.

**Table 2. Survival of the *T. canis* and *A. suum* eggs during anaerobic composting**

| Exposure (days) | Demaged eggs ($\bar{x}$ % ± SD) *A. suum* | *T. canis* |
|---|---|---|
| **0 (control)** | 15.52 ± 1.82 | 6.12 ± 1.15 |
| **35** | 19.22 ± 1.42 | 31.41 ± 14.97** |
| **60** | 24.09 ± 3.66 | 41.06 ± 12.88** |
| **110** | 28.60 ± 1.45* | 70.18 ± 0.76*** |
| **150** | 36.53 ± 4.15* | 56.06 ± 0.72*** |

Differences between control and examinated group significant at the level S:
* — $P < 0.05$; ** — $P < 0.01$; *** — $P < 0.001$


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**Table 1. Physico-chemical properties of the organic material during anaerobic composting**

| | Exposure (days) 0 | 35 | 60 | 110 | 150 |
|---|---|---|---|---|---|
| pH | 7.83 ± 1.02 | 8.14 ± 0.02 | 8.79 ± 0.23 | 8.35 ± 0.05 | 7.73 ± 0.02 |
| Dry matter (%) | 63.68 ± 12.00 | 66.46 ± 2.00 | 73.61 ± 1.10 | 58.83 ± 0.23 | 63.49 ± 1.75 |
| IM* | 72.48 ± 17.92 | 78.11 ± 1.99 | 71.75 ± 2.87 | 60.33 ± 8.95 | 77.08 ± 4.70 |
| OM* | 27.52 ± 17.92 | 21.89 ± 1.99 | 28.25 ± 2.87 | 39.67 ± 8.95 | 22.92 ± 4.70 |
| $NH_4^+$ * | 9.26 ± 7.01 | 5.71 ± 1.39 | 31.93 ± 10.20 | 28.74 ± 2.32 | 17.64 ± 1.98 |
| $N_t$* | 21510.20 ± 3802.34 | 11273.68 ± 1667.36 | 18558.29 ± 4013.61 | 30512.24 ± 4893.06 | 27793.39 ± 792.36 |
| $P_t$* | 1102.51 ± 184.14 | 1572.93 ± 97.78 | 1600.41 ± 293.48 | 909.52 ± 64.36 | 1223.47 ± 61.90 |
| C:N | 10.23:1 | 14.86:1 | 10.54:1 | 11.27:1 | 6.61:1 |

* — $mg.kg^{-1}$

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# THE INFLUENCE OF AIR POLLUTANTS ON CHANGES IN SOME BLOOD AND LIVER PROFILE PARAMETERS OF THE POLAR FOX

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## ABSTRACT

**Investigations were conducted on a farm housing about 50 polar foxes (*Alopex lagopus*) to determine the impact of gaseous pollutants on blood and liver profile parameters. Twelve animals caged in a pavilion system made up the control group (6 females and 6 males) and the offspring (6 females), kept in a chamber with controlled air flow, constituted the experimental group. The activity of ALP, AST and ALT were increased insignificantly in the experimental group of foxes.**

**Key words: gas pollutants; liver profile; polar fox (*Alopex lagotus)***

## INTRODUCTION

Under undesirable environmental conditions, apart from the stress resulting from decreased welfare, many systems of organisms may be inactivated. The action of some environmental stressors (gas pollutants) may result in some changes in physiology, reproduction and behaviour as observed in some studies (1, 5, 6).

## MATERIAL AND METHODS

The investigations were carried out to observe the influence of gaseous pollutants released from a fur bearing animal farm on the health of polar foxes. Over the experimental period fifty polar foxes (*Alopex lagopus*) were housed on a farm in south-eastern Poland. The animals were caged in a pavilion system. Six males and six females made up the control, while the offspring (sx females) was kept in a chamber with controlled air flow and constituted the treatment group. Gases in the air were monitored for both groups using colorimetric and gas chromatography methods (1).Throughout the experiment, the foxes were supplied the same diet corresponding to standards for fur animals and suitable for their age. The animals were under veterinary and animal husbandry supervision.

Blood for examination was collected three times from the foot vein *(vena saphena parva)*. Respective diagnostic kits (Cormay and Medonic) were used to determine selected parameters in blood and blood serum of foxes.

The data were processed statistically using variance analysis for the nonorthogonal triple cross classification at weighed restrictions.

## RESULTS AND DISCUSSION

The feeding and maintenance conditions of animals are vital for their health status, efficiency and reproduction parameters. Air pollution as one of the microclimate factors results in disturbances of homeostasis and, subsequently, in reduced animal welfare.

