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EXPERIMENTAL CYPERMETHRIN TOXICITY IN RABBITS — A CLINICAL AND PATHO-ANATOMICAL STUDY

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ABSTRACT

Cypermethrin (synthetic pyrethroid pesticide) was administered orally to New Zealand white rabbits of either sex for a period of fifty days to evaluate the toxic effects in non-target organisms. The rabbits received cypermethrin dissolved in soybean oil (1:2 ratio) at a dose of 140 mg.kg^{-1} b.w. per day equivalent to 1/10 of the LD_{50}. Clinically, dullness, anorexia, reduced feed and water intake, diarrhoea, hyperirritability, salivation, coarse tremors, movement of eyeballs, dilation of pupils, weakness, paralysis of hind limbs, gasping and tonic-clonic convulsions were observed. Decrease in body weight was observed in the treatment group in comparison to the control group. The carcasses of the rabbits of the treatment group appeared emaciated, anaemic with subcutaneous oedema and gelatinization of fat in the fat depots.

In the treatment group, the liver was the most affected organ followed by the stomach, intestines, kidneys, lungs, heart, spleen, gonads, brain, pancreas, adrenals and thyroid glands.

Broadly, at the initial stage vascular congestion and focal haemorrhages were observed in the affected organs. In the terminal stages the lesions were characterised by hepatomegaly with a granular appearance, catarrhal enteritis, sub-capsular haemorrhages in kidneys and pneumonic changes in the lungs. Histopathological examination revealed vascular congestion, hydropic degeneration and leukocytic infiltration in the affected organs at the initial stages. At the terminal stage of toxicosis, coagulative necrosis, perivascular/periductal fibrocellular reaction along with mononuclear cellular infiltration in liver, mucosal eruptions with inflammatory reaction in the gastrointestinal tract and hyalinization of tubular epithelium in the kidneys were observed.

The lymphoid population in the splenic follicles and Peyers patches was depleted. The seminiferous tubules showed degeneration/denudation of the epithelium and reduction in the number of matured spermatids.

Key words: cypermethrin; oral toxicity; pathological findings; rabbits

INTRODUCTION

Pesticides are widely used as common health cover agents for the control of ectoparasites and vector borne diseases of domestic and laboratory animals. Synthetic pyrethroids occupy an important position among commonly applied pesticides because of lower residual toxicity. Cypermethrin is a highly active synthetic pyrethroid insecticide, which intensively controls a wide range of pest species in agriculture, animal breeding and the household.

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This pesticide can enter the human or animal system either directly or as an environmental pollutant (1). Though the absorption of cypermethrin from digestive tract is rapid (18), acute toxic effects in human and animals are relatively lower because of the rapid biotransformation and excretion in the form of non-active metabolites, mostly in urine (16). In mammals and insects, it produces a neurotoxic effect by affecting the axon of the neurons of the peripheral and central nervous system and interacting with the sodium ion transportation system through the cellular membrane (28, 19). Cypermethrin also exerts suppressive effects on the immunological system (7, 22, 27). Subacute toxicity of cypermethrin in mice causes decreased bactericidal activity of neutrophils (18). Chronic exposure of human or animals to pyrethroid pesticides may also result in endocrine disturbances (8).

The aim of the study was to assess the clinical, gross and histopathological alterations in various organs in rabbits following per-oral administration of cypermethrin for a period of fifty days.

MATERIALS AND METHODS

Thirty-two New Zealand white rabbits of either sex and aged between one and two months were procured from the Laboratory Animal Research Division, Indian Veterinary Research Institute (IVRI), Bareilly, India and were individually housed in stainless steel cages. Feed and water were provided *ad libitum*. These rabbits were maintained on a toxin free base diet supplied by Feed Processing Plant, IVRI, along with green fodder (Burseem), until they gained a body weight of about 1 to 1.5 kg. Prior to the commencement of the study, the rabbits were dosed with sulphadimidine powder mixed in their feed @ 0.2 $g.kg^{-1}$ for five to six days to prevent coccidiosis. The body weights of the animals were recorded and were divided at random into three groups — two controls, each comprising eight animals and one experimental comprising sixteen animals. Control Group I was administered soybean oil of pharmaceutical grade used for suspending pyrethroid, while Control Group II was kept only on a base diet. For the experiment, a technical grade (chemically pure — min. 97 %) of Cypermethrin was procured from M/s Montari Industries Ltd., Chandigarh, India. Based on a pilot study conducted as per Weil (29), the LD_{50} value of cypermethrin for rabbits was calculated to be 1430 $mg.kg^{-1}$ body weight (b.w.). Cypermethrin @ 1/10 of LD_{50} dose (140 $mg.kg^{-1}$ b.w.), dissolved in soybean oil at 1:2 ratio was administered *per os* daily to the rabbits for a period of fifty days. The animals in Control Group I were administered only soybean oil at the same time as the experimental animals.

The rabbits were observed regularly for clinical signs, feed and water intake. At each interval of ten days, the body weights of all the animals were recorded and two animals from the treatment group and one each from the control groups were sacrificed. All sacrificed and spontaneously dead rabbits were subjected to detailed necropsy examination. Representative tissues from the brain, lungs, heart, liver, stomach, intestines, spleen, kidneys, gonads (testes/ovaries), pancreas, adrenals and thyroid glands were collected in a 10 % buffered formol saline for detailed histopathological studies. The tissues were processed and paraffin sections (4—6 µm thickness) were stained with haematoxylin and eosin by standard procedures (6).

RESULTS

Clinical Signs

The control rabbits did not manifest any abnormal signs during the study period. In the cypermethrin treated group, the signs of toxicity were noticed from the 20th day onwards and were initially characterized by dullness, reduced feed and water intake, diarrhoea, hyperirritability followed by salivation, coarse tremors, movement of eyeballs, dilatation of pupils and weakness in the later stages. Towards the terminal stage of the experiment, paralysis of hind limbs, gasping and tonic-clonic convulsions were observed.

A decrease in the body weight was observed in the treatment group in comparison to the control group. In the treated group, there was a mortality of four animals (25 %) on the 31th, 36th, 43rd, 46th day respectively. At the end of 50th day, the animals in the treatment group showed poor body condition.

Gross Pathology

The rabbits of the treated group that died during the experimental period were emaciated and had moderate degree of congestion of the liver, kidneys, gastrointestinal tract (GIT) and brain. In the sacrificed animals, emaciation, anaemic appearance of the carcass, patchy alopecia, pronounced subcutaneous oedema and gelatinization of mesenteric fat was observed. Diffuse congestion and focal haemorrhages were seen in all the visceral organs. Liver, stomach, intestines and kidneys were the most affected organs followed by lungs, heart, spleen, gonads and brain.

Hepatomegaly with a granular appearance, rounded borders and focal congestion was observed from the 40th day onwards (Fig. 1). The gall bladder was slightly distended with thick bile along with mucosal congestion and oedema. The mucous membranes of the stomach and intestines were thickened, congested and oedematous with minute focal erosions. The renal lesions were prominent from the 30th day onwards, characterized by congestion and focal sub-capsular haemorrhages. The lungs were moderately congested and oedematous with accumulation of frothy exsudate in the bronchi in the later stages. The heart showed focal haemorrhages on the epicardium from the 40th day onwards. The spleen was moderately congested. The brain showed moderate degree of meningeal congestion at the end of the experimental toxicosis. The testes, ovaries, pancreas, adrenals and thyroid glands showed mild congestion after forty days of toxicosis.

Histopathology

In the treatment group, the liver was the most affected organ followed by stomach, intestines, kidneys,

Fig. 1. Liver — Enlarged with a rough granular appearance along with focal areas of haemorrhages and necrosis

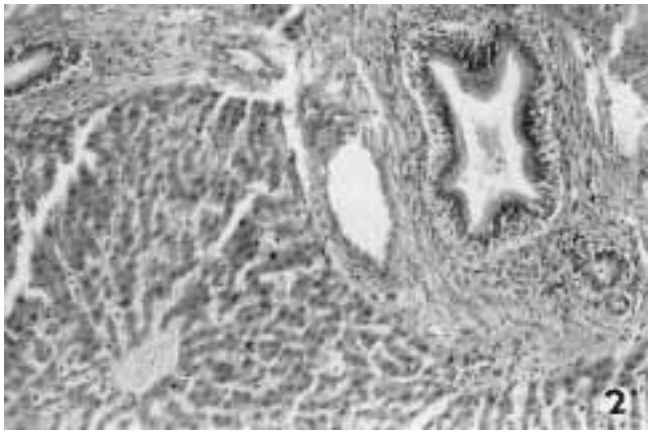

Fig. 2. Liver — Thickened portal area with periportal and perivascular fibrosis and mononuclear infiltration. H & E: × 190

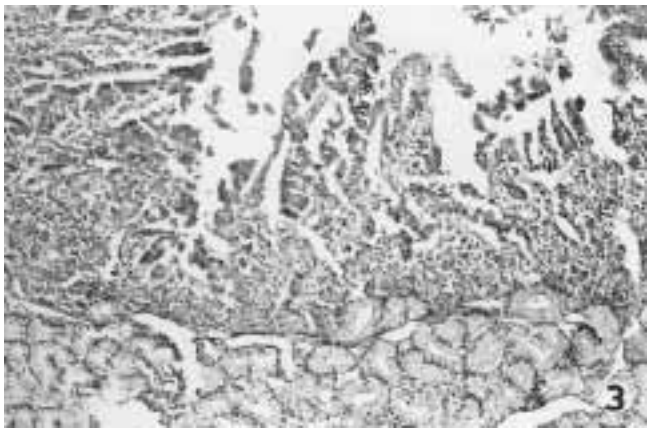

Fig. 3. Intestine — Degeneration of villous epithelium and hyperactivity of mucous glands. H & E: × 150

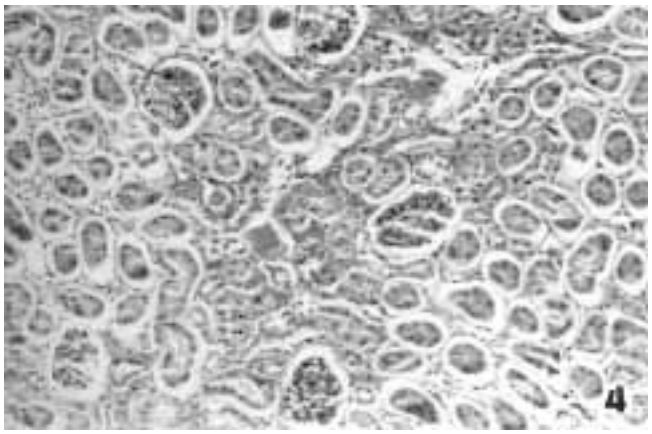

Fig. 4. Kidney — vascular congestion, focal haemorrhages and degeneration of the tubular epithelium. H & E: × 190

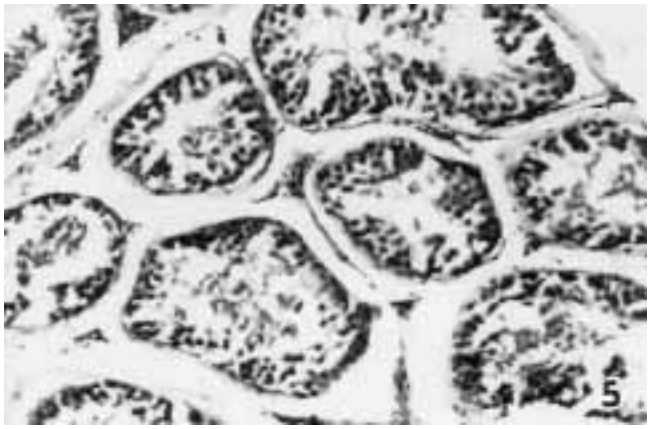

Fig. 5. Brain — Vascular congestion, a widened Virchow-Robin's space with perivascular oedema. H & E: × 190

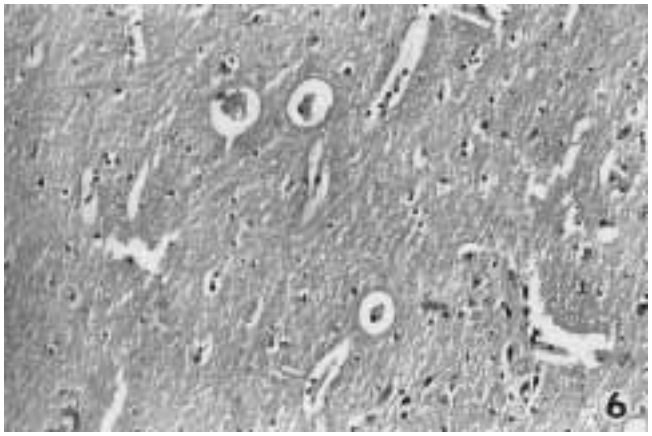

Fig. 6. Testes — Degeration/ denudation of lining epithelium with reduced spermatid population in seminiferous tubules. H & E: × 210

lungs, heart, spleen, gonads, brain, pancreas, adrenals and thyroid glands. The liver revealed generalized vascular congestion, swelling of the hepatocytes with varying degree of hydropic degeneration and mild fatty changes up to the 30th day. From the 40th day onwards, areas of coagulative necrosis were observed around the central veins. The portal areas were engorged and there was hyperplasia of the bile duct epithelium with perivascular/ periductal mononuclear cellular infiltration. A moderate degree of portal fibrosis with focal infiltration of mononuclear cells (Fig. 2) was observed at the terminal stage of toxicosis.

In GIT, initially there was thickening of the mucosa with engorged blood vessels, haemorrhages, focal heterophilic inflammatory reaction and hyperplasia of the mucus glands followed by degeneration/desquamation of the villous epithelium at the later stage. On the 50th day, the superficial epithelium of the gastric and duodenal mucosae showed denudation, erosions along with mild mononuclear cell infiltration in the submucosa (Fig. 3). The lymphoid follicles in the Peyers patches showed depletion of lymphoid cells.

In the kidneys, initially there was vascular congestion throughout the parenchyma followed by focal areas of haemorrhages and degeneration of the tubular epithelium (Fig. 4) in the later stage. After forty days of toxicosis, the renal tubules showed marked degenerative and necrotic changes in lining epithelium. Subsequently there was hyalinization of the tubular epithelium at the terminal stage of the experiment. The Bowman's spaces of glomeruli were also widened.

The lungs revealed congestion, haemorrhages and oedema with marked aggregation of mononuclear cells in the peribronchial areas. A mild level of haemorrhages and fatty changes was observed in the cardiac muscles until the 40th day. By the 50th day, focal mononuclear cells infiltration were observed in the myocardium. The spleen showed vascular congestion, mild distension of sub-trabecular sinuses. After forty days, mild capsular sclerosis along with haemosiderosis was observed. The splenic follicles appeared compressed due to the pressure of extravasated red blood corpuscules (RBC's) in the parafollicular areas. The follicles in the cortex showed degeneration and depletion of lymphoid cells. There was marked proliferation of reticuloendothelial (RE) cells in follicles and parafollicular areas.

The seminiferous tubules showed mild degeneration, detachment and denudation of epithelium along with reduction in the population of matured spermatids (Fig. 5) in the later stage of toxicosis. Vascular engorgement and mild degenerative changes in the follicular epithelium was observed in the ovaries. The brain, at the terminal stage showed a widened Virchow-Robin space along with mild perivascular oedema (Fig. 6) along with neuronal degeneration, mild satellitosis and focal glial nodule formation. The adrenal glands at the later stage of toxicosis showed capsular sclerosis, mild vacuolar degeneration of the cells in the *zona fasciculata* and mild vascular congestion in the medulla. The thyroid glands, initially revealed congestion of the blood vessels followed by a mild thickening of interfollicular space at the later stage of toxicosis. Subsequently, at the terminal stage, the follicles showed diminished colloid matter in the lumen and the acinar lining epithelial cells appeared degenerated. Diffuse vascular congestion was observed in the pancreas at the terminal stage of experiment.

DISCUSSION

The decrease in the body weight of rabbits of treatment group observed in the present study can be correlated with the reduced feed/water intake and also prolonged diarrhoea. The oral feeding of cypermethrin to Wistar rats for thirteen weeks 540 $mg.kg^{-1}$ caused decreased growth, which was correlated with decreased feed intake (31). The occurrence of diarrhoea may be due to the direct effect of cypermethrin on cholinergic nerve endings (15, 30) of the gastro-intestinal tract, which in turn increase its peristaltic movement and catarrhal enteritis.

The manifestation of nervous symptoms indicated the involvement of central nervous system (CNS) in cypermethrin toxicosis. It has been reported that the good solubility of pyrethroids in fats facilitates their absorption, spread in the body and their penetration into the nervous tissue resulting in nervous signs (5, 17). Kagan *et al. (14)* have suggested that inhibition of cholinesterase activity of the erythrocyte, liver and brain is responsible for the induction of toxic signs due to pyrethroid pesticide toxicosis.

The clinical symptoms observed in the present study corresponded with earlier reports on cypermethrin toxicosis in laboratory animals (1, 7, 11). In the experimental group, marked vascular changes in the liver, kidneys, heart, spleen, lungs and brain were observed with greater severity after thirty days of toxicosis. Changes in GIT were characterized by diffuse congestion, oedema, mucosal thickening and haemorrhages with focal erosions.

The irritant effect of pyrethroids including cypermethrin is well known (26, 4) and the changes in the gastro-intestinal epithelium might be due to the direct effect of the pesticide (23). The vascular changes are indicative of cypermethrin induced endothelial injury. Further decreased synthesis of clotting factors following hepatic damage may also have contributed to the vascular changes observed in this study.

Similar findings have also been reported earlier following pyrethroid pesticide toxicosis in goats (23, 20). Hepatic lesions observed in this study concurred with earlier findings following cypermethrin toxicity (13, 1, 23). Toukhy and Girgis (10) described an inhibitory effect of cypermethrin on the activity of the total ATP-ase in rat livers, which may disturb the active transport of Na^+, K^+ and Mg^+ ions leading to pathological changes in hepatocytes.

Studies on freshly isolated hepatocytes following cypermethrin toxicity from rats of either sex have indi-

cated reduced cell viability and increase in leakage of aspartate transaminase and alanine transaminase (10).

Although the nephrotoxic effects of cypermethrin has been reported to be low due to its rapid biotransformation and excretion (16), the changes observed in this study indicate that kidney is also a target organ of the toxic action of cypermethrin. These findings are in agreement with Ahmed (1), Luty et al. (17) following cypermethrin toxicosis in rats. Luty *et al.* (18) have also reported microfocal mononuclear cellular infiltration in liver and kidneys and parenchymatous degeneration of hepatocytes following subacute toxicity with cypermethrin in mice.

The pneumonic changes in the lungs could be due to the lymphotrophic-lymphotoxic immunosuppressive effect of the pesticide (7). Studies on adult Sprague-Dawley rats fed with cypermethrin at 18.93 and 39.66 mg/animal/day for twelve weeks caused an increase in weight of testes, vascular congestion, haemorrhages and a significant accumulation of connective tissue surrounding the seminiferous tubules, which contained a large number of immature spermatids demonstrating the adverse effect of pesticide on fertility and reproduction in male rats (9). Similar changes were observed in the present study in testes of the treatment group except for the connective tissue proliferation around the seminiferous tubules.

Pyrethroids can interact competitively with androgen receptors and sex hormone binding globulin (SHBG) causing disruption of the endocrine system by mimicking the effect of the female hormone, estrogen leading to lowered sperm counts (8, 12). Lesions in the brain observed in the present study corresponded with the findings of Ahmed (1); Tondon (24) in rats and Tamang et. al. (23) in goats following cypermethrin toxicosis. These changes might be due to anoxic damage and interference with energy metabolism of the functional cells (21) and can be correlated with clinical manifestation of nervous signs. Inhibition of acetylcholinesterase due to synthetic pyrethroid toxicosis leads to higher concentration of acetylcholine in brain affecting adversely the functions of nerve cell in brain substance (3).

The present study concludes that continuous and prolonged oral administration of cypermethrin @1/10 LD50 dose level beyond forty days results in cumulative toxicosis leading to patho-anatomical alterations in liver followed by kidneys, heart, spleen, stomach/intestines and brain and the experimental design in rabbits can be suitably utilized as a model for studying effect of pesticide in other species of animals, birds and human beings.

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A PHARMACOKINETIC STUDY OF SULPHADIMIDINE (SULPHAMETHAZINE) ALONE AND AFTER ITS POTENTIATION IN RABBITS

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ABSTRACT

The influence of trimethoprim administration as a potentiator of sulphadimidine in the blood plasma of twenty-four rabbits was investigated. The four groups of rabbits (A, B, C, D) were administered sulphadimidine. Group A – 30 mg.kg^{-1} b.w. orally (p.o.), Group B – the same dose intramuscularly (i.m.), Group C – 100 mg.kg^{-1} b.w. p.o., Group D – the same dose i.m. The other four groups of rabbits (AP, BP, CP, DP) were administered sulphadimidine with trimethoprim in a dose 30 mg.kg^{-1} b.w. of sulphadimidine and 6.4 mg.kg^{-1} b. w. of trimethoprim p.o. or i.m. (groups AP and BP, respectively) and 100 mg.kg^{-1} b.w. of sulphadimidine with 21.3 mg. kg^{-1} b.w. of trimethoprim p.o. and i.m. (groups CP and DP, respectively). Blood samples were withdrawn at one, three, six, nine and twenty-four hours after each administration of medication. The comparison of sulphadimidine levels in the experimental groups revealed a statistically significant increase ($P < 0.05$) only in the groups with higher doses of potentiated sulphadimidine, which can be ascribed to the influence of the drug interaction in the place of binding of agents on plasmatic proteins of rabbit. However, further investigation is proposed.

Key words: rabbit; sulphadimidine; trimethoprim

INTRODUCTION

Sulphonamides are one of the oldest groups of antimicrobial compounds. They were discovered as derivatives of the azo-dye Prontosil (sulphanilamide). Sulphonamides have been in clinical use for nearly seventy years and their mechanism of action is well-known. They act as antimetabolites of folic acid of micro-organisms, which must intracellularly synthesize their own folic acid. In this way, by competitive substitution of p-aminobenzoic acid, sulphonamides interrupt the metabolic pathway of the normal production of RNA, protein synthesis and microbial replication mechanisms.

Sulphonamides have little effect on mammalian cells, because they can utilize preformed folic acid.

Susceptible organisms include many bacteria, coccidia, chlamidia and protozoal organisms including *Toxoplasma* spp. (11). Sulphonamides are used in veterinary medicine to treat both microbial and protozoal infections of the urinary, gastrointestinal and central nervous systems. Because they are bacteriostats, the patient's own resistance and body defences must be encouraged concurrently with sulphonamide therapy.

Sulphonamides have been largely displaced in therapy by antibiotics in recent years, but interest in the sulphonamides continues, especially after the discovery of their anticarbonic anhydrase, diuretic, hypoglycaemic, and antithyroid activity among others. The large number of structurally novel sulphonamide derivatives have ultimately been reported to show substantial protease inhibitory properties (12).

Sulphadimidine (sulphamethazine) is one of the widely used systemic sulphonamides in many species of animals such as

cattle, horses, swine, poultry, small ruminants and rabbits. It is used in drinking water, as a feed additive and in injectable form by itself and in combination with other antimicrobial drugs.

The combinations of sulphonamides with other antimicrobial drugs from the group of diaminopyrimidines – most commonly with trimethoprim – are termed "potentiated sulphonamides". The mechanism of diaminopyrimidines action can by explained by the block of synthesis of tetrahydrofolic acid from dihydrofolic acid thanks to the competitive inhibition of the enzyme dihydrofolate reductase. The effect of the combination of sulphadimidine with trimethoprim is characterized by the two step blocking of folic acid metabolism in pathogens and the combination becomes bactericidal and so increases the spectrum of antimicrobial activity.

The absorption rates of different sulphonamides are proportional to the water solubilities of drugs. Soluble sulphonamides are widely distributed throughout the body and into many soft tissues, including cerebrospinal and synovial fluids. Sulphonamides show great variability in the extent of their binding to plasma proteins with respect to individual drugs and species.

The aim of our work was to study the influence of sulphadimidine potentiation with trimethoprim upon its pharmacokinetics after oral and intramuscular administration in two different doses in the blood plasma of rabbits.

MATERIAL AND METHODS

The experiments were carried out on twenty-four female rabbits of the breed *Chinchilla gigantica* with average weight 3.64 kg, one year old, divided into eight experimental groups designated as groups A, B, C, D, and AP (P = potentiated), BP, CP and DP, with three animals in each group.

The animals were kept individually in cages. The standard hygiene in the housing corresponded to the principles of The European Convention on Animal Protection (9).

The animals were given a commercial food mixture and drinking water *ad libitum.*

In groups A, B, C, and D was administered *sulphadimidinum natricum* only, for *group A* in the dose 30 mg.kg^{-1} b. w. orally in the form of 10 % water solution, for *group B* the same dose in the form of 20 % injectable solution intramuscularly into the thigh muscles of the animals. *Group C* was administered sulphadimidine in the dose 100 mg.kg^{-1} b.w. orally and in group D the same dose of the same drug, but intramuscularly.

In groups AP, BP, CP and DP sulphadimidine was administered potentiated with trimethoprim in the pharmaceutical preparation Duon plv. a. u. v. containing *sulphadimidinum natricum* 20 g and *trimethoprimum* 4 g in 100 g of preparation orally and in the pharmaceutical preparation Duon inj. a. u. v. containing *sulphadimidinum natricum* 37.6 g and *trimethoprimum* 8 g in 100 ml of injectable solution for intramuscular administration. *Group AP* was administered sulphadimidine in a dose 30 mg.kg^{-1} b. w. in combination with trimethoprim in a dose 6.4 mg.kg^{-1} b. w. orally in 10 % concentration, in *Group BP* the same dose of sulphadimidine was administered with trimethoprim as an intramuscular injection. *Group CP* was given sulphadimidine in a dose 100 mg.kg^{-1} b. w. with trimethoprim in a dose 21.3 mg.kg^{-1} b. w. orally, and *Group DP* was administered the same dose of potentiated sulphadimidine in the form of intramuscular injection.

Blood samples were collected from v. auricularis marginalis at one, three, six, nine and twenty-four hours after administration of the drugs. The blood plasma obtained after fifteen minutes centrifugation at 3000 rot/min was used for the determination of the directly reacting sulphadimidine concentration using the colorimetric method described by Berecký (3). The colour intensity of the samples was evaluated photocolorimetrically using a Specol 11 (Jena, Zeiss) digital spectrophotometer at wavelength 470 nm.

The results were analyzed by the program Statgrafic with PC computer Celeron – Graphics Series G 70 fm.

RESULTS

The results of our experiments are presented in figures 1 and 2. After the oral administration of medicines (Fig. 1) the highest concentrations of directly reacting sulphadimidine were measured 3 hours after administration and reached after a dose 30 mg.kg^{-1} b. w. 0.78 ± 0.55 mg.100 ml^{-1} blood plasma. After a dose 100 mg.kg^{-1} of nonpotentiated and potentiated sulphadimidine it was 1.80 ± 0.74 and 2.83 ± 1.28 mg.100 ml^{-1} blood plasma, respectively. The increase between groups C and CP was statistically significant ($P < 0.05$). The peak of sulphadimidine concentration after the smaller dose was only 0.36 ± 0.06 mg.100 ml^{-1} blood plasma in the first hour after the administration of the medicine. The most pronounced decrease of sulphadimidine concentration was observed in the time interval three to six hours and after twenty-four hours the mean concentration was 3.9 times smaller than at peak concentration. Our results demonstrated that the plasma concentration of sulphadimidine in groups after the administration of potentiated sulphadimidine were relatively higher.

After the intramuscular administration of medicines (Fig. 2 the maximal concentrations of directly reacting sulphadimidine) were registered in first hour of experiment. The concentration was after a dose 30 mg. kg^{-1} b. w. of nonpotentiated and potentiated sulphadimidine 0.53 ± 0.43 and 0.33 ± 0.16 mg in 100 ml of blood plasma, respectively. After the highest dose 100 mg.kg^{-1} the concentration of sulphadimidine was 3.23 ± 1.56 and 4.08 ± 1.14 mg.100 ml^{-1} blood plasma, respectively, but the increase was not statistically significant. The most pronounced decreases in sulphadimidine concentrations were between the first and third hour of experiment. The mean level of sulphadimidine after twenty-four hours was nine times smaller, than in the first hour. After the higher dose 100 mg.kg^{-1} b.w. there was at this time also a higher level of sulphadimidine in the group after the simultaneous administration of sulphadimidine and trimethoprim.

No significant changes in clinical status of animals during the experiments were registered.

DISCUSSION

To follow up previous studies (2, 4, 5) we made an experimental effort to study the pharmacokinetics of sulphadimidine and its combination with potentiator trimethoprim in rabbits. The rabbit has a special importance for kinetical studies of sulphonamides, because it has the highest binding of sulphonamides to proteins in blood plasma. High protein binding markedly increases the half-life of sulphonamides, however, only the non-ionized and non-protein-bound part of the drug is pharmacologically active (11).

Table 1. The binding of some sulphonamides to plasmatic proteins in different species of animals in % (13)

Species of the animal	Sulpha-dimidine	Sulpha-diazine	Sulpha-merazine	Sulpha-mono-methoxine
Cattle	**80**	**15**	**55**	**55–60**
Horse	**60**	**20**	**25–35**	**55–60**
Pig	**80**	**25**	**55**	**55–60**
Dog	**60**	**25**	**25–35**	**55–60**
Poultry	**25**	**35**	**55**	**45**
Rabbit	**90**	**85**	**95**	**95**

Sulphonamides are excreted through the kidneys, in faeces, bile, milk, sweat and tears as a parent compound or as metabolites.

The pharmacokinetic aspects of sulphadimidine depends on many factors, such as the species, the age, the weight, sex, the health of animal, the method of administration, the dose and the frequency of drug administration.

In the *in vitro* tests difference due to sex was evident in the metabolisation of sulphadimidine, when in male rat hepatocytes a significantly higher hydroxylation activity was observed and acetylation activity was higher in females (15).

For the elimination of the influence of species and sex, rabbits of the breed *Chinchilla gigantica were used in our experiment, only females, aged one year, the weight of animals was in the range of 3.1–4.5 kg.*

The simultaneous occurrence of disease also exerts an influence upon the pharmacokinetics of the drug. The clearance of sulphadimidine was decreased markedly and the half-lives of the drug were increased in Streptococcus suum infected pigs (17). The half-life of sulphadimidine was prolonged significantly also after experimental infection with *Fasciola gigantica* in sheep, clearance was decreased five and nine weeks after infection (8). In rabbits the pharmacokinetics of sulphadimidine dependent on health was studied after infection with *Pasteurella multocida* and the results suggest that the blood concentration of sulphadimidine was affected (16). The study of sulphadimidine tissue residues shows that in rabbits infected with *E. stiedai* the withdrawal period was three days longer than in healthy rabbits (1).

However, our animals were clinically examined and clinically healthy animals were used for the experiment only.

Acetylation, glucuronide conjugation and aromatic hydroxylation occurring to the varying degress are the pathways by which sulphonamides are metabolized in animals (11). In a comparative *in vitro* study sulphadimidine metabolism was investigated in rat, goat, sheep and cattle hepatocytes. In goats, sheep and cows the hydroxylation pathway is important, whereas in rat acetylation is predominant (14). The binding of sulphadimidine to pig plasma proteins is weak and the main binding protein is albumin (10).

Potentiated sulphonamides have the desirable property of reducing the MIC of sulphonamides, which results in smaller doses and thereby a reduction in the total dose of drug administered to the animal (6). The simultaneously administered drugs interact with sulphadimidine at different levels. The *in vitro* interactions are known as incompatibility, the well known physico-chemical properties of drugs allow us to predict these interactions. Interactions which develop within the organism are more complex and require experimental studies. Two drugs may interact *in vivo* in their reabsorption, distribution, binding to blood proteins, passage across cell membranes, bio-transformation, as well as during their elimination from the organism (4). For example after the premedication of horses by flunixin the sulphadimidine elimination half-life decreased by 21 % (7).

In our experiment a significant increase of directly reacting sulphadimidine was observed only after oral administration of the highest dose of potentiated sulphadimidine (simultaneous administration of 100 mg of sulphadimidine and 21.3 $mg.kg^{-1}$ b. w. of trimethoprim), which is probably caused by a competitive interaction of sulphadimidine and trimethoprim in binding with blood plasma proteins. As after intramuscular administration of the same doses the results were not statistically significant, some further investigations are proposed.

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THE EFFECT OF FORAGE PARTICLE SIZE ON CELLWALL-FRACTION DIGESTIBILITY IN SHEEP (A short communication)

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ABSTRACT

In a cross-over trial with six wethers the prediction was tested that forage particle size would not affect the digestibility of crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF). However, it appeared that reducing particle size decreased the digestibily of NDF and ADF. Possibly, an increase in rumen passage rate after the reduction in forage particle size caused the observed decrease in apparent fibre digestibility.

Key words: ADF; crude fibre; digestibility; NDF; particle size; sheep

INTRODUCTION

It is generally accepted that the digestibility of crude fibre from roughage is reduced when pelleted concentrates are incorporated into the ration of lactating cows (2). The decrease in crude fibre digestibility might be explained, at least partly, by a reduced rumen pH which inhibits the activity of cellulolytic bacteria (2, 3).

The observed decrease in rumen pH can be explained by either an increased rate in fermentation and/or a reduced saliva production. However, rumen pH was not affected in dry cows when they were fed iso-energetic rations containing 60 *versus* 0 % concentrate (6) or 20 *versus* 2 % corn starch (5).

Thus, at a low plane of nutrition rumen pH does not seem to respond to the physical form or the chemical composition of the ration. Consequently, the digestibility of crude fibre may not be affected by pelleting the whole ration when fed at a low plane of nutrition. In other words, forage particle size may not influence the apparent digestibility of crude fibre. This suggestion was put to the test in the current experiment.

MATERIALS AND METHODS

Six, two year-old, ruminally fistulated Dutch Black Blaze wethers, with a mean initial body weight of 75 kilograms were used. The trial had a 22 × 22 day cross-over design. The sheep were randomly assigned to the order of the two treatments. During the experiment, the sheep were housed individually in pens with a layer of wood shavings as bedding, or in metabolism cages with a slatted floor.

During the experimental periods, the sheep were fed either 200 grams of concentrate and 700 grams of artificially dried grass (Control) or 900 grams of a completely pelleted ration (Test). The ingredient and analyzed composition of the diets are shown in Table 1.

On an energy basis (NE_l) the rations provided a sufficiency for maintenance. The sheep were fed twice daily in two equal portions at 9:00 and 16.00. During the experiment the sheep were allowed *ad libitum* access to water. The experimental feeds were sampled during each period, ground and subsequently

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Table 1. The ingredient and analyzed composition of the experimental rations

	Control	Test
Ingredient composition		
	(g.day^{-1})	
Artificially dried grass	700	–
Pelleted concentrate	200	–
Pelleted whole ration	–	900[1]
Analyzed composition of whole ration		
	(g. kg^{-1} dry matter)	
Crude protein	211	210
Crude fibre	235	213
Crude fat	48	62
Neutral detergent fibre	531	440
Acid detergent fibre	239	226
Lignin	24	24

[1] — The test ration contained 700 grams of artificially dried grass and 200 grams of concentrate, feedstuffs were ground (≤ 1 mm), mixed, and pelleted

stored in a sealed jar at room temperature. During the last eight days of each experimental period, faeces were collected quantitatively. The twenty-four hour collections were stored at –18 °C in plastic bags.

At the end of each experimental period, faeces collections were pooled per wether and mixed thoroughly. Two samples, each representing 20 % of total faeces from each wether for each period, were dried for five days at 60 °C, ground and stored in a sealed jar at room temperature. On day 22 of each experimental period, rumen fluid pH was recorded prior to feeding at 8.30 and after feeding at 10.00, 11.00 13.00, 15.00 and 17.00.

The nitrogen contents of feedstuffs were determined by the macro-Kjeldahl method; a factor of 6.25 was used to convert grams of N into crude protein. Ether extracts of the feedstuffs were prepared; the solvent was evaporated and the crude-fat residue was weighed. The crude fiber contents and the contents of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the feedstuffs and faeces were estimated using the Fibertec System M2 (Tecator, Stockholm, Sweden). The accuracy of each assay run was monitored using a commercial reference sample (hay powder, CRM 129; Community Bureau of Reference, Brussels, Belgium). The combined within and between-run precision of the determinations was ≤ 3 % (coefficient of variation).

RESULTS

The digestibility (% of intake) of crude fibre, NDF and ADF was significantly lower after feeding the test ration (Table 2). Mean post-feeding values of rumen pH were numerically lower when the test ration was fed, but the difference with the control was not statistically significant (Table 2).

DISCUSSION

In contrast to our suggestion, pelleting of the whole ration significantly reduced the digestibility of crude fibre, NDF and ADF which was not related to rumen pH. It might be speculated that pelleting of the whole ration increased the passage rate of feed particles, thereby effectively reducing degradation time in the rumen. Consequently,

Table 2. The intake, faecal excretion and digestibility of crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF) and mean post-feeding rumen pH in sheep (n = 6) after feeding the experimental rations

	Control	Test			
	Mean	SE	Mean	SE	P-value*
Crude fibre					
Intake (g.day^{-1})	193	nd	173	nd	nd
Faecal excretion (g.day^{-1})	73	7.1	87	5.5	0.028
Digestibility (% of intake)	62.0	3.68	49.8	3.17	0.003
NDF					
Intake (g.day^{-1})	437	nd	357	nd	nd
Faecal excretion (g.day^{-1})	117	9.3	136	8.1	0.028
Digestibility (% of intake)	73.2	2.13	61.9	2.23	0.001
ADF					
Intake (g.day^{-1})	197	nd	184	nd	nd
Faecal excretion (g.day^{-1})	81	7.2	94	5.5	0.028
Digestibility (% of intake)	58.6	3.64	49.0	2.97	0.008
Rumen fluid pH	6.41	0.094	6.22	0.059	0.209

* — Students paired *t*-test

nd — not determined because the animals were fed a restricted amount of feed

more feed particles may have escaped rumen fermentation when the test ration was fed (1, 2, 8).

It has been shown that hindgut fermentation may compensate for a reduced rumen fermentation when particle size of the ration is reduced (7). Clearly, an increase in hindgut fermentation cannot be excluded when the test ration was fed, but it did not completely counteract, if any, the reduction in rumen fermentation because there was a reduction in total digestive-tract digestibility of crude fibre, NDF and ADF. It has been shown that the activity of cellulolytic bacteria is inhibited when rumen pH becomes < 6. (2,9). In our study, rumen pH did not drop to such low values. The current observation that rumen pH is not lowered to values < 6 after the feeding of a completely pelleted ration, is corroborated by earlier observations of Ram *et al*. (4). It may be suggested that the rate of absorption of volatile fatty acids (VFA) was in proportion with the rate of production of VFA. Alternatively, it can be speculated that baseline production of saliva may have been high enough to prevent a drop in rumen pH.

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THE LOCALIZATION OF S-100 PROTEIN IN THE BOVINE SPLEEN

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ABSTRACT

An indirect immunohistochemical method was used to demonstrate S-100 protein in the bovine spleen. For this purpose a rabbit antiserum and immunoperoxidase technique was used. Three components were positive for S-100 protein – follicular dendritic cells and interdigitating cells, nerve fibres and endothelial cells. The presence of the nerve bundles was observed in the splenic hilus and capsule, fine nerve fibres in the splenic trabeculae and in the white pulp. In the hilus the main trunk of nerve fibres were seen accompanying the main blood vessels, whereas in the trabeculae preferentially fine nerve fibres were seen singly distributed among the smooth muscle cells. In the white pulp S-100 protein nerve fibres were observed both in T-lymphocytic area and in B-lymphocytic area. The fine nerve fibres were located around the central artery of the periarteriolar lymphatic sheath and lymphatic follicles. A specific immunoreaction was found in the dendritic cells located both in the periarteriolar lymphatic sheath and germinal centers of secondary lymphatic follicles. Besides, S-100 protein was expressed in the endothelial cells of the blood vessels in all portions of the splenic blood circulation.

Key words: bovine spleen; immunohistochemistry; S-100 protein

INTRODUCTION

A positive reaction to S-100 protein has been found in the lymphatic organs of a number of mammalian species. Various types of cells have been detected by this protein. Ramis *et al.* (24) have made an immunohistological and enzyme histochemical study of pig lymphoid tissue. S-100 protein was detected in follicular dendritic cells (FDCs) in the lymph nodes and spleen. The authors observed the expression of S-100 protein mainly in the T-lymphocytic area, but also in some follicles of the spleen. Cocchia *et al.* (8) have found that S-100 protein was confined to the FDCs in the lymph nodes and spleen, which are known to be exclusively associated with B-lymphocytes in secondary follicles. Liu *et al. (19)* have characterized FDCs as stromal cells unique to primary and secondary lymphoid follicles.

A specific immunoreaction product was found in the distinctive cell types located in the germinal centers and adjacent mantle region of secondary follicles. Chen and Steinman (9) and Mandel *et al.* (20) have reported that these cells were associated exclusively with germinal centers. Atoji *et al.* (2) have observed S-100-immunoreactive giant macrophages in the lymphoid tissues of the guinea pig. S-100-immunoreactive giant cells were dendritic in shape with one or two extending cell processes located at the marginal zone of the spleen.

S-100 protein has also been used to demonstrate the nerve components in the lymphatic organs, which is considered to be a specific antibody for the glial elements of nervous tissue (6, 7). Numerous researchers have paid attention to its distribution to nervous components in the lymphatic organs

after using histochemical methods (1, 12, 13, 14, 18, 23, 24) or electron microscopic methods (11). In these studies the presence of autonomic nerves in the specific regional sites of the mammalian primary and secondary lymphoid organs has been demonstrated. Recently, immunohistochemical methods and S-100 protein antibody were used to demonstrate the innervation and circulatory splenic system (21,22,28).

The present paper reports on the immunohistochemical distribution of S-100 protein in the bovine spleen. The S-100 protein antiserum was used to provide information about the location of the nerve fibres and the follicular dendritic cells.

MATERIAL AND METHODS

The spleen of six cattle were used in this study. Samples of tissues were fixed in 0.1 mol phosphate-buffered 10 % formaldehyde for twenty-four hours at room temperature, dehydrated and embedded in paraffin. Sections of thickness 5 μm were prepared and processed by the avidin-biotin-peroxidase complex (ABC) method (15). Sections were deparaffinised in xylene and treated with l.5 % hydrogen peroxide in methanol for thirty minutes to eliminate the endogenous peroxidase reaction. Sections were pretreated with 3 % H_2O_2 to reduce endogenous activity and preincubated with 2 % goat serum to mask nonspecific binding sites. Sections were then incubated overnight with the first polyclonal antiserum to bovine S-100 protein antibody (Immunotech), dilution 1:100. Afterwards, the sections were washed in PBS, incubated with biotinylated secondary antibody and after-incubated with avidin-biotin-peroxidase complex (Vectastain, ABC kit; Vector, Burlingame CA, USA). Then the sections were washed with PBS and visualised with 0.05 % 3´.3´-diaminobenzidine (DAB) and 0.03 % H_2O_2 (v/v). Some sections were counterstained with Mayer's haematoxylin. Negative controls were obtained by omitting the primary antibody.

RESULTS

The S-100 protein positive nerve bundles were observed in the hilus area where they, together with blood vessels, entered the spleen. Smaller nerve bands and fine nerve fibres were seen in the splenic capsule running longitudinally in connective tissue components (Fig. 1). Finer nerve bands were located just beneath the capsule. Fibres entered the spleen parenchyma with the trabeculae nerve. In the trabeculae, fine nerve fibres run among the smooth muscle cells and connective tissue fibres. More positive nerve fibres were seen located in their peripheral area (Fig. 2).

Inside the parenchyma the expression of S-100 protein positive nerve fibres was observed next to the trabeculae in the surrounding parenchyma. The accumulation of the nerve fibres in relation to blood vessels was confined to the T-lymphocytic area and B-lymphocytic area. Inside the T-lymphocytic area the presence of fine nerve fibres was seen next to the arterial wall inside the PALS. Inside the B-lymphocytic area nerve fibres were seen more conspicuously and were present around the *a. centralis* of the lymphatic follicles. The nerve fibres made a remarkable ring on the periphery of the central artery. In the red pulp no continuous nerve fibres were found though punctuate profiles were found in the red pulp

In an additional location, outside the nervous system, S-100 protein was detected in the follicular dendritic cells (Fig. 3). A specific immunoreaction product was found in this distinctive cell type located both in the PALS and in germinal centers and adjacent mantle region of secondary follicles. No immunoreaction was detected in the other cell types present in secondary follicles as small lymphocytes, lymphoblasts, macrophages, plasma cells or stromal cells present outside the secondary follicles.

A strong positive reaction was seen in the endothelial cells of the blood vessels. This reaction allowed us to follow the distribution of the blood vessels inside the splenic parenchyma. The strongest reaction for S-100 protein was seen in the central follicular arteries and branches of the penicillar arteries (Fig. 4).

DISCUSSION

Three components were positive for S-100 protein-nerve fibres, FDCs and endothelial cells of blood vessels. The affinity of S-100 protein to Schwann cells in the peripheric nerve fibres allowed us to follow the distribution of the nerve fibres inside the lymphatic tissue in the bovine spleen. Immunostaining with S-100 protein demonstrated the presence of thick nerve bands and fine nerve fibres. In the bovine spleen thick nerve bands were seen in the hilus next to the splenic artery and their branches. The accompanying branches of the splenic artery nerve fibres entered the splenic parenchyma and with the blood vessels were distributed in the white pulp. Most of the spleen nerve fibres positive for S-100 protein were found in the perivascular area, particularly the trabecular arteries and arterioles of the white pulp. This way of nerve distribution has also been described in other animal species (26, 27).

The presence of a positive reaction to S-100 protein in the red pulp was not found constantly, though many dott-like and fibrillar positive structures were observed in the red pulp. These structures were as equally stained as the nerve fibres in the area of the white pulp. For this reason we suppose that fine nerve fibres were also present in the red pulp.

An additional location for S-100 protein, outside the nervous system, was confined to the interdigitating cells and to follicular dendritic cells. The IDCs are known to be exclusively associated with T-lymphocytes of the PALS while the FDCs are confined to B-lymphocytes of the primary and secondary follicles of the spleen. In the bovine spleen the reactive cells in T-lymphocytic area were less numerous than in the B lymphocytic area. The central position of the FDCs, similar to human, rat

Fig. 1. Section through the capsule (C) and adjacent zone. Thicker bands and fine nerve fibres are seen inside the capsule (× 280)

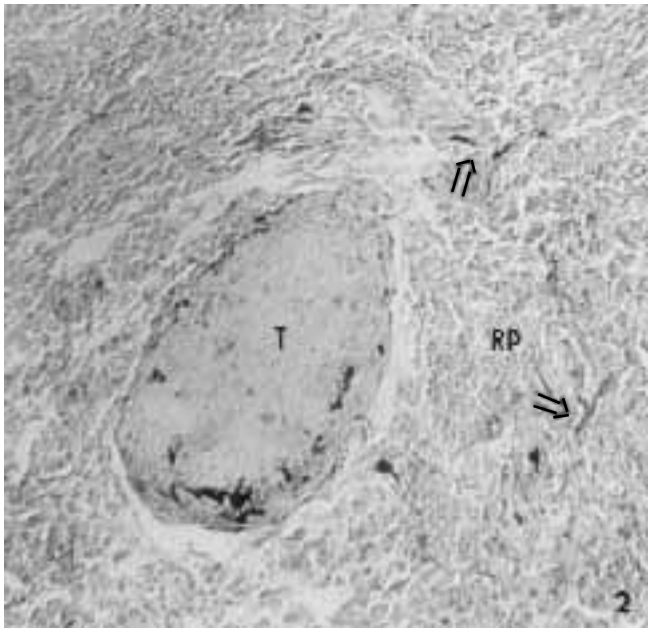

Fig. 2. Section though the splenic trabeculae (T) and the surrounding red pulp (RP). Fine nerve fibres are located at the periphery of the trabeculae. In the surrounding red pulp short fine nerve fibres are seen *(double arrows)* (× 280)

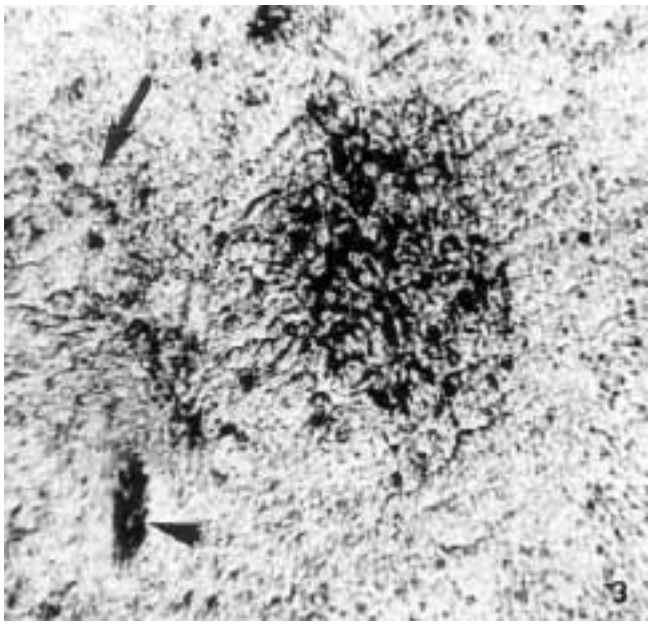

Fig. 3. Section through the lymphatic follicle. A strong positive reaction in the follicular dendritic cells is in the germinal center. A few positive cells (arrow) are in the mantle zone. Positive reaction is also in the endothelial cells of the surrounding arteriole *(arrowhead)* (× 345)

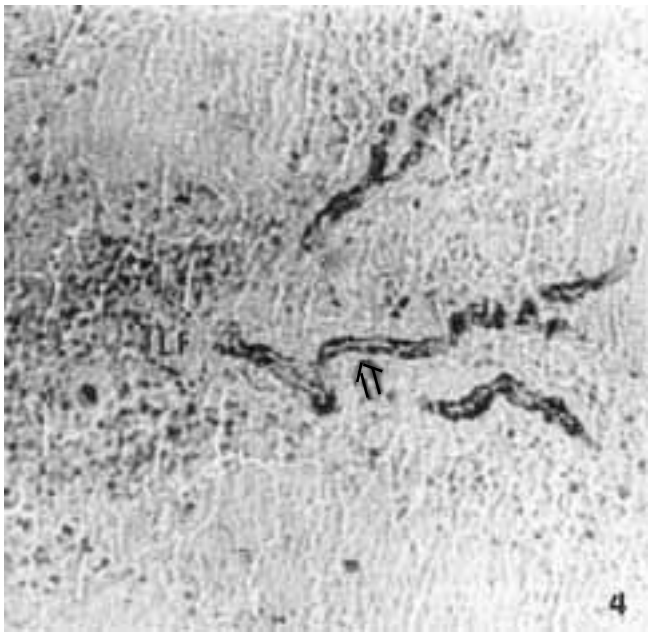

Fig. 4. Section through the lymphatic follicle (LF) and branching penicillar arterioles. Endothelial cells of the arterioles are strongly positive *(double arrows)* (× 280)

and mice spleens, has been observed by Kaiserling *et al.* (17).

According to Eikelenboom *et al.* (10) the follicles contain follicular dendritic cells, which are capable of arresting antigen-antibody complexes at the cell surface. In mammals the antigen-retaining dendritic reticular cells have been found exclusively in the light zone of the follicle center where they are often in close connection with capillaries. Though the presence of FDCs in the germinal center of lymphatic follicles was constant, the presence of FDCs in the mantle zone was not always confirmed. Chen and Steinman (9) and Man-del *et al.* (20) have reported that these cells are associated exclusively with germinal centers. Cocchia *et al. (8)* have noted that S-100 protein is confined to the FDCs both in the lymph node and the spleen. The authors have found a specific immunoreaction product in the distinctive cell types located in both the germinal centers and adjacent mantle region of secondary follicles. The high level of S-100 protein-positive in the B-lymphocytic area of lymphatic follicles that we observed in the bovine spleen was confined to follicular dendritic cells in the central area both of primary and secondary lymphatic follicles.

Carbone et al. (5) in human lymphoid tissues have made a comparative immunohistologic study of the cell density and distribution pattern of follicular dendritic reticulum cells within their follicular micro-environments (germinal centers and mantle zones). Their results consistently indicate that DRCs positive for S-100 protein are confined to the central and pericentral portion of germinal centers. In the bovine spleen, the presence of the FDCs in this area was seen to be constant and only a few FDCs were found in the marginal zone of the follicle. Despite this finding we hypothesise that their presence in this area is not stable and reflects the reactive state of the splenic follicle. In our study, as in the study of Chen and Steinman (9) and Mandel *et al.* (20), the immnunoproduct was widely distributed in both nucleoplasm and cytoplasm. No immunoreaction was detected in the other cell types present in secondary follicles as small lymphocytes, lymphoblasts, macrophages, plasma cells or stromal cells present outside the secondary follicles as they have been observed by Ramis *et al.* (24) and Atoji *et al.* (2).

Other S-100 protein positive structures were the endothelial cells of the blood vessels. This reaction was seen in all types of blood vessels. Atoji and Suzuki (3) have observed in the spleen immunoreactivity in the endothelial cells of the trabecular artery, central artery, trabecular vein and lymph vessels. These findings suggest that S-100 protein of the vascular system is perhaps related to the flow of lymph and blood, namely to the blood cells transported inside the blood vessels.

Imai and Yamakawa (16) have described that in each of five follicular zones the FDCs have distict ultrastructural features, reflecting the different three-dimensional structures and functions of these zones. It could indicate that different types of these cells may exist. New evidence suggests the presence of two types of dendritic cells within germinal centers in the human spleen (19). It is becoming increasingly clear that dendritic cells are not a homogenous cell population, but comprise different subpopulations that differ in ontogeny and function. In the bovine spleen it was not possible to identify more types of reactive cells of FDCs.

Dendritic cells which are present in many tissues, play a critical role in the initiation of the immune response by presenting antigens to T- and B-lymphocytes (25). Caminschi *et al.* (4) have characterized the dendritic cells as potent antigen presenting cells that activate naive T-cells. According to Carbone *et al.* (5) consistent heterogenity of the labelling patters appears to suggest a possible in situ immunoheterophenotypic grouping of dendritic follicular cells, and the concept of their possible heterogeneity appears to be corroborated. Liu *et al.* (19) have stated that recirculating resting B-cells migrate through the FDCs networks, whereas antigen-activated B-cells undergo clonal expansion within the FDCs networks in T-cell-dependent fashion, thereby generating germinal centers. There is evidence, derived from cultures *in vitro,* indicating that FDCs contribute directly to the survival and activation of peripheral B-cells. Finally, FDCs appear to be involved in the growth of follicular lymphomas and in the pathogenesis of HIV infection.

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THE DISTRIBUTION OF S-100 PROTEIN IN THE CAT THYMUS

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ABSTRACT

The present study deals with the presence of S-l00 protein positive structures in adult cat thymuses detected by using the immunohistochemical method. An attempt was made to follow this protein in relation to the interdigitating cells and Hassall's corpuscles. A strong positive reaction to S-l00 protein was found in the interdigitating cells present in a different density in the medulla zone and in close relationship to Hassall's corpuscles. The size and shape of the positive cells varied, some of them resembling giant macrophages. A faint to moderate reaction to S-l00 protein was found in the epithelial cells of Hassall's corpuscles and membranous cystic structures localized in the medulla region. Differences in the reactions of Hassall's corpuscles were remarked in unstained core and stained peripheral epithelial cells. A positive reaction was expressed by the nerve fibres surrounding the larger and smaller blood vessels localized in the interlobular trabeculae and in the *medulla* close to Hassall's corpuscles.

Key words: cat thymus; immunohistochemistry; S-l00 protein

INTRODUCTION

S-l00 protein was first described in the nervous tissue (13) and later in various other tissues including the lymphatic tissue. It has been identified in the human lymph node (18), in the rat lymph node and spleen (9). S-100 protein has been localized in the pig and horse spleen (19), sheep spleen (l1), in canine and caprine lymph nodes (17), in the bovine lymph node and spleen (2) and in various lymphatic organs.

Studies dealing with the presence of S-l00 protein in the thymus have been carried out mainly on humans. S-l00 protein positive interdigitating cells have been described in the normal thymus (20), Down's human thymi (l) and bovine foetus (4). In animal species a positive reaction to S-l00 protein has been observed in the *cortex* and the *medulla.* In the pig thymus positive immunoreaction has been localized in epithelial reticular cells, in myeloid cells, polynucleated giant cells and some macrophages. In the *medulla,* besides the epithelial cells of Hassall's corpuscles, positivity to S-l00 protein has also been observed in some lymphocytes (16). In the guinea pig thymus, S-100 immunoreactive giant cells have been described by Atoji *et al. (3).*

The present paper report is on the immunohistochemical distribution at a light microscopic level of S-l00 protein-positive cells in the cat thymus.

MATERIAL AND METHODS

The experiment was performed on five cats, two to four years old. The samples of thymi were fixed in 10% formaldehyde in 0.1 mol phosphate buffer for twenty-four hours at room temperature and routinely embedded in paraffin. Serial sections were cut at 5 μm thickness and mounted on slides coated with poly-L-lysine. The sections were deparaffinised and rehydrated

for immunostaining using the avidin-biotin-peroxidase complex (ABC) method (8). The sections were pretreated with 3 % H_2O_2 in PBS to reduce endogenous activity, preincubated with 2 % goat serum to mask unspecific binding sites. Afterwards the sections were incubated at 4 °C overnight with a polyclonal antiserum for S-l00 protein (Sigma) diluted 1:100. The sections were washed twice in a phosphate-balanced salt solution (PBS) and then incubated with biotinylated anti-rabbit IgG secondary antibody for forty-five minutes washed in PBS and then incubated with avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector, Burlingame Calif., USA). After washing with PBS peroxidase activity was visualized with 0.05 % 3´.3´-diaminobenzidine (DAB) and 0.03 % v/v H_2O_2. Some sections were counter-stained with Mayer's haematoxylin. Thereafter the sections were dehydrated in ethanol and mounted with synthetic resin (DPX; Fluka, Switzerland). Negative controls were performed by omitting the primary antibody.

RESULTS

A positive reaction to the S-l00 protein in the cat thymus was expressed in the medullary region and was related to the interdigitating cells (IDCs) and epithelial cells of Hassall's corpuscles. The immunoreactive IDCs occur singly or in groups containing a few cells and were distributed irregularly in the *medulla* generally not far from Hassall's corpuscles. No rule for their location in relation to Hassall's corpuscles was observed. The positive interdigitating cells were stellate in shape and displayed a homogenous appearance. In some cells a positive reaction was seen throughout the cytoplasm and the nucleus in others a positive reaction only in the cytoplasm was observed (Fig. 1). Besides, the round bodies, larger than IDCs, occasionally expressed a positive reaction to the S-l00 protein (Fig. 2). The intensity of the reaction in these bodies was similar to the IDCs. The round bodies were localized in the medullary region together with IDCs generally next to or inside Hassall's corpuscles.

A weak to moderate reaction to S-l00 protein was observed in Hassall's corpuscles; these showed differing intensities in the reaction of the epithelial cells involved in their structure. A positive cystic formation showing a peripheral membranous character and unstained vesicular content was also observed (Fig. 3). The peripheral zone of Hassall's corpuscles was more stained in comparison to the central area, which was only faintly stained or even unstained (Fig. 4). Moreover, the immunostaining for the S-100 protein was localized in the nerve fibres running in the interlobular connective tissue in close contact with the blood vessels. On some occasions the presence of nerve fibres was observed in the periphery of Hassall's corpuscles (Fig. 4). Individually, some positive nerve fibres were present inside the parenchyma entering the lymphocytes (Fig. 5). No positive reaction by the lymphocytes to this antibody was recorded.

DISCUSSION

A positive reaction to S-l00 protein in the cat thymus was linked mostly with interdigitating cells localized in the medullary region. Similar distribution was observed in the human thymus (l5) when S-l00 positive cells in the thymic medulla were in a close relationship with Hassall's corpuscles. In the foetal and young human thymus, S-l00 protein positive interdigitating reticular cells were mainly in the medulla with some scattered elements in the *cortex* (20). In the cortex of the pig thymus, whereas a positive immunoreaction to S-l00 protein was found in some macrophages in the *medulla* besides the epithelial reticular cells and those of Hassall's corpuscle, also in the polynucleated giant cells and in some lymphocytes (l6).

The size and morphological characteristics of the round bodies we observed in the cat thymus are different from the giant macrophages or polynucleated giant cells described by Atoji *et al.* (3). In the human thymus localization Hassall's corpuscles are mainly in the cortico-medullary zone and it was only occasionally possible to find IDCc around or adhering to Hassall's corpuscles (6, l4).

The medullary region, some for S-l00 protein-positive cells were found inside Hassall's corpuscles (l) or inside the cystic encapsulated formations in the young human. Their location in the cat thymus is similar to that described by Aita *et al.* (1) in Down's human thymus, who have observed IDCs adhering closely to the external epithelial sheath of Hassall's corpuscles or located inside them. Inside the cystic formation we also observed Hassall's corpuscles in the cat thymi. We found, that the position of positive cells in the cat, was exclusively in the medullary zone next to Hassall's corpuscles and in rare cases inside them. Variability in the shape and structure of Hassall's corpuscles and differences in the amount of positive cells accompanying them were noted in the cat.

Bornemann and Kirchner (5) have characterized IDCs in the human thymus as star-like cells with cytoplasmic processes. This characterization can also be applied to the cat thymus where the immunoreactive cells revealed a similar shape, but in a few cases the cells seemed round, not star-like in shape. Morphologically similar cells, as we also observed in the cat thymus, have been described in the white pulp of the human spleen (7) and in the human thymic *medulla* (l2).

In our study the presence of giant macrophages positive to the S-l00 protein in the corticomedullary zone was not observed, on the other hand Atoji *et al.* (3) have described this type of cell.

The interdigitating cells revealed some specific features which may be related to their function. From the specific distribution of IDCs in relation to Hassall's corpuscles observed in the cat we can deduce a particular interaction between IDC and Hassall's corpuscles.

We detected a strong positive reaction in the nerve fibres accompanying the blood vessels localized in

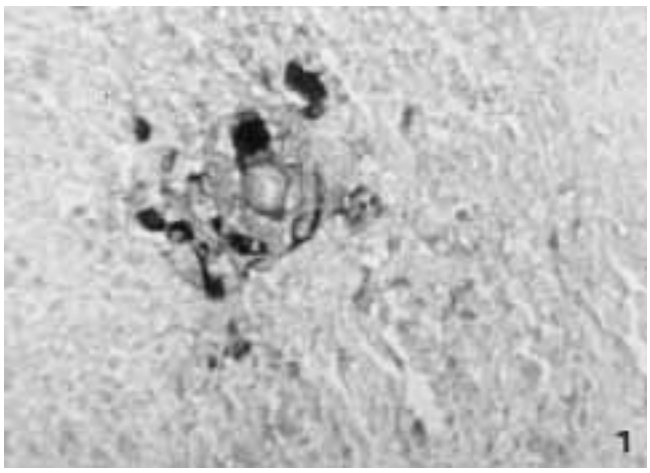

Fig. 1. Strongly positive interdigitating cells are localized at a short distance from Hassall's corpuscle (× 350)

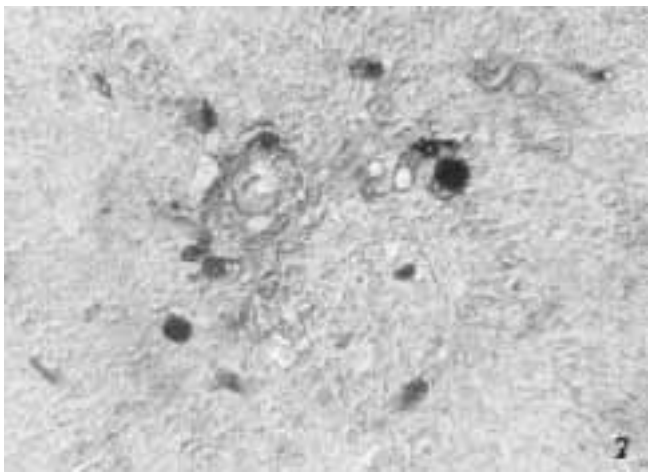

Fig. 2. A few positive interdigitating cells and round dense bodies are situated in the area of developing Hassall's corpuscle (× 420)

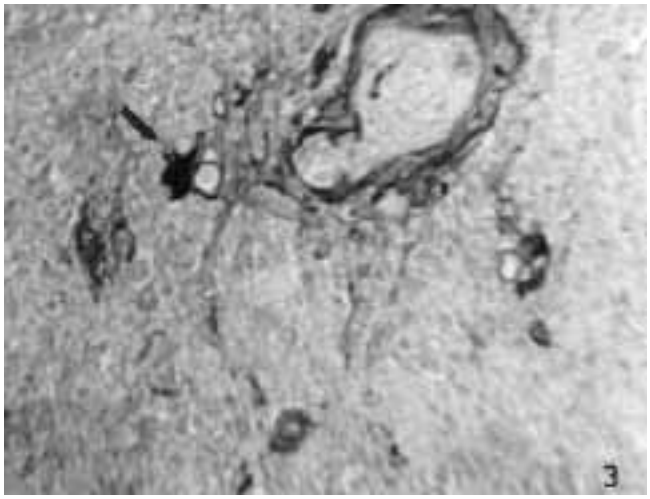

Fig. 3. A large cystic formation showing moderately positive peripheric coat and negative central area is seen. Not far from the corpuscle a few stellate cells are seen (*arrow*) (× 350)

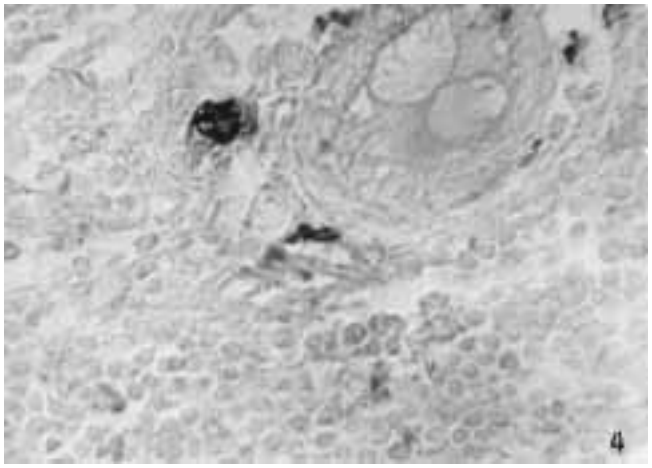

Fig. 4. A few positively stained nerve fibres are seen in the periphery of a large Hassall's corpuscle (× 700)

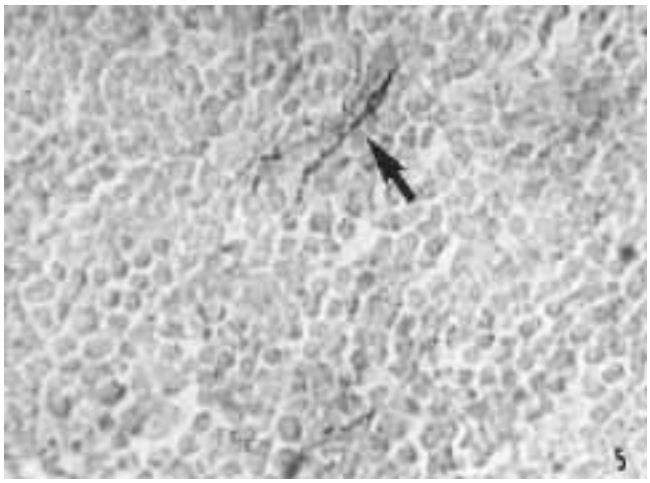

Fig. 5. Positive reaction on the nerve fibre inside the cortical area (*arrow*). Nerve fibre entering the lymphocytes (× 700)

the interstitial tissue and in the cortical and medullary parenchyma. The presence of nerve fibres in this area is expected and has also been observed in the sheep thymus (12). It is stated that the parenchymal nerves may affect lymphopoiesis and lympholysis by controlling the secretory activity of epithelial reticular cells. Results from Williams and Felten (21) have suggested that both noradrenergic and histamine are available to lymphocytes in the thymus and spleen and thus provide morphological evidence for neural modulation in immune activity *in vivo.*

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CHANGES IN METABOLIC AND IMMUNOLOGICAL PARAMETERS IN GERM-FREE AND CONVENTIONAL PIGLETS AFTER THE APPLICATION OF OIL WITH AN ENHANCED CONTENT OF n-3 POLYUNSATURATED FATTY ACIDS (PUFA)

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ABSTRACT

Oil with n-3 polyunsaturated fatty acids (PUFA) was applied to suckling conventional (n = 11; 6 + 5) and germ-free piglets (n = 8; 4 + 4). The application of oil with PUFA resulted in a significant increase in the actual counts of B-lymphocytes in the peripheral blood ($P < 0.05$) of germ-free piglets. The indices of the phagocyte activity of neutrophils were slightly increased. The indices of the phagocyte activity of potentially phagocytic cells were during the whole time of the experiment higher in the experimental group (EG). Total counts of leukocytes were higher in EG. Higher individual titres of specific antibodies were recorded in EG after vaccination. In the EG of the piglets the level of growth factors was significantly higher ($P < 0.05$), determined on the basis of the somatomedin in the blood serum. Biochemical indices showed a significant increase in the level of γ-linolenic (GLA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the blood serum and at the same time the level of arachidonic acid (AA) decreased. n-3 PUFA affects fatty acid metabolism and prostaglandin synthesis. This can be a factor in the therapy of inflammatory processes and in reducing the risk of infectious diseases in piglets.

Key words: fatty acids metabolism; immunity; n-3 PUFA; piglets

INTRODUCTION

Recently, great attention has been paid to essential polyunsaturated fatty acids (PUFA) and their effect on the nutrition of piglets. Essential PUFAs, acting as structural components of cellular membranes and as precursors for eicosanoid production, are important modulators of humoral and cell-mediated immune reactions. The composition of PUFAs in the cells of the immune system indicates their potential effect on the biological receptors, signal transduction and lymphocyte proliferation. The n-3 PUFAs intervene in prostaglandin synthesis. This leads to a decrease in the aggregation of thrombocytes. Prostaglandins and leucotriens have an effect on immune functions and inflammatory reactions. The n-3 PUFAs pass the lipogenase pathway, whereby mediators with reduced inflammatory activity are formed; on the other hand, n-6 PUFAs are metabolised by cyclo-oxygenase and pro-inflammatory metabolites arise (14).

The mechanism of n-3 PUFA action on the plasmatic lipids has not been explained yet. The n-3 PUFAs are expected either to decrease the rate of apoprotein B synthesis (9) or formation of VLDL triacylglycerols (7). An increased intake of n-3 PUFA decreases the level of triacylglycerides, VLDL (very low density lipoproteins), cholesterol and low density lipoproteins (LDL) in the blood plasma of experimental animals. In the plasma lipids, the representation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is increased at the expense of arachidonic acid (AA) (8). In some studies an increase in the high density lipoprotein (HDL) cholesterol is described, while the effect of DHA was more pronounced than that of EPA (9).

The aim of this study was to find out the effect of oil with n-3 PUFAs on some metabolic and immunological changes in the organism of piglets. As well, the production of growth hormones and formation of eicosanoids were observed using the analysis of blood serum of piglets and comparison of results of these examinations was carried out in experiments with conventional and germ-free animals.

MATERIALS AND METHODS

The experiments were carried out on conventional and germ-free piglets of the same breed. Eleven conventional piglets (Landrace x Slovak White Improved crossbreds) at the age of four days, weighing 1.2–2.0 kg were divided into two groups (control: n = 5; experiment n = 6). Eight germ-free piglets, obtained by hysterectomy, bred in sterile isolators, were divided into experimental (n = 4) and control (n = 4) groups.

During the thirty five day period following birth, the conventional piglets (n = 6) of the experimental group were given oil containing n-3 PUFAs orally at a dose of 100 mg. kg^{-1}/day. The oil dose was increased according to the weight measured at weekly intervals. The composition of the oil is in Table 1. The control group was given a physiological solution. All the animals (experimental and control) were immunised intramuscularly with 1 ml of vaccine against Aujeszky's disease (INVAK G1 inj. a.u.v., Mevak, Inc., Nitra, The Slovak Republic) in order to determined the effect of n-3 PUFA on the formation of specific antibodies.

Table 1. Chemical composition of the oil

5g of oil contains g	
Total n-6 PUFA	**0.1**
Total n-3 PUFA	**1.0**
Total unsaturated FA	**2.6**
Total saturated FA	**0.9**
Cholesterol	**0.005**

Germ-free experimental piglets (n = 4) were orally administered the oil with increased contents of n-3 PUFA at an amount of 0.5, 1.0, and 1.5 ml/day in weeks 1, 2, and 3 for the twenty-one days following birth. The control animals were administered a physiological solution in the same dose instead of oil. The food ration consisted of dry, full-fat milk (Sunar, PMV Hradec Králové, The Czech Republic) diluted with water at a ratio 1 : 9 (Table 2). The piglets were fed six times daily. Blood samples were taken from the venous sinus of the piglet eye at weekly intervals.

Lipids were extracted from the serum and modified to bromphenylesters of fatty acids by the method of derivation in acetonitrile (10). Bromphenylesters of fatty acids were determined using the HPLC method (Spectra Physics SP 8 700, Santa Clara, CA, USA) with UV VIS detector at 254 nm.

The growth hormone somatomedin C (IgF_1) was determined in the blood serum by the RIA method.

Subpopulations of T-lymphocytes (CD4, CD8) and B-lymphocytes were assayed quantitatively on the flow cytometer using mouse anti-pig monoclonal antibodies by the indirect immunofluorescence method and measured by FAC-Scan (Becton Dickinson, Germany). Primary monoclonal antibodies used in experiments were: CD4 (74-1-4, IgG2b, 1:25), CD8 (76-2-11, IgG2a, 1:25) all from from the Czech Academy of Sciences, Prague, Czech Republic; IgM (Kl39.3El, IgG2a, 1:25) from the University of Bristol, Great Britain. A dot plot of 10,000 cells was used for the analysis, obtained by forward and side scattering of the physical character of the lymphocyte population. The results are therefore expressed as a percentage of the lymphocyte population positive for specific MoAbs. The absolute number of lymphocytes was calculated by differential counts of leukocytes.

Evaluation of the functional activity of phagocytes in the peripheral blood of piglets was carried out by the iodo-nitro-tetrazolium reductase test (11). The functional activity of lymphocytes was determined after the mitogenic activation of lymphocytes by phytohemagglutinin (PHA, Sigma) using the migrating-inhibitory test (MIT) under agarose (1).

The Student *t-test was used for statistical evaluation.*

Table 2. Representation of fatty acids in milk SUNAR

Acid	% of volume	Acid	% of volume
Butyric	**9.5**	**Stearic**	**7.6**
Caproic	**4.1**	**Arachic**	**1.8**
Caprylic	**0.8**	**Myristelic**	**0.9**
Capric	**3.2**	**Palmitic**	**4.3**
Lauric	**2.9**	**Oleic**	**22.4**

RESULTS

Germ-free animals served as an optimal experimental model of digestion physiology and lipid metabolism. By comparison of the results of experiments with germ-free and conventional animals it was possible to observe more pronounced differences in the individual immune parameters, especially in germ-free animals.

The growth factors, found in the blood serum of piglets using the RIA method by somatomedin measurement, indicate a significant increase in the experimental group compared to the control groups of animals ($P < 0.05$; Fig. 1).

In the peripheral blood of conventional piglets in the experimental group, the mean counts of leukocytes (week 3) and lymphocytes (weeks 1–3, Table 3) were slightly increased, whereas no essential differences were found between conventional and germ-free piglets.

Examination of CD4 and CD8 positive cells revealed a significant increase in the absolute number in the experimental group at the third sampling in germ-free piglets. CD4 and CD8 positive subpopulations of lymphocytes showed values in the experimental group of conventional piglets without statistically significant differences between experimental and control group (Table 4).

The subpopulation of B-lymphocytes measured by the expression of IgM molecules showed increased values in

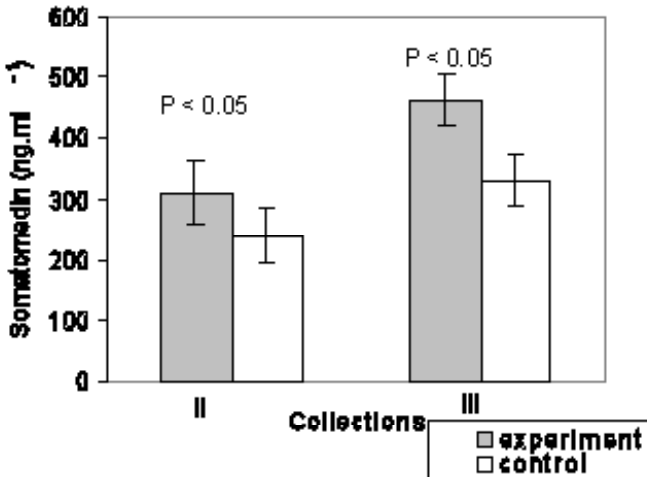

Fig. 1. The growth factor in the blood of conventional piglets

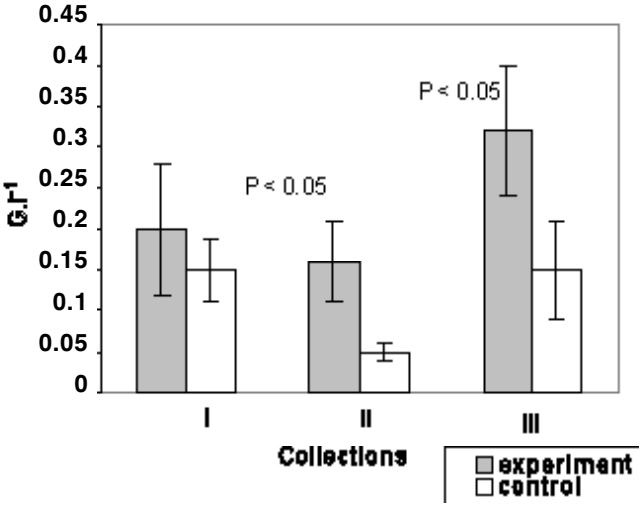

Fig. 2. IgM positive lymphocytes in germ-free piglets

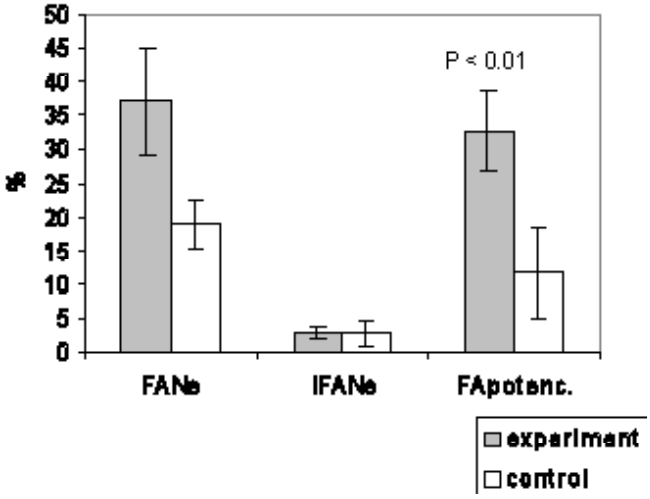

Fig. 3. Phagocyte activity in germ-free piglets

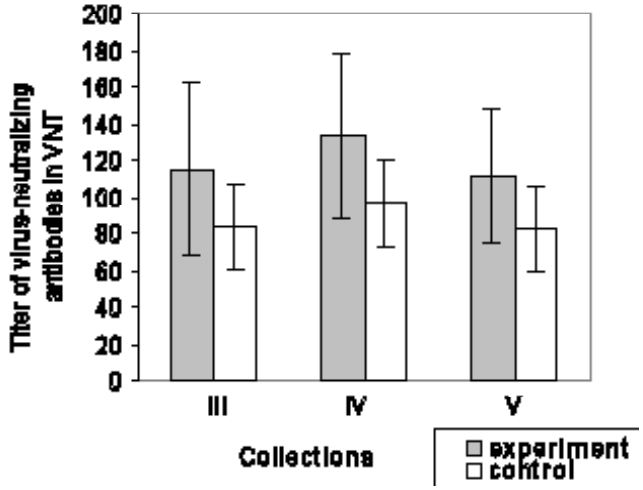

Fig. 4. Titres of neutralizing serum antibodies against Aujezsky virus (VNT) in conventional piglets

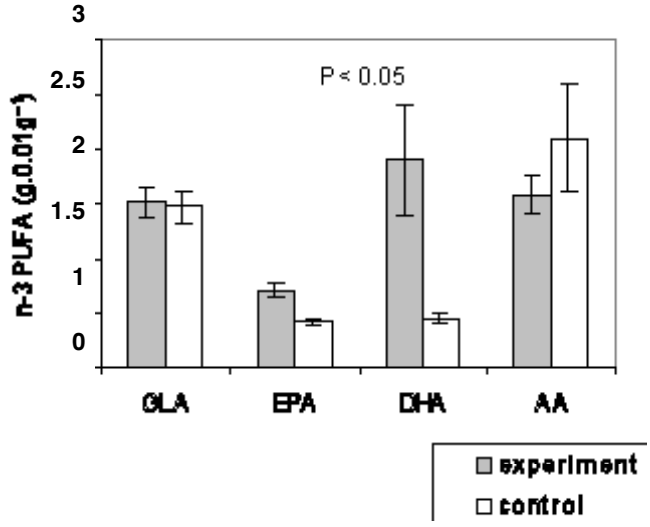

Fig. 5. n-3 PUFA in the blood of germ-free piglets

both the experimental conventional and germ-free piglets during the whole experiment. In the second and third collection in germ-free piglets the difference between the experimental and control group was statistically significant ($P<0.05$; Fig. 2).

The phagocyte activity of neutrophils (FANe) in the peripheral blood in germ-free piglets in the first two weeks of oil administration was not significantly influenced. However, in the third week of the experiment there was a significant increase of FANe in the experimental group of piglets ($P<0.01$) in comparison with the control group (Fig. 3).

In conventional animals, the level of specific immunity in a relationship to antibodies against Aujeszky's disease was tested after vaccination with Inavak G 1 (Mevak, Nitra). In first two weeks after application of the vaccine to piglets a low titre of neutralizing antibodies was recorded in some animals. Following re-vaccination an increase in the neutralizing titres of antibodies occurred

Table 3. The mean counts of leukocytes (Lc) and lymphocytes (Ly) in the peripheral blood of piglets

	Germ-free piglets			
	Lc		Ly	
Collections	E	C	E	C
1	7.05 ± 1.09	6.97 ± 1.30	3.83 ± 0.64	2.73 ± 0.98
2	5.78 ± 1.67	8.23 ± 2.52	3.84 ± 1.00	2.99 ± 0.70
3	8.06 ± 0.67	7.73 ± 2.28	5.49 ± 1.19	3.70 ± 075
	Conventional piglets			
	Lc		Ly	
Collections	E	C	E	C
1	8.35 ± 2.22	7.12 ± 1.80	5.60 ± 1.38	4.81 ± 1.22
2	12.73 ± 4.04	9.10 ± 2.53	8.76 ± 3.80	5.67 ± 2.13
3	12.13 ± 1.94	11.46 ± 1.17	6.71 ± 1.74	6.09 ± 1.10

Legend: Lc — leukocytes, Ly — lymphocytes, E — experimental group, C — control group

Table 4. The effect of n-3 PUFA supplementation on the absolute number of CD4 and CD8 subpopulation of lymphocytes in the peripheral blood of germ-free and conventional piglets (means ± SD)

	Germ-free piglets			
	CD4 ($1.10^9.l^{-1}$)		CD4 ($1.10^9.l^{-1}$)	
Collections	E	C	E	C
1	1.71 ± 0.33	1.18 ± 0.50	0.74 ± 0.20	0.55 ± 0.29
2	1.42 ± 0.35	0.98 ± 0.31	0.89 ± 0.32	0.57 ± 0.29
3	1.28 ± 0.02	1.09 ± 0.08*	1.79 ± 0.49	0.84 ± 0.24*
	Conventional piglets			
	CD4 ($1.10^9.l^{-1}$)		CD4 ($1.10^9.l^{-1}$)	
Collections	E	C	E	C
1	1.70 ± 0.39	1.40 ± 0.32	1.36 ± 0.32	1.02 ± 0.29
2	2.96 ± 1.14	2.23 ± 0.94	3.50 ± 2.13	1.81 ± 0.72
3	2.92 ± 0.99	3.03 ± 0.47	2.37 ± 0.74	2.05 ± 0.48

Legend: CD — cluster of differentiation, E — experimental group, C — control group, * — $P < 0.05$

in both the experimental and control groups of piglets. In all three collections after re-vaccination, higher mean titres of specific antibodies were recorded in the experimental group of piglets compared to the control group of animals. The differences in the mean titres in both groups were not statistically significant regarding the great variability of titres of specific antibodies in individual piglets of both groups. The individual titres of some animals in the experimental group reached values 1:512, while in the control group it was 1:128 maximally (Fig. 4).

After administration of n-3 PUFA to the germ-free experiments, increased levels of fatty acids in the serum of piglets were found. The level of EPA in the experimental group increased 1.3-times compared to the control group. The level of DHA was significantly increased 1.7-times ($P<0.05$) in comparison with the control animals, and concentration of AA decreased 1.5-times compared to the control (Fig. 5).

DISCUSSION

The experiment aimed at the observation of biochemical, haematological and immunological changes in the organism of piglets caused by supplementation of oil

with increased content of n-3 PUFA. The experiments were carried out on conventional and germ-free piglets. Germ-free piglets were used as an optimal experimental model of the digestive tract physiology and lipid metabolism. The representation of gamma-linolenic acid and its metabolites in the colostrum is related to a low activity of desaturating enzymes in suckling piglets that are not able to synthesize higher PUFAs from the precursor PUFAs at the beginning of their life (3).

Conventional piglets had a higher intake of PUFA from the colostrum and mother milk than germ-free piglets from cow milk, which could have contributed to the differences in the results between these two groups. The amount of growth factor somatomedin was higher in both experimental groups than that in the control groups and this resulted in the higher weight of experimental animals compared to the controls. These results are in agreement with the previous study of Kašteľ *et al.* (10).

The administration of n-3 PUFA could favourably influence the treatment of inflammatory processes and increase the resistance of piglets against infections. A significant increase in the mean values of B-lymphocytes, some subpopulations of T-lymphocytes as well as a slight increase in the immunological parameters observed and in the specific antibodies after vaccination testify to this hypothesis.

In the peripheral blood of the germ-free piglets of the experimental group, there were slightly increased mean counts of leukocytes and lymphocytes. CD4 cells on the third sampling in germ-free piglets showed a significant increase. During the experiment, the subpopulation of CD8 positive cells was slightly increased over the experimental conventional piglets while in germ-free piglets the differences between the animals of control and experimental groups were larger, and in the third sampling they were significant. Sanderson *et al.* (13), Yaqoob and Calder *(15)* reported a decrease in the lymphocyte proliferation in the animals fed the diet with increased amount of n-3 PUFA was demonstrated in rodents, chickens and humans (4, 2).

The administration of oil with increased amount of n-3 PUFA to conventional and germ-free piglets induced the increase in the concentration of docosahexaenoic acid and decrease in arachidonic acid in the experimental group compared to the control; this leads to a decreased production of prostaglandins in coincidence with the literary data (5). An increased supply of n-3 PUFA leads in the plasma lipids to increasing the representation of APA and DHA at the expense of AA (8). The mechanism of n-3 PUFA action on the plasma lipids has been unsolved yet. Eicosapentaenoic acid and other n-3 PUFA extrude arachidonic acid from the membranous phospholipids, and so they influence the lipid metabolism (6).

In conclusion it can be confirmed that administration of n-3 PUFA in suckling piglets led to:

– an increase in the level of n-3 PUFA in the blood serum,

– changes in production of eicosanoids,

– an increase in numbers of CD4 and CD8 subpopulations of T-lymphocytes and IgM presenting lymphocytes in peripheral blood,

– an increase in the specific postvaccinal antibodies to Aujeszky's disease,

– an increase of FANe in the experimental group of piglets in comparison with the control group ($P < 0.01$),

– an increase in the level of growth factor somatomedin ($P < 0.05$),

– an increase in the concentrations of GLA, EPA and DHA in the blood and decrease in AA.

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MORPHOMETRIC STUDIES OF THE CRANIO-FACIAL REGION OF THE WEST AFRICAN DWARF GOAT IN NIGERIA

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ABSTRACT

This study involved the measurement of some parameters in the craniofacial region of the West African Dwarf Goat (WADG). The mean weight of the head was 0.74 kilograms. The length of the *rima oris* and space between the median canthi were 8.69 centimetres and 8.81 centimetres, respectively. The right palpebral fissure and external nare were longer than the left. The eyeball weight was 6.87 grams; the left eyeball was heavier than the right. Females had significantly heavier heads and body weights than the males. The right palpebral fissure was longer than the left in females but the reverse was the case in males. These findings will serve as baseline information in the study of the head of WADG and in comparative anatomy with other breeds.

Key words: craniofacial region; West African Dwarf Goat

INTRODUCTION

The WADG are present in all of humid Africa from the Southern Sudan to the West Coast. They make up about 38 % of the estimated 38 million goats in the West African humid zone. They are important in the socio-economic life of most rural dwellers and are managed basically through the extensive system (3, 12).

There is currently a paucity of information on the cranio-facial anatomy of this breed of goat (8, 9, 11).

This study involved the investigation of the morphometry of some indices in the head of the WAD goat.

MATERIALS AND METHODS

A total of 30 clinically healthy WAD goats were used for the study. Preparation of samples and determination of indices

1. Head weight: The heads were severed at the occipito-atlantal joint and weights taken using a “Five goat” spring scale.
2. Rima oris length: This was taken using a twine from the ends of the lateral commisures of closed lips. The linear measurement of the twine was then taken.
3. Orbit circumference: A twine was employed to get the circumference of the bony orbit beneath the skin. Linear measurement of the twine was then taken.
4. Palpebral fissure lengths: These were taken from the median to the lateral *canthi* of the eye.
5. Distance between the median *canthi:* This was measured as the distance between the median *canthi* of the left and right eyes.
6. Length of external nares: The external naves were compressed medio-laterally from *philtrum* before they were measured.
7. Removal of eyeball: The eyeballs were removed by enucleation (6) and put in a 0.9 % saline solution.
8. Eyeball weight: The eyeballs were weighed in nylon surgical gloves (whose weight was first determined) on a “microwa-swiss” weighing machine; the weight difference was then taken.

RESULTS

The result of the parameters measured in the goat heads are shown in Tables 1—2 presented as mean ± SD. The results were compared using the Student *t* test at 0.05 % level of significance.

Legend

RO: *Rima oris* lenght
RPT: Right palpebral fissure length
LPF: Left palpebral fissure length
REN: Right external nares length
LEN: Left external nares length
DMC: Distance between median *canthi*
WOH: Weight of head
WOA: Weight of animal

Table 2. The eyeball weights and orbit circumference of WADG

Observation	Mean Values
Total Eyeball Weight (g)	6.87 ± 2.01
Right Eyeball Weight (g)	6.86 ± 1.97
Left Eyeball Weight (g)	7.00 ± 2.19
Female Eyeball Weight (g)	5.73 ± 1.69
Male Eyeball Weight (g)	7.66 ± 2.14
Total Orbit Circumference (cm)	10.92 ± 0.83
Right Orbit Circumference (cm)	10.87 ± 0.84
Left Orbit Circumference (cm)	10.97 ± 0.87

The mean weight of the head of WADG was 0.74 ± 0.13 kilograms while that of the animal body was 10.48 ± 510 kilograms. The right palpebral fissure was longer than the left as were the right external nares compared to the left. These observations were however not statistically significantly ($p > 0.05$). The mean distance between the median *canthi* was 8.81 ± 1.08 centimetres while the *rima oris* length was 9.69 ± 1.64 centimetres.

The female WADG had significantly higher body weights and longer pelpebral fissures than their male counterparts ($p < 0.05$).

The mean right palpebral fissures of the females were longer than the left but the reverse was the case in the males. The females also had wider mean left external nares than the right but the males had equal lengths of both nares

The mean eyeball weight of WADG was 6.87 ± 2.01 kilograms. The left eyeball was heavier than the right likewise the males had heavier eyeballs than the females.

Animals above twelve kilo body weight had significantly higher values ($p < 0.05$) in all parameters observed than animals below this body weight.

DISCUSSION

The mean weight of head of the WADG was 0.74 ± 0.31 kilograms. This is smaller than that of Red Sokoto goats (0.90 ± 0.16 kilograms) obtained in the same environment (9). Both breeds, however, had the same relative weight of 0.07 indicating that percentage head to body weight between goat breeds could be fairly constant. The palpebral fissure lengths of 2.16 ± 0.25 centimetres and 2.14 ± 0.28 centimetres for right and left eyes respectively were smaller than those of Red Sokoto goats in which the fissures had equal length (2.50 ± 0.17 centimetres). The mean of the palpebral fissure lengths between the WADG and the Red Sokoto was however not much different from each other as it has been observed (13) that the lengths of palpebral fissures was fairly constant among breeds. The distance (8.81 ± 1.08 centimetres) between the median canthi (DMC) was shorter in the WADG than the 10.19 ± 0.66 centimetres of Red Sokoto goats (9). The DMC was also shorter than the distance between the orbits (15.14 centimetres and 17.28 centimetres) in bovine and equine respectively but longer than the 3.55 centimetres obtained in dogs and the 2.30 centimetres in cats (4). These morphometric findings are consistent with the fact that animals that are hunted prey (herbivores, for example, ruminants and horses) have wider faces with more laterally placed eyes than their hunters or predators (2).

The results in this study indicate that although they have similar relative head weight, the WADG is smaller than the Red Sokoto goat. They also have a narrower eye bridge and shorter eyelids.

Table 1: Some craniofacial indices in the WADG

Observation	Total n = 30	Female n = 22	Male n = 8	Animals >12kg n = 20	Animal<12kg n = 10
RO (cm)	8.69 ± 1.64	8.45 ± 2.08	7.63 ± 0.93	9.93 ± 1.75*	7.66 ± 1.00
RPT (cm)	2.16 ± 0.28	2.20 ± 0.29	1.85 ± 0.15	2.37 ± 0.18*	1.91 ± 0.18
LPF (cm)	2.14 ± 0.28	2.13 ± 0.31	1.86 ± 0.18	2.34 ± 0.19*	1.89 ± 0.17
REN (cm)	3.19 ± 1.47	2.80 ± 1.55*	2.06 ± 092	4.15 ± 1.42*	2.00 ± 0.83
LEN (cm)	3.18 ± 1.46	2.81 ± 1.55*	2.06 ± 0.92	4.16 ± 1.38*	2.01 ± 0.90
DMC (cm)	8.81 ± 1.08	877 ± 1.03	7.96 ± 0.52	9.79 ± 0.68*	8.25 ± 0.87
WOH (kg)	0.74 ± 0.31	1.57 ± 0.22	0.49 ± 0.19	1.06 ± 0.19*	0.53 ± 0.19
WOA (kg)	10.48 ± 5.10	11.19 ±5.48	6.75 ± 1.49	16.45 ± 3.13*	6.88 ± 2.57

* — Significant at $p < 0.05$

The mean weight of head of the females was significantly higher than that of the males. This could be due to a higher bone and muscle mass. The longer palpebral fissures, external nares and *rima oris* obtained in females in this study could be size dependent as similarly observed by (9) in Red Sokoto goats. Females because they are kept longer for reproductive purposes, even when they attain market weight, are usually older and heavier at any given time on the farm (11). The female WADG also had a longer DMC and thus have wider spaced eyes than males.

Slight asymmetry was seen in between the left and right palpebral fissures and external nares in this work irrespective of sex and weight. Similar asymmetry was been reported between the two halves of the bodies of animals (14).

Animals above twelve kilos in body weight had significantly heavier heads. They also had a wider face, larger external nares, longer palpebral fissures and lips, all of which tends to suggest that to a large extent the parameters observed in this study are size dependent.

The mean eyeball weight of WADG in this study was 6.87 ± 2.01 grams. This is heavier than the 6.77 grams and 3.00 grams obtained in man and rabbits, respectively but lighter than the 15.09 grams, 7.25 grams and 29.29 grams obtained for sheep, pig and cattle (5), and the 100.89 grams, 14.2 grams and 10.79 grams obtained for horse and large and small dogs respