FOLIA VETERINARIA

The scientific journal of the UNIVERSITY
OF VETERINARY MEDICINE IN KOŠICE
— The Slovak Republic

ISSN 0015-5748

1
XLIX • 2005

UNIVERSITY
OF VETERINARY MEDICINE
IN KOŠICE

EDITORIAL BOARD

FOLIA VETERINARIA is issued by the *University of Veterinary Medicine* in Košice (UVL); address: Komenského 73, 041 81 Košice, The Slovak Republic (tel.: +421 55 633 51 03, fax: +421 55 633 51 03, E-mail: Simkova@uvm.sk).

The journal is published quarterly in English (numbers 1—4) and distributed worldwide.

Subscription rate for 1 year is 200 Sk, for foreigners 80 euros. Orders are accepted by *The Department of The Scientific Information — The Library of The University of Veterinary Medicine, Košice* (UVIK); the subscription is accepted by the National bank of Slovakia in Košice (at the account number mentioned below).

Bank contact: *National bank of Slovakia*, 040 01 Košice, Strojárenská 1, our account number: 19-1924-512/0720.

FOLIA VETERINARIA, vydáva *Univerzita veterinárskeho lekárstva* v Košiciach (UVL), Komenského 73, 041 81 Košice, Slovenská republika (tel.: 055/633 51 03, fax: 055/633 51 03, E-mail: Simkova@uvm.sk).

Časopis vychádza kvartálne (č. 1—4) a je distribuovaný celosvetove.

Ročné predplatné 200 Sk, pre zahraničných odberateľov 80 eur. Objednávky prijíma *Ústav vedeckých informácií a knižnice Univerzity veterinárskeho lekárstva v Košiciach* (UVIK); predplatné Národná banka Slovenska v Košiciach (na nižšie uvedené číslo účtu).

Bankové spojenie: Národná banka Slovenska, Košice, Strojárenská 1, číslo príjmového účtu: 19-1924-512/0720.

Tlač: **EMILENA**, Čermeľská 3, 040 01 Košice
Sadzba: **Aprilla,** s.r.o., Hlavná 40, 040 01 Košice

Registr. zn. 787/93

For basic information about the journal see
Internet home pages: www.uvm.sk

Indexed and abstracted
in AGRIS, CAB Abstracts

FOLIA VETERINARIA

PUBLISHED BY
THE UNIVERSITY OF VETERINARY MEDICINE
KOŠICE, THE SLOVAK REPUBLIC

Folia veterinaria
Vol. 49, 2005

VYDALA UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA
KOŠICE 2005

FOLIA VETERINARIA, 49, 1, 2005

CONTENTS

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 49, 1: 3, 2005

EDITORIAL

THE SCIENTIFIC SYMPOSIUM AGROCHEMICALS AND MORPHOGENESIS

A scientific symposium *Agrochemicals and morphogenesis* was held at the University of Veterinary Medicine (UVM) in Košice on December 3, 2004. This Symposium was organized by the Institute of Anatomy and Institute of Biology of the UVM. Its opening ceremony was led Professor J. Danko, DVM, PhD and the Vice Rector of UVM Professor E. Pilipčinec, DVM, PhD and the symposium was moderated by Professor F. Lešník, DVM, DSc.

The main aim of the symposium was to discuss and explain the influence of some agrochemicals, such as xenobiotics, on health including animal morphogenesis.

Lectures discussed environmental genotoxicity estimates, the effect of carbamates on reproductive and immunological systems, some metabolic profiles, biochemical parameters, respectively (e.g., antioxidant enzymes, haematological pictures) and the adrenergic and BuChE-positive innervation of the rabbit spleen.

The Proceedings of the reports of the contributions (18) were published in the Slovak language with English abstracts by UVM (Košice, The Slovak Republic 2004, ISBN 80-8077-014-X) with the following contents:

Lešník, F.: A medical view of agrochemicals.

Antal, J., Maraček, I.: The effect of carbamates on the reproductive system of domestic animals.

Dianovský, J., Šiviková, K.: Environmental genotoxicity estimates in animals.

Velesová, M., Váczi, P., Šutiak, V., Neuschl, J., Korének, M.: Glycine, its properties and effects on organisms.

Lenártová, V., Holovská, K., Sobeková, A., Holovská, K., Jr.: The effect of pollutants on organisms and the possible routes of their elimination.

Siroťáková, M., Schmidtová, K.: Microscopic pictures of adrenergic and BuChe positive innervation of the spleen in rabbits after application of bendiocarbamate.

Mojžišová, J.: Changes of the immunological and haematological parameters in rabbits after the application of bendiocarbamate.

Piešová, E., Danko, J.: The activity of bendiocarbamate in the rabbit bone marrow micronucleus.

Tučková, M.: Changes in some biochemical parameters of rabbits induced by bendiocarbamate.

Cigánková, V., Naď, P.: The effect of the structure of kidneys in Japanese quail.

Sobeková, A., Holovská, K., Lenártová, V., Flešárová, S.: The effect of bendiocarbamate on the activities of antioxidant enzymes in some organs of rabbits

Pošivák, J., Lazar, G., Lešník, F., Valocký, I., Horňáková, Ľ., Čurlík, J.: Changes in number and quality in ovine embryos influenced by PCB.

Bálent, P., Točková, J.: Molecular-biological methods in the identification of opportune pathogens.

Marettová, E., Danko, J., Bátor, R., Šulla, I., Flešárová, S.: The distribution of S-100 proteins in the rabbit thymus after application of bendiocarbamate.

Čokašová, D., Lešník, F.: A study of the influence of bendiocarbamate.

Horňáková, Ľ., Lazar, G., Pošivák, J., Lešník, F., Valocký, I., Šťavová, Ľ.: The evaluation some analysis of ovine oocytes matured *in vitro* after PCB application.

Šulla, I., Bátor, R., Ucekaj, N., Marettová, E., Flešárová, S.: The effect of the bendiocarbamate on the organism of tame rabbits.

Dorko, F., Flešárová, S., Šulla, I.: The effect of bendiocarbamate on the lymphopoetic organs of rabbits.

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FOLIA VETERINARIA, 49, 1: 4—6, 2005

A MEDICAL VIEW OF AGROCHEMICALS (A Review)

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ABSTRACT

One group of xenobiotics are agrochemicals including approximately 700 registered pesticides that can be related to many health disorders. Besides acute intoxication these can also cause motor and sensory disorders (e. g., Parkinson's disease), carcinogenic diseases, reproduction disorders, immunosuppression and allergic states, etc. From a medical view these exogenous foreign environmental compounds command great respect. The animal eukaryotic multicellular organism is a complicated multistage process at the molecular, physiological and morphological levels. Various negative regulating factors can pass into their processes. Teratogenesis and consequently malformation, eventually death of embryo or foetus can be a result. Some pesticide action of cholinesterase inhibitors, which prevent or inhibit cholinesterase from cleaning up unused acetylcholine also belong to this group. Many of these xenobiotics are certified or assumed to be carcinogenic, embryotoxic or foetotoxic and teratogenic chemical compounds. The subject of our study in terms of influence on morphogenesis is the anticholinesterase insecticide bendiocarbamate, which increases the incidence of total lymphoreticular tumours in males of rats.

Key words: bendiocarbamate; cholinesterase inhibitors; morphogenesis; morphogens; neurotransmitters; organic pollutants; pesticides; xenobiotics

INTRODUCTION

Agrochemicals, as chemicals used in agriculture, include a broad range of products used for the nutrition of plants, protection of fruits, protection and nutrition of animals. There are pesticides, substances used as repellents or destroyers of all sorts of plant and animal pests (2). At present, among more than 700 registered pesticides around the world, there are herbicides, insecticides, fungicides rodenticides, nematocides, bactericides, algicides, molluscicides, ascaricides, etc. (17). They are exogenous foreign substances, xenobiotics, with various consequences for living organisms. Their effect depends mainly from substance activity, from their metabolizing, respectively.

It is known that pesticides can cause acute intoxication or carcinomas. Many of these suppress the immune system which increases its susceptibility to virus, bacteria and parasitic infections and to tumours. It is known that farmers are the a population with a high risk of Hodgkin's disease, melanomas, multiply myeloma and leukaemia.

Many agrochemicals (xenobiotics) including pesticides, in the liver through detoxication can be transformed to reactive compounds with mutagenic, carcinogenic or teratogenic metabolites (so-called biotransformation).

At present, in the world there are extremely toxic xenobiotics including some pesticides classified as persistent organic pollutants (POPs):

1. Aldrin – a pesticide that is rapidly converts to rodent carcinogen – dieldrin, toxic substance for man, birds and fish.

2. Chlordane – a broad-spectrum insecticide, which may

affect the human immune system. It is classified as a possible human carcinogen, which in rodents increases the incidence of hepatocellular carcinomas.

3. Dichlorodiphenyltrichloroethane (DDT) – a pesticide causing chronic health problems of man and in mouse carcinogenesis.

4. Dieldrin – an insecticide, toxic substance, with teratogenic activity, its carcinogenic property in animals is not completely certified.

5. Dioxines – the most toxic anthropogenic genotoxicants with mutagenic, teratogenic and carcinogenic activity. These compounds can arise in the manufacture of pesticide derivate from chlorophenoxyacetic acid and from the burning of hospital and municipal waste, turf, coal and wood.

6. Endrin – an insecticide, rodent carcinogen, which causes the bone marrow hyperplasia in dogs and hyperplasia or tumour proliferation in other tissues.

7. Furans – a common source with dioxines and polychlorinated biphenyls, rodent carcinogens.

8. Heptachlor – an insecticide, possible human carcinogen. It is believed that it is responsible for the decline of several wild bird populations.

9. Hexachlorobenzene (HCB) is used in seed treatment and against parasitic fungi, up to now it has been practically detected in all types of food-stuff.

10. Mirex – one of the most stable and persistent insecticides.

11. Polychlorinated biphenyls (PCBs) – industrial very stable lipophilic contaminants of the global ecosystem with mutagenic and carcinogenic activity, probable human carcinogens (they are connected with hepatocarcinoma and kidney carcinoma). In children suckling milk with high concentration of PSB we can observe a lower level of physical and mental development (they decreasing production of the thyroid hormones). These compounds also cause reproduction disorders and suppression of the immune system.

12. Toxaphene (Polychlorocamphene) – insecticide, toxin with relatively strong carcinogenic activity for rodents (1, 5, 7, 8, 11, 13, 16).

A lot of pesticides can persist in the environment's soil and water for more than several years after their use (e.g., toxaphene and endrin for up to 12 years, DDT 10–15 years, mirex 10–20 years, etc. (1, 13, 17). And so it is no surprise that the majority of food of plant and animal originin our daily consumption contains residual pollutants.

Many pesticides, mainly insecticides are introduced among cholinesterase inhibitors, which commonly have an intimate relation to animal morphogenesis.

MORPHOGENESIS

The biological process of morphogenesis is a process in which living systems produce forms and structures through mechanical and biological factors including morphogens. These are developmental signals that exert specific effects on receptive cells depending on their concentration. Morphogenes can be neurotransmitters when they act as dose-dependent morphogenetic signals in neural and non-neural tissues (3). A well-known neurotransmitter is acetylcholine (ACh), a concentional synaptic neurotransmitter, chemical substance, which neurons in the brain use to communicate with one another. Acetylcholinesterase (AchE) enzyme normally degrades unused ACh, by breaking it into its components – acetate and choline.

CHOLINESTERASE INHIBITOR (ChEI)

ChEI is a group of chemical compounds, which inhibits ACh hydrolysis by AchE, thereby ACh accumulates in the reactive locations of a living organism. This beneficial effect we can observe in types of "memory-drugs" used to treat Alzheimer's disease and other causes of dementia, when neurons are damaged (10). However, some insecticidal products have the same property.

At present, we know three isoforms of AchE:

– **AChE – S** (the synaptic soluble form): main amphipatic membrane-bound multimeric enzyme in brain and muscle.

– **AChE – R:** soluble monomeric "readthrough" in the embryonic and tumour cells and is induced under psychological, chemical and physical stress.

– **AChE – E:** glypiated dimmers bonded with the erythrocyte membrane (6).

Several neurotransmitters may coexist together inside individual neurons, where usually one neurotransmitter is accompanied by one or more neuropeptide(s). "Prenervous" neurotransmitter present in the same embryonic cells may act as multifunctional regulators, which is the additional important property of neurotransmitters (2). From this view neurotransmitters can affect in the same way as the morphogens that control the positive regulation of embryo development.

PESTICIDES, CHEI AND MORPHOGENESIS

Great numbers of known pesticides on the basis of carbamate (CA) or organophosphates (OPs) belong to group of ChEI.

For example, Carbaryl (CA), Coumaphos (OP), Cythioat (OP), Dichlorvos (OP), Diazinon (OP), Fenitrothion (OP), Fenthion (OP), Iodofenphos (OP), Phosmet (OP) and Propoxur (CA) are ChEI. Many of these are foetotoxic, embryotoxic, respectively, and/or teratogenic, eventually carcinogenic (e.g., Carbaryl, Phosmet, Propoxur; 9, 15).

Some insecticides based on OP, except ChEI, can also cause motor and sensory disorders. On the other hand, some pesticides can be triggers of serious disease, such as Parkinson's disease, because pesticides may impair the neurons.

BENDIOCARBAMATE

Bendiocarbamate (bendiocarb) is a carbamate insecticide, which also belongs among the ChEIs. Up to now, in the organotoxic (acute and chronic toxicity) and ecotoxic properties, moreover reproductive, teratogenic, mutagenic and car-

cinogenic effects of this insecticide have been studied. However, from the studies involved no teratogenic effect or influence to morphogenesis, respectively has been established. But it has been observed in certain conditions an increase of the incidence of total lymphoreticular tumours (lymphosarcoma, reticulum cell sarcoma, lymphatic and myeloid leukaemia) in all treated male rats (4).

Every carcinogenesis and teratogenesis is connected with an initiation and promotion step and with a common accumulated mutation. Therefore bendiocarbamate is a suitable model for study from multiple views, including its influence on animal morphogenesis. Into this process we can also include the lack of apoptosis (programmed or physiological cell death), whose values are not sufficiently transparent (12).

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Received January 31, 2005

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FOLIA VETERINARIA, 49, 1: 7—9, 2005

THE EFFECT OF POLLUTANTS ON ORGANISMS AND THE POSSIBLE ROUTES OF THEIR ELIMINATION (A Review)

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ABSTRACT

Environmental pollutants can generate the formation of oxygen radicals. These radicals then alter and damage the biomolecules and stimulate oxidative stress. The evolutionary survival process has provided aerobic organisms with antioxidant defense systems to neutralize the oxidative effect of oxygen and its reactive metabolites. Antioxidant enzymes such as superoxide dismutases, peroxidases and catalase also belong to the antioxidant scavenging systems. These enzymes are strategically compartmentalized in subcellular organelles within the cell to provide maximum protection. Environmental pollutants can influence the activity of antioxidant enzymes by sudden increases in oxygen metabolites or by control over the genetic expression of antioxidant enzymes.

Key words: antioxidant enzymes; oxidative stress; reactive oxygen radicals

INTRODUCTION

Environmental contamination such as polluted air and rivers, xenobiotics used in agriculture stimulate questions how the organisms can eliminate the toxic effect of these pollutants. Pollutants can exert a cytotoxic effect *via* the production of reactive oxygen species (ROS). These highly reactive compounds can damage most biomolecules, namely lipids, proteins and DNA (8, 11).

CHARACTERIZATION OF REACTIVE OXYGEN SPECIES

Aerobic metabolism entails the production of ROS, which are derived from normal physiological and metabolic processes, hence there is a continuous requirement for the inactivation of a variety oxidants, also called prooxidants. Substances that neutralize the potential ill effect of free radicals are generally grouped in what is described as the antioxidant defense system (4, 9). Their task is to protect cellular homeostasis from oxidative disruption by free radicals. An imbalance in favor of prooxidans, potentially leading to damage, has been called "oxidative stress" (12).

Molecular oxygen can be reduced to water. The intermediate steps of oxygen reduction are formation of superoxide anion, hydrogen peroxide and the hydroxyl radical, corresponding to the steps of reduction by one, two and three electrons respectively:

$O_2 + e^- \rightarrow \mathbf{O_2^{\bullet -}}$ — superoxide anion radical
$O_2 + H_2O \rightarrow \mathbf{HOO^\bullet} + OH^-$ — perhydroxyl radical
$HO_2^\bullet + e^- + H \rightarrow \mathbf{H_2O_2}$ — hydrogen peroxide
$H_2O_2 + e^- \rightarrow \mathbf{HO^\bullet} + OH^-$ — hydroxyl radical

Oxygen radicals, in combination with other atoms or larger molecules, can occur as alkoxyl (RO•) or peroxyl (ROO•) radicals e.g. in lipids.

Superoxide radical

The superoxide radical is unique in that it can lead to the formation of many other reactive species, including hydrogen

peroxide, and perhydroxyl radicals. For example, protonation of $O_2^{\bullet-}$ leads to the formation of HOO•, making it a much stronger oxidative species than $O_2^{\bullet-}$ itself. Superoxide radical also interacts with H_2O_2 to generate the singlet oxygen molecule, another reactive oxygen species (6).

Hydroxyl radicals

The hydroxyl radical is considered potentially the most potent oxidant encountered in biological systems, because of its extremely short half-life. It readily reacts with a variety of molecules such as those found in organic lipids by removal or addition of hydrogen to unsaturated bonds (6). Cellular generation may occur by several routes as the Fenton reaction or Haber-Weiss reaction.

Hydrogen peroxide

The dismutation of $O_2^{\bullet-}$ by superoxide dismutase is a major source of hydrogen peroxide. H_2O_2 itself is not reactive enough to oxidize many organic molecules. Nevertheless, it is a biologically important oxidant, because of its ability to generate highly reactive hydroxyl radicals through interaction with transition metals (1). Hydrogen peroxide can diffuse through biological membranes relatively easily because it has no charge.

DEFENSE AGAINST OXIDATIVE STRESS

The evolutionary survival process has provided aerobic organisms with mechanisms to neutralize the oxidative effects of oxygen and its reactive metabolites and prevent oxidative stress. To provide maximum protection, cells contain a variety of substances capable of scavenging many different species of free radicals. They are strategically compartmentalized in subcellular organelles. The cooperation between different antioxidants is very important for effective protection. Antioxidant action includes the prevention of ROS formation, interception of ROS once formed and repair of ROS induced damage. Preventive antioxidation involves channeling an attacking species into less harmful products (13). Interception of ROS includes deactivation or transferring the radicals' action from a lipophylic to a hydrophilic phase. Cells are equipped with several lines of antioxidant defense, both enzymatic and nonenzymatic, preventing or minimizing generation of ROS.

Enzymatic systems

The antioxidant enzymes are unique in cofactor requirements and cellular location. All cells in eukaryotic organism contain powerful antioxidant enzymes: superoxide dismutases (Cu, Zn-SOD, Mn-SOD), catalase (Cat) and peroxidases (Se-dependent GSHPx and Se-independent GSHPx). SODs convert superoxide radical to hydrogen peroxide, while catalase and peroxidases convert hydrogen peroxide to water. In such a way two toxic compounds — superoxide radical and hydrogen peroxide — are converted to nontoxic compound — water. Indirect antioxidant functions carried by enzymes are a back-up function, e.g. the replenishment of glutathione from glutathione disulfide by glutathione reductase (GR) and transport and elimination of reactive compounds by glutathione-S-transferases (10).

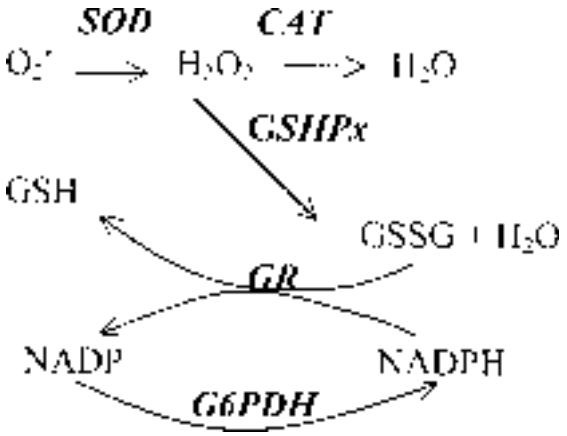

Fig. 1. The cooperation of antioxidant enzymes

The cooperation of antioxidant enzymes is shown in Fig. 1.

The subcellular organelles localization of these enzymes is very important. Generally we can say that Mn-SOD is localized in the mitochondria, Cu, Zn-SOD in cytoplasm, Cat in peroxisomes and GSHPx in many subcellular compartments. All these enzymes exist in several isoforms. The reason for an isoform existence is to reduce oxidative stress in different cell compartments.

Superoxide dismutase (SOD, EC 1.15.1.1) is the primary defense, because this enzyme prevents the further generation of free radicals. It catalyses dismutation of superoxide anion to hydrogen peroxide and exists in virtually all aerobic organisms and all subcellular structures. SOD are classified into classes depending on the metal ion content on the active side: Cu, Zn-SOD, Mn-SOD and Fe-SOD. Two last are structurally similar, Cu, Zn-SOD is different. All studied prokaryotic organisms contain Mn-SOD or Fe-SOD, or both. Cu, Zn-SOD is found only in certain bacteria and chloroplasts. Eukaryotic algae and protozoa possess Mn-SOD and Fe-SOD but not Cu, Zn-SOD. Cu, Zn-SOD has been found in all higher eukaryotes within the animal kingdom, as has Mn-SOD; while Cu, Zn-SOD is cytosolic, Mn-SOD is found in the mitochondria (2).

Catalase (Cat, H_2O_2: H_2O_2 oxidoreductase, EC 1.11.1.6), a haeme protein with Fe^{3+} in the active centre, is ubiquitously distributed in tissues of all species. It catalyses the decomposition of H_2O_2 to water and oxygen. In many cases, the enzyme is localized in peroxisomes where oxidases, the main source of hydroperoxides, are also found (3).

Glutathione peroxidases (GSHPx, glutathione: H_2O_2 oxidoreductase, EC 1.11.1.9; EC 1.11.1.12) catalyse the reaction of hydroperoxides with reduced glutathione to form oxidized glutathione disulfide and the reduction product of the hydroperoxide. There are two types of GSHPx, selenium dependent and selenium independent. Se-dependent GSHPx contains one Se atom in the active centre, which participates

in the catalytic reaction and thus protect against radical damage by reducing peroxides according to their increasing lipofility: H_2O_2, hydroperoxides of fatty acids, phospholipids, cholesterol, triglycerides and reduced cholesterol. Se-independent GSHPx contains also Se atom, but it does not take part in the catalytic reaction. The preference substrates are organic hydroperoxides. It is believed that it is identical with glutathione-S-transferase which detoxifies xenobiotics by conjugation with glutathione (5).

The antioxidant enzyme activity can be regulated by concentration of ROS in cells, or controlled over their genetic expression. Different stress conditions evoke induction or inhibition of antioxidant enzymes activity. So we can assume, that they are also the mediators of the gene regulation of these enzymes. Such regulation has been well described in microorganisms. Antioxidant enzyme regulation in tissues of higher animals depends on many diverse factors, including organ specificity, the developmental stage, prevailing hormone profile, and the availability of active site cofactors. Many tissues show separate and unique responses to factors that regulate antioxidant enzymes. To elucidate their role in the defense mechanisms in higher organisms against environmental pollutants, further studies are necessary.

ACKNOWLEDGEMENT

This investigation was supported by Grant agency VEGA 2/3064/23.

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Received January 31, 2005

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FOLIA VETERINARIA, 49, 1: 10—14, 2005

THE EFFECT OF BENDIOCARBAMATE ON THE ACTIVITIES OF ANTIOXIDANT ENZYMES IN SOME ORGANS OF RABBITS

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ABSTRACT

The activities of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and the contents of thiobarbituric acid reactive substances (TBARS) were determined in the liver, kidney, spleen and thymus of rabbits after exposure to bendiocarbamate. In the liver, the activities of SOD and CAT were not affected by bendiocarbamate. The activities of GSHPx-cum and GSHPx-H_2O_2 were significantly decreased on the 3rd and the 10th days of experiment. A significant increase in the activities of SOD, CAT, GSHPx-cum and GSHPx-H_2O_2 were observed in the spleen. In the thymus, activities of SOD and CAT were significantly increased and GSHPx-cum and GSHPx-H_2O_2 were significantly decreased. In the kidney, the activity of SOD was significantly increased and the activities of CAT and GSHPx-H_2O_2 were significantly decreased. The rabbits' exposure to bendiocarbamate did not affect the contents of TBARS in the kidney and spleen. In the liver and thymus, the contents of TBARS were significantly increased in experimental groups compared to the control. Our results showed that the organ's response to bendiocarbamate is various and may depend on specific organ damage and their protective abilities.

Key words: antioxidant enzymes; bendiocarbamate; kidney; liver; spleen; thymus

INTRODUCTION

All living cells can be exposed to toxic concentrations of different environmental pollutants by consumption of contaminated feed and water.

Bendiocarbamate (2,2-dimethyl-1,3-benzodioxol-4-yl-N-methyl carbamate) is a carbamate insecticide. In agriculture it is used against a variety of insects, especially those in the soil. Like other carbamate insecticides, bendiocarbamate kills insects and causes poisoning in animals by inhibiting the enzyme, acetylcholinesterase (AChE) which normally functions to degrade acetylcholine in nerve synapses. Bendiocarbamate is degraded *via* hydrolysis of the methyl carbamate and heterocyclic compounds 2,2-dimethyl-1,3-benzodioxol-4-ol or by oxidation on N-hydroxymethylbendiocarbamate and 5,8 or 7-hydroxy bendiocarbamate. The rate of degradation of bendiocarbamate is pH-dependent, rapidly in alkaline media, and more slowly in neutral and acidic media (1).

Some studies have demonstrated that many chemical compounds such as pesticides, herbicides, metals, many organic compounds can induce oxidative stress by enhancing reactive oxygen species (ROS) in tissues (14, 13, 3, 16, 11). ROS may be generated by redox cycling of biotransformed organic molecules (20). ROS possess the potential to damage either cellular or organelle membranes and macromolecules, causing lipid peroxidation and nucleic acid and protein alterations. This damage is responsible for cell death during oxidative stress (18).

The bendiocarbamate is transformed into more polycyclic compounds. These different chemical structures can display

their toxicity and lead to the increased formation of ROS. The concentration of ROS have to be controlled by several defense mechanisms, which also involve antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidases. Their induction reflects a specific response to pollutants (5). Therefore, the effect of bendiocarbamate on the activities of antioxidant enzymes has been determined in the liver, kidney, spleen, thymus-tissues of rabbits with a high metabolic capability and detoxifying capacity.

MATERIALS AND METHODS

Animals and diets

The rabbits (hybrid Hyla, clinically healthy, male, 50 days old) were fed with standard diet Norm-typ-0-10 (the control group — C). The rabbits in the experimental group were fed the same diet as in the control group and plus received *per os* 5 mg bendiocarbamate.kg^{-1} b.w./13 days and then the same dose of bendiocarbamate were administred 11 weeks but only every other day (LD_{50} 35–40 mg.kg^{-1}). The rabbits were killed (n = 6) on the 3rd, 10th, 30th and 90th days of experiments and samples of liver, kidney, spleen and thymus were taken.

Preparation of tissue extracts

The liver, kidney, spleen and thymus were washed two times with a cooled physiological solution, cut into pieces and homogenized in Ultra-Turrax homogenizer to make a 25 % (w/v) homogenate in 5 mmol.l^{-1} Tri-HCl buffer, pH 7.8, containing 0.15 mol.l^{-1} KCl, 1 mmol.l^{-1} Na_2EDTA and 2 mmol.l^{-1} GSH. Homogenates were centrifuged for 60 minutes at 105 000 × *g* using a Beckman L8-60 ultracentrifuge, and were stored at -50 °C until use for later assays. All procedures were performed at 4 °C.

Enzyme assays

Superoxide dismutase (SOD, EC 1.15.1.1) as measured according to Flohé and Őtting (8), at 550 nm (25 °C), through the inhibition of cytochrome reduction using xantine-xantine oxidase $O_2^{\cdot -}$ generating system. One unit of SOD was defined as the amount of enzyme that inhibits the rate of cytochrome c reduction by 50 %, under the conditions specified.

Catalase (CAT, EC 1.11.1.6) was assayed at 30 °C by monitoring the decrease of absorbance of H_2O_2 at 240 nm (19).

Glutatione peroxidase was measured according to Flohé and Günzler (6) in a coupled assay with glutathione reductase using cumene hydroperoxide (EC. 1.11.1.12) or H_2O_2 (EC. 1.11.1.9) as substrates (37 °C, 340 nm).

Lipid peroxide formation was measured as malondialdehyde and other aldehydes, by reaction with thiobarbituric acid yielding colored products named thiobarbituric acid reactive substances (TBARS) that absorb at 535 nm (9). The content of TBARS was expressed in absorbance/mg of protein.

Protein concentration was determined by method of Bradford (2) using bovine serum albumin as the standard.

Enzyme activity was expressed in U.mg^{-1} protein as a mean value of six individual samples.

All reagents of the highest purity, were from Sigma, Merck and Boehringer.

Statistics

The results are means ± S.E.M. Statistical analysis were done by Student's *t*-test with a significance level of $p < 0.05$; 0.02; 0.01; 0.001.

RESULTS AND DISCUSSION

The specific activities of antioxidant enzymes (SOD, CAT and GSHPx) of the control groups in the rabbit organs are given in Table 1. The activities of SOD and CAT were high, especially the activity of SOD in the spleen and the activity of CAT in the kidney, which points to their important role in these organs.

It is known that oxidative stress induces or enhances the activity of antioxidant enzymes. The primary antioxidant protection is provided by the enzyme SOD. SOD is a scavenger of superoxide radicals, which are converted to H_2O_2 (15). Our experiments showed that the three month administration of bendiocarbamate did not affected the activity of SOD in the liver. A significant increase in SOD activity was observed in the kidney, spleen and thymus. In the kidney, the specific activity of SOD was significantly increased on the 10th, 30th and 90th days, in the spleen on the 3rd and 90th days and in the thymus on the 3rd day of experiment (Fig. 1).

Catalase and glutathione peroxidase are the predominant enzymes in regulating and controlling intracellular H_2O_2 concentrations. Catalase is especially effective at

Table 1. The specific activities of the antioxidant enzymes

Enzyme	Enzyme activity (U.mg^{-1} protein) liver	kidney	spleen	thymus
SOD	33.1 ± 0.4	40 ± 10	120 ± 10	35 ± 6
CAT	114 ± 9	274 ± 8	54 ± 2	28 ± 2
GSHPx-cum	1.2 ± 0.1	0.51 ± 0.06	0.26 ± 0.03	0.374 ± 0.009
GSHPx-H_2O_2	0.94 ± 0.07	0.85 ± 0.08	0.17 ± 0.02	0.281 ± 0.007

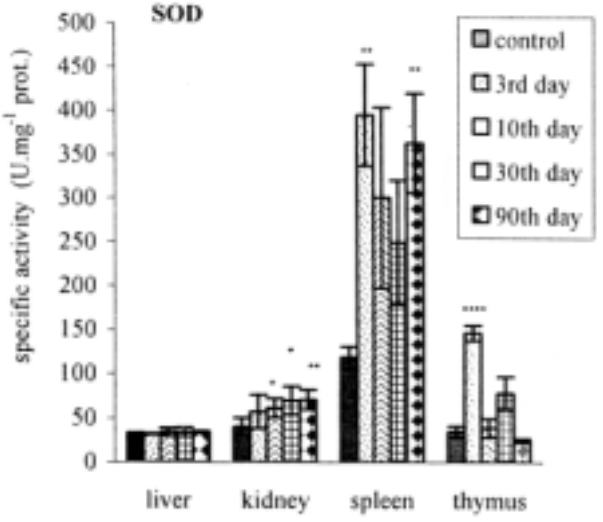

Fig. 1. Superoxide dismutase activity in the tissue extracts of the rabbits' liver, kidney, spleen and thymus after exposure to bendiocarbamate. The assays were performed as described in Materials and methods. The results are expressed as means ± SEM

*** — p < 0.05; ** — p < 0.02; *** — p < 0.01; **** — p < 0.001; asterisks represent significant differences between the control group and the experimental group**

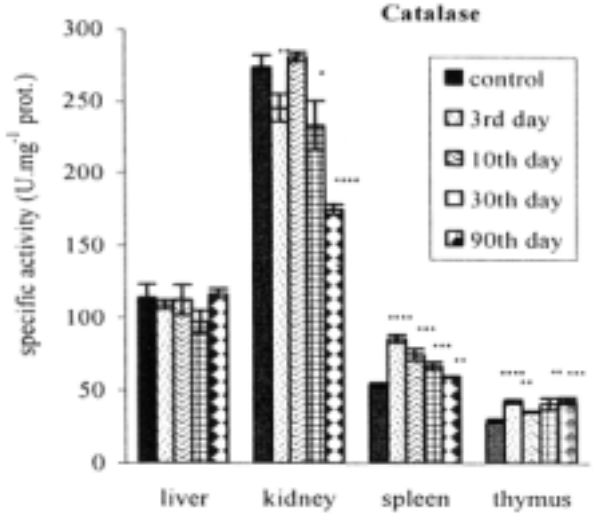

Fig. 2. Catalase activity in the tissue extracts of the rabbits' liver, kidney, spleen and thymus after exposure to bendiocarbamate. The assays were performed as described in Materials and methods. The results are expressed as means ± SEM

*** — p < 0.05; ** — p < 0.02; *** — p < 0.01; **** — p < 0.001; asterisks represent significant differences between the control group and the experimental group**

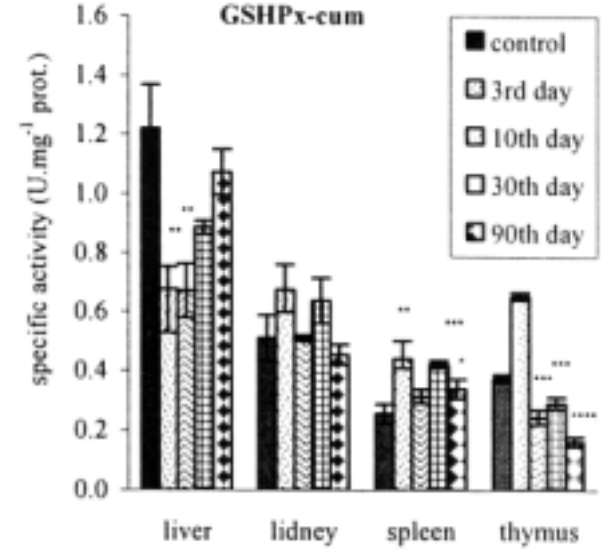

Fig. 3. Glutathione peroxidase activity with cumene hydroperoxide as substrate in the tissue extracts of the rabbits' liver, kidney, spleen and thymus after exposure to bendiocarbamate. The assays were performed as described in Materials and methods. The results are expressed as means ± SEM

*** — p < 0.05; ** — p < 0.02; *** — p < 0.01; **** — p < 0.001; asterisks represent significant differences between the control group and the experimental group**

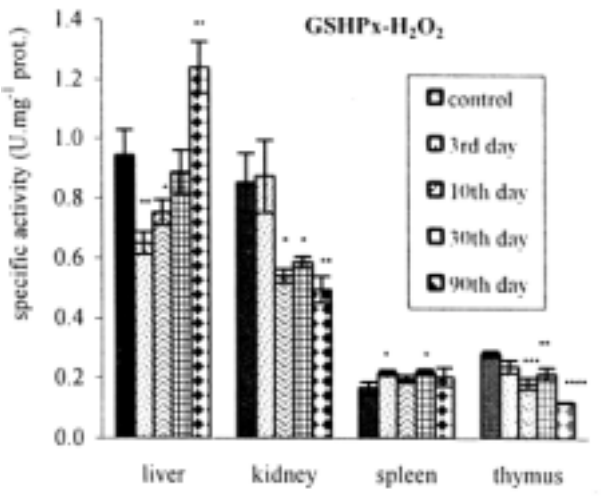

Fig. 4. Glutathione peroxidase activity with H_2O_2 as substrate in the tissue extracts of the rabbits' liver, kidney, spleen and thymus after exposure to bendiocarbamate. The assays were performed as described in Materials and methods. The results are expressed as means ± SEM

*** — p < 0.05; ** — p < 0.02; *** — p < 0.01; **** — p < 0.001; asterisks represent significant differences between the control group and the experimental group**

a high concentration of H_2O_2 and glutathione peroxidase is capable of utilizing hydroperoxides and metabolizing H_2O_2 when its concentration is low (4).

Similarly, as with SOD in the liver, CAT activity did not show significant changes in the experimental groups compared with the control. A significant increase in CAT activity was observed in the experimental groups in the spleen and the thymus. In the kidney on the 30th and 90th days a significant decrease in CAT activity was observed (Fig. 2).

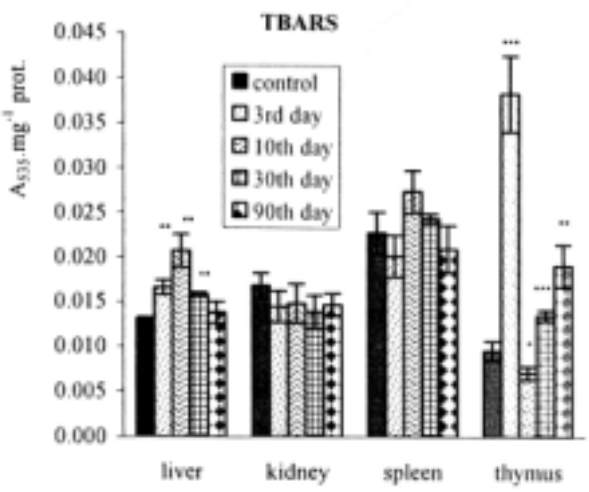

Fig. 5. Content of the TBARS in the tissue extracts of the rabbits' liver,kidney, spleen and thymus after exposure to bendiocarbamate. The assays were performed as described in Materials and methods. The results are expressed as means ± SEM

*** — p < 0.05; ** — p < 0.02; *** — p < 0.01; **** — p < 0.001; asterisks represent significant differences between the control group and the experimental group**

GSHPx plays a major role in protecting cells from oxidative damage, especially lipid peroxidation of biological membranes (7). Several studies have shown important differences in the activity of glutathione peroxidase in animals after exposure to xenobiotics. Like other authors, we have also observed the inhibition of GSHPx activity after bendiocarbamate administration (10, 12). The activities of GSHPx-cum and GSHPx-H_2O_2 were significantly decreased on the 3rd and 10th days in the liver and on the 3rd, 10th and 90th days in the thymus after exposure to bendiocarbamate. In the kidney, only the activity GSHPx-H_2O_2 was significantly decreased on the 10th, 30th and 90th days of experiment. The activity of GSHPx-cum was nearly the same in experimental groups when compared to the control. In contrast, in the spleen a significant increases in the activities of GSHPx-cum (3rd, 30th, 90th days) and GSHPx-H_2O_2 (3rd and 30th days) were observed in experimental group compared to the control (Figs. 3, 4).

The present study has shown the differences in the activities of antioxidant enzymes between the experimental and control groups as a biochemical response to bendiocarbamate administration in rabbits. The changes in the activities of these enzymes have led us to study whether the bendiocarbamate could stimulate an oxidative stress. One of the markers of the oxidative damage of membrane lipids is the TBARS content in tissues (17). Compared with the control, the TBARS contents were significantly increased in the experimental groups in the liver and thymus. The rabbits' exposure to bendiocarbamate did not affect the contents of TBARS in the kidney and spleen (Fig. 5).

Our results have shown that the organ's response to bendiocarbamate is different and may depend on a specific organ's damage and its protective abilities. The changes in the activities of SOD, CAT, GSHPx and the contents of TBARS indicate that bendiocarbamate increases the formation of ROS. Therefore we can assume that the toxicity of bendiocarbamate and its transformed metabolites could be influenced not only by reversible inhibition of AChE, but also by ROS, which may be generated by redox cycling of biotransformed metabolites of bendiocarbamate.

ACKNOWLEDGEMENT

This investigation was supported by Grant Agency VEGA 2/3064/23.

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Received January 31, 2005

FOLIA VETERINARIA
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FOLIA VETERINARIA, 49, 1: 15—18, 2005

ENVIRONMENTAL GENOTOXICITY ESTIMATES IN ANIMALS (A Review)

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ABSTRACT

The opportunities for veterinary medicine for complex environmental genotoxicity estimates (caused by herbicides, fertilising agents and industrial contaminants) through the health status and reproductive disturbances of farm animals are discussed in the article. The herds of pastured farm animals — cattle, sheep, goats or horses, eventually are proposed as acceptable biological material, because of long-term contact with environmental genotoxins which cannot be simulated in laboratory conditions. An increase of pesticide resistance in housefly, blowfly and tick populations as well as the increased virulence of microbial and viral pathogens are considered as primary indirect criteria for environmental genotoxicity. The frequency of reproductive failures, hereditary diseases and a significantly higher number of developmental abnormalities in the newly-born within the same species bred under different environmental conditions are considered as serious indirect indicators of environmental genotoxicity. Evidence of similar types of tumours in different species living in a contaminated area also contributes as helpful information. Micronuclei test (MN), chromosomal aberrations (CA), sister chromatide exchanges (SCE) and fluorescent *in situ* hybridization (FISH), eventually are recommended as a direct methods for the estimation of environmental genotoxicity.

Key words: environment; genotoxicity; home animals

INTRODUCTION

Almost all well developed countries apply the standard genotoxic program to prevent the spread of genotoxic agrochemicals and industrial contaminants in agriculture and their accumulation in the environment. Multi-stepped approaches including three genotoxic categories — mutagenicity, teratogenicity and cancerogenicity are required for this aim (2, 30). Because of laboratory, fur or farm animals that are used for such purposes, genotoxicity should be taken as a serious veterinary research field.

The opportunity to estimate environmental genotoxicity according to the health status of animals and reproductive disturbances seem to be the proposing screening for environmental protection and human health safety as a final stage in the food chain.

The populations of wild or pastured farm animals provide the optimal biological model for a complex environment genotoxicity estimate that cannot be achieved in laboratory conditions. The importance of such a model is given by the cumulative effect and interactions of different compounds in many thousands of such combinations that counter the genotoxic effect of partial components (11, 14).

INDIRECT CRITERIA FOR GENOTOXICITY ESTIMATION

The screening of indirect criteria for environmental genotoxicity according to Wurgler and Krammers

(31) has to be taken into account as preliminary markers in a genotoxic study in wild-living or pastured animal populations. These are:

a) Increase in resistance to pesticides

This mechanism of environmentally induced changes in the genetic constitution of population is detectable in veterinary practice as the increase in resistance to insecticides in houseflies, blowflies and ticks. This phenomenon could be explained by the establishment of resistant sub-populations resulting from the recombination of genes still viable in the population, or from *de novo* reproducible mutants induced by environmental mutagenic drift.

This process resulting in the increased resistance of 225 insect species against the five most frequent groups of insecticides over a period of eighteen years has been described by M e y e r (24).

See the following table:

Insecticides	Year 1976	1994
DDT	212	212
HCH	175	223
Organophosphates	82	178
Carbamates	25	153
Pyrethroids	—	27

b) Increased virulence of pathogens

Bacterial resistance to antibiotics (except of the plasmid mediated drug resistance), indifferent to the vaccines actually used, and the rise of new viral serotypes not known up to date, represents common cases of environmentally induced changes in the population make-up, detectable in veterinary practice (8, 19).

c) Reproductive failures or sterility

This is the most important aspect of environmentally induced changes in the population's genetic make-up in terms of economic consequences. The lethal point mutations, chromosome aberrations in gametes, interruptions of embryonic or foetal development due to the influence of environmental genotoxins, lead to sterility or to microabortions and may, in this way, reduce potential fertility (3, 9). The reduction of the reproductive potency of free-living animals or population of pastured animals compared to animal species in different environmental conditions is a relevant criterion for environmental genotoxicity.

d) Increase in hereditary coded diseases

If a new mutation is transmitted to live born offspring and does not drastically reduce reproductive fitness it may enter the animal gene pool. Depending on its phenotypic effect in heterozygous carriers, it may persist for a few or many generations and will contribute to the genetic load and related genetic disease (13). The estimated average persistence for free crossed population is as follows:

— autosomal dominant or X-linked mutation — five generations,
— recessive mutations — many generations,
— mutations of complex inheritance — ten generations,
— structural chromosome changes:
  a) balanced — five generations,
  b) unbalanced — three generations,
— numerical chromosomal change — one generation (17).

Therefore, the statistically increased frequency of inherited diseases by contrast with comparable population of the same animal species living in different environmental condition, is a further criterion for the evidence of environmental genotoxicity (26, 27).

That means the screening of hereditary coded defects is a long-term process that involves different generations living in a given area.

e) Increase in developmental abnormalities in the newly-born

The teratogenic potency of the environment is expressed through inborn abnormalities and developmental defects arising in different ways. There are gametic or somatic mutations at gene or chromosome level, and/or alterations of embryonic development due to the inactivation of relevant enzymes (21, 22). Developmental abnormalities (monstrosities) are compulsorily reported and registered for cattle especially in well-developed countries (4).

The statistically significant increase in the total amount of developmental abnormalities detectable at birth, independently of their other classifications, is an important indirect criterion for the accumulation of environmental genotoxicity.

f) Evidence of the similar types of tumours in various species

An interesting fact, for this criterion is that in fish tumours the same type of oncogenes is activated as in neoplasmas of mammals and man (6). Laboratory experiments have detected that the exposure of rainbow trout embryos to rodents hepatocarcinogens (aflatoxin B_1, dimethylnitrosoamine, benzo(a)pyrene, N-methyl-N-nitrosoamine) showed the induction of well-differentiated hepatocellular carcinomas (23). This aspect is also utilisable as a preliminary factor in environmental genotoxicity in veterinary practice, but the direct application of such a test is dependent on an extremely massive genotoxic influence elsewhere as the period for clinical expression of a detectable tumour is greater than an interval of a generation in the herd.

DIRECT TESTS FOR GENOTOXICITY ESTIMATION

The following methods can detect environmentally caused chromosomal damage. They can be used as direct indices of environmental genotoxicity, because of genotoxic influence detection on DNA holders — chromosomes.

a) Micronuclei

Micronuclei (MN) are formed by condensation of acentric chromosomal fragments, or by chromosomes that are left behind (lagging of chromosomes) during anaphase movement. The presence of micronuclei can therefore be taken as an indicator of the previous existence of chromosomal aberrations.

Micronuclei can be scored in peripheral lymphocytes or in bone marrow cells after exposure to potential genotoxins. To make evident micronuclei cells have to go through mitosis. Also the evidence of polychromatic erythrocyte frequency in 1,000 scored erythrocytes, derived from one animal or person of the tested group, is one frequent modification of this test for routine genotoxicity evaluation. The nuclei-staining compounds (May-Grünwald or Giemsa staining modifications) visualise the chromosomal fragments in erythrocytes in this modification (20).

The MN frequency in somatic cells of animals living in different feeding and environmental condition, and/or differences in MN at the beginning and by the end of the pasture season provides noteworthy indices for the genotoxicity of environmental factors.

b) Chromosomal analysis

Chromosomal *in vitro* analysis is a compulsory test for all newly developed compounds that are intended for use as agrochemicals, pesticides conserved agents or remedies (18). The CA (chromosomal aberration)-test is required as a preventive genotoxic test prior to their licensing for public use (12).

This method is also suitable for detecting balanced chromosomal aberrations causing fertility errors (7), and for environmental genotoxicity estimates of pastured animals continuously exposed to environmental genotoxins in the long term. The four per cent of aberrant cells which is considered as a marker for heavy genotoxic influence is, by the end of pasture season, more than twice greater for animals in contaminated districts (28).

c) Sister-chromatide exchanges

A sister chromatide exchange is the cytological manifestation of DNA breakage and rejoining at apparently homologous sites on the two chromatides of a single chromosome. The mechanism of SCE induction is not fully understood, but several studies have demonstrated that the frequency of SCE increases when cells of animals or humans are exposed to known mutagens or carcinogens (5). A SCE is efficiently induced by those substances that form the covalent adducts to DNA or otherwise interfere with DNA metabolism or repair. Consequently, SCE is one of the most frequent genotoxic tests — T u c k e r *et al.* (29).

It could be used as an environmental genotoxicity estimate as a final test in cases of doubled procedure i.e. screening the long term exposure to the environment on the pasture, and a precise experiment where contaminated forage, water or industrial pollutants were applied to animals for 60 or 90 days (1, 15).

d) Fluorescent *in situ* hybridisation (FISH)

The FISH technique enables the visualisation of whole chromosomes and their structural re-arrangements. Most significant balanced chromosomal aberrations are especially detectable by this method (7). Also the loci or DNA sequences influencing or regulating cellular division (i.e. centromeric or telomeric) can be marked by this method (10). Markers of quantitative trait loci (QTL) i.e. k kazein, b lactoalbumin, F factor in Boorola sheep and phylogenic diversity among different animal species (16, 25) are detectable by the method mentioned.

CONCLUSIONS

The genotoxic effect of the environment can be expressed through the increase of mutagenicity, carcinogenicity or teratogenicity. The three tests have been considered as suitable detectors for all of these categories.

These are: micronuclei test, chromosome aberrations and sister chromatide exchanges.

Because of the first full contact with the environment, pastured farm animals might be used as a biological material for its complex genotoxicity. The optimal time for such screening is the end of the pasture season, because of different feeding during the winter-time (consumed feed could be taken from a different area, not contaminated by industrial pollutants, agrochemicals, mycotoxins, etc.). The chromosomal damages induced by the environment can be expressed by micronuclei. This is the simplest method for monitoring genotoxicity. About 30 animals are proposed as a sample for revealing statistical significance. The achieved value has to be compared to an untreated group of animals or to their previous status before contact with environmental contaminants.

The chromosome aberrations and SCE are more difficult methods, therefore the number of investigated units is proposed as a reduced 10—15 animals.

The SCE frequency must be statistically compared between treated and untreated groups of animals or to their previous status as mentioned for the micronuclei test.

We conclude that FISH is a discriminating technique in hereditary health control and/or environmental genotoxicity detection.

ACKNOWLEDGEMENT

Article was supported by grants No. 1/1367/04 and 1/1271/04 VEGA MŠ SR.

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Received January 31, 2005

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FOLIA VETERINARIA, 49, 1: 19—23, 2005

MICROSCOPIC PICTURES OF ADRENERGIC AND BuChE-POSITIVE INNERVATION OF THE SPLEEN IN RABBITS AFTER ADMINISTRATION OF BENDIOCARBAMATE

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ABSTRACT

Innervation of the spleen in rabbits after application of bendiocarbamate was studied. Butyrylcholinesterase (BuChE)-positive nerve components of the organ were visualized by the direct thiocholine method and the adrenergic nerve components by the glyoxylic acid histofluorescence method. Adrenergic and BuChE-positive nerve components enter the organ in a common bundle with arteries in all experimental and control animals. In the organ they form characteristic periarterial and periarteriolar plexiform arrangements, which are especially conspicuous around the *aa. centrales* running through the white pulp. Then, nerve fibres extend away from these plexuses into adjacent layers of trabeculae further into marginal layers of the periarterial lymphatic sheath (PALS) as well as into the mantle zone of follicles. Several scattered periarteriolar and individual nerve fibres can be seen also in the marginal sinuses and cords of the red pulp. In the fibrous capsula of the organ adrenergic and BuChE-positive nerve fibres can be seen, which have an evident connection with the trabecular and parenchymal nerves of the organ. Microscopic findings of density, topography and distribution of BuChE-positive nerve profiles in experimental and control animals are in principle the same. On the other hand, pictures of adrenergic nerve fibres in experimental animals, after the bendiocarbamate administration compared with controls, appeared diffuse and dim in all topography.

Key words: adrenergic nerve; bendiocarbamate; BuChE-positive nerve; rabbit; spleen

INTRODUCTION

From the great number of published data on the nerve supply of the various compartments of the primary and secondary lymphoid organs in various mammals it is evident that innervation of the spleen is not an object of only marginal interest. The presence of autonomic nerve fibres in the parenchyma of lymphoid organs established an anatomical link between the brain and the immune system in order to translate central neural processes into chemical signals that can influence specific functions of the immune system.

Visualisation of adrenergic, AChE-positive, and BuChE-positive nerve components of an organism has proved to be of great importance not only in the mapping of adrenergic and cholinergic components of an organism, but also in the study of their quantitative and qualitative changes under various pathological circumstances, including the consequences of neural lesions induced by the effect of different toxic substances.

Bendiocarbamate is a chemical agent assigned mainly for the destruction of insect pests. Its toxicity is evident after the consumption of contaminated food, but also it acts as a contact poison. It blocks AChE, after around 24 hours there is a return to the normal status, because its accumulation in mammalian tissue does not occur. The toxic effects of this substance are varied. As it inhibits the activities of cholinesterases it results in the accumulation of ACh, this transmitter is not

hydrolysed and can accumulate in the reactive sites of the organism (8, 6). Such induced effects of ACh accumulation are equivalent to the continual stimulation of cholinergic synapses through CNS and PNS (3, 9, 11). The most frequent accompanying symptom of its toxicity is a decrease in the activity of AChE and BuChE in the blood (4), further a disorder of the ion regulation between the outside and inside environment of cells, whereby formation of action potentials on the neural terminations is impaired (1, 5, 7).

Regarding these facts, we have made an effort to find out in experiments with rabbits, whether after application of bendiocarbamate, which acts as a reversible inhibitor of AChE, there are changes in the shape of adrenergic and BuChE-positive neural components.

MATERIAL AND METHODS

Twenty-five rabbits of both sexes, weighing 1.5–3.5 kg b.w. were examined. Bendiocarbamate was administered *per os* at a dose of 5 mg.kg^{-1} in this way: 5 animals were administered ampoules of bendiocarb every day. The other animals (20) received this substance at the same dose every second day. Subsequent samplings were carried out according to the programme at two weeks intervals. All animals were anaesthetized with pentobarbital (40 mg.kg^{-1}, i.p.). Their spleens were removed from the experimental and 10 control animals. For visualisation of BuChE-positive nerve structures a direct thiocholine method for cytochemical evidence of AChE according to Badawi and Schenk (2) was used; BW 284 c 51 (Sigma) was used as a blocker of specific AChE. Excisions from organs were fixed in 4 % formaldehyde at 4 °C for between 2 and 10 hours. Sections of 16 µm were cut on a cryostat, incubated in medium (pH 5.5) for 2–4 hours at 37 °C. Individual sections were mounted on glass slides. Adrenergic nerve profiles were visualised using the fluorescent histochemical method with glyoxyl acid according to Schvalev and Zhuckova (10). Cryostat sections (15–20 µm) were incubated in 2 % glyoxylic acid (Sigma) incubation medium then dried with a hair drier and kept at 100 °C. Individual sections were mounted in non-fluorescent immersion oil and examined. Microscopic examinations and photo documentation were performed using Jenalumar 2 (Zeiss, Jena).

RESULTS AND DISCUSSION

Microscopic pictures of BuChE-positive innervation of the spleen

BuChE-positive nerve plexuses have been found to enter the spleen in the common bundle with *a. lienalis* and its branches. Then, in the organ they pass together with their branches in the form of periarterial and periarteriolar plexuses through the trabeculae as well as white pulp. From these nerve plexuses the fibres extend away, which within PALS pass in the scope of the marginal zone between the white and red pulp.

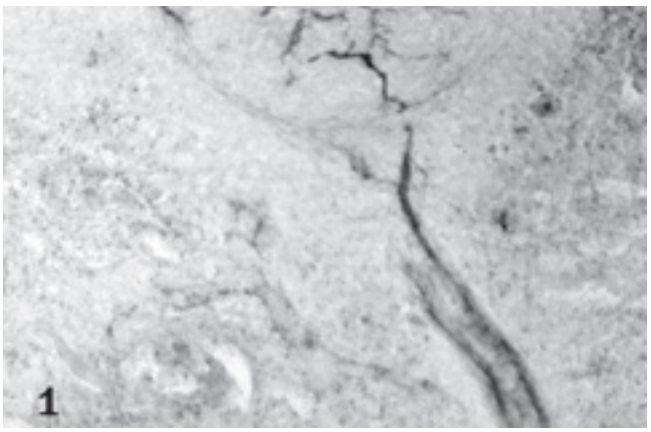

Fig. 1. BuChE-positive thicker nerve profiles and adventitial plexiform nerve formations in the vicinity of the follicle. Very fine linear BuChE-positive non-neural profiles may be seen also as crossing the follicle (control animal). Magn. × 250

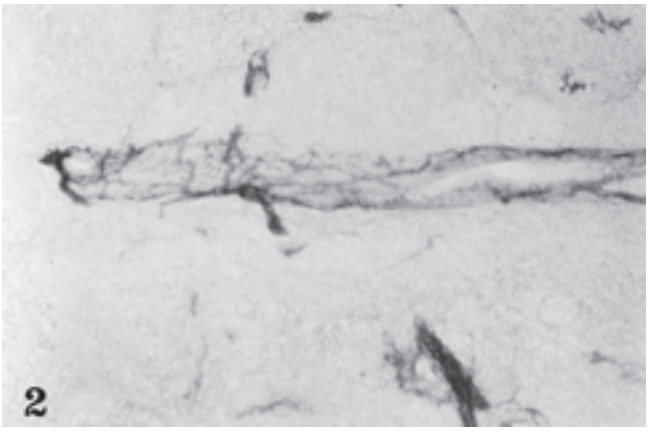

Fig. 2. Plexiform arrangement of nerve fibres around the arteriolae running in the septum. (experimental animal). Magn. × 250

Dense BuChE-positive neural profiles with an abundant content of coloured reaction product accompany *aa. centrales,* passing through the white pulp. BuChE-positive neural fibres with a small amount of coloured reaction product were also recorded in the marginal zone of follicles, or in the course of *aa. interfoliculares.* In the spleen as well as in other lymph organs, BuChE-positive nerve fibres do not penetrate the centre of follicles.

Even if BuChE-positive innervation of the white pulp in PALS is not very apparent, the presence of periarterial or periarteriolar, and individual nerve fibres is seemingly evident on the borderline of the white and red pulp. A sporadic occurrence of delicate neural profiles can be seen in the marginal sinuses and cords of red pulp. Some BuChE-positive nerve fibres run through the trabecula and capsule of the organ without showing connections with vascular branches.

Innervation of the wall of large and larger veins is segmented and very poor. Nerve fibres can be also seen in the fibrous capsule of the organ and their connection with parenchymal and trabecular nerves is quite evi-

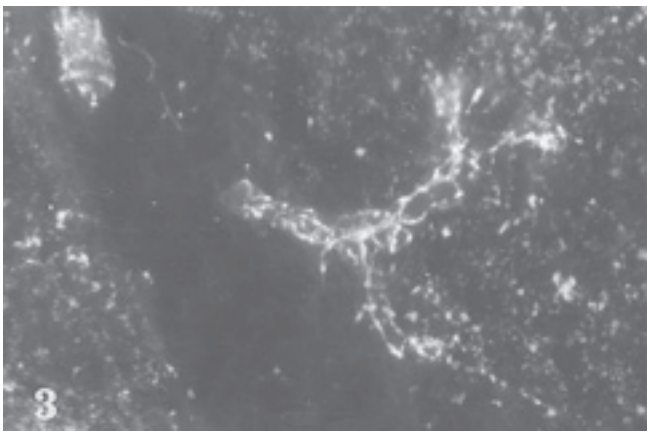

Fig. 3. Richly innervated fine arteriolar twigs whose branches tend towards the surroundings parenchyma of the organ (control animal). Magn. × 250

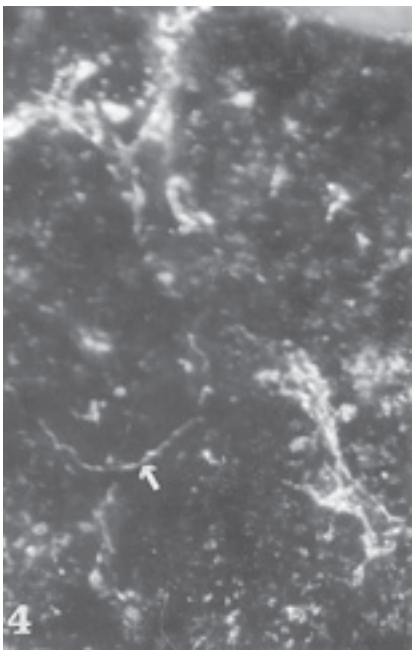

Fig. 4. Intrasplenic nerve components lying in the subcapsular parts of the parenchyma. Solitary nerve fibres in the red pulp are very rarely (*arrow*) in the control animal. Magn. × 250

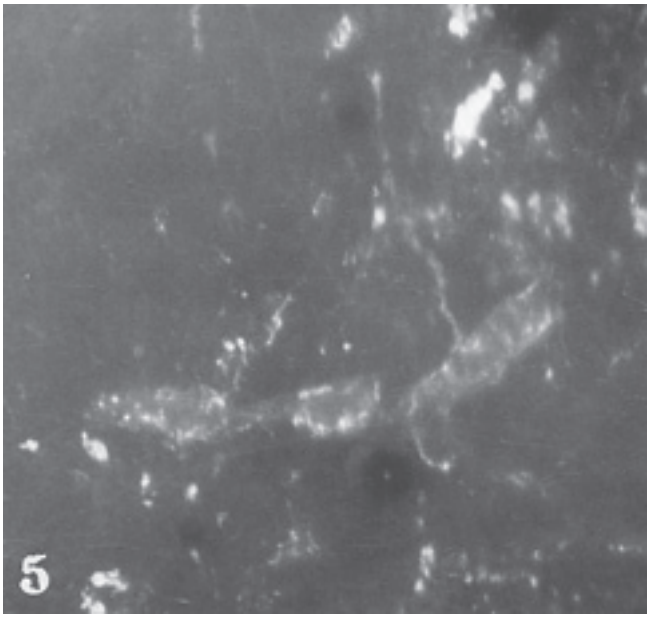

Fig. 5. Relatively thinner adrenergic nerve plexuses around the arteriolae on the borderline of PALS and the red pulp. The diffusion of the fluorofore and weaker intensity of the fluorescence of the nerve profiles are evident, while the fluorescence of varicosities is usually intensive enough (experimental animal). Magn. × 250

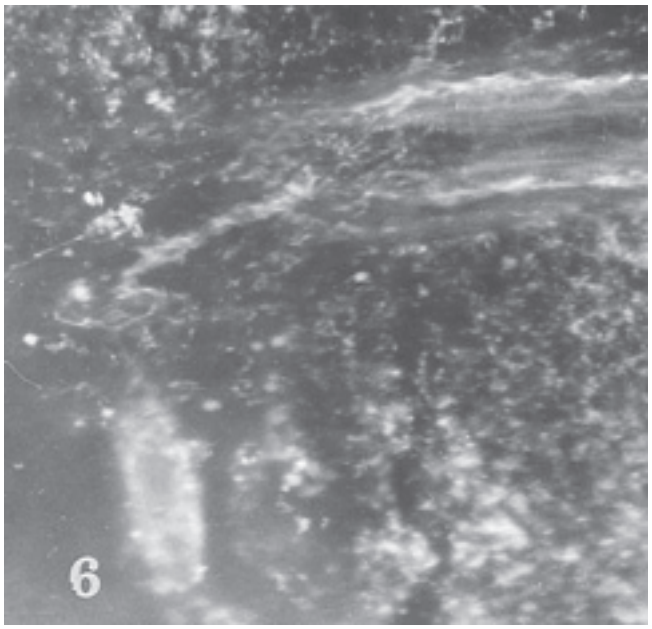

Fig. 6. The evidently dim and weaker intensity of the fluorescence may be seen in the periarterial and periarteriolar topography (experimental animal). Magn. × 250

dent. In addition, BuChE-positive non-neural structures with a variable content of the reaction product in the lymph follicles can be seen, which are similar in shape to the reticulin skeleton.

No substantial changes were recorded after comparison of the density, distribution and topographic connections of BuChE-positive neural components in the spleen of rabbits given bendiocarbamate with the findings in the control animals. The greatest density of BuChE-positive neural profiles, with an abundant amount of coloured reaction product was always found in the same topography, i.e. round *aa. centrales*, PALS, marginal zone of follicles as well as in the organ capsule (Figs. 1, 2). There are also similar findings of BuChE-positive neural fibres with a relatively smaller content of coloured reaction product in the periarterial or periarteriolar, and individual fibres lying in the marginal sinuses and cords of the red pulp. Our findings confirm data that bendiocarbamate inhibits AChE, but not BuChE in the organ examined.

Microscopic pictures of adrenergic innervation of the spleen

Post-ganglionic adrenergic neural plexuses enter the spleen like BuChE-positive in a common bundle with the arteriae. In the organ they pass through the *trabeculae* in the shape of perivascular plexiform aggregations of carrying neural fibres with the addition of thicker and thinner neural fascicles as well as more delicate plexuses consisting mainly of pre-terminal and terminal varicose fibres, lying in a close contact with the external side of the muscular media layer, marked as "adventitial plexuses."

In this topography, however, relatively numerous individual neural fibres extend away from the periarterial or periarteriolar neural plexuses that ramify, and often they do not display direct connections with the perivascular nerves. Intensively fluorescent neural profiles accompany the central arteriae running through the white pulp. In their further course, they pass through PALS in the form of linear aggregations in the marginal zone between the white and red pulp. Moreover, specifically fluorescent neural profiles are also recorded in the marginal zone of the follicles. The entry of adrenergic neural fibres into the germinal centre of follicles was not observed.

The patterns of arrangement and density of neural fibres in PALS are quite variable. In the marginal sinuses and cords of red pulp, only modestly represented dispersed delicate neural profiles can be seen. The walls of large and larger veins are innervated only partially and rather poorly. In the fibrous capsule of the organ there are brightly fluorescent neural fibres whose connections with the trabecular or parenchymal nerves are mostly evident. Adrenergic innervation of the spleen in the control and experimental animals is substantially the same, however, in the experimental animals all the neural structures in the given topographies are illustrated dimly (Figs. 3, 4, 5, 6).

Functional changes after administration of anticholinesterases are also characterised by disorders of the metabolic processes of the acid-base balance regulation to which the accumulation of lactate and pyruvate especially contribute (while pyruvate is known to play an important role as the main precursor of acetylic groups of ACh in the brain). Depolarisation of biological membranes and hypokaliemia occurs due to the effect of accumulated ACh (1, 8, 9). In the early stage of toxicity, an acceleration of the pulse frequency and increase in blood pressure often occur that later change vice versa (8, 11). These symptoms could be a sign of the combined impairment of nicotinic and muscarinic regulatory mechanisms that on the periphery cause a release of catecholamines from the adrenal glands, or sympathetic fibres. At the same time, manifestation of some stimulating effects occurs (8), which in the visualisation of adrenergic neural profiles is displayed in the diffusion of a neurotransmitter into the nerve surroundings.

It is well known that on the surface membranes of various subpopulations of lymphoid cells there are more specific receptors for some classical neurotransmitters as well as for more peptides. There are data (7) that a released neurotransmitter can influence the functions of T-, and B-lymphocytes as well as the process of taking up the antigen by the dendritic cells. Lymphocytes isolated from the spleen, thymus, lymph nodes as well as from peripheral blood contain muscarinic and nicotinic cholinergic receptors with different functions and biochemical effects (7). Moreover, adrenergic and BuChE-positive neural fibres in the spleen are very close to, or in contact with migrating lymphocytes or plasmatic cells and macrophages, and arterioles and in this way they participate in the local modulation of humoral and cellular responses of the organism, and indirectly immunological responses also.

CONCLUSION

Based upon the microscopic findings of BuChE-positive innervation of the spleen in rabbits administered bendiocarbamate at a dose of 5 $mg.kg^{-1}$ b.w., it may be stated that this substance does not substantially influence the microscopic visualisation of these neural structures. On the other hand, examination of adrenergic neural structures in the organ observed, are in agreement with the data from the literature (8) that due to the impairment of nicotinic receptors a release of catecholamines from the sympathetic nerves occurs. Morphologically, these changes in our material are visualised in the form of phenomena of the neurotransmitter diffusion into the neighbourhood of adrenergic neural fibres and their fascicles.

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Received January 31, 2005

FOLIA VETERINARIA
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FOLIA VETERINARIA, 49, 1: 24—26, 2005

THE ACTIVITY OF BENDIOCARBAMATE IN THE RABBIT BONE MARROW MICRONUCLEUS ASSAY

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ABSTRACT

Bendiocarbamate is a systemic insecticide, effective as a contact and an ingested poison. It is active against many public health, industrial and storage pests. Like other carbamate insecticides, it is a reversible inhibitor of cholinesterase. In the present study, micronucleus (MN) formation in rabbit bone marrow was determined after both three and ninety daily applications of bendiocarbamate (5 mg.kg^{-1}) *per os*. Results showed that exposure to insecticide did not cause significant increases in the frequencies of MN in any of the tested groups. Depression of bone marrow proliferation was evident in reduction of PCEs after three days of exposure and attained statistical significance ($P < 0.05$).

Key words: bendiocarbamate; bone marrow; micronuclei; rabbit

INTRODUCTION

Interest in pesticide toxicity has particularly increased in recent years due to the increasing evidence of carcinogenic, mutagenic and teratogenic effects in experimental animals and exposed humans (4).

Bendiocarbamate is a carbamate insecticide that is effective against a wide range of nuisance and disease vector insects. In agriculture it is used against a variety of insects (5), especially those in the soil. Absorption through the skin is the most likely route of exposure. Like other carbamate insecticides, bendiocarbamate is a reversible inhibitor of cholinesterase (an essential nervous system enzyme). The oral LD_{50} for the bendiocarbamate is 34 to 156 mg.kg^{-1} in rats, 35 to 40 mg.kg^{-1} in rabbits, and 35 mg.kg^{-1} in guinea pigs. A two-year study with rats showed a wide range of changes in organ weights, blood, and urine characteristics, as well as an increase in the incidence of stomach and eye lesions (1). No reproductive effects were seen in a three-generation study with rats (1). Carbamates generally are excreted rapidly and do not accumulate in mammalian tissue. If exposure does not continue, cholinesterase inhibition and its symptoms reverse rapidly.

In this study we evaluated the genotoxic potential of bendiocarbamate after exposure in rabbits through a erythrocyte bone marrow assay.

MATERIALS AND METHODS

Chemicals

Bendiocarbamate (CAS 22781-23-3), chemical name: 2,3-isopropylidene-dioxyphenyl methyl carbamate; structural formula: $C_{11}H_{13}NO_4$.

Foetal bovine serum (Sigma, St. Louis, MO, USA).

Animals

Male and female hybrid Hyla rabbits (Kamenná Poruba, the Slovak Republic) used in the assay were housed in rabbit units. They received a standard rabbit diet and water *ad libitum*. The environment of all the animals' rooms was maintained within a temperature range of 15–20 °C and a relative

Table 1. The groups of rabbits used in the micronuclei assay

Control		After 3 days		After 90 days	
No. of animal/ sex	Body weight (kg)	No. of animal/ sex	Body weight (kg)	No. of animal/ sex	Body weight (kg)
28 F	2.43	10 F	1.29	3 F	3.16
31 M	2.78	11 F	1.35	35 F	3.34
41 M	2.67	12 F	1.32	44 M	3.95
42 M	3.00	23 M	1.50	47 M	4.05
	37 M	1.00	51 F	3.38	
Group Mean ± SD	2.72 ± 0.24		1.29 ± 0.18		3.58 ± 0.39

F — female, M – male

humidity range of 40–70 %.

Bone marrow micronucleus assay in rabbits

The group sizes used in the micronucleus assay are shown in Table 1. The dose of bendiocarbamate was 5 $mg.kg^{-1}$ body weights *per os* every day for the first 13 days and then was agent administered to the animals every second day. The rabbits were killed humanely (a lethal injection of anaesthetic agent) after three and ninety days. A portion of bone marrow from the thoracic rib was flushed and resuspended in 1.5 ml of foetal bovine serum. The bone marrow suspension was spun and smears were prepared and stained with May-Grünwald and Giemsa. Each slide was assessed for the presence of micronucleated polychromatic erythrocytes (MNPCEs) among 1000 polychromatic erythrocytes (PCEs). The ratio of PCEs to normocytes (NCEs) was determined among 1000 cells.

Micronuclei analysis

The frequency of MN in bone marrow was detected according to Mac G r e g o r *et al.* (6).

Data were summarized as the mean number of MNPCEs for 1000 PCEs. In addition, the toxicity in bone marrow was determined as ratio between PCEs and NCEs. The staining must allow clear discrimination between PCEs and NCEs. However, this is somewhat subjective since the transition in maturation is a continuous process. The normal ration is about 1 : 1. An increase in NCEs signals a cytotoxic effect. Similarly, at longer sample intervals, an increase in PCEs signals a stimulation of proliferative activity due to an early phase of cell depletion.

Student's *t*-test was used to compare MNPCEs data and PCEs to NCEs rations between treated and control groups for statistical significance.

RESULTS

The results of rabbit bone marrow micronucleus test after exposure to the bendiocarbamate are summarized in Table 2.

For each group, the average incidence of MNPCEs as well as the PCEs/NCEs ratio are shown.

In vivo administration of 5 $mg.kg^{-1}$ body weight of insecticide did not increase the incidence of $MNPCE_s$ in bone marrow of any tested groups.

Cytotoxic insult to bone marrow frequently impairs the proliferating and maturational abilities of erythroid cells. Typically, a ratio of enucleate, immature poly-

Table 2. Frequencies of micronuclei in rabbit bone marrow treated with bendiocarbamate

Control			After 3 days			After 90 days		
No. of animal	MNPCEs/ 1000PCEs	PCEs/NCEs ratio	No. of animal	MNPCEs/ 1000PCEs	PCEs/NCEs ratio	No. of animal	MNPCEs/ 1000PCEs	PCEs/NCEs ratio
28	0	1.071	10	0	0.450	3	1	0.607
31	1	0.680	11	1	0.394	35	0	0.872
41	0	0.933	12	0	0.473	44	1	0.841
42	0	0.904	23	0	0.607	47	0	0.966
			37	0	0.708	51	0	0.948
Group Mean ± SD	0.25 ± 0.5			0.25 ± 0.5[a]			0.40 ± 0.5[a]	
PCEs/NCEs ± SD		0.89 ± 0.16			0.53 ± 0.13*			0.85 ± 0.14[a]

MNPCEs — micronucleated polychromatic erythrocytes, PCEs — polychromatic erythrocytes, NCEs — normochromatic erythrocytes
SD — standard deviation; a — no significant difference; * — statistical significance ($P < 0.05$)

chromatic erythrocytes (PCEs) to mature normochromatic erythrocytes (NCEs) is used to assess cytotoxicity in the micronucleus (MN) assay. Depression of bone marrow proliferation was evident in the reduction of PCEs after three days of exposure and attained statistical significance ($P < 0.05$).

DISCUSSION

To our knowledge, there are very few published reports available describing the cytogenetic or genotoxic effects of bendiocarbamate on animal cells. Our present genotoxicity data for the bendiocarbamate are a part of more extensive collaborative study (7, 8, 9).

The micronucleus test in erythrocytes of mouse bone marrow has been proposed as a screening test by Boller and Schmid (2) and Heddle *et al.* (4). The frequency of micronuclei can be most easily evaluated in young erythrocytes shortly after the main nucleus is expelled. These young erythrocytes are termed polychromatic (PCEs) and are distinguished from mature normochromatic (NCEs) erythrocytes by their different staining properties. With a combination of Giemsa and May-Grünwald staining the PCEs stain bluish to purple due to their high content of RNA in the cytoplasm. In contrast, the NCEs stain reddish to yellow and are also slightly smaller than PCEs.

Published mutagenic, carcinogenic and teratogenic studies show that the bendiocarbamate is harmless (1). In a micronucleus test, groups of male mice were treated with technical bendiocarbamate i.p. for two days. There was no significant increase in the frequency of polychromatic erythrocytes containing micronuclei or change in polychromatic/normochromatic ratio in any treated group.

In conclusion, the results presented in this paper show that bendiocarbamate is unable to raise the incidence of micronuclei in rabbit bone marrow significantly, at least in our tested conditions.

ACKNOWLEDGEMENT

This study was supported by grants No. 1/1367/04 and No. 1/1271/04 of the Ministry of Education and Science of the Slovak Republic and by National Reference Laboratory for Pesticides of UVM Košice.

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Received January 31, 2005

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 49, 1: 27—31, 2005

THE INFLUENCE OF BENDIOCARBAMATE ON SOME PARAMETERS OF HOMEOSTASIS IN RABBITS

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ABSTRACT

Our investigations focused on the effect of bendiocarbamate (BDC) on some biochemical parameters in the blood serum of 50-day old experimental rabbits (n = 35). The rabbits were administered BDC at a dose of 5 mg.kg^{-1} bw daily for the first 13 days and then every other day. Altogether seven blood samples were withdrawn by heart puncture. The zero blood sample was taken three days before the first administration of BDC and the 1st sample three days after its administration. The following 3 samples were collected at ten day intervals and an additional 2 at thirty day intervals. The control group consisted of 11 rabbits. The blood serum of rabbits was examined for alkaline phosphatase (ALP), alanine transferase (ALT), γ-glutamyl transferase (GGT), cholesterol (CHOL), and serum bile acids (SBA). The dynamics of enzyme activities determined after the administration of BDC was similar in both groups with one statistically significant ($P < 0.05$) peak between the 4th and 6th samples (37th and 97th days of experiment). At the end (97th day) of the experiment, the activities of ALP and GGT in the treated group were increased and that of ALT was decreased compared to the control. Increased levels of SBA and CHOL were observed in experimental rabbits during the administration of BDC. Maximum levels of SBA and cholesterol were determined at the 3rd and 6th samples, resp. The differences between experimental and control rabbits were statistically significant ($P < 0.05$) for both parameters.

Key words: bendiocarbamate; bile acids; cholesterol; enzymes; rabbits

INTRODUCTION

The use of agrochemicals in agricultural practice is one of the important ways for xenobiotics to penetrate the food chain. Depending on the production and origin, they may contain high levels of many toxic elements. Despite the fact that the ecological problems arising from these toxic substances are becoming increasingly important and complex they have not been studied sufficiently. We lack information on the consequences of their action on the health of humans and animals. To resolve the questions of environmental pollution related to these substances we must adopt a complex approach encompassing pedological, botanical, veterinary, hygiene-toxicological aspects as well as those related to the quality of the environment and health risk assessment. These substances interact constantly with each other and may also react with organic compounds present in the outer environment and in the internal environment of living organisms.

From the point of view of crop protection the use of pesticides frequently becomes unavoidable. With regard to the environment it is desirable to have the biggest possible body of detailed knowledge about the short- and long-term effects of pesticides.

Bendiocarbamate (BDC) is an effective carbamate insecticide used in households, food stores and agriculture. Metabolic studies concerning BDC have been carried out on plants, animals and soil. It was observed that in the majority of plant species the principle metabolites are formed as glycoside conjugates of phenol NC 7312 and N-hydroxymethyl bendiocarbamate. The principle metabolite in rat urine is phenol which is eliminated as beta-glucuronide and sulphate conjugate.

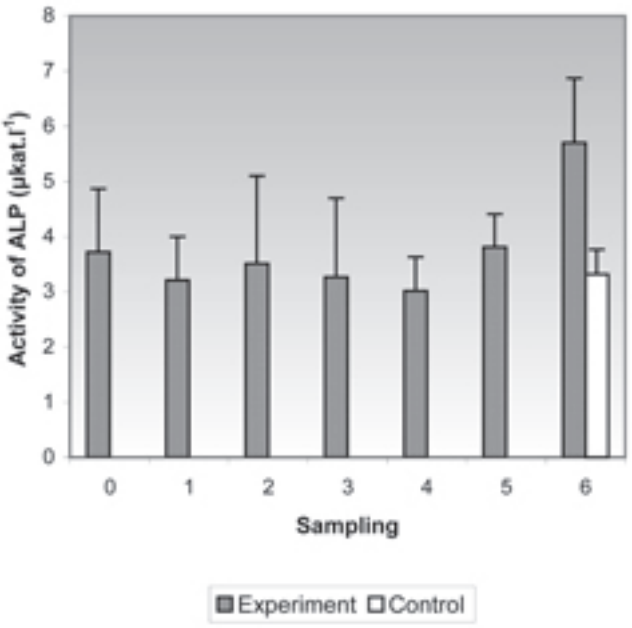

Fig. 1. The activity of ALP in blood serum of rabbits after bendiocarbamate administration

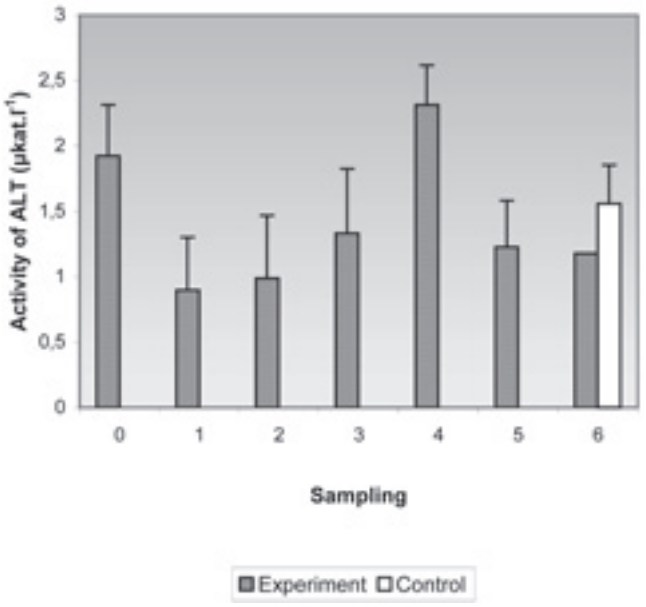

Fig. 2. The activity of ALT in blood serum of rabbits after bendiocarbamate administration

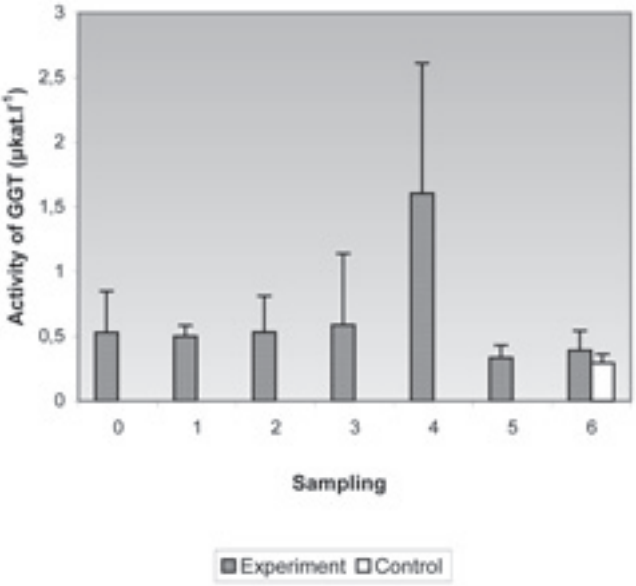

Fig. 3. The activity of GGT in blood serum of rabbits after bendiocarbamate administration

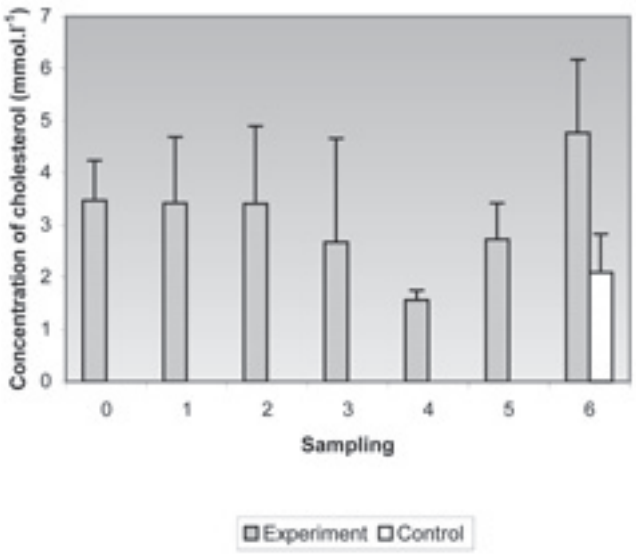

Fig. 4. The concentration of cholesterol in blood serum of rabbits after bendiocarbamate administration

A similar state of affairs can be observed in rabbits. Only small amounts have been detected in their faeces. The BDC is unable to raise the incidence of micronuclei in rabbit bone marrow significantly (10). No accumulation has been observed in mammals. The changes in the activity of superoxide dismutase, catalase, glutathione peroxidase and levels of thiobarbituric acid reactive substances in the liver, kidney, spleen and thymus do not point to rapid metabolism and elimination of BDC (11). Prolonged administration of BDC to rabbits results in marked suppression of functional activity of lymphocytes and phagocytes associated with leukopenia, neutropenia and relative lymphocytosis (9).

The study of BDC influence on the vitality of unicellular eukaryotic organism protozoa cultivated in hay medium showed that the effect of BDC differs from that in multi-cellular eukaryotic animal organisms (2).

The results of distribution of S-100 protein positive structures in the rabbit thymuses studied by the immunohistochemistry method after BDC administration indicated that BDC failed to produce any changes in the positivity of S-100 protein (8).

Changes in biochemical, haematological and other parameters of the health status of humans and animals call for investigation of the long-term effects of low doses of these substances.

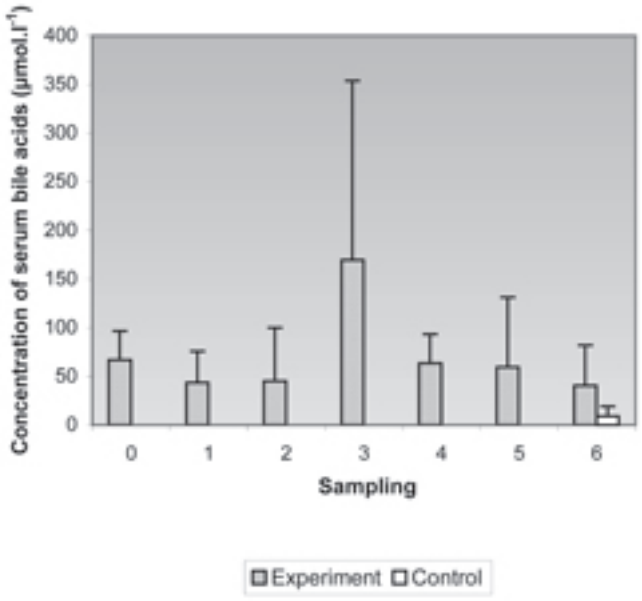

Fig. 5. The concentration of the serum bile acids of rabbits after bendiocarbamate administration

MATERIALS AND METHODS

The effect of bendiocarbamate on some serum biochemical parameters was studied in an experimental group consisting of thirty-five 50-day old rabbits of hybrid Hyla, obtained from a private breeder (Kamenná Poruba). The rabbits were fed *ad libitum* complete granulated mixed feed (feed registration number 7313/a, manufacturer BIOFER assoc., Prešov, manufacturing plant Ve ký Šariš) for fattening rabbits. During the first 13 days of the experiment the rabbits were administered 5 mg.kg^{-1} bw of bendiocarbamate daily and then every other day. The zero sample was taken three days before the first administration of BDC. Three days after receiving the first BDC dose the first blood sample was withdrawn. Samples 2 to 4 were taken at ten day intervals and 5 and 6 at thirty day intervals. The control group, which consisted of 11 rabbits, was fed *ad libitum* the same mixed feed as the experimental group.

The blood serum of rabbits was examined for alkaline phosphatase (ALP), alanine aminotransferase (ALT), α-glutamyl transferase (GGT), cholesterol (CHOL) and serum bile acids (SBA). Blood enzymes and cholesterol were determined by means of Bio-La test kits made by Pliva – Lachema a. s. Brno (The Czech Republic) and serum bile acids by Randox Laboratories Ltd. kits (United Kingdom). Blood samples were obtained by heart puncture. The results obtained were evaluated by Student *t*-test.

RESULTS

The results obtained indicated that the activities of enzymes ALT, ALP and GGT exhibited similar dynamics after administration of BDC. The activity of ALP (Fig. 1) in the first blood sample showed a slight decrease and after that it followed an almost linear course with a pronounced peak at the sixth sample significantly different ($P < 0.05$) compared to zero, second to fourth sample and the control group (Table 1).

After administration of BDC the activity of ALT (Fig. 2) showed an insignificant decrease at the first sample and then increased gradually up to a significant peak ($P < 0.05$) at the fourth sample. After reaching maximum the activity decreased slowly in the period of fifth and sixth samples, the differences being significant ($P < 0.05$) in comparison with the maximum. The activity of ALT in the control and experimental group differed significantly ($P < 0.05$) at the second sample (Table 1).

The activity of GGT (Fig. 3) resembled that of ALP and ALT as it decreased insignificantly at the first sample and then it showed a moderate increase following almost a linear course. Similarly as with ALT it increased significantly ($P < 0.05$) at the fourth sample and then it decreased abruptly during the fifth and sixth samples even below the starting level. The difference compared

Table 1. The activities of some blood serum enzymes in rabbits during bendiocarbamate administration

		Experimental group Samplings							Control group
Days of experiment		**0 0**	**1 7**	**2 17**	**3 27**	**4 37**	**5 67**	**6 97**	
ALP	$\bar{x}$	3.72*	3.21	3.51*	3.27*	3.02*	3.81	5.78*	3.32*
(µkat.l^{-1})	**SD ±**	1.15	0.79	1.59	1.43	0.61	0.60	1.17	0.45
ALT	$\bar{x}$	1.925*	0.898	0.988*	1.333	2.314*	1.228*	1.180*	1.558*
(µkat.l^{-1})	**SD ±**	0.650	0.389	0.403	0.480	0.493	0.303	0.354	0.296
GGT	$\bar{x}$	0.529*	0.499	0.533	0.588*	1.604*	0.334	0.389	0.293*
(µkat.l^{-1})	**SD ±**	**0.132**	**0.085**	**0.279**	**0.551**	**1.008**	**0.097**	**0.154**	**0.069**

Legend: ALP — alkaline phosphatase, ALT — alanine aminotranspherase, GGT — γ-glutamyl transferase, * — $P < 0.05$

Table 2. Blood serum cholesterol and bile acid levels in rabbits during bendiocarbamate administration

		Experimental group Samplings							Control group
		0	1	2	3	4	5	6	
CHOL	$\bar{x}$	3.47*	3.42	3.41*	2.67*	1.56*	2.73 *	4.77*	2.09*
(mmol.l^{-1})	**SD ±**	0.77	1.27	1.49	1.99	0.19	0.69	1.40	0.74
SBA	$\bar{x}$	66.9*	43.4	45.02	169.36	63.4*	59.6	40.5	9.0*
(µmol.l^{-1})	**SD ±**	29.7	32.2	54.9	84.4	29.9	71.7	41.3	9.9

Legend: CHOL — cholesterol, SBA — serum bile acids, * — $P < 0.05$

to the control was significant at zero and fourth samples (Table 1). The serum cholesterol level (Fig. 4) showed a tendency to decrease after the zero sample and reached a significant minimum ($P < 0.05$) at the fourth sample. Then it increased gradually to the fifth sample (67th day of experiment) ($P < 0.05$) and abruptly to the sixth sample (97th day of experiment) ($P < 0.05$). Except for an insignificant difference in cholesterol level between the control rabbits and minimum at the fourth sample higher levels of cholesterol were observed in the control animals throughout the experiment (Table 2).

The level of serum bile acids after administration of BDC (Fig. 5) showed a marked peak at the third sample and significant ($P < 0.05$) decrease at the fourth sample. Then the bile acids decreased gradually and at the sixth sampling reached a level lower than the starting one. Serum bile acids of experimental rabbits were higher in all samples compared to the control. The differences between experimental and control serum bile acid levels were significant at the zero and fourth samples (Table 2).

DISCUSSION

Bendiocarbamate is defined as a strong inhibitor of cholinesterase (CHE) in various animals and in humans and this has been confirmed by many authors. Administration of BDC to mice resulted in reduction of CHE in their blood (4). Kemp and Hounsell (6) have observed that whole blood is more sensitive to the inhibitory effect of BDC on CHE than blood plasma. Acute oral toxicity in mammals differed between genera and species but not between sexes. Other enzymes were studied less frequently although they are also affected by pesticides.

Although sublethal doses of pesticides resulted in a significant decrease in some enzymatic activities (AST, ALT, ALP) in the liver and brain of rabbits, blood plasma showed an increased activity (3) which correlates with our observations of ALP and GGT activities in rabbit sera. On the other hand, serum ALT in our experimental rabbits was increased only at two samples compared to the control. Kaur and Dhanju (5) have described significant changes in the activity of alkaline phosphatase and aminotransferases in blood plasma of rats administered subchronical doses for prolonged time.

Similar significant increase in the level of total lipids, cholesterol and triglycerides in blood plasma was caused by other pesticides (14). Warner, Adock and Pearce (12) have carried out repeated measurements of haematological and biochemical parameters in blood and urine and observed significant changes in some of them (cholesterol, total proteins). Increased blood cholesterol was found also in our experiment on rabbits. The study of mechanism of cyclooxygenase inhibition under the action of carbamate insecticides conducted by Krug, Hamm and Berndt (7) in humans has provided similar results to the studies of other authors, i.e. that these insecticides inhibit the metabolism of arachidonic acid through their action on cycloxygenase, which supports the assumption that BDC affects in this way the metabolism of fats. This has been confirmed by our observation of cholesterol dynamics.

Afzal *et al.* (1) have detected increased levels of bile acids in rabbits caused by paraquat. This observation was proved in our experiment, which dealt also with the dynamics of serum levels of bile acids following administration of BDC.

In conclusion, on the basis of these authors, one can conclude that similar changes in the activity of enzymes can result from the action of both carbamate and organophosphate pesticides. Administration of bendiocarbamate can cause an increase in the activity of alkaline phosphatase, γ-glutamyl transferase, blood serum cholesterol and bile acids in experimental rabbits compared to the control group.

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Received January 31, 2005

FOLIA VETERINARIA
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FOLIA VETERINARIA, 49, 1: 32—35, 2005

CHANGES OF THE IMMUNOLOGICAL AND HAEMATOLOGICAL PARAMETERS IN RABBITS AFTER THE APPLICATION OF BENDIOCARBAMATE

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ABSTRACT

The effect of bendiocarbamate application (5 mg. kg^{-1} b.w.) on the haematological and immunological parameters in rabbits was evaluated. The total leukocyte cell count, erythrocyte cell count, differential cell count were determined over a period of three months of bendiocarbamate application and compared with those in healthy animals. The immunotoxic effect was evaluated by a test of the ingestion ability of phagocytes (phagocytic activity and index of phagocytic activity) and the proliferation activity of lymphocytes after mitogen stimulation. A significant decrease of total leukocytes, lymphocytosis and neutropenia were found after bendiocarbamate application. The functional activities of phagocytes (expressed as phagocytic activity) and lymphocytes (proliferative activity) were significantly suppressed in rabbits treated with bendiocarbamate compared with those in the control groups and the values before the experiment.

Key words: bendiocarbamate; blastogenesis of lymphocytes; phagocytosis; rabbits

INTRODUCTION

Animals' immune system reacts to various exogenous stimuli through the changes in reactions in their immune subsystems. The optimal functioning of the immune system and immunoregulatory balance are dependent on mutual interference among immune reactions. A decrease of the reactions of the immune system following influence of exogenous factors results in immunosuppression, i.e. permanent or temporary immune dysfunction leading to the increased susceptibility of organisms to pathogenic agents.

Pesticides belong to the substances that can cause significant modulation of the immune mechanisms in man and in animals (5). The immune system reacts more sensitively than other systems to the chemicals even in very low concentrations (15). Bendiocarbamate is a carbamate insecticide that has an effect as a reversible cholinesterase inhibitor. Besides its beneficial effect in insect control and consequent reduction of the spread of infectious diseases transmitted by vectors, bendiocarbamate can affect some biochemical (9, 17) and immunological parameters (4), reproduction (1) in man and animals. In the clinical picture of the intoxication of rabbits with bendiocarbamate strong diarrhoea, vomitus, expressive dehydratation, decrease of body weight, alopecia appeared (16).

In this study we present the effect of long-term bendiocarbamate application on the immune system and basic haematological parameters in rabbits.

MATERIAL AND METHODS

Animals. 35 experimental rabbits, hybrid Hyla, aged 50 days from a private owner, kept in standard cages, fed by a complete feed mixture (BIOFER, reg. No. 7313/A). Eleven rabbits, hybrid Hyla, aged 50 days from a private owner served

as the control group. The control animals were kept and fed in the same way as the experimental group.

Bendiocarbamate application. Bendiocarbamate was applied to the experimental animals at a dosage of 5 kg^{-1} b. w. *per os* for the first 13 days daily, later the interval of application was prolonged to every second day until the end of the experiment.

Blood collection. Blood was taken by heart puncture to the tubes containing heparin. Sampling 0: – before the first bendiocarbamate application, sampling 1: – day 4 after bendiocarbamate application, sampling 2—4: – in the interval of 10 days, sampling 5—6: – at an interval of one month.

Parameters evaluated. In experimental and control animals the following haematological parameters were examined: total leukocyte and erythrocyte counts using common haematological methods, differential cell count from 200 cells from a blood smear stained using May-Grünwald, Giemsa-Romanowski staining.

The evaluation of parameters of cellular immunity: *Blastogenic response of blood lymphocytes to mitogens.* Lymphocytes were separated from venous blood on the Ficoll density gradient (Pharmacia Biotech Ab, Sweden). The viability of the isolated cells was determined by trypan blue exclusion and exceeded 97 %. Most (> 95 %) isolated cells were mononuclear cells. The cultivation (culture medium contained 10 % of autologous serum), mitogen stimulation and the measurement of blastogenic response of lymphocytes by the fluorescence method were performed according to N a k a n i s h i *et al.* (12). Concanavalin A (Con A, Sigma Chemical Co., USA) was used for stimulation in the concentration 25 $mg.ml^{-1.}$ The level of the blastogenic response of the lymphocytes was expressed as the stimulation index (SI). The SI was calculated according to the formula SI = (a – c)/(b – c), a = mean FI (fluorescence intensity) with mitogen, b = mean FI without mitogen, c = background FI. The FI was measured by a spectrofluorometer (Jasco fp-550, Japan).

The phagocytic activity of blood leukocytes was examined as described by V ě t v i č k a *et al.* (18). 0.1 ml of fresh heparinized blood (5 u of heparin.1 ml^{-1} of blood) was mixed with 0.05 ml of 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 mm, Artim Prague, Czech Republic) and incubated for one hour at 37 °C with occasional shaking. The phagocytic activity (PA) of leukocytes (Le) was expressed as the percentage of the leukocytes phagocytizing 3 or 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 the number of potentially phagocytizing Le).

Statistical analysis. Results were expressed as a mean and standard deviation. The significance of differences was evaluated by the Student *t*-test.

RESULTS

The results of the immunological and haematological examination in rabbits after bendiocarbamate application and in the control animals are shown in Table 1. In haematological examination, a decrease of total leukocyte count that was significantly different from control group in samples 1, 3—6, a significant increase of the percentage ratio of lymphocyte and related decrease of the neutrophil percentage in comparison to the control group as well as sample 0 (before bendiocarbamate application) were found Significant changes were confirmed in samples 3 and 5 in eosinophils proportion comparing with control and sample 0. Total erythrocyte count was not markedly influenced by bendiocarbamate application.

The functional status of the immune system cells had been altered already by the first sample after bendiocarbamate application. The most significant changes were found in phagocytic activity and the index of phagocytic activity of leukocytes that was significantly lower in the experimental group and until the end of the experiment remained at a lower level in comparison to sample 0 and the control group. The ability of rabbit lymphocytes to react to mitogen by proliferation expressed as stimulation index (SI) significantly decreased after bendiocarbamate application in sample 2 and these

Table 1 . Some haematological and immunological parameters in rabbits after bendiocarbamate application

Sampling	Lc x 10^3	Ec x 10^6	Ly %	Mo %	Ne %	Eo %	PALe %	IPALe	SI
0	4.57 ± 1.04	5.31 ± 0.59	69.25 ± 6.6	1.01 ± 1.78	23.25 ± 3.3	2.75 ± 4.32	29.0 ± 17.46	6.1 ± 1.41	1.8 ± 0.4
1	3.11 ± 0.81*	5.86 ± 0.66	89.17 ± 3.6*a	2.50 ± 1.26	7.33 ± 3.7*a	1.01 ± 1.15*a	6.0 ± 4.04*a	4.4 ± 2.69*a	1.2 ± 0.4
2	3.51 ± 2.85	5.25 ± 0.51	80.15 ± 8.2*a	0.57 ± 0.73*	14.43 ± 7.46*a	4.86 ± 2.80	15.6 ± 6.93*a	5.8 ± 2.49	1.1 ± 0.2*a
3	3.22 ± 2.27	5.73 ± 0.65	77.83 ± 6.7*a	1.67 ± 1.49	11.83 ± 4.26*a	8.67 ± 5.56*a	15.5 ± 4.07*a	4.4 ± 2.24*a	0.9 ± 0.3*a
4	3.91 ± 0.55*	5.08 ± 0.73	81.20 ± 2.99	2.6 ± 1.74	13.0 ± 3.29*a	3.21 ± 2.0	13.4 ± 2.73*a	4.7 ± 1.33*a	0.9 ± 0.2*a
5	3.34 ± 1.27*	4.62 ± 0.30	80.41 ± 1.85*a	2.6 ± 0.8	9.8 ± 4.21*a	7.20 ± 3.87*a	11.4 ± 5.64*a	4.1 ± 2.74*a	1.0 ± 0.3*a
6	3.48 ± 0.66*	6.62 ± 0.33	82.67 ± 3.40*a	2.3 ± 1.89	8.33 ± 3.21*a	6.67 ± 3.68	8.83 ± 3.58*a	4.6 ± 3.06*a	0.8 ± 0.1*a
Control	6.2 ± 1,74	5.52 ± 0.54	73.0 ± 3.34	1.86 ± 0.64	22.86 ± 3.98	2.29 ± 1.75	23.43 ± 14.76	9.27 ± 1.76	1.9 ± 0.2

Legend: Lc — total leukocytes, Ec — erythrocytes, Ly — lymphocytes, Mo — monocytes, Ne — neutrophils, Eo — eosinophils, PA — phagocytic activity, PI — phagocytic index, SI — stimulation index
* — $P < 0.001$ (*versus* control), a — $P < 0.001$ (*versus* sampling 0)

altered values persisted until the end of our observation comparing value at the beginning of the experiment (sample 0) and the control group.

DISCUSSION

The optimal functional status of the immune system is the decisive factor in limiting the susceptibility of an organism to pathogenic agents. Any alteration of particular immune components has serious consequences for health conditions in the affected individual. Pesticides belong to the substances that can interfere with normal functions of the immune cells, negatively influencing humoral and cellular immunity (2) and the incidence of tumors (10).

Phagocytosis is the first defence line against foreign infectious factors. Its alteration results in a higher incidence of infections, occurrence of the diseases caused by opportunistic pathogens, presence of recurrent diseases resistant to therapy. The immunosuppressive effects of pesticides were already confirmed *in vitro* condition in dichlofluanide, simazine and triallate on the metabolic activity of phagocytes (14). Cypermethrin and endosulphane application in chicken showed an immunotoxic effect on macrophage functions (7). Similarly, the inhibitory effect of endosulphan on blood phagocyte function in sheep has been presented by P i s t l *et al.* (13).

Our findings are in an agreement with the currently published results and also confirm the significant immunosuppressive effect of bendiocarbamate on the functional status of blood phagocytes. Proliferation ability evaluation after mitogen stimulation is a suitable method for the determination of functional status of this cell population (3). Carbamate pesticides including bendiocarbamate are capable of destructive effect on lymphocyte functions, which was unequivocally confirmed in the evaluation of the negative effect of ziram and maneb on cytotoxic NK cells functions (18).

Intoxication by the herbicide bentazon in sheep resulted in a decrease of lymphocyte reactivity to mitogen activation, but did not influence the functions of phagocytes (11). The alteration of the proliferative activity of lymphocytes after stimulation with mitogen Con A, that was confirmed in our observation is in agreement with the results mentioned above. On the other hand atrazine exposition in mice did not significantly influence lymphocyte stimulation by mitogens (6). Our results can confirm the suppressive effect of long-term bendiocarbamate application on the functional status of lymphocytes and phagocytes in rabbits *in vivo* conditions.

Many authors have presented the different susceptibility of phagocytes and lymphocytes to various pesticides dependent on the different chemical structure, and mechanism of their effect. Similarly dose and time-dependence of response was reported (7).

According to the results presented in this study we can conclude that long-term bendiocarbamate application in rabbits at a dosage of 5 kg^{-1} b. w. resulted in the significant suppression of the functional activity of phagocytes and lymphocytes, leukopenia, neutropenia and lymphocytosis.

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Received January 31, 2005

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 49, 1: 36—39, 2005

THE SUSCEPTIBILITY OF *Campylobacter jejuni* STRAINS TO SELECTED ANTIMICROBIAL AGENTS

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ABSTRACT

Campylobacteriosis has recently appeared in significant- and widespread gastroenteritic diseases. During 2003 and 2004, 121 *Campylobacter jejuni* strains isolated from poultry and 85 strains isolated from human patients were assessed in our laboratory. These strains were tested for their susceptibility to tetracycline, nalidixic acid, chloramphenicol, erythromycin and ciprofloxacin by agar dilution. The greatest number of strains were resistant to ciprofloxacin and nalidixic acid. 12 % of the strains isolated from poultry were resistant to tetracycline, 31 % to nalidixic acid, 0 % to chloramphenicol, 1 % to erythromycin and 59 % to ciprofloxacin. 8 % of the strains isolated from humans were resistant to tetracycline, 29 % to nalidixic acid, 1 % to chloramphenicol, 7 % to erythromycin and 69 % to ciprofloxacin. Multiresistance was predominantly represented by the combination of ciprofloxacin and nalidixic acid in strains isolated both from poultry and from humans. It can be concluded from these results that the incidence rate of resistant strains in poultry is similar to that in humans, which could indicate campylobacteriosis is transmitted to humans. The results correspond with the situation in antimicrobial resistance in surrounding countries.

Key words: antimicrobial agents; *Campylobacter jejuni;* humans; poultry; resistance

INTRODUCTION

Campylobacteriosis together with salmonellosis have appeared as the most frequent cause of gastroenteritic diseases in the Czech Republic for the last few years.

The incidence rate of campylobacteriosis was about 200 cases per 100,000 inhabitants in 2003, this has recently been considered constant. The most commonly reported *Campylobacter* infections are in children between one and four years old. In the course of a normal year, the typical graph of the occurrence of campylobacteriosis has a distinctive peak in August. Animal products, mainly poultry and raw milk, have been indicated to be a primary source of infection (11). *Campylobacter jejuni* has been the main etiologic agent.

The increasing resistance of *Campylobacter jejuni* as an etiologic agent to selected types of antibiotics has been observed in connection with the widespread use of antimicrobial agents both in animal husbandry and human treatment. The resistant strains can cause problems in the treatment of campylobacteriosis. In the Czech Republic, no research team has observed antimicrobial resistance in *C. jejuni* systematically. Special regulations, where requirements for the monitoring of antiobiotic resistance are given, specify conditions and methods of monitoring.

Owing to the epidemiological importance of the agent and in view of the fact that the resistance has been observed not only in Europe, a methodology for resistance assessment has been introduced to the research establishment and since 2002 antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* has been monitored. This report gives an assessment of

the antibiotic resistance of *Campylobacter jejuni* strains isolated from poultry and human patients.

MATERIALS AND METHODS

121 *Campylobacter jejuni* strains isolated from poultry and 85 strains from human patients were assessed during 2003 and 2004. Poultry samples were obtained at slaughterhouses and in stores and shops by swabs from the surface of poultry. Human strains were isolated from patients suffering from enteritis in the course of nation-wide monitoring and were tested in cooperation with SZU Prague.

Examinations were carried out by cultivation methods and biochemical tests according to CSN ISO 102 72. Specific identification and the differentiation of thermophilic *Campylobacter* by PCR/RFLP (4) were carried out. A 491 bp long PCR product was obtained by amplification of the variable parts of the 23S rRNA gene. *Campylobacter jejuni* was identified by digestion with *AluI* endonucleases.

The agar dilution method was used in testing the susceptibility of *Campylobacter jejuni* (*C. jejuni*) strains to selected antimicrobial agents. This method is based on an evaluation of the lowest antibiotic concentration that inhibits the growth of the examined germs (MIC).

According to the NCCLS M31-A2 method (10), the MIC value for tetracycline ≥ 4 μg.ml^{-1}, nalidixic acid ≥ 32 μg.ml^{-1}, chloramphenicol ≥ 32 μg.ml^{-1}, erythromycin ≥ 8 μg.ml^{-1} and ciprofloxacin ≥ 1 μg.ml^{-1}. The Mueller-Hinton agar (Mueller-Hinton agar; Oxoid, England) supplemented with 5 % sheep blood (Sheep blood; Bioveta, Czech Republic) of pH 7.2—7.4, in a concentration of Ca ions 20—25 mg.l^{-1} and Mg ions 10—15 mg.l^{-1} was used.

The prepared medium was on Petri dishes mixed with solutions of antimicrobial agents of determined concentration. The five following antimicrobial agents were chosen: tetracycline (Tetracycline hydrochloride; Sigma-Aldrich, Germany), nalidixic acid (Nalidixic acid sodium; Sigma-Aldrich, Germany), chloramphenicol (Chloramphenicol; Sigma-Aldrich, Germany), erythromycin (Erythromycin; Sigma-Aldrich, Germany) and ciprofloxacin (Ciprinol 100; Krka, Slovenia). The antibiotics were diluted to a final concentration 128; 64; 32; 16; 8; 4; 2; 1; 0.5; 0.25 mg.l^{-1}, ciprofloxacin to concentration 32; 16; 8; 4; 2; 1; 0.5; 0.25; 0.125; 0.063 mg.l^{-1}. The Petri dishes prepared in this way were inoculated with 2 μl bacterial suspension.

Inoculum was prepared from a 48-hour culture and the suspension with a final cell number 10^{4-5}.ml^{-1} was prepared by measurements of turbidity by McFarland. Incubation was carried out at 38 °C in an anaerostat in atmosphere containing 5 % O_2 and 10 % CO_2 for 48 hours. The collection strain of *Campylobacter jejuni jejuni* ATCC 33560 was used as a control.

The assessment of the susceptibility by NCCLS M31-A2 (10) followed the incubation of the samples. According to the recorded values, the strains were divided into susceptible (S) and resistant (R) strains.

RESULTS

The samples were collected and tested from January 2003 to the end of July 2004. 121 *Campylobacter jejuni* strains isolated from poultry and 85 from humans were tested during the given time. Table 1 shows the results of the assessment of the resistance in *C. jejuni* strains isolated from poultry and humans. In the strains isolated from poultry, the highest resistance was recorded for ciprofloxacin – 59 % (71) tested strains were resistant. The second highest resistance was confirmed for nalidixic acid – 31 % (38) resistant strains were recorded. 12 and 10 % (15 and 12) strains showed resistance to tetracycline and erythromycin. No *C. jejuni* strain isolated from poultry was resistant to chloramphenicol.

Table 1. Resistance of *C. jejuni* strains isolated from poultry and humans to selected antibiotics

Antimicrobial agent	Strains isolated from poultry (%)	Strains isolated from humans
Tetracycline	15 (12)	7 (8)
Nalidixic acid	38 (31)	25 (29)
Chloramphenicol	0 (0)	1 (1)
Erythromycin	12 (10)	6 (7)
Ciprofloxacin	71 (59)	59 (69)

In the strains isolated from humans, there was a similar situation. The highest number of tested *C. jejuni* strains (69 % – 59) was resistant to ciprofloxacin. In 29 % (25) of the strains, resistance to nalidixic acid was found. Only 8 % (7) of the tested strains isolated from humans were resistant to tetracycline and 29 % (25) to erythromycin. Resistance to chloramphenicol was confirmed in one strain (1 %).

Comparing the resistance in strains isolated from poultry and from humans, both poultry and human isolates of *C. jejuni* were resistant mostly to ciprofloxacin. In human isolates, resistance is higher than in poultry isolates. About one third of the strains isolated from poultry and humans were resistant to nalidixic acid. On the other hand, strains isolated from poultry showed a slightly higher resistance to tetracycline (12 %) and erythromycin (10 %) compared to human isolates (8 % to tetracycline and 7 % to erythromycin). Unlike poultry isolates, we confirmed one *C. jejuni* strain from humans, which was resistant to chloramphenicol.

In addition, multi-resistance to the given antimicrobial agents was observed in the tested strains. Due to the high number of strains resistant to ciprofloxacin and nilidixic acid, the highest multi-resistance in *C. jejuni* strains isolated both from poultry and humans was recorded in this combination of antimicrobial agents: 24 (20 %) strains isolated from poultry and 19 (22 %) from humans. None of the poultry or human isolates had any

significantly occurring multi-resistance. Table 2 gives the complete results of the multi-resistant strains.

Table 2. Multi-resistance of *C. jejuni* strains isolated from poultry and humans to chosen antibiotics

Multi-resistance to:	Strains isolated from poultry	Strains isolated from humans
NAL/CIP	24	19
NAL/ERY	0	1
ERY/CIP	1	2
TET/CIP	0	4
TET/NAL	2	0
TET/ERY	2	0
TET/ERY/CIP	1	1
NAL/ERY/CIP	1	1
TET/NAL/ERY/CIP	4	0
TET/NAL/CIP	4	2

NAL — nalidixic acid, TET — tetracycline, ERY — erythromycin, CIP — ciprofloxacin

DISCUSSION

The use of antibiotics in human medicine and in veterinary practice is commonly widespread. About one half of the antimicrobial agents produced annually in the world are estimated to be consumed for veterinary purposes. Antibiotics have been used for pet treatment; they have been given to livestock and to animals reared in aquacultures. Antibiotics have not been used only for treatment, but also for sub-therapeutic purposes, mainly in livestock and aquacultures (12).

The frequent use of antibiotics, mainly in low doses, results in the resistance of pathogenic organisms. This situation is generally known, so the administration of antimicrobial agents to livestock was significantly reduced, in many cases was totally forbidden in individual European countries. However, misuse of antibiotic administration to food animals cannot be excluded as well as poor observance of the protective terms in using antimicrobial agents for treatment purposes. That is why the EU has established a requirement to observe particular zoonosis and to monitor resistance to antimicrobial agents in member states. Our laboratory has been dealing with the evaluation of microbial resistance in *Campylobacter* spp. since 2002.

The high resistance to quinolonic antibiotics, ciprofloxacin and nalidixic acid, has been observed both in strains isolated from poultry and from humans. More than one half of the strains were resistant to ciprofloxacin – in poultry 59 % and in humans 69 %. This is consistent with the situation in Europe, where resistance in poultry isolates was found to be 53 in Italy (11) and 55 % in Germany (1). German authors have described a similar situation in human isolates with 45 % of *C. jejuni* strains resistant to ciprofloxacin (8). In Switzerland (5) and Ireland (9), low numbers of resistant strains in poultry (1.6 % and 3 %) have been recorded.

About 30 % of the strains have been found to be resistant to nalidixic acid. This value is comparable with the situation in Ireland (3) – 21 % of resistant strains in poultry isolates.

Other authors have recorded higher values. Simply because of increasing resistance to nalidixic acid, its use in treatment has been gradually stopped. The high rate of *C. jejuni* strains resistant to nalidixic acid causes difficulties in the identification of isolated strains. A test for the resistance to nalidixic acid has been one of methods for differentiation *C. jejuni* and *C. coli*. When the number of resistant strains is higher, species identification of *Campylobacter* spp. strains can be incorrect. Standardization of strains by DNA analysis can solve this situation.

In strains isolated from poultry and humans, we determined 12 % and 8 % resistance to tetracycline, which is a significantly lower value than that determined by other authors. 10 % of the strains isolated from poultry showed resistance to erythromycin, which corresponds to the values published in Italy (11) – 12.5 %, and in Ireland (3) – 10 %. In contrast, another study from Ireland (9) confirms resistance to erythromycine only in 4 % of the strains isolated from poultry.

Only one strain isolated from humans was resistant to chloramphenicol, all strains isolated from poultry were susceptible to chloramphenicol. Similarly, none of the cited authors has observed chloramphenicol resistance.

Antibiotic resistance in the Czech Republic can be considered to be similar to the situation in the surrounding countries. It is very interesting that ciprofloxacin resistance has been observed more frequently as compared to nalidixic acid resistance, even though ciprofloxacin as a derivative of nalidixic acid was introduced due to its stronger antimicrobial effect. A different antibiotic policy connected with political and economic changes in the country could be one of the possible explanations.

The observed rate of tetracycline and erythromycin resistance was significantly lower compared with other authors. This can be explained by a different pattern of antimicrobial use in the past, when antimicrobials were administered in significantly lower doses in the Czech Republic than in the surrounding countries. Despite restriction on the use of antimicrobial agents in Europe, their administration to food animals not only in treatment but also in prophylaxis is still allowed in the USA.

CONCLUSION

The aim of this study was to discover the situation with regard to antibiotic resistance of *C. jejuni* isolated from poultry and from humans in the Czech Republic in 2003 and 2004 and to compare the results with the situation in other countries. Strains isolated both from poultry

and from humans showed the highest resistance to ciprofloxacin – 59 %, resp. 69 %. About 30 % of the strains isolated from poultry and from humans were resistant to nalidixic acid. Except for the study from Ireland (3), the other authors show higher values. The situation in antibiotic resistance to tetracycline and erythromycin in the Czech Republic was evaluated as favourable.

Based on these results, we can state that antibiotic resistance of *C. jejuni* isolated from poultry and from humans has been developing in a similar way in our country to the other countries monitored, with lower resistance to some antibiotics observed. The high resistance to ciprofloxacin is alarming. With regard to ever an increasing epidemiological significance as well as significance for hygiene and the harmless use of meat and meat products, the monitoring of the situation with regard to antibiotic resistance by *Campylobacter jejuni* strains should continue.

ACKNOWLEDGEMENT

The project was supported by the NAZVA QD1192 and QF3085 grants.

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Received October 11, 2004

FOLIA VETERINARIA
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FOLIA VETERINARIA, 49, 1: 40—44, 2005

THE EFFECT OF CADMIUM ON FATTY ACID ABSORPTION ACROSS THE RUMEN EPITHELIUM IN SHEEP

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ABSTRACT

The effect of cadmium as one of the heavy metals on acetate and propionate absorption across the rumen epithelium was investigated. In the experiment rumen walls obtained from adult Merino sheep between two and four years old were used. Acetate and propionate absorption was observed on an apparatus constructed in our laboratory. Determination of the acids was carried out by gas chromatography (Perkin-Elmer 8500). Absorption of acetate (50 mmol.l^{-1} of Thyrode's solution) across the rumen epithelium when used separately without the addition of cadmium was higher than in combination with propionate (50 + 15 mmol.l^{-1} of Thyrode's solution). The addition of five milligrams of cadmium to separately applied acetate into the vessels with this solution (50 ml) resulted in a significant decrease in its absorption rate. However the addition of twenty and fifty milligrams of cadmium caused a marked increase in its absorption across the rumen epithelium. All three concentrations of cadmium added to combined acetate and propionate in Thyrode's solution caused increased acetate absorption. A different effect of cadmium was seen with propionate (15 mmol.l^{-1} of Thyrode's solution) absorption across the rumen epithelium. However addition of all three doses of cadmium decreased propionate absorption across the rumen epithelium both in a separate and an acetate combined application. It can be stated that cadmium inhibited the absorption rate of both acids across the rumen epithelium of sheep.

Key words: absorption; acetate; cadmium; propionate; rumen epithelium

INTRODUCTION

Acetic and propionic acid belong to the short-chain fatty acids (SCFA) which are a usual part of the rumen content. They are produced during the fermentation of plant materials in the forestomach of ruminants. In the rumen epithelium they are metabolized or absorbed into the blood stream. Acetic acid is utilized as a source of energy, propionic acid is the only short-chain fatty acid which is glucogenic. It is quantitatively the most important precursor of glucose (8, 9, 10). Practically all propionic acid is utilized in the liver and does not reach the peripheral blood stream (13).

Cadmium is a heavy metal and often contaminates the environment from where it enters the animal organism in food. Although it is an essential micro-element for organisms it readily reacts with a number of active molecules of proteins, phospholipids, purines, nucleic acids and enzymes changing their activity which is the cause of its toxic effect (12, 16, 11). The toxic effect of cadmium is related to the amount supplied to the organism (6, 19, 14). The aim of this experiment was to investigate the effect of cadmium on acetate and propionate absorption across the rumen epithelium.

MATERIAL AND METHODS

In the experiment the rumen walls of eight adult Merino sheep between two and four years old were used. The animals were individually housed in sheds and given a diet consisting of meadow hay *ad libitum* and 200 grams of ground barley per animal and day. The animals had free access to water and lick salt. Immediately after slaughter and bleeding the entire digestive tract was removed from the abdominal cavity and brought to the laboratory. There the rumen was separated, its content removed and washed with lukewarm water. Sac slices were cut from the dorsal ruminal, its mucosa separated from the muscular layer and transferred into a glass vessel containig saline solution.

Acetate and propionate absorption was observed on an apparatus that had been constructed in our laboratory (Fig. 1). It consisted of a large round glass vessel with a diameter of 45 centimetres. On the bottom there was a stand with several round openings through which water penetrated to reach the required level of the water bath. The stand served to hold four identical glass vessels with a diameter of 10 centimetres. The latter were filled with 150 ml of Thyrode's solution with pH 7.4 (serous side). This solution matched the blood stream, i.e. the environment into which the fatty acids are absorbed from the rumen in the mucous-serous direction.

Into each of these vessels Janette's syringes, fastened to the laboratory stands, were directed and adjusted. After having removed the plug an opening of an area of 13.2 cm^2 arose on the syringe which was covered with a slice of the rumen mucosa. The individual mucosal slices were bandaged with a surgical silk so that the mucous side was directed into the syringe and the serous side into the vessel containing Thyrode's solution. The original opening at the other end of Janette's syringe was used to insert of a funnel through which the syringe was filled with 50 ml of Thyrode's solution containing the combined fatty acids (acetate in amount 50 $mmol.l^{-1}$ and propionate in amount 15 $mmol.l^{-1}$ of Thyrode's solution) with pH 6.9 (mucous side) and with the cadmium in amounts of 5, 20 and 50 mg as $CdSO_4$. Each combination was tested six times (n = 6).

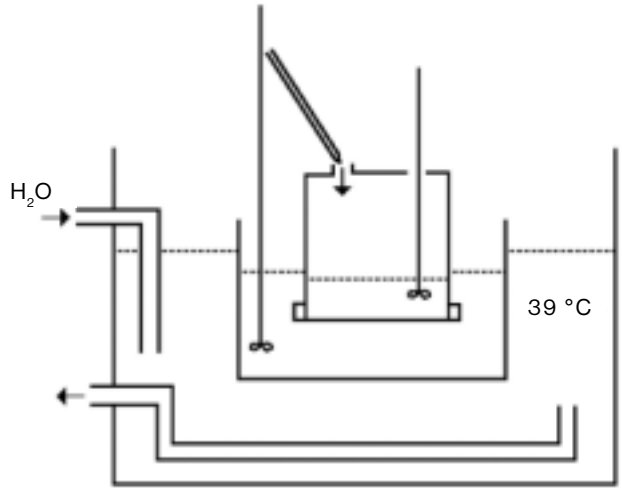


Fig. 1. The device for measuring of acetate and propionate absorption across the rumen epithelium

The solutions in the vessels and syringes were stirred by a thin stainless blender connected to 1.5 V small motors and bubbled through with oxygen containing 5 % CO_2. The water in the bath was continuously stirred throughout the experiment by means of a through-flow thermostat maintained a constant temperature of 39 °C. After one hour the experiment was finished and 4 ml samples were taken from the individual vessels and syringes into plugged test-tubes and after the addition of a 1ml standard solution of crotonic acid the fatty acids were determined by gas chromatography (Perkin-Elmer 8500).

Statistical analysis: The means of the individual parameters were compared using the Tukey-Kramer multiple comparison test (GraphPad Instat Software, Inc., San Diego, USA).

The differences from the means marked in the graphs indicate standard error (S.E.).

RESULTS

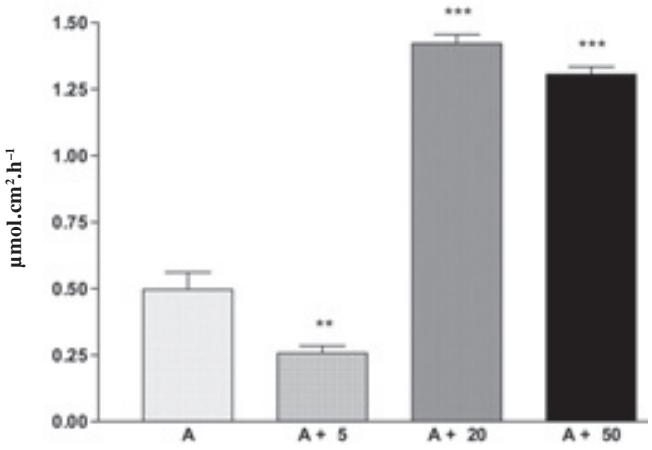
Comparing acetate (50 $mmol.l^{-1}$ of Thyrode's solution) absorption across the rumen epithelium applied alone or combined with propionate (15 $mmol.l^{-1}$ of Thyrode's solution) a higher absorption for the separate form was indicated (Figs. 2 and 3). The addition of five milligrams of cadmium resulted in a significantly higher acetate absorption in where it was combineed with propionate compared to acetate alone. There were no differences in acetate absorption between both acetate applied forms after the addition of 20 and 50 mg of Cd into the Thyrode's solution (50 ml).

The effect of Cd on the absorption of acetate had a significant affect on both forms of acetate. All doses of cadmium increased acetate absorption apart from the addition of five milligrams of Cd in the case of alone acetate when absorption temporarily decreased.

The different affect of cadmium on propionate absorption through the rumen epithelium was observed. The comparisons of cadmium influence on the absorption of propionate applied alone and combined with acetate are presented in Figures 4 and 5. It follows that cadmium decreased propionate absorption across the rumen epithelium in both applied forms of propionate alone and combinated with acetate. The depression of absorption was significant in all cadmium concentrations used. The tendency of propionate absorption when it was applied alone was to increase with an increased amount of Cd and where there was a combined application of propionate with acetate it fell.

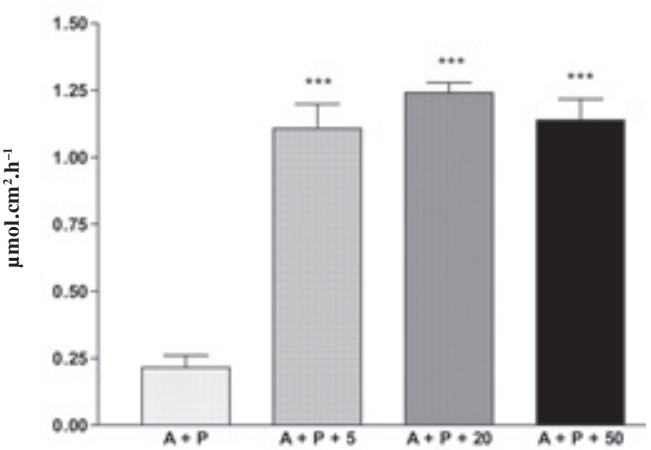
DISCUSSION

Cadmium belongs to the group of heavy metals the effect of which on living organisms in higher doses is unfavourable. The industrial utilization of components containing cadmium has in the recent years gianthered pace. The mobilization and speed of transport of this



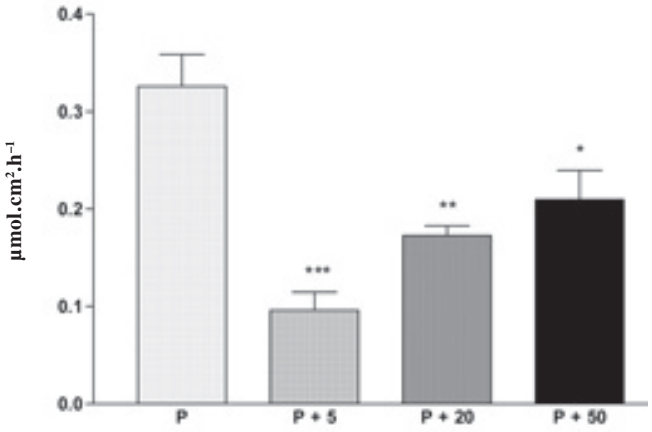
— P < 0.01; *— P < 0.001; A — acetate
5, 20, 50 — cadmium (mg)

Fig. 2. Acetate absorption across the rumen epithelium when separately administered after the addition of cadmium



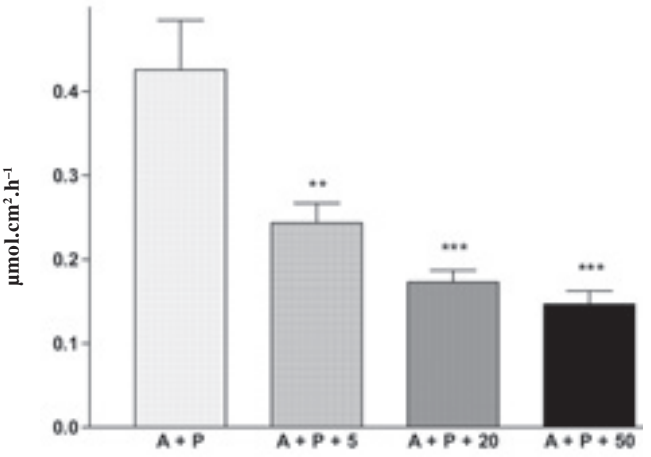
***— P < 0.001; A — acetate; P — propionate 5,
20, 50 — cadmium (mg)

Fig.3. Acetate absorption across the rumen epithelium administered in combined form (A + P) after the addition of cadmium



*— P > 0.05; **— P < 0.01; ***— P < 0.001
P — propionate; 5, 20, 50 — cadmium (mg)

Fig. 4. Propionate absorption across the rumen epithelium when separately administered after the addition of cadmium



— P < 0.01; *— P < 0.001; A — acetate
P — propionate; 5, 20, 50 — cadmium (mg)

Fig. 5. Propionate absorption across the rumen epithelium in combined form (A + P) after the addition of cadmium

component highly exceed the possibilities of its natural recycling which results in an increased rate of cadmium deposit in living organisms (5). This is also the reason for the effect of cadmium being given great attention in the investigation of its effect on living organisms at the present time (7, 4, 17, 1, 2, 20).

Ruminants can be exposed to the effect of heavy metals by the consumption of contaminated food and water. The digestive tract of animals, but also other organs, are thus subjected to the direct effect of this group of xenobiotics. Since cadmium is an essential micro-element decreased milk production in ruminants and retarded growth of young, muscular weakness, decreased motility and death have been observed after an insufficient supply of cadmium. Increased cadmium supply up to 0.3 mg.kg^{-1} dry matter eliminated these symptoms (3). In the case of cadmium supplementation is possible, but its negative effect from a higher supply to the organism is of greater importance. The effect of cadmium on the microbial population and SCFA production in the gastrointestinal tract of ruminants has been considered in some papers (15, 18).

These facts led us to the idea of examining the manifestations of graded cadmium doses on the permeability rate of the mucous membrane in the rumen with the two most important short-chain fatty acids in the digestive tract of ruminants i.e. acetic and propionic acid.

In our experiments in observing the acetate absorption rate across the rumen epithelium an interesting finding was recorded. When separate acetate (50 mmol.l^{-1} of Thyrode's solution) only was applied its absorption rate across the membrane was significantly higher as compared to an application of the same amount of acetate but in combination with propionate (15 mmol.l^{-1} of Thyrode's solution). However, no support has been found

in so far known experimental work described in the literature, but we do not assume that the presence of propionate in the solution applied could decrease acetate absorption to such a great extent. We rather endorse the opinion that this phenomenon could have been caused by some other factors which, with the technique of observation of SCFA absorption, we could not take into account.

As stated in the results the acetate absorption rate across the epithelium was lower when acetate was applied together with propionate than it was after the separate application of the latter. The addition of five milligrams of Cd to the Thyrode's solution with acetate slightly decreased the transport of acetate across the ruminal mucosa but the absorption rate showed a marked increase after A + P application. When higher doses of cadmium were added (20 and 50 mg) in this solution the increased acetate absorption rate remained at approximately the same level. It can be stated that cadmium at the concentration used had a strongly depressive effect on the acetate absorption rate across the rumen epithelium of sheep. It is difficult to unambiguously explain the reasons for this finding.

One of the possible explanations of this phenomenon could be the fact that cadmium readily enters into interactions with proteins and phospholipids, which are important components of the cell membranes. In this way destruction of the epithelial components with a subsequent increase in the transport of not only fatty acids but also other components might occur.

Propionate absorption across the rumen epithelium was similar to that found with acetate differing only very slightly when applied separately or in combination with acetate. With the individual concentrations of cadmium a significant decrease of the propionate absorption rate was observed both after its separate or combined application. It can thus be stated that while the acetate absorption rate across the rumen epithelium was markedly increased by cadmium the absorption rate of propionate was decreased. This finding could perhaps be explained also by the different acetate and propionate metabolism in the wall of the gastrointestinal tract.

It is assumed that the luminal and basolateral epithelial surfaces differ as to the permeability of both forms of SCFA, and that the transport of SCFA can be explained by maintaining the electrochemical gradient for the hydrogenous ions between the epithelial cell content and the solutions on both sides of the membranes. SCFA transport from the rumen into the blood can therefore be explained not only by the diffusion, but also by the fact that the process in question is affected by the pH of the lumen and both the cell content and the intercellular SCFA metabolism.

The assumed destruction of the rumen epithelium, which might be the reason of the increased acetate absorption rate after the addition of cadmium was not manifested in a similar way with propionate. Reversely, a decreased absorption rate of the latter was observed.

As it seems other interactions might also have occurred between the epithelium and propionate i.e. a higher metabolization rate in the epithelium and a larger molecule might have played a role as compared to acetate.

CONCLUSIONS

The acetate absorption rate across the rumen epithelium when applied separately without the addition of cadmium was higher than for a combined application with propionate.

The addition of five milligrams of cadmium into the vessels with 50 ml of Thyrode's solution with acetate alone (50 $mmol.l^{-1}$) manifested itself in a significantly decreased absorption rate. On the other hand, the addition of cadmium at an amount of 20 mg and 50 mg to the same amount of this solution caused a pronounced increase of its absorption across the rumen epithelium.

The addition of cadmium at all three concentrations into the vessels with 50 ml of Thyrode's solution with acetate + propionate (50 + 15 $mmol.l^{-1}$) manifested itself in an increased acetate absorption rate.

The addition of cadmium at all three doses decreased the propionate absorption rate across the rumen epithelium after both separate and combined applications with acetate.

ACKNOWLEDGEMENTS

The study was supported by funds from the Grant Agency for the Ministry of Education (2/8044/21) and the Slovak Academy of Sciences (2/3058/23).

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Received October 20, 2004

FOLIA VETERINARIA
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FOLIA VETERINARIA, 49, 1: 45—47, 2005

DIETARY FISH OIL VERSUS SUNFLOWER OIL LOWERS PLASMA TRIGLYCERIDE CONCENTRATIONS IN HEALTHY DOGS

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ABSTRACT

The question addressed was whether the intake of fish oil would reduce plasma triglyceride concentrations in dogs. Diets containing either fish oil or sunflower oil were fed to six healthy, adult dogs in a parallel trial. Blood samples were collected at the beginning and at the end of the three week feeding period and the fatty acid composition and the concentration of plasma lipids were determined. A significant lowering of plasma concentrations of triglycerides was found after feeding the diet supplemented with fish oil instead of sunflower oil.

Key words: dietary fish oil; dogs; plasma triglycerides; sunflower oil

INTRODUCTION

There is evidence for beneficial health effects of n-3 polyunsaturated fatty acids in humans and animals. Fish oil, which is rich in eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) diminishes clinical signs in dogs with idiopathic pruritis (1). In cats, the ingestion of high amounts of EPA may counteract the progression of chronic renal failure (2). In humans, the intake of n-3 polyunsaturated fatty acids in the form of fish oil may decrease the risk of cardiovascular disease (3) and prostate cancer (4).

In this experiment with dogs, the influence of feeding a diet rich in fish oil on the plasma concentrations of cholesterol and triglycerides was evaluated. In humans, the consumption of fish oil lowers plasma triglycerides (5), but in cats such an effect could not be established (6). In dogs with induced kidney failure, fish oil in the diet has been shown to lower plasma cholesterol and triglycerides (7). As far as we know, no data are available for healthy dogs. Thus, in this study six healthy adult dogs were fed diets containing either sunflower oil or fish oil during a period of three weeks according to a parallel design. The fatty acid composition and concentrations of plasma lipids were measured.

MATERIALS AND METHODS

Dogs and diets

Six adult Groenendaler dogs were used. The dogs were kept as pets and were housed with their owner. There were five females, one of which was ovarihysterectomized, and one male dog. They were aged 7.2 ± 3.9 years (mean ± SD).

Prior to the experiment the dogs were fed a commercial dry feed (Life Time Petfood, Van der Klei Diervoeders, Herveld, The Netherlands) According to the guaranteed analysis panel the composition was as follows: crude protein 26 %; crude fat 15 %, crude ash 7.4 %; crude fibre 2.6 %. During the experiment, the experimental diets were fed and each dog received a slice of brown bread as a treat daily. Four times during the three week's experimental period each dog was given a hardboiled egg. The experimental diets were fed once a day. Left-overs, if any, were given again the next day. For each dog a separate bag with the experimental diet was used. The bags were weighed at the beginning and at the end of the three week trial in order to determine food intake. The dogs were weighed at the beginning and at the end of the experiment. All dogs remained healthy during the experiment.

The experimental diets were based on a commercial dry feed for dogs (Carocroc 26/15, Vobra BV, Loosbroek, The Netherlands). The base diet was in the form of meal and the sprayed fat in the commercial version of this diet was replaced by the test oils (sunflower oil or fish oil). After addition of the oils, the complete diet was pelleted. Fish oil was

used as a source of n-3 polyunsaturated fatty acids and sunflower oil as a source of the n-6 poly-unsaturated fatty acid, linoleic acid (C18:2 n-6). The calculated intrinsic fat content of the diets was 6.9 g.100 g^{-1} and the added amount of variable fat was 12.5 g.100 g^{-1}. The analysed composition of the diets is given in Table 1.

Table 1. Analysed concentrations of macronutrients and fatty acid composition of the experimental diets

Nutrient	Sunflower oil diet	Fish oil diet
Macronutrients (g.100 g^{-1})		
Dry matter	91.6	91.3
Crude protein	24.5	24.6
Crude fibre	2.4	2.4
Crude ash	6.3	6.1
Crude fat	18.1	17.9
Fatty acid (g.100 g^{-1} fatty acids)		
14:0	0.2	2.7
16:0	10.5	14.0
18:0	4.5	3.5
18:1 n-9	31.7	23.7
18:2 n-6	48.0	16.2
18:3 n-6	0.0	0.1
18:3 n-3	0.9	1.9
20:4 n-6	0.2	0.5
20:5 n-3	0.0	4.2
22:5 n-3	0.0	1.9
22:6 n-3	0.0	6.5

Fatty acids are presented in shorthand notation: C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1 n-9, oleic acid; C18:2 n-6, linoleic acid; C18:3 n-3, alpha-linolenic acid C20:4 n-6, arachidonic acid C20:5 n-3, eicosapentaenoic acid; C22:5 n-3, docosapentaenoic acid; C22:6 n-3, docosahexaenoic acid

Blood sampling and chemical analysis

Blood sampling took place at the start and at the end of the three week feeding period. Blood samples of approximately 5 ml were taken by jugular venepuncture into heparinized tubes. The blood was centrifuged as soon as possible and the plasma was harvested and stored at -20 °C for further analysis. Fatty acid analysis was performed by capillary gas-liquid chromatography using a flame ionisation detector, a chromopack column (Fused Silica, No. 7485, CP.FFAPCB 25 m × 0.32 mm, Chromopack, Middelburg, The Netherlands) and H_2 as carrier gas. Plasma lipids were extracted (8) and fatty acid methylesters were produced (9) for analysis. Plasma total cholesterol, total phospholipids and total triglycerides were determined with commercial test combinations (Roche Diagnostics, Almere, The Netherlands) and the Cobas Bio centrifugal analyser (Roche Diagnostics, Basel, Switzerland). Diet samples were analysed for dry matter, crude protein, crude fibre and crude ash according to the Weende method.

Statistical analysis

The values are expressed as means ± SD. Diet effects were evaluated with Student's *t*-test. The means and SD's were calculated in Microsoft Excel.

RESULTS

Food intake and body weight

There were no significant differences in intake of the two experimental diets. Intake of the fish-oil diet was 0.37 ± 0.03 kg.day^{-1} and of the sunflower oil diet it was 0.37 ± 0.01 kg.day^{-1}. At the end of the experimental period an increase in body weight was found in all six dogs. Body weight at the beginning of the experiment was 23.7 ± 2.6 kg for the dogs fed with the fish-oil diet. The mean increase in weight was 0.67 ± 0.29 kg. The initial weight of the dogs fed the diet with sunflower oil was 21.5 ± 1.5 kg. The mean increase in weight was 1.17 ± 0.29 kg.

Composition of plasma lipids

The fatty acid composition of plasma lipids is given in Table 2. Consumption of the diet with fish oil was associated with significantly greater proportions of EPA and DHA in plasma lipids. The intake of fish oil instead of sunflower oil reduced the percentage of arachidonic acid (C20:4 n-6) in plasma lipids significantly. The intake of sunflower oil significantly increased the percentage of linoleic acid.

Concentration of plasma lipids

Table 3 shows that plasma cholesterol concentrations fell significantly ($P < 0.05$; paired Student's *t* test) irrespective of the type of diet. There were changes in plasma concentrations of triglycerides: in the fish-oil group the level had decreased whereas in the sunflower-oil group it had increased. The change in plasma triglycerides

Table 2. Fatty acid composition of plasma total lipids

Fatty acid (g. 100 g^{-1} fatty acids)		*Sunflower oil diet*	*Fish-oil diet*
14:0	Initial	0.3 ± 0.06	0.4 ± 0.07
	Change	-0.1 ± 0.05	0.3 ± 0.08
16:0	Initial	12.6 ± 0.66	13.4 ± 0.99
	Change	-2.4 ± 0.61	-1.6 ± 0.74
18:0	Initial	17.0 ± 1.09	17.8 ± 0.92
	Change	-1.4 ± 1.24	-2.6 ± 1.55
18:1 n-9	Initial	11.3 ± 1.03	11.7 ± 1.04
	Change	-0.2 ± 1.00	-1.4 ± 0.84
18:2 n-6	Initial	24.2 ± 0.74	24.0 ± 2.80
	Change	10.7 ± 1.78	-3.3 ± 2.65*
18:3 n-3	Initial	0.3 ± 0.06	0.4 ± 0.04
	Change	0.1 ± 0.06	0.2 ± 0.07
20:4n-6	Initial	21.4 ± 1.91	20.5 ± 0.62
	Change	-3.7 ± 1.21	-10.2 ± 0.61*
20:5n-3	Initial	0.5 ± 0.03	0.5 ± 0.09
	Change	0.4 ± 0.06	9.6 ± 0.67*
22:5 n-3	Initial	2.2 ± 0.08	2.0 ± 0.45
	Change	-0.9 ± 0.08	1.1 ± 0.45
22:6n-3	Initial	0.9 ± 0.11	0.9 ± 0.16
	Change	-0.2 ± 0.05	6.6 ± 0.28*

Values are means ± SD (n = 3). * — Statistically significant ($P < 0.05$) *versus* the change in the group fed sunflower oil

differed significantly ($P < 0.05$) between the two treatment groups. Phospholipid concentrations were not affected by the type of oil in the diet.

Table 3. Plasma concentrations of cholesterol, triglycerides and phospholipids

Plasma constituent ($mmol.l^{-1}$)		Sunflower oil diet	Fish oil diet
Cholesterol	Initial	8.13 ± 0.38	6.30 ± 1.85
	Change	-2.07 ± 0.36	-1.32 ± 0.41
Triglycerides	Initial	0.66 ± 0.15	0.74 ± 0.05
	Change	0.18 ± 0.09	-0.18 ± 0.12*
Phospholipids	Initial	4.34 ± 0.03	3.99 ± 0.38
	Change	-0.28 ± 0.21	-0.39 ± 0.12

Values are means ± SD (n = 3). * — Statistically significant ($P < 0.05$) *versus* the change in the group fed sunflower oil

DISCUSSION

After consuming the fish-oil diet, which was rich in EPA as well as in DHA, there was considerable incorporation of these fatty acids into the plasma lipids. After consuming the sunflower oil diet, which is rich in linoleic acid, a high content of linoleic acid in plasma lipids was observed. These effects would have been anticipated on the basis of controlled studies (6). Thus, it can be concluded that compliance of the owners was adequate.

The purpose of this study was to find out whether the intake of n-3 polyunsaturated fatty acids in the form of fish oil would reduce the levels of triglycerides, as is well-known in humans. Indeed, the dogs that were fed the diet with fish oil instead of sunflower oil showed a significant decrease in the plasma concentration of triglycerides. This outcome is consistent with a study in which menhaden fish oil was shown to lower plasma concentrations of triglycerides in dogs with 15/16 nephrectomy as a model for patients with renal insufficiency (7).

A considerable decrease in arachidonic acid in plasma lipids was observed after feeding the fish-oil diet. The fish-oil diet contained approximately 2.5 times more arachidonic acid as did the sunflower oil diet (Table 1), but the animals that were fed the latter diet only showed a slight decrease in the arachidonic acid content of plasma lipids. This is not consistent with another study (10) showing no significant changes in the concentration of arachidonic acid in plasma phospholipids. Sunflower oil contains high levels of linoleic acid, which in the body is converted to arachidonic acid whereas fish oil contains high levels of n-3 fatty acids, which inhibit the conversion of linoleic acid into arachidonic acid (6). This could explain why in the present study the fish-oil diet reduced the percentage of arachidonic acid in plasma total lipids.

Feeding the experimental diets produced a decrease in plasma cholesterol concentrations, but this effect cannot be readily explained because the composition of the pre-experimental diet probably differed from the experimental diets with regard to multiple components. In addition, a time effect cannot be excluded. It is likely however, that the diet change had influenced plasma cholesterol concentrations. Plasma cholesterol in dogs can be modulated by diet (11), but the impact on health remains unknown. Likewise, it is not known whether the observed decrease in plasma triglycerides after fish oil consumption has any practical relevance.

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Received July 26, 2004

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FOLIA VETERINARIA, 49, 1: 48—51, 2005

THE EFFECT OF *Bacillus licheniformis* ON HAEMATOLOGICAL PARAMETERS IN CALVES

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ABSTRACT

The aim of the study was to evaluate the effect of *Bacillus licheniformis* administration on haematological parameters, levels of minerals and some metabolic indices in blood of calves. We also compared the phagocytic activity of neutrophils between groups. Eighteen calves, 7—8 days old, were included in the trial and they were divided into three groups. Experimental groups were given 200 ml of *Bacillus licheniformis* culture twice a day for ten days. Calves in the first group (n = 6) got a culture at the concentration of 3.10^8 ml^{-1} and the second experimental group (n = 6) got a culture at the concentration of 3.10^7 ml^{-1}. The control group (n = 6) got only milk. Blood samples were collected on the 0th, 2nd, 5th and 10th day of the experiment.

***Bacillus licheniformis* significantly increased the level of haemoglobin, values of packed cell volume (PCV), number of erythrocytes on day 10 of the trial. The level of total cholesterol in the blood of the experimental groups was steady while cholesterol in the control group increased on day 10, which was expressed significantly compared with the experimental groups. Application of *Bacillus licheniformis* did not influence the index of the phagocytic activity of neutrophils and level of minerals in the blood.**

Key words: *Bacillus licheniformis*; calves; haematological parameters; metabolic indices

INTRODUCTION

In order to reduce the morbidity of calves, the main efforts should be focused on the diarrhoeic syndrome. Diarrhoeic syndrome results not only in direct losses due to death of the animals, but also indirect losses – loss of weight, expensive therapy, and weight gains lower by up to 20 % during reconvalescence. Diseases of the digestive system are the most frequent cause of non-profitability in the rearing of calves and in Slovakia they contribute to total losses by 20–40 %. There are various causes of diarrhoea, the most common of dietary, bacterial, viral, or parasite origin. Among the bacteria of diarrhoea *E. coli* and *Salmonella* spp., between viral *Corona* and *Rota* viruses, and among parasites COCCIDIA and *Cryptosporidium* spp. are the most frequent causes.

In evaluating the occurrence of disease, the percentage of sick animals should be considered. When over 20 % of animals are sick, the period is critical. The critical period in calf diarrhoea is spring from March to May.

In the past, in the prevention of diarrhoea, antibiotic premixes were frequently used. However, because of the required reduction of antibiotic residua in food, pre- and probiotics are more promising. Although there are many results on probiotics and their effect in the prevention of gastrointestinal disorders much less is known about the influence of probiotics on the values of metabolic indices in blood of animals. Our work was aimed at the study of the effects of probiotic culture of *Bacillus licheniformis* in the potential of calves' non-specific immunity, and the examination of selected indices of haematological and mineral profiles.

MATERIAL AND METHODS

The experiments were performed in the II Clinical Department for Internal Diseases of UVM Košice. In the experiments, 18 calves (Holstein x Slovak spotted crossbreds, 7—8 days old) were used, divided into three groups. The first experimental group (n = 6) was given *Bacillus licheniformis* culture at a concentration of 3.10^8 ml^{-1} and the second experimental group (n = 6) was given a culture of *Bacillus licheniformis* at the concentration of $3.10^7.ml^{-1}$ in the milk; the control group (n = 6) was fed only milk.

A lyophilised culture of *Bacillus licheniformis* was prepared at the Department of Biochemistry and Biotechnology of the Agronomic Faculty at SAU Nitra after culturing of bacteria *Bacillus licheniformis*, strain CCM 7/79 from a Czechoslovak collection of microorganisms. The culturing medium consisted of 0.5 % maize starch, % dried milk, 0.2 % yeast autolysate, and wort extract. Culturing was done in fermenting equipment (MBR Sulzer, Switzerland) at 37 °C and initial pH 8.5. After centrifugation of the medium obtained by fermentation, the sediment was lyophilised with 18.10^6 *Bacillus licheniformis* per gram of lyophilisate.

Prior to administration, either 0.1 g or 0.01 g of the lyophilisate was mixed with 1 l of milk and cultured at 37 °C for 10 hours. The final culture, which contained 3.10^{11}, respectively 3.10^{10} bacteria per l of milk, was given to calves in the first experimental group and the second group at the dose of 200 ml twice a day.

Blood samples were collected from jugular vein on 0th, 2nd, 5th, 10th days.

Phagocytic activity (PhA) was estimated with the use of L-hydroxymethyl-metacrylate particles according to T o m a n *et al.* (7). Haematological indices were analysed by automatic cell analyser SERONO 150 Plus (Switzerland). Blood concentrations of mineral elements were analysed by flame absorption spectrophotometry (A Analyst 100, Perkin Elmer, U.S.A.). The level of total cholesterol was analysed by automatic analyser ALIZE (Lisabio, France) with the use of Bio Merieux kits (France). The health state of the animals was checked every day. The results were analysed by Student's *t*-test.

RESULTS

In the first experimental group, haemoglobin (Hb) levels increased insignificantly from 8.8 $g.dl^{-1}$ to 9.3 $g.dl^{-1}$ during the experiment and in the 2nd experimental group increased from 7.9 to 8.1 $g.dl^{-1}$. In the control group Hb level decreased significantly from 9.15 $g.dl^{-1}$ (0 th) to 7.5 $g.dl^{-1}$ (10th d). We recorded significant differences between the groups on the 10th day (the 1st experimental group – 9.3 $g.dl^{-1}$; control group – 7.5 $g.dl^{-1}$).

During the experiment, the values of packed cell volume (PCV) were quite steady. While in the 1st experimental group PCV ranged from 0.29 to 0.31 1.1^{-1}, in the 2nd experimental group and in control group PCV slightly decreases at the end of experiment. On the 10th day we found significant difference among the groups.

At some sampling intervals, the numbers of erythrocytes (Ec) even exceeded the reference values. Higher Ec numbers we recorded in the first group, in which they increased from 8.7 $T.l^{-1}$ to 9.1 $T.l^{-1}$ and persisted till the end of the experiment. In the 2nd group the number of Ec was steady over the whole period of the trial. On the other hand, in the control group Ec decreased from 9.5 $T.l^{-1}$ to 7.6 $T.l^{-1}$. In the control group, significant decrease was observed on the 10th day. Between the groups, significant difference was observed on the 10th day.

The numbers of leukocytes (Leu) increased in all groups during the experiment. In the first group. Leu number gradually increased from initial 10.6 $G.l^{-1}$ up to 15.1 $G.l^{-1}$ on the 10th day of the experiment, in the 2nd group from 8.6 $G.l^{-1}$ to 15.5 $G.l^{-1}$. In the control group

Table 1. Haematological profile in calves

Parameter	0th day	2nd day	5th day	10th day
Hb – 1st exp. group ($g.dl^{-1}$)	8.8 ± 1.07	9.46 ± 0.84	9.05 ± 0.92	9.3 ± 1.03[a]
Hb – 2nd exp. group ($g.dl^{-1}$)	7.88 ± 1.07	7.4 ± 2.16	8.6 ± 0.83	8.12 ± 1.38
Hb – control group ($g.dl^{-1}$)	9.15 ± 1.69	8.06 ± 2.08	8.5 ± 0.96	7.5 ± 1.32[a]
PCV – 1st exp. group ($l.l^{-1}$)	0.29 ± 0.02	0.31 ± 0.02	0.31 ± 0.04	0.31 ± 0.03[a, b]
PCV – 2nd exp. group ($l.l^{-1}$)	0.28 ± 0.13	0.25 ± 0.06	0.28 ± 0.03	0.24 ± 0.03[a]
PCV – control group ($l.l^{-1}$)	0.31 ± 0.07	0.25 ± 0.05	0.29 ± 0.04	0.24 ± 0.03[b]
Er – 1st exp. group ($T.l^{-1}$)	8.7 ± 0.92	9.2 ± 1.06	9.1 ± 0.94	9.6 ± 1.18[a, b]
Er – 2nd exp. group ($T.l^{-1}$)	7.3 ± 1.55	7.2 ± 1.5	7.3 ± 1.3	7.5 ± 0.87[a]
Er – control group ($T.l^{-1}$)	9.6 ± 1.68	7.44 ± 1.35	7.7 ± 1.23	7.6 ± 0.65[b]
MCV – 1st exp. group	33.8 ± 2.12	33.5 ± 1.66	33.6 ± 1.8	32.5 ± 1.49
MCV – 2nd exp. group	33.8 ± 1.08	33.7 ± 1.12	33.75 ± 1.42	32.2 ± 2.15
MCV – control group	32.7 ± 2.84	33.6 ± 2.36	33.68 ± 2.77	30.1 ± 2.6
Leu – 1st exp. group ($G.l^{-1}$)	10.9 ± 4.5	13.65 ± 3.37	12.3 ± 3.6	15.1 ± 6.2
Leu – 2nd exp. group ($G.l^{-1}$)	8.58 ± 0.89	10.4 ± 1.5	10.7 ± 0.39	15.5 ± 6.9
Leu – control ($G.l^{-1}$)	11.2 ± 6.65	11.8 ± 5.4	11.3 ± 5.5	15.2 ± 8.8

[a, b] — significant differences between groups

Table 2. Values of phagocytic activity and cholesterol

Parameter	0th day	2th day	5th day	10th day
PhAneu – 1st exp. group (%)	44.1 ± 9.6	41.0 ± 9.3	43.4 ± 9.5	44.7 ± 5.9
PhAneu – 2nd exp. group (%)	48.9 ± 10.0	41.8 ± 6.9	45.2 ± 9.1	45.3 ± 4.0
Phaneu – control group (%)	44.4 ± 7.6	40.3 ± 14.4	45.8 ± 6.7	44.4 ± 4.9
Chol – 1st exp. group (mmol.l^{-1})	1.58 ± 0.31	1.57 ± 0.21	1.58 ± 0.09^{a}	1.87 ± 0.14^{a}
Chol – 2nd exp. group (mmol.l^{-1})	1.87 ± 0.4	1.87 ± 0.4	1.94 ± 0.32^{a}	1.7 ± 0.32^{b}
Chol – control group (mmol.l^{-1})	1.59 ± 0.23	1.73 ± 0.31	1.87 ± 0.45	2.37 ± 0.4$^{a, b}$

PhA neu — phagocytic activity of neutrophils; Chol — cholesterol
$^{a, b}$ — significant differences between groups

Leu numbers were constant up to the 5th day of experiment and then increased suddenly on day 10. We found no significant difference (Table 1).

The phagocytic activity of neutrophils (PhAneu) was steady during the experiment without any significant differences between the groups. In all groups PhAneu decreased to 40—41 % on 2th day and later increased to 44—45 %. During the whole experiment we did not record any significant differences. In the experimental groups, steady cholesterol (Chol) concentrations were recorded with the initial value 1.58 mmol.l^{-1}, respectively 1.87 mmol.l^{-1}. This concentration persisted until the 10th day without any significant differences. In the control group, cholesterol levels gradually increased from initial 1.59 mmol.l^{-1} until the end of experiment. We revealed significant differences on the 10th day between experimental groups and the control group (Table 2).

In all groups, calcium (Ca) concentrations varied, at some sampling intervals significantly. At the beginning of the experiment, the lowest Ca concentrations (2.24 mmol.l^{-1}) were recorded in the first experimental group compared with 2.47 mmol.l^{-1} in the second group and 2.35 mmol.l^{-1} in the control group. On the 5th day, a decrease in Ca concentrations was observed in all groups. Significant differences between the 1st and the 2nd experimental groups were observed only on 0th day of the experiment. In the first group, Ca concentration was 2.24 mmol.l^{-1} and in the 2nd group 2.47 mmol.l^{-1}; the next sample revealed no significant differences.

During the experiment, magnesium (Mg) concentrations ranged within the reference limits in all groups without any significant difference. Phosphorus (P) concentrations slightly decreased in all groups during the experiment. In the first group, the highest P concentration was recorded on the 0th day – 2.9 mmol.l^{-1} and then P levels gradually decreased to 2.64 mmol.l^{-1} on the 10th day, however the decrease was insignificant. In the 2nd experimental group P slightly decreased from 3.0 (day 0) to 2.73 (day 10). In the control group, P levels ranged from 2.9 to 2.56 mmol.l^{-1} (Table 3). All animals were healthy over the whole period of the experiment.

DISCUSSION

Haematological indices (Hb, PCV, Ec) were significantly more favourable in the second experimental group compared with the control group. This is related to the use of probiotics, which improve haematopoiesis. Our results are similar to these reported by H e r i c h *et al.* (1), who found a significant increase in PCV and Hb values (compared with the controls) after 10 days of *Lactobacillus casei* administration to newborn piglets. Even if H ú s k a (2) reported a significantly higher mean corpuscular volume (MCV) of erythrocytes in pigs after administration of *Lactobacillus reuteri* in our trial we did not observe significant differences in MCV between groups. K o u d e l a *et al.* (4) have observed favourable effects of *Enterococcus faecium* administra-

Table 3. Concentrations of minerals in the blood of calves

Parameter	0th day	2nd day	5th day	10th day
Ca – 1st exp. group (mmol.l^{-1})	2.24 ± 0.1^{a}	2.55 ± 0.12	2.13 ± 0.06	2.18 ± 0.06
Ca – 2nd exp. group (mmol.l^{-1})	2.47 ± 0.1^{a}	2.55 ± 0.1	2.21 ± 0.07	2.18 ± 0.05
Ca – control group (mmol.l^{-1})	2.35 ± 0.15	2.53 ± 0.06	2.24 ± 0.07	2.2 ± 0.05
P – 1st exp. group (mmol.l^{-1})	2.9 ± 0.22	2.7 ± 0.4	2.8 ± 0.29	2.64 ± 0.26
P – 2nd exp. group (mmol.l^{-1})	3.04 ± 0.55	2.86 ± 0.25	2.83 ± 0.12	2.73 ± 0.18
P – control group (mmol.l^{-1})	2.96 ± 0.24	2.92 ± 0.23	2.9 ± 0.29	2.56 ± 0.3
Mg – 1st exp. group (mmol.l^{-1})	0.88 ± 0.16	0.97 ± 0.14	0.84 ± 0.1	0.78 ± 0.1
Mg – 2nd exp. group (mmol.l^{-1})	1.05 ± 0.1	1.0 ± 0.11	0.89 ± 0.12	0.91 ± 0.21
Mg – control group (mmol.l^{-1})	0.92 ± 0.14	0.98 ± 0.05	0.86 ± 0.08	0.82 ± 0.05

tion on increasing of Hb concentrations in piglets and poultry.

In young, growing animals, concentrations of minerals strongly depend on their content in the milk. In our experiment Ca, P and Mg levels were within the physiological range and significant differences between groups were not observed.

Cholesterol concentrations slightly increased on day 10 in the first experimental group and in the control group. We have discovered significant differences in the control group in comparison with experimental groups. Xiao *et al.* (8) have found that *Bifidobacterium longum* strain BL1 significantly decreased serum concentration of total cholesterol, low-density lipoprotein cholesterol and triglycerids in rats. On the other hand, administration *Streptococcus thermophilus* did not decrease these indices. Pereira and Gibson (6) suppose that in hyperlipidemic subjects, any effects that do occur result primarily in reductions in cholesterol, whereas in normal lipidemic subjects, effects on serum triglycerides are the dominant feature. Klaver and Van der Meer (3) have reported decreasing cholesterol levels after administration of *Lactobacillus acidophilus* related to changes of micelles of cholesterol and its subsequent precipitation by bile acids.

Mojžišová *et al.* (5) have reported a significant increase in leukocyte phagocytic activity in fattening bulls after one-month's administration of *Lactobacillus plantarum.* However, our experiment lasted a shorter time and therefore the immunostimulating effects of the probiotic could not be demonstrated.

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Received October 12, 2004

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 49, 1: 52—55, 2005

PRODUCTION AND UTILIZATION OF CAMEL MEAT IN JIJIGA AND SHINILE ZONES OF SOMALI NATIONAL REGIONAL STATE, ETHIOPIA

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ABSTRACT

The purpose of this study was to provide base line information on production and utilization of camel meat in Jijiga and Shinile zones of Somali Regional National State, Ethiopia. Eighty-four households from four districts of Jijiga and Shinile were interviewed using a structured questionnaire. In addition, a nine-year's and a thirteen-year's data were used to determine the *per capita* consumption of camel meat in Dire Dawa and Jijiga towns, respectively. It was found that camel meat is highly relished by the sample respondents. It is consumed either boiled or fried. Among the offals, the intestine, rumen, lung, spleen, tongue and feet were not consumed due to cultural taboos. The intestinal fat was used as oil for cooking. In the rural areas of Jijiga and Shinile zones, out of the 80 camels slaughtered *per annum*, 43.7 % were slaughtered at religious occasions, whereas 45.0 % were emergency slaughtered due to disease problems. *Per capita* consumption of camel meat in Dire Dawa and Jijiga towns was found to be 0.51 kg and 0.82 kg, respectively. If only Muslim communities in the two towns were considered, the *per capita* consumption of camel meat would have been much higher than the obtained result. In order to extend the consumption of camel meat from a few days to several months, pastoralists have used traditional meat preserving methods. The most common methods were found to be *Olobe* and *Mofo* preparations. The camel meat market study result showed that the price of hump was much higher compared to the other part of the carcass. (25 Birr/kg, 16 Birr/kg, respectively; 8.567 Birr = $1).

Key words: camel; consumption; market; meat; one-humped; pastoralists; production; processing

INTRODUCTION

Camel production systems in Eastern Ethiopia are typically geared towards the output of calves and milk for human consumption. The production of meat though is important, yet subsidiary to the calf-milk operation. As a result, camel meat has a limited contribution to the total availability of animal protein in camel rearing and other regions of the country. In most camel rearing areas, camels slaughtered for meat are those animals, which remain after the best male has been selected for breeding and others have been castrated and selected as riding or draught animals (2). Non-producing females are also slaughtered (!0).

Much of the malnutrition seen in the developing world is the result of relying too much on a single staple food. Camel meat and its products can be concentrated sources of high quality protein and their essential amino acid composition compensate for deficiencies in the staple food. Camel meat resembles beef in taste, appearance, colour, texture and palatability (9, 4, 6). Meat from young camels is tasty and resembles veal (20).

Camel has relatively high feed conversion efficiency in the arid and semi-arid conditions. The male camel carcass weight ranges from 270 kg to 600 kg (8). The Somali camel, especially the "Benadir" type, however, is large and heavy. Its mature body weight ranges from 500 kg to 800 kg (5). The dressed carcass percentages of camels are in the range of 45 to 55 % and exceptionally up to 60 %. Total carcass composition is about 66 % muscle, 19 % bone, and 14 % fat with the fat being mainly from the hump. Lean meat in the camel has more moisture and less fat than beef (8). The proportion of edible meat in a camel body is comparable to cattle but the fat content is far lower than the beef (3). A b u t a r b u s h

Table 1. Camel meat parts usually consumed and not consumed

Parts	Mode of consumption	Remark
Carcass		
Lean meat	Fried or boiled and eaten with or with out local 'bread', rice or sorghum	Usually processed
Fat meat	Fried or boiled and eaten with or with out local 'bread', rice or sorghum	Usually processed
Bone	The marrow separated and eaten with lean or fat meat	
Organs/viscera		
Liver	Fried and eaten alone or with local 'bread'	
Kidney	Fried and eaten alone or with local 'bread'	Could be processed
Heart	Fried and eaten alone or with local 'bread'	Could be processed
Intestine	Cultural taboo	Some people consume it
Rumen	Cultural taboo	
Lung	Cultural taboo	
Others (spleen, intestinal fats)	Cultural taboo	The intestinal fat is used as oil during cooking
Others		
Tongue and feet	Cultural taboo	

and Dawood (1) have reported that camel meat has a lower cholesterol content than beef and mutton. Camel meat, therefore, could be the healthiest option.

The flavour and juiciness of camel meat are not only enjoyed by nomads and camel owners, but it has been scored as high as or better than beef by tasting panels in the Arab states (8). Camel meat is fast gaining popularity, particularly in Australia where camel meat is now available in many supermarkets.

Although, camel has been prized by Jijiga and Shinile pastoralists through the ages for being the most efficient source of milk and meat, so far information is lacking on the meat production potential of camel in the Jijiga and Shinile zones. The purpose of this study was, therefore, to provide basic information on the production and utilization of camel meat in the Jijiga and Shinile zones of Ethiopia.

MATERIALS AND METHODS

Sampling procedure

Based on their representative nature, with respect to the proportion of camel herding households, and seasonal livestock movement pattern and camel types, two districts, Jijiga and Kebribeyach from the Jijiga zone and Shinile and Errer from the Shinile zone, were selected. Throughout this paper, the Jijiga and Kebribeyah districts are collectively referred as the Jijiga zone while the Shinile and Errer districts are referred as the Shinile zone. It should be noted that the selection of the specific study areas was dictated by a host of factors. Factors such as accessibility and security, as well as transportation, financial and time constraints limited the survey to the Jijiga and Shinile zones.

The Jijiga and Shinile zones were further divided into six sub-sites based on the homogeneity of the sub-sites. A total of 27 villages, 16 in Jijiga and 11 in Shinile, were randomly selected from the six sub-sites. Further, from the 27 villages, a total of 84 households, 53 in Jijiga and 31 in Shinile, were sampled.

Data collection and analysis

Single-Visit-Formal-Survey Methods (9) were adopted to collect data on camel herd demographic characteristics and camel meat production and utilization. Various data collection techniques and tools such as questionnaire, record sheets, and progeny history examinations were used during information gathering. Moreover, discussions with elders, ethnic leaders and development agents were made in all the selected sites. The discussions were particularly useful in gaining more insight into community beliefs and attitudes towards camel keeping.

The authors administered the questionnaire and filled in the record sheets. The collection of information was made at the villages/settlements before the animals were released or at watering points. Pre-testing of the questionnaire and the record sheets was done and on the basis of the information obtained from the pre-test, appropriate modifications were made to both the questionnaire and record sheets. Local ethnic leaders who were known to the respondents were used to interpret and explain the questions in the course of individual interviews and discussions. The ethnic leaders were found to be quite useful in creating ease of communication between the researchers and the respondents. The first author stayed with the camel herders to get better access to information and to win their confidence.

Group discussions and individual interviews were performed to investigate camel meat processing mechanisms. In addition, a nine-year and a thirteen-year data of abattoirs were used to determine the *per capita* consumption of camel meat in Jijiga and Dire Dawa* towns, respectively.

* — Most of the camel supply to Dire Dawa comes from Shinile

RESULTS AND DISCUSSION

Throughout the paper, the term camel meat utilization is used in the sense that it includes camel meat consumption, processing, and marketing.

Camel meat consumption has been going on in Eastern Ethiopia for centruries. It is immensely popular on the tables of most of the Muslim communities of the region. The parts of camel meat, which are consumed and not consumed, are indicated in Table 1. The major part of the camel meat consumed by the herders is the carcass.

As per the information obtained from the respondents, the number and reason for slaughtering of camels in rural areas during the year 1996 are shown in Tables 2 and 3, respectively. Although camel meat is highly relished by the pastoralists, it is not available in everyday meals. This is probably due to the cost it incurs, which is not usually affordable by many households. It also probably indicates survival strategies of camel owning households that killing camels for meat production squeezes the "herd capital". In many camel dependent households, camels are regarded as assets of the family that must be maintained for future generations. According to this study, the overall mean of slaughtered camels per household in 1996 was around 0.88.

Table 2. Total camels slaughtered during the last 12 months by zone (1996)

Category	Jijiga (n = 41)[4]	Shinile (n = 39)	All (n = 80)
Male	46.3	41.0	43.8
Female	46.3	59.0	52.5
Castrated	7.4	0	3.7
Total (%)	100	100	100

n — refers to number of camels slaughtered

In the rural areas of Jijiga and Shinile out of the total 80 slaughtered camels, 43.7 % were slaughtered on religious occasions, whereas 45.0 % were emergency slaughtered due to disease problems (Table 3). An insignificant proportion of households slaughtered camel in other occasions. It is, therefore, possible to conclude that in the study areas camels have a special significance in religious ceremonies and disease is the major threat to camel production.

The annual average meat supply and *per capita* camel meat consumption in kg is given in Table 4. However, if only the Muslim population in Dire Dawa and Jijiga towns was considered, the *per capita* consumption would have been higher than the obtained result.

Meat is a highly perishable product and soon becomes unfit for consumption and a possible hazard to public health through microbial multiplication, chemical changes and breakdown by indigenous enzymes. To curtail this problem, pastoralists have used traditional methods of meat preservation. The most common methods are *Olobe* and *Mofo** preparations.

Table 3. Reasons for slaughtering camels during the 12 months of 1996

Reason for slaughtering	Out of total slaughter Jijiga (n = 41)	Shinile (n = 39)	All (n = 80)
Home consumption	0	2.6	1.3
Religious ceremonies	41.5	41.1	43.7
Due to disease	48.7	41.1	45.0
Butchery (in village)	4.9	10.3	7.3
Others	4.9	0	2.5
All (%)	100	100	100

Table 4. *Per capita* meat consumption in Dire Dawa and Jijiga towns

Towns	Meat type and kg/year Camel meat	Beef	*Per capita* consumption (kg/person/year) Camel meat	Beef
Dire Dawa	105.746	1,391,285	0.51	6.71
Jijiga	164.506	431,327	0.82	2.15

— slaughterhouse data from July 1984/85 to July 1995/96 from Dire Dawa, and July 1887/88 to 1995/96 from Jijiga municipality were taken

— most of the camel supply to Dire Dawa town comes from Shinile

Olobe (*Odka*, *Lukemet, Mukemet, or Kumebis*) preparation

Olobe is the most common traditionally processed product of camel meat. There may be a slight difference in the methods of preparing *Olobe* among different societies. In preparing *Olobe,* the lean (non-fatty) meat is sliced into thin strands then sun dried. The fatty part of the meat, mainly the hump fat, is melted in a separate pan. Then the sliced dried lean meat is cut into pieces and added to the already melted fat and then fried. According to the purpose of processing, it is possible to add some spices and butter. If *Olobe* is made for a marriage ceremony, fruit such as dates are added.

Olobe can be stored as the fat and lean mixed or separately in tightly closed tin or traditionally made equipment. The lean meat separated from the fat is called *Muremure.* This form of *Olobe* is usually sold in the market. *Olobe* can be processed either by male or female. According to the pastoralists, *Olobe* can be consumed safely for months. There are traders in Jijiga who export *Muremure* to Hargeyisa (Somalia).

* — Words, which are written in italic are in the Somali language

Mofo(Solayise)

Mofo is the other processed meat produced by the pastoralists. A hole is made on the ground. Fire is made in the hole. After the wood is completely burnt, a wooden grill is placed above the fire. Lean meat, which has been sliced and slightly dried mixed with fat is packed with green leaves or grass and placed on the wooden grill. Then it is covered with soil. By doing so the meat is cooked and dried. After a few hours it is taken out and stored in similar ways to *Olobe*. Men usually make *Mofo*. According to the pastoralists, preparation of *Mofo* allows the meat to be preserved for a long time, so that consumption can be postponed from a few days to several months, which made it ideal to take on long trips. *Olobe* and *Mofo* can consumed after warming or as they are taken out from the storage material. Further study is required to determine the hygienic status of *Olobe* and *Mofo.*

Marketing of camels

The economic value of camels cannot be denied. They are able to use marginal land and browse plants that are rejected by other animals to efficiently produce a marketable product. In the studied area, there were five main towns for marketing camel meat (Table 5). Camel meat was more expensive in the towns of Jijiga and Dire Dawa. This is because of the domination of the Muslim community in the two towns as opposed to Harar. Compared with beef, camel meat has a reasonable price (Table 5). In Jijiga, traders do not weigh the camel meat so they try to guess by size. Of the camel meat, the price of the hump is higher. The hump is a mound of fatty tissue from which the animal converts fat into energy, when there is a shortage of feed.

Table 5. Camel meat prices in major towns of Eastern Ethiopia

Towns	Price of camel meat (Birr/kg)				
	Wet season		Dry season		Beef
	Meat	Hump	Meat	Hump	(Birr/kg)
Jijiga	15—18	25	16	25	17—20
Harar	10	15	6—7	15	15
Dire Dawa	16	–	16	–	20
Shinile	10	25	7	25	15

Income from the sale of live camels makes a relatively high contribution to the economy of households of the studied area. Camel marketing occurs in towns like Dire Dawa, Jijiga, Shinile. It also occurs in the pastoralist settlements/villages, where the traders go to the herd. The actual process of selling camels seems to involve the traders in examining the camels and then agreeing the price with the owners of the camel. Whether they purchase camel for meat or other purposes, buyers scrutinize each animal from head to toe. The hump and the teeth, generally the camel's body condition determines its value. Females are worth more because they breed.

According to T e z e r a and T a f e s s e (cited by 7), the average price for male and female camels between 1991 and 1993 at three livestock markets of Eastern Ethiopia was 754 Birr and 573 Birr, respectively. The same authors reported that only 30 % of the total camels that had brought to the markets were sold. On the contrary, in the present study only few households, 9.4 % in Jijiga and 12.9 % in Shinile, reported the market as a constraint in camel production.

Many camels, especially young camels, cross the boarder with illegal traders to be sold abroad. That this happens may be due to the relatively high price most pastoralists get by selling their camels to illegal buyers. The price for a medium sized camel in the illegal market was reported to be around 1,200 Birr.

AKNOWLEDGEMENTS

The study was funded by European Camel Project. The authors would like to thank Professor Dr. H. B r u c k e n e r, Dr. Z. F a r a h, and Dr. S. K a p p e l e r for the initiation of this study. The authors are very grateful to the management of the Alemaya University for providing facilities.

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Received July 6, 2004

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 49, 1: 56—60, 2005

ANATOMICAL BODY STRUCTURES IN PRACTICAL USE — BODY CONDITION SCORING IN SHEEP (A Review)

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ABSTRACT

***Regio presternalis, regio hypochondriaca, processus costarius, regio tuberis coxae, regio articulationis coxae, regio tuberis ischiadici, regio clunis, regio sacralis, regio glutea, fossa paralumbalis* and *regio analis* are used to evaluate the body condition score of sheep. Condition scores range from 1, a very thin sheep with no fat reserves, to 5, a severely over-conditioned sheep on a 1—5 scale system. Ideal condition scores fall in the range 2.5—3.5. Age, the reproduction cycle and the influence of feeding principles on body condition scores are discussed.**

Key words: anatomical feature; body condition scoring; fat reserve; sheep

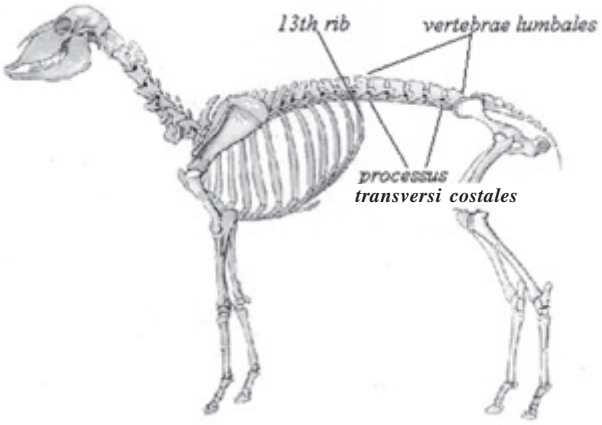

Fig. 1. The most important skeletal marks in evaluating the body condition of a sheep

INTRODUCTION

Perhaps one of the most important management skills of sheep producers is the ability to body condition score their animals and track progress towards achieving a desired degree of fatness to meet a given reproductive goal in a herd.

Body condition scoring (BCS) is an index of the degree of fatness expressed in anatomical features (Fig. 1) of an animal that can be viewed by the naked eye. Essentially, BCS is a systematic process of attempting to visualize the degree of underlying skeletal features that can be detected by observing the animals (6, 10).

Every dairy producer has sheep that are too fat or too thin for their stage of lactation. Failure to recognize these sheep and take action costs dearly in disease treatments, lost milk production, and decreased fertility (13).

Body condition is a reflection of the body fat reserves carried by the animal (Tab. 1). These reserves can be used by the sheep in periods when it is unable to eat enough to satisfy its energy needs. In high producing sheep, this normally happens during early lactation, but it may also happen when sheep get sick, when they are fed with poor quality feeds, or the feed intake is restricted (10, 12, 15). After a period of weight loss, the sheep should be fed more than their requirements to restore normal body condition.

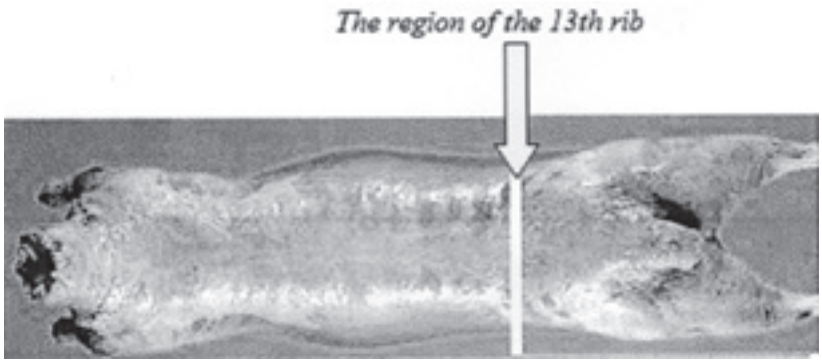

Fig. 2. The position of the 13th rib for evaluating the body condition score by palpation

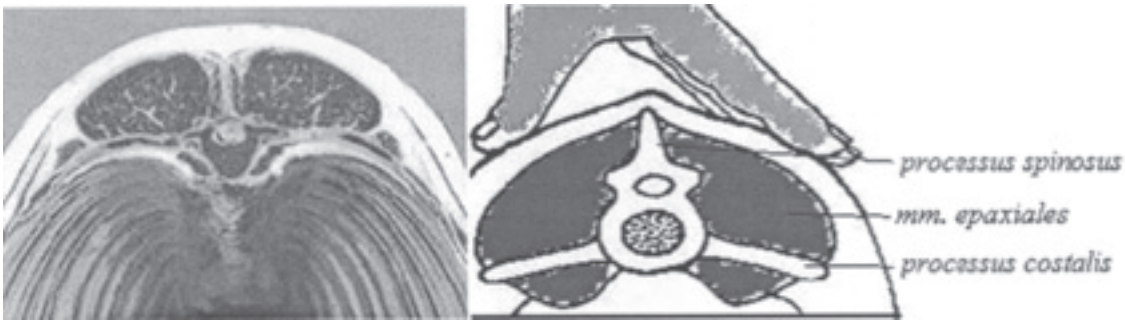

Fig. 3. The composition of the palpated region at the 13th rib transversal section

The condition and nutritional status of livestock can be measured by live weight (LW) change and body condition scoring. LW change, using LW scales, is the most accurate way of measuring the condition of livestock except during late pregnancy or when the gut-fill varies between each weighing. At these times, condition scoring is a useful, additional method to LW change (1, 11, 16).

Where LW scales are not available, condition scoring is the best alternative. Visual appraisal is unreliable and is easily confused by gut-fill, length of fleece and pregnancy.

Condition scoring can be used (13):

— To assess whether more feed is needed to maintain or increase condition and LW; and

— In meat production systems, where a particular carcass finish is desired by the consumer.

— Carcass finish is assessed as a fat score (2), which is directly related to the condition score of the live animal, for instance, carcass fat score 3 is equivalent to condition score 3.

The most frequently used region to evaluate by palpation is the 13th rib transversal section (Figs. 2 and 3).

Condition scoring is done by feel. Accuracy improves with practice. When feeding for survival or for maintenance of body condition during periods of feed shortage, the livestock should be maintained at a condition score of 2. Below condition score 2, wool production in sheep is likely to be affected, with the development of tender fleeces. At condition score 1 or below the animal is emaciated and its long-term production may be reduced. In the breeding ewe or doe, condition scores near 3 are desirable. Lower scores will result in fewer lambs or kids being born, lower birth weights and thus lower survival rates.

THE PROCEDURE TO EVALUATE A CONDITION SCORE

Condition score is independent of body size. Animals of small lambs and of shipper wither size can have the same body condition score. Condition score measures the amount of meat and fat over the bones of the animal not the size of the animal (4, 5, 13).

The animal should be standing in a relaxed position. It should not be tense, crushed by other animals or held in a crush. If the animal is tense it is not possible to feel the short ribs and get an accurate condition score.

Locate the 13th rib, which is the last in a sheep (Fig. 2.). Using your thumb and fingers, try to palpate and feel the *processus spinosus* of *vertebrae lumbales* with the thumb and the end of *processus costalis* with the finger tips immediately behind the last rib.

Feel the muscle and fat cover around the ends of *prosessus costalis* and over the *processus spinosus*. Try to feel the extent of the *mm. epaxiales* (Fig. 3).

The degree of roundness of the ends of the bones, the amount of tissue between the bones and the extent of *mm. epaxiales* determines the condition or finish of the animal condition score.

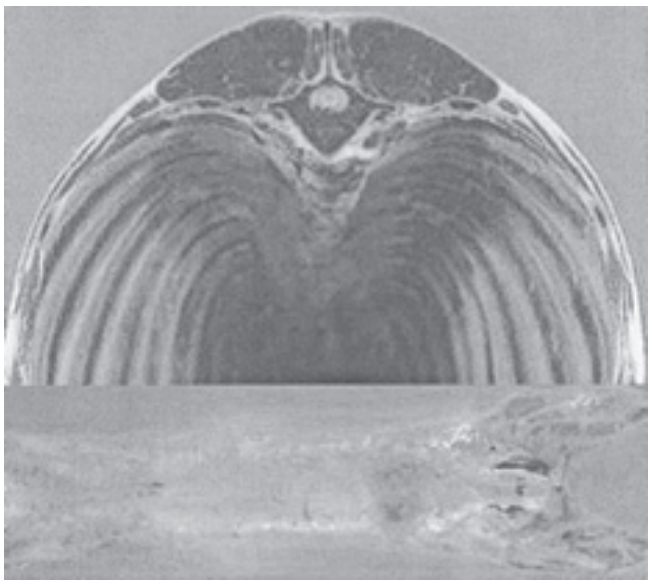

Fig. 4. The transversal and the full length back of a slaughtered sheep body (Notice the thickness of the subcutaneous fat is almost none)

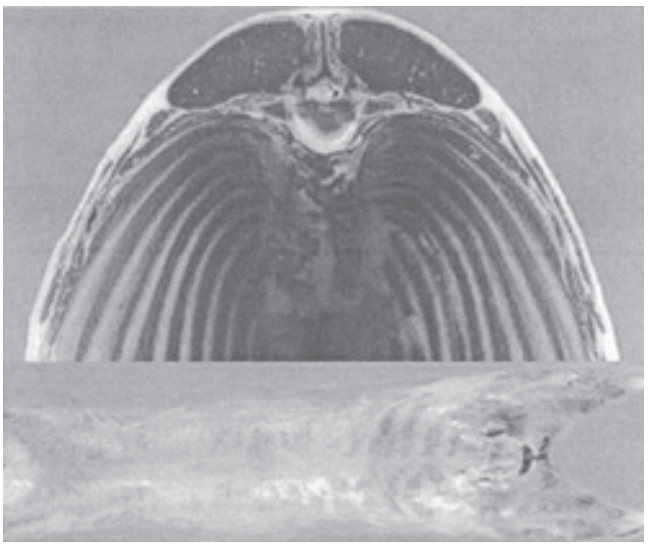

Fig. 5. The transversal and the full length back of a slaughtered sheep body (The thickness of the subcutaneous fat is noticeable)

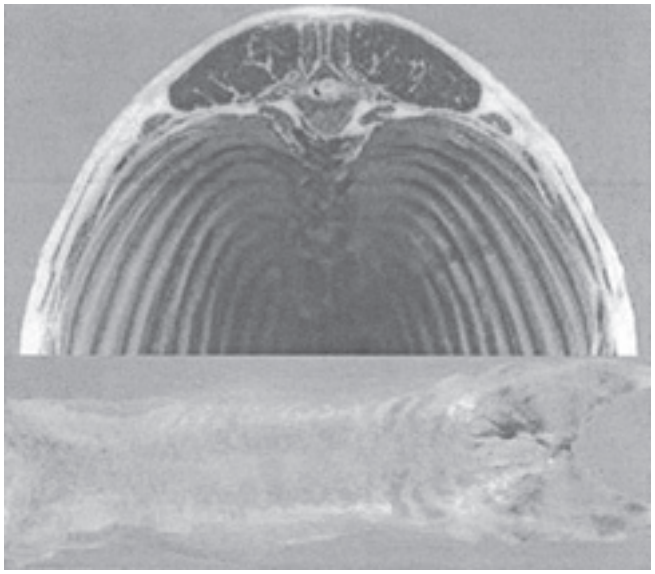

Fig. 6. The transversal and the full length back of a slaughtered sheep body (Note the thickness of the subcutaneous fat all over is fair)

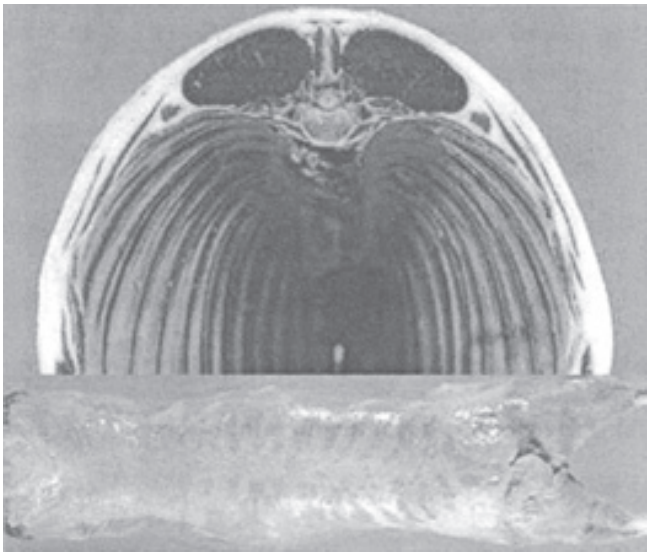

Fig. 7. The transversal and the full length back of a slaughtered sheep body (Note the thickness of the subcutaneous fat is bulky)

THE SHEEP CONDITION SCORES

Score 0: The animal is emaciated. It is in an extremely poor condition. There is no fat cover under the skin. You do not palpate the surface of *mm. epaxiales,* when your thumb is moving down from the backbone to the end of the *processus costalis.* There is only little tissue between the *processus spinalis* of the *vertebrae lumbales* or *processus costalis.*

Score 1: The *processus spinalis* is prominent and sharp, the ends of the *processus costales* are sharp and easy to press between, over and around and *mm. epaxiales* are thin, the surface is likely to feel hollow (Fig. 4).

Score 2: The *processus spinosus* is sharp and prominent. The *mm. epaxiales* are covered with little fat but it is full. The *processus costales* are smooth and relatively rounded (Fig. 5).

Score 3: The *processus spinosus* is smooth and rounded. Each of the *processus spinosus* can be felt only with pressure. The *processus costales* are smooth and well covered with fat. The *mm. epaxiales* are covered with fat too. You need to insert pressure to feel all of the mentioned compositions (Fig. 6).

Score 4: The *processus spinosus* can be distinguished only with pressure. The *processus costales* cannot be felt. *Mm. epaxiales are full with a thick fat cover (Fig. 7).*

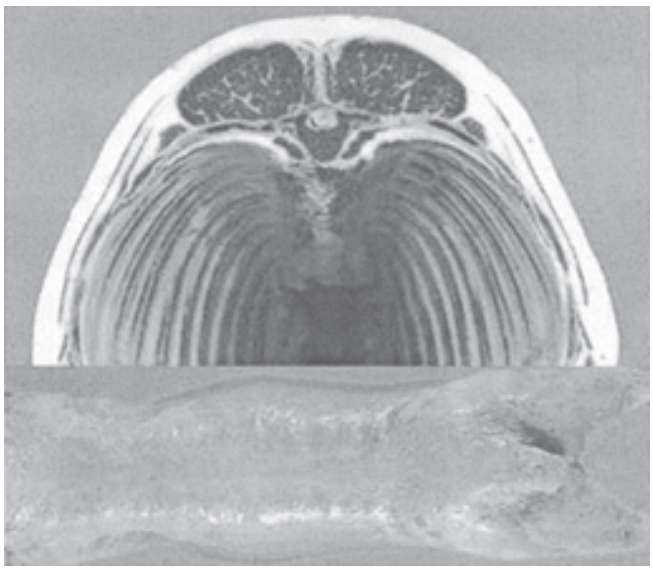

Fig. 8. The transversal and the full length back of a slaughtered sheep body (Note the thickness of the subcutaneous fat is extremely bulky)

Score 5: The *processus spinosus* cannot be distinguished. There is a depression between the fat, where the spine would normally be felt. The *processus costales* cannot be distinguished. The *mm. epaxiales* cannot be felt even, when firm pressure is inserted due to a thick layer of fat (Fig. 8).

The system includes everything from emaciated sheep to those that are grossly obese due to over-feeding or being non-productive. In most typical sheep flocks, over 90 percent of the sheep should have a body condition score of two, three, or four. The intermediate half scores are helpful when the condition of the animal is not clear. It is recommended that half scores be used between two and four (10, 13).

Other than practical experience, there is little available research comparing condition scores with performance. The majority of the research reported has dealt with the relationship of body condition score at breeding to ovulation rate and subsequent lambing percentage (Table 1). Generally, the better the body condition score at mating, the higher the ovulation rate and therefore the higher the potential lambing percentage (8, 9). However, ewes with a condition score greater than 4 at breeding tend to have a higher incidence of infertility. Ewes with a condition score less than 3 at breeding will be more responsive to the effects of flushing than those with condition scores of 3.0—3.5 at mating.

Two research trials conducted by Oregon State University found that ewe body condition score at lambing had an effect on total kilograms of lamb weaned per ewe. Ewes with a body condition score of 3 to 4 at lambing lost fewer offspring and weaned more kilograms of lamb than those with a condition score of 2.5 or less (3).

In one study, ewes with a body condition score of 4 at lambing had a total weight of lamb weaned per ewe that was 82 percent greater than ewes with a body condition score of 2.5. The total weight weaned was 42 kg comparing to 23 kg per ewe. The increase in total weaning weight was due to improved lamb survival and heavier weaning weights (8).

In the other study, there was a 33 percent difference in total weight of lamb weaned (24 *versus* 32 kg per ewe) between ewes with pre-lambing body condition scores of 2.5 to 3.5. This increase in kilograms of lamb weaned was primarily due to improved lamb survival for offspring from the ewes with the higher body condition score (14).

Table 1. Some suggested condition score optimal values for the various stages of the production cycle

Production cycle	Optimum stage score
Breeding	3—4
Early Mid Gestation	2.5—4
Lambing: Singles	3.0—3.5
Lambing: Twins	3.5—4
Weaning	2 or <

The scores suggested above should allow for optimum productivity in highly prolific ewes. On average, a difference of one unit of condition score is equivalent to about 13 percent of the live weight of a ewe at a moderate (3—3.5) body condition score. Thus, a ewe with a maintenance weight of 55 kg would need to gain approximately 7.5 kg to go from a body condition score of 2.5 to 3.5.

CONCLUSION

Body condition scoring is a subjective way to evaluate the status of a sheep flock. It is a potential tool for producers to increase production efficiency in their flocks. In sheep meat industries body condition scores of 2—3 well finished without being fat are desirable. Condition score one animals are unfinished because muscle development is poor, in the other hand animals at condition scores 4 and 5 are too fat to be accepted for further processing.

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Received July 2, 2004

BOOK REVIEW

Robert, H. Dunlop and Charles-Henri Malbert (eds.): *Veterinary Pathophysiology*. 1st edition, 2004, Iowa State Press Blackwell Publishing, 540 pages, 55 tables and 250 figures and photographs, 18 x 26 cm, hardcover, ISBN 0-8138-2826-0, Price: $99.99; Ł65.00, 27 contributors.

A long-awaited textbook in veterinary pathophysiology has been published in English. The previous textbook on veterinary pathophysiology was written and published almost sixteen years ago (edited by Wayne F. R o b i n s o n and Clive R. R. H u x t a b l e : *Clinicopathologic Principles for Veterinary Medicine* in 1988) and there was an urgent need to apply the principles of homeostasis and behavioural adaptation to advance understanding of pathophysiology in clinical disease.

The textbook *Veterinary Pathophysiology* offers students and clinicians a comprehensive introduction to understanding the interactions between organs and functional systems that underlie infectious and metabolic diseases.

Veterinary Pathophysiology is divided into thirteen chapters each with appropriately numbered parts. Individual chapters consist of a short introduction in physiology, anatomy and histology and description of activation of normal regulatory mechanisms responding to abnormal circumstances associated with a particular disease in various domesticated animals. The text is accompanied with tables, figures, photographs and flow charts.

The *first chapter* (20 pp.) describes the physiology and pathophysiology of the internal environment.

The *second chapter* (53 pp.) describes the pathophysiology of cellular regulation, cell death, and cancer. Normal mechanisms of cellular behaviour, growth, and proliferation control and the complex issues of cell signaling form the basis of Part 1 of this chapter and describe normal cellular physiology with special reference to the control of the cell cycle. In Part 2, the central role of the cell cycle in the development of the malignant phenotype is described. Part 3 reviews the etiology of cancers in domestic animals. Part 4 describes molecular pathology and the new technologies that are enabling us to define the genetic basis of cancer and improve diagnosis. In Part 5, the interaction of the malignant phenotype with the host immune system along with the role of the immune system are described. In Part 6, the clinical manifestations of cancer, the biological basis of conventional therapies, and some of the new genetic approaches to treatment are described.

The *third chapter* (31 pp.) describes host-pathogen interactions. Part 1 of this chapter reviews the bewildering variety of organisms that have adopted an infectious or parasitic way of life. In the Part 2, host and environmental factors relevant to infectious diseases are considered separately.

The *fourth chapter* (31 pp.) describes pathophysiology of the gastrointestinal tract. The origin of gastrointestinal dysfunction involves disturbances in the neurohumoroimmune control of the gut function.

The *fifth chapter* (33 pp.) describes pathophysiology of the respiratory tract. Part 1 of this chapter reviews pathophysiology of obstructive respiratory disorders. Part 2 deals with the functional effects of restrictive pulmonary diseases. In Part 3, the pathophysiology of vascular pulmonary diseases is described. Part 4 describes environmental respiratory disease in domestic animals.

The *sixth chapter* (35 pp.) describes the pathophysiology of cardiovascular disease. In the Part 1, the cardiovascular system as a biological control system is described. Part 2 of this chapter reviews extracardiac peripheral circulation and shock.

The *seventh chapter* (45 pp.) describes the pathophysiology of the reproductive system. In Part 1 of this chapter, mammalian sex determination is described. Part 2 reviews the pathophysiology of female reproduction and Part 3 describes the pathophysiology of male reproduction.

The *eighth chapter* (17 pp.) deals with the pathophysiology of muscle disease.

The *ninth chapter* (58 pp.) describes the pathophysiology of the central nervous system. In Part 1, infectious-inflammatory diseases of the central nervous system are described. Part 2 describes the pathophysiology of noninfectious neurological diseases. Part 3 reviews mechanisms of central nervous system homeostasis.

The *tenth chapter* (32 pp.) describes welfare, stress and behaviour in pathological conditions.

The *eleventh chapter* (28 pp.) deals with the pathophysiology of the liver.

The *twelfth chapter* (75 pp.) describes the pathophysiology of endocrine homeostasis. In Part 1, the pathophysiology of calcium-phosphorus metabolism and bone is described. Part 2 reviews metabolic disorders of calcium-phosphorus homeostasis. Part 3 deals with the pathophysiology of the thyroid gland and Part 4 describes the pathophysiology of the endocrine endocrine pancreas.

The *thirteenth chapter* (32 pp.) describes the pathophysiology of homeostatic and toxic disorders. This chapter deals with aspects that have a particular focus involving the nervous system that are attributable to or involve nutritional, metabolic, or toxic factors. Often these types of condition involve multiple organ systems and their interactions. In Part 1, the pathophysiology of metabolic balance is described. Part 2 reviews the pathophysiology of plant toxicants. Part 3 deals with the pathophysiology of some non-plant toxicants. Part 4 describes the pathophysiology of spongiform encephalitis.

The *Veterinary Pathophysiology* should be included in the group of textbooks that enrich veterinary medicine. It clearly emphasizes the importance of pathophysiology in the veterinary medicine curriculum.This book will be invaluable for clinicians, instructors and veterinary students interested in a deeper understanding of animal diseases.

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(FOLIA VETERINARIA, 49/2005, 61)