By means of gas chromatography higher concentrations of gaseous pollutants were determined in the enclosed space compared to those in the pavilion cages.

**Table 1. Morphological parameters of control and experimental foxes**

| Collection site | RBC ($10^6$.mm$^{-3}$) | | HCT (%) | | HGB (g.dl$^{-1}$) | | WBC ($10^6$.mm$^{-3}$) | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | SD | $\bar{x}$ | SD | $\bar{x}$ | SD | $\bar{x}$ | SD |
| Females in cages | 9.18 | 0.76 | 41.25 | 3.97 | 16.2 | 1.17 | 9.56 | 1.51 |
| Females in chamber | 8.62 | 1.03 | 40.92 | 4.86 | 15.71 | 2.01 | 10.80 | 1.93 |

RBC — red blood cells; HCT — packed cell volume; HGB — haemoglobin; WBC — white blood cells

According to the monitoring of air pollutants their levels were affected by the sampling site, temperature and air flow. Throughout the experimental period the highest levels of organic sulphur compounds were recorded for methyl mercaptan and dimethyl disulphide. Of aldehydes hexanal (122.43 mg.m$^{-3}$) and pentanal (154.27 mg.m$^{-3}$) predominated. Acetaldehyde (69.23 mg.m$^{-3}$) and butanol (148.73 mg.m$^{-3}$) exceeded the permissible mean values per calendar year (5, 6, 7).

The presence of gaseous contaminants in the air influenced the morphological parameters and activity of enzymes. In the experimental group lower values were recorded for RBC and HCT and higher for WBC in comparison with the control group (Table 1).

The mean ALP level in females reached 47.03 U.l$^{-1}$ in the control and 51.23 U.l$^{-1}$ in females from the treatment group (Table 2). The slight increase in ALP in animals stressed by pollutants was insignificant. Although the ALP activity in both groups was higher than the reference values, it remained within the standard values for canines (2, 3, 4).

The mean AST level was 36.80 U.l$^{-1}$ in females in cages and 36.81 U.l$^{-1}$ in those in the chamber (Table 2). The AST activity surpassed slightly the reference values (2).

**Table 2. Mean activity of ALP, AST and ALT of experimental and control foxes**

| Enzyme | Experimental females | | Control females | |
|---|---|---|---|---|
| | $\bar{x}$ | SD | $\bar{x}$ | SD |
| Alkaline phosphatase ALP (U.l$^{-1}$) | 51.23 | 21.47 | 47.03 | 17.61 |
| Asparaginine aminotransferase AST (U.l$^{-1}$) | 36.81 | 2.62 | 36.45 | 3.26 |
| Alanine aminotransferase ALT (U.l$^{-1}$) | 56.27 | 3.71 | 51.90 | 2.85 |

The mean ALT activity in females from the treatment group was a little higher (56.27 U.l$^{-1}$) compared to the females from the control group (51.90 U.l$^{-1}$) (Table 2). No statistically significant differences were detected between the groups.

Irritation of the respiratory tract was observed in the experimental foxes manifested by excessive accumulation of mucus (6).

In conclusion, investigation of polar foxes exposed to volatile gaseous pollutants has not provided an explicit answer regarding their influence on morphological parameters and the activity of selected enzymes.

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# THE LEVEL OF SOME PLASMA OXIDATIVE STATE INDICES IN FARMED MINKS AND THE HISTOPATHOLOGICAL PICTURE OF THEIR INTERNAL ORGANS

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## ABSTRACT

**Investigations were carried out for one year on sixty scanbrown farmed mink yearlings (thirty experimental, thirty control) intended for slaughter with the aim of determining some blood plasma indices, considered to be markers of the oxidative state (glutathione peroxidase GPx, glutathione reductase, superoxide dismutase EC-SOD, total antioxidant status and proteins). The experimental group was supplemented with preservative and antioxidant additives. Ten slaughtered animals from each group were subjected to *postmortem* and pathomorphological examination. Internal organs essential for digestion-absorption processes and metabolism were examined histopathologically. The feed supplementation caused differentiation of histopathological changes in the liver of the animals. No pathological changes were recorded in the mink alimentary tract.**

**Key words: antioxidant; feed; minks; pathological histology; preservatives**

## INTRODUCTION

Excess of aerobic radicals is hazardous to animal health owing to their high nonspecific reactivity. It may lead to an imbalance between antioxidants and prooxidants in favour of oxidation that induces oxidation stress. Numerous studies have reported a relationship between oxidation stress and health disturbances (4, 9, 10). The sources of free radicals seem to be vital as they developed in an organism under endo- and exogenous conditions. The latter include nutrition, ultraviolet radiation and environmental pollution (2, 3).

The objective of the present paper was to determine the values of some blood plasma markers considered the oxidation state indices in one-year old farmed minks.

## MATERIAL AND METHODS

The investigations were performed on a mink farm in south-eastern Poland (approx. 500 scanbrown females). Sixty one-year old minks intended for slaughter were included in the experiment. Blood was collected in the fourth year from the heart. After weaning, the animals were divided into two groups (I — experimental, II — control), thirty minks in each. Both groups were fed a diet of the same composition and energy level but group I was supplemented with sodium pyrosulphite (0.2—0.3 % ready feed) and antioxidant Hadox (0.15—0.20 g per kg ready feed). Vitamin-mineral premix Guyofox was added to the feed at a dose of 1.0 $kg.t^{-1}$ ready feed.

Determinations in plasma: glutathione reductase according to Akerboom and Sies (1) activity of superoxide dismutase by the method of Misra and Fridowich (8), glutathione peroxidase activity and total antioxidant status with a diagnostic test of RANDOX, and the level of soluble protein by the method of Lowry *et al.* (5) using a two-beam spectrophotometer CE 7200 CECIL.

Ten slaughtered animals from each group were subjected

**Table 1. Quantitative composition of feed (%) supplied to minks and energy value per 1 kg feed**

| Product | Aug. – 15th Sept. | 16th Sept. – 10th Nov. | 11th Nov – slaughter |
|---|---|---|---|
| COD | 4.0 | 7.0 | 5.0 |
| SPRAT X | 15.0 | – | – |
| Poultry parts | 42.0 | 50.0 | 55.0 |
| Horse stomach | 10.0 | 10.0 | 13.0 |
| Meat-bone meal | 8.0 | 8.0 | 6.0 |
| Animal fat | 3.0 | 2.0 | 1.0 |
| Crude wheat meal | 9.6 | 9.6 | 12.0 |
| Bran | 2.0 | 2.0 | 2.0 |
| Boiled potatoes | – | 5.0 | – |
| Water | 6.0 | 6.0 | 6.0 |
| Guyfox | 0.1 | 0.1 | 0,1 |
| Sodium pyrosulphite | 0.28 | 0.28 | – |
| RENDOX | 0.02 | 0.02 | – |
| Energy content Kcal.kg$^{-1}$ | 1700 | 1670 | 1690 |
| **% EM** | | | |
| Proteins | 35.1 | 33.3 | 33.0 |
| Fat | 52.4 | 52.5 | 52.2 |
| Carbohydrates ($CH_2O$) | 12.5 | 14.2 | 14.8 |

COD — fillet scraps (heads, backs); SPRAT X — all fish; Poultry parts — heads, paws, bowels

to *postmortem* and pathomorphological examinations. The internal organs essential for digestion-absorption processes and metabolism (stomach and duodenum, small and large intestine, liver, kidneys) were examined histopathologically.

The results were analysed statistically using the ANOVA method.

## RESULTS AND DISCUSSION

Antioxidative enzymes play a vital role in the defensive system against attack of free radicals. They include, among others, superoxide dismutase (EC-SOD) and glutathione peroxidase (GPx). The above mentioned enzymes metabolise free radicals into less toxic or nontoxic products. EC-SOD is sometime termed a "locally specific" protective enzyme (2). Throughout the investigations, the EC-SOD level reached 0.1207—0.2440 U.l$^{-1}$ in group I and 0.1255–0.2410 U.l$^{-1}$ in group II.

Glutathione peroxidase is an adaptive enzyme the activity of which increases in response to the oxidation stress (10). It catalyzes the reduction of hydrogen peroxide and organic peroxides. Glutathione participates in the reconstitution of damaged cell components. The mean levels of glutathione peroxidase in minks ranged from 0.2015—0.2720 U.l$^{-1}$ in group I and 0.1583—0.2760 U.l$^{-1}$ in group II. A number of studies have investigated forms of glutathione peroxidase in human cells and in animals fed high-energy, high-protein feed (6, 7). Carnivorous fur animals emit some tens of odourous substances, particularly sulphoorganic compounds, ketones, aldehydes, alcohols and aliphatic and aromatic hydrocarbons which increase the risk of oxidation stress resulting from air pollution (7, 8, 10).

A response to oxidation stress manifests itself with a definite antioxidation state that can be presented as the total antioxidant status (TAS). This antioxidative marker in minks varied between 0.0063 and 0.0088 U.l$^{-1}$ in group I and 0.0069 and 0.00910 U.l$^{-1}$ in group II. The mean values of soluble proteins were in the range 61.91—87.52 mg.ml$^{-1}$.

The feed supplementation with a preservative and antioxidant caused a differentiation in the histopathological changes in the liver of animals of both groups. The kidneys showed slight epithelial steatosis of order I convulated tubules. No pathological changes were recorded in the mink alimentary tract. Preservatives decrease the pH of feed inhibiting microbe development. Autooxidation of feed lipids can be neutralized by some chemical compounds. Their antioxidative activity is higher when they are employed early enough to preserve the

**Table 2. The mean values of blood oxidation stress parameters of mink (n = 60)**

| Parameter | Collection I | | Collection II | | Collection III | | Collection IV | |
|---|---|---|---|---|---|---|---|---|
| | E | C | E | C | E | C | E | C |
| Glutatione peroxidase U.l$^{-1}$ | 0.2720 | 0.2760 | 0.2210 | 0.1583 | 0.2015 | 0.1736 | 0.2422 | 0.2680 |
| Glutatione reductase U.l$^{-1}$ | 0.0016 | 0.0016 | 0.0010 | 0.0012 | 0.0022 | 0.0024 | 0.0022 | 00023 |
| Superoxide dismutase U.l$^{-1}$ | 0.2440 | 0.2410 | 0.1207 | 0.1629 | 0,2091 | 0.1255 | 0.1878 | 0.0850 |
| Total antioxidant status U.l$^{-1}$ | 0.0077 | 0.0073 | 0.0063 | 0.0073 | 0.0088 | 0.0091 | 0.0113 | 0.0069 |
| Proteins mg.ml$^{-1}$ | 79.38 | 75,43 | 85.08 | 85.51 | 87.52 | 84.87 | 56.98 | 61.91 |

E — group I (experimental); C — group II (control)

feed. Antioxidants are absorbed well from the alimentary tract and deposited in the adipose tissue, skin, liver, stomach, heart and kidneys (10). Owing to their high chemical activity, their maximum allowed doses should not be exceeded.

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# CHANGES IN THE ACTIVITY OF SELECTED ADAPTIVE ENZYMES IN LABORATORY RATS IN RELATION TO SEX DURING PROLONGED FASTING

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## ABSTRACT

**Male (60 animals) and female (60 animals) rats of Wistar SPF breeding were examined to determine the effect of prolonged fasting (0—7 days) on changes in the activity of selected adaptive enzymes: tyrosine aminotransferase (TAT), tryptophan-2-3-dioxygenase (TO), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the liver. The results of the experiment suggested that some of the changes were related to the sex of experimental animals.**

**Key words: alanine aminotrasferase; aspartate aminotransferase; fasting; liver; rat; tyrosine aminotransferase; tryptophan-2-3-dioxygenase**

## INTRODUCTION

The existence of life depends on the intake of food which ensures continuous and adequate supply of energy. Both components of food intake qualitative and quantitative are important. The organism can adapt to a wide range of nutritional conditions and to extreme bioclimate factors (3, 8). The present study investigated the extent of changes in the activity of selected adaptive enzymes in laboratory rats of both sexes during 0—7 days of fasting.

## MATERIAL AND METHODS

The experiment was carried out on adult rats – males and females – of Wistar SPF breeding, of initial body weight 160 ± 10 g. During the experiment, the animals were kept in plastic cages with perforated floor to prevent coprophagy. They were supplied tap water *ad libitum.* Analyses were carried out on days 0, 1, 2, 3, 5 and 7 of fasting. The activity of tyrosine amino transferase (TAT, E.C. 2.6.1.5.) was determined by the method of (2) and that of tryptophane-2-3-dioxygenase (TO, E.C. 1.13.1.11.) by the method of (7). The liver was examined for the activity of aspartate aminotransferase (AST, E.C. 2.6.1.1.) and alanine aminotrasferase (ALT, E.C. 2.6.1.2.) using BIO-La test kits (LACHEMA Brno). The activities of enzymes were reported per mg of proteins which were determined according to (9). The results obtained were evaluated statistically using non-paired *t*-test. Eight to ten animals were examined in individual groups. The experiment was carried out in winter.

## RESULTS AND DISCUSSION

The acitivity of TAT (Fig. 1) in the liver of fasting male rats was increased ($p < 0.05$) significantly between days 1 and 5 of fasting. The female rats showed a significant increase ($p < 0.01$) in this parameter between days 2 and 5 of fasting. The comparison of results between

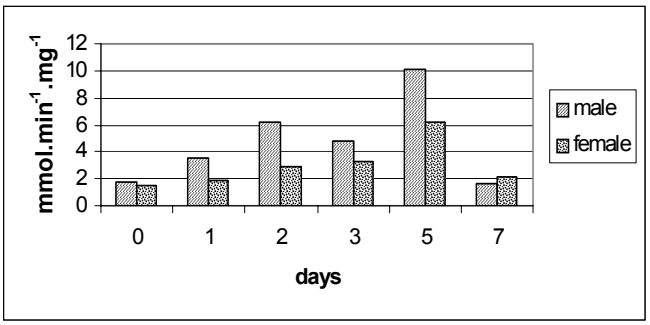


**Fig. 1. Changes in the activity of tyrosine aminotransferase in rat liver**

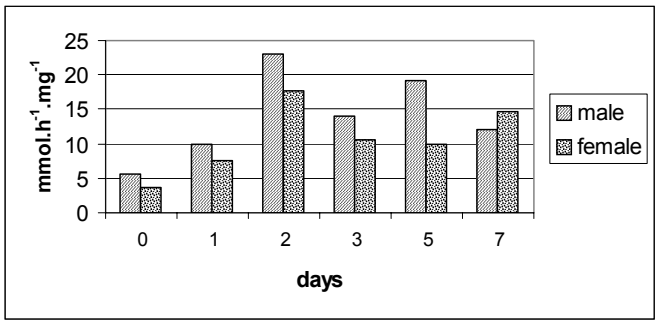


**Fig. 2. Changes in the activity of tryptophane-2-3-dioxygenase in rat liver**

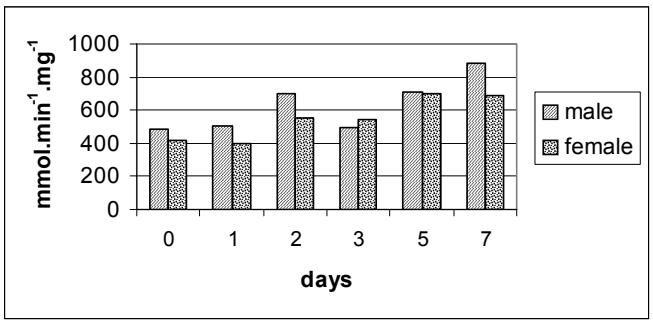


**Fig. 3. Changes in the activity of alanine aminotransferase in rat liver**

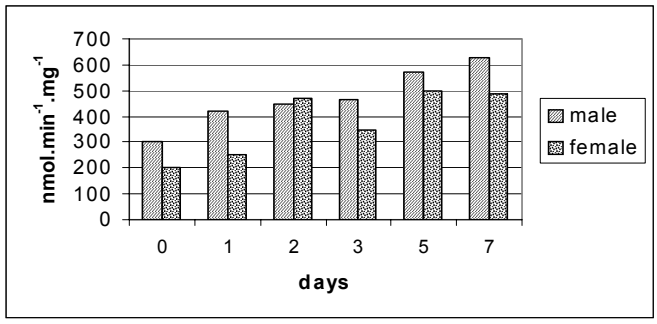


**Fig. 4. Changes in the activity of aspartate aminotransferase in rat liver**

sexes showed a decrease in female with the exception of the terminal period (7 day). The decrease was significant only on days 2 and 5 of fasting.

The activity of TO (Fig. 2) was increased in festing animals in comparison with well-fed animals. The difference between sexes was significant on days 2 and 5 when an increased activity of this enzyme was recorded in male rats ($p < 0.05$, $p < 0.01$, resp.).

The activity of ALT (Fig. 3) in the liver of fasting male rats was increased throughout the experimental period. The increase was significant on days 2, 5 and 7. The female rats showed an insignificant decrease on day 1 of fasting. In the remaining period a significant increase was recorded. The mutual comparison showed decreased activity of ALT in female rats with the exception of day 3.

The activity of AST in the liver (Fig. 4) of male rats was increased significantly throughout the period of observation and in the female rats between days 2 and 7 of fasting. In comparison with the males the activity of AST was decreased except for day 2. The decrease was significant on day 1 of fasting ($p < 0.01$).

The arrangement of the experiment followed the aim to verify possible differences in the changes of activity of selected adaptive enzymes in laboratory rats of both sexes during prolonged fasting when the organism makes an effort to attain new balance between the intake and expen diture of energy. It was based on the knowledge that some adaptive enzymes, in our case TAT and TO, exhibit changes in their activity relatively early while others, such as ALT and AST, require longer time to adapt to the stressors. Such stressors include also tha fasting during which the organism must adapt to new, considerably changed conditions. Because these conditions mean that no food is supplied to the organism, all life functions must be maintained through utilization of body reserves.

An increase in the activity of adaptive enzymes is caused by higher than 3-fold increase in serum cortico-sterone which confirms the stressful effect of fasting (5, 6, 10).

Although the dynamics of changes in the activity of observed enzymes showed no marked differences, the response to prolonged fasting differed between the sexes, particularly in the initial and terminal periods.

The results obtained show that the differences in metabolic response of laboratory animals to prolonged fasting depend not only on sex but also on the age, starting body weight, strain, and the season in which the experiment was conducted. In addition to that the changes can be affected considerably also by the composition of feed during early ontogenesis and immediately before the experiment (1).

According to the up-to-date results, the scope of problems related to the fasting of laboratory animals has not yet been completely elucidated.

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# WELFARE FROM THE VIEWPOINT OF THE EARLY WEANING OF PIGLETS

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## ABSTRACT

**The relationship between pig weaning and their welfare status under large-scale breeding conditions was studied. Two forms of weaning were compared: 35 days (standard; control group) and 21 days (early). $F_1$ (Landrasse x White Improved) animals were used. Previously tested methods were used to compare the welfare status of piglets. The animals were observed between the ages of five and eight weeks for one hour in a 24 hour cycle at three-minute intervals. Five forms of animal behaviour were registered. Abnormal activities were more frequent in early weaned piglets as a result of the absence of maternal stimuli in early ontogenesis. Early weaning affected motoric behaviour, the lying of animals, playfulness and fighting. Our results indicated behavioural maladaptation as a result of social deprivation in early weaned animals and implied decreased welfare.**

**Key words: early ontogenesis; pig; social deprivation; welfare status**

## INTRODUCTION

Animal welfare is defined as physiological and psychological harmony between animal and its environment (1). Basic criteria of good welfare of animals are good health and the normal repertoire of behaviour.

Early ontogenesis is an important phase of animal development with regard to subsequent integration of biological, physiological and psychological structures of an adult individual. Absence of specific environmental stimuli during this period causes retardation of individual behavioural forms. This applies particularly to the relationship mother – the young (5). Isolation of animals resulted in their excitation and the dysfunction of brain structures (2).

In large-scale production units piglets are normally weaned at the age of five weeks (10) but recently early weaning at 21 days of age has been practised increasingly (12, 3).

Our study was aimed at the determination of behavioural criteria for comparison of standard and early weaning, the determination of the risk associated with early weaning and the analysis of the prospects from the early weaning of piglets.

## MATERIAL AND METHODS

Our study was carried out on 144 $F_1$ (Landrasse x White Improved) piglets (Tab. 1) kept under standard conditions in pens of dimensions 2.80 × 2.80 metres with rubber surface. Local heating was used to ensure a temperature of between 25 and 27 °C for early weaned piglets.

The animals in individual series were selected once a month from several hundreds of newborn piglets to obtain uniform groups with regard to sex and body weight. Observations were carried out by two people on piglets between the age of five and eight weeks for one hour in a 24 hour cycle at three-minute intervals and verified by a third person concentrating on seven behavioural criteria: 1 — standing (all forms of motoric activity); 2 — lying; 3 — playing/fighting; 4 — exploration of other piglets; 5 — exploration of the environment;

6 — acceptance of food; 7 — drinking. Statistical significance was determined by the Wilcoxon' test.

**Tab. 1. Piglets included in the experiment**

| Series | Standard weaning (35 days) | Early weaning (21 days) |
|---|---|---|
| 1 | 8 | 9 |
| 2 | 10 | 10 |
| 3 | 9 | 8 |
| 4 | 8 | 10 |
| 5 | 8 | 10 |
| 6 | 9 | 8 |
| 7 | 9 | 9 |
| 8 | 10 | 9 |
| Total | 71 | 73 |

## RESULTS AND DISCUSSION

The ethological knowledge concerning the welfare of piglets after weaning is insufficient as the individual excitation characteristics of this animal species and psychosomatic relations (PSE syndrome, *appoplexis cordis*) together with the complexity of methods and specifics of early ontogenesis of individuals further complicate the picture.

Our results are summarised in Tab. 2. No significant differences between the groups were observed in digestive behaviour and drinking (criteria 6 and 7).

Our investigations showed that insufficient contact with the mother affected behavioural motivation mechanisms and increased social tension (Tab. 2; crit. 1—5). According to M a r x (7) the lying of animals indicates comfort or emotional tension and decrease in its frequency points to some discomfort (crit. 2). Playfulness/fighting (crit. 3) was evaluated as a complex in order to avoid some inaccuracy related to definition of motoric activities of the adaptation type (8, 14, 13). During the suckling, uptake of milk takes about one minute and the remaining dominating phases include massage and after-massage (12). These activities were also disturbed in early weaned piglets and were compensated by increased mutual exploration (crit. 4). Exploration of the environment also differed significantly (crit. 5).

Our results point to a significant increase in abnormal behavioural forms in early weaned piglets. This supports the hypothesis of the deprivation of maternal stimuli and decreased animal welfare. They also point to the necessity of studying this problem in a wider context including environmental factors (4, 9, 11, 6), clinical ethology, behavioural genetics and others. It should also be stressed that ethological relations between early ontogenesis of pigs and their welfare are essential for their health and the ethical aspects of animal rearing.

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**Tab. 2. Frequency of observed behavioural forms at two types of weaning**

| Behaviour criterium | Standard weaning W1 | W2 | W3 | Weeks total | Early weaning W1 | P | W2 | P | W3 | P | Weeks total | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 12.2 | 13.3 | 12.3 | 12.6 | 98.9 | +++ | 98.8 | +++ | 98.8 | +++ | 99.1 | +++ |
| 2 | 83.2 | 80.8 | 84.9 | 82.6 | 11.2 | +++ | 14.3 | +++ | 13.8 | +++ | 13.1 | +++ |
| 3 | 3.9 | 6.4 | 5.7 | 5.3 | 58.8 | +++ | 59.9 | +++ | 63.4 | +++ | 60.7 | +++ |
| 4 | 1.2 | 0.9 | 1.4 | 1.1 | 19.8 | +++ | 20.3 | +++ | 21.6 | +++ | 25.5 | +++ |
| 5 | 1.5 | 1.3 | 1.5 | 4.3 | 18.7 | +++ | 18.9 | +++ | 19.8 | +++ | 19.3 | +++ |
| 6 | 50.2 | 46.4 | 48.6 | 48.4 | 51.20 | 0 | 47.0 | 0 | 50.3 | 0 | 49.5 | 0 |
| 7 | 12.1 | 13.7 | 16.0 | 14.2 | 13.8 | 0 | 14.3 | 0 | 17.2 | 0 | 15.1 | 0 |

Significance of differences: +++ — $P < 0.01$; ++ — $P < 0.02$; + — $P < 0.05$; 0 — insignificant

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# THE TESTING OF THE EFFICACY OF SELECTED DISINFECTANTS UNDER LABORATORY CONDITIONS AND THE ECOLOGICAL ASPECTS OF THEIR APPLICATION CONCERNING ENVIRONMENTAL IMPACTS

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## ABSTRACT

**The effectiveness of the disinfectant preparations Persteril, Pedox and Peroxid Aktiv K, based on persteril, was tested under laboratory conditions on bacterial strains *E. coli, S. aureus*. Testing was carried out by a suspension test, using concentrations of the active ingredient from 0.01 to 2.0 % and exposure time 5, 30 and 60 minutes, and by a dilution test at concentrations from 0.02 and 2.0 %. The results were compared with those obtained for Chloramin B, based on active chlorine. Good devitalisation effects on tested bacteria were observed for all three preparations. Persteril provided the best results in both the tests and was followed by Pedox. The effectiveness of Peroxid Aktiv K was close to that of Chloramin B but required longer exposure time.**

**Key words: chloramin B; disinfection effectivenes; Pedox; Peroxid Aktiv K; Persteril**

## INTRODUCTION

Disinfection is a process during which the pathogenic microorganisms are removed or inactivated by chemical or physical means. An ideal disinfectant should have a broad spectrum of antimicrobial activity and should be nontoxic and nonirritating. The testing of disinfectants under laboratory conditions is very important with regard to the determination of their correct concentration and other aspects of their use. It forms the basis of selection of disinfectants in the food industry and may prevent undesirable environmental consequences.

It is well known that synthetic chemical disinfectants frequently have a negative effect on the environment. Due to their toxic properties, they may disturb the aquatic ecosystem, air and soil and penetrate to food chain negatively affecting both the environment and human health. Therefore it is very important what quantity and concentration of these substances enters the environment and how quickly the substances decompose or how long they accumulate. In the case of chemical disinfectants no serious disturbance of natural cycles is expected (except for emergencies in production or during transport).

The study focused on the laboratory investigation of the effectiveness of the disinfectant preparations Pedox, Peroxid Aktiv K and Persteril, based on peracetic acid, in comparison with Chloramin B, based on active chlorine.

## MATERIAL AND METHODS

Tests were carried out on the nonstable disinfectants Pedox, Peroxid Aktiv K and Persteril with peracetic acid as an active ingredient and a stable disinfectant Chloramin B with organically bound active chlorine. Bactericidal effectiveness was evaluated under laboratory conditions by a suspension and dilution test (1) on standard strains from the group of Gram-negative *E. coli* and Gram-positive *S. aureus*.

The suspension test is used to determine the bactericidal exposure of increasing concentrations of the disinfectants tested in comparison with a well known disinfectant.

**Table 1. Results of the suspension test performed on *E. coli***

| Preparation | Exposure (min) | Concentration (%) 0.01 | 0.1 | 0.5 | 1.0 | 2.0 |
|---|---|---|---|---|---|---|
| Pedox | 5 | + | - | - | - | - |
| | 30 | + | - | - | - | - |
| | 60 | - | - | - | - | - |
| Peroxid Aktiv K | 5 | + | + | - | - | - |
| | 30 | + | - | - | - | - |
| | 60 | + | - | - | - | - |
| Persteril | 5 | - | - | - | - | - |
| | 30 | - | - | - | - | - |
| | 60 | - | - | - | - | - |
| Chloramin B | 5 | + | - | - | - | - |
| | 30 | + | - | - | - | - |
| | 60 | + | - | - | - | - |

**Table 2. Results of the suspension test performed on *S. aureus***

| Preparation | Exposure (min) | Concentration (%) 0.01 | 0.1 | 0.5 | 1.0 | 2.0 |
|---|---|---|---|---|---|---|
| Pedox | 5 | + | - | - | - | - |
| | 30 | + | - | - | - | - |
| | 60 | + | - | - | - | - |
| Peroxid Aktiv K | 5 | + | + | - | - | - |
| | 30 | + | + | - | - | - |
| | 60 | + | - | - | - | - |
| Persteril | 5 | - | - | - | - | - |
| | 30 | - | - | - | - | - |
| | 60 | - | - | - | - | - |
| Chloramin B | 5 | + | + | - | - | - |
| | 30 | + | - | - | - | - |
| | 60 | + | - | - | - | - |

**Table 3. Dilution test performed on *E. coli***

| Preparation | Concentration (%) 0.02 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 | 1.8 | 2.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pedox | - | - | - | - | - | - | - | - | - | - | - |
| Peroxid Aktiv K | + | - | - | - | - | - | - | - | - | - | - |
| Persteril | - | - | - | - | - | - | - | - | - | - | - |
| Chloramin B | + | - | - | - | - | - | - | - | - | - | - |

**Table 4. Dilution test performed on *S. aureus***

| Preparation | Concentration (%) 0.02 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 | 1.8 | 2.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pedox | - | - | - | - | - | - | - | - | - | - | - |
| Peroxid Aktiv K | + | - | - | - | - | - | - | - | - | - | - |
| Persteril | - | - | - | - | - | - | - | - | - | - | - |
| Chloramin B | + | - | - | - | - | - | - | - | - | - | - |

The dilution test is used to determine the bactericidal concentration of the tested preparation in comparison with a well known disinfectant

## RESULTS AND DISCUSSION

Results obtained by the suspension test on *E. coli* and *S. aureus* strains are presented in Tables 1 and 2, respectively. The results indicate that Persteril showed the greatest effectiveness on Gram-positive and Gram-negative bacteria. It ensured their devitalisation at 0.01 % concentration and five minutes exposure. Pedox rated second while the effectiveness of Peroxid Aktiv K showed similar effectiveness as Chloramin B but required a longer exposure time.

Tables 3 and 4 show the results of the dilution test performed with the same disinfectants. In this test Persteril and Pedox showed the highest effectiveness and devitalised both tested species at concentration of 0.02 %. Peroxid Aktiv K and Chloramin B were effective at the concentration of 0.2 %.

To ensure the safety of food of animal origin it is essential to control the microbial contamination of incoming raw materials and additive substances and prevent secondary contamination of all materials and intermediate products during their handling and processing (5, 2, 6). Cleaning and disinfection are the basic processes used to remove and devitalise harmful and undesirable micro-organisms and the requirements on preparations used for this purpose are very demanding. They should have good devitalisation effects already at low

concentrations and should not leave residues nor put an additional load on the environment (3). Although all disinfectants pose some risk to the environment (biodegradability, wastewaters, packaging materials), the selection of the most suitable ones and their proper handling can decrease this risk to a minimum.

The disinfectants tested appear prospective for use in food industry as they show high effectiveness already at very low concentrations and do not leave harmful residues. Our results are supported by those of other authors (4) who reported that disinfectants based on hydrogen peroxide and peracetic acid were very effective also on micro-organisms in biofilms which is a frequent problem in food industry facilities.

## REFERENCES


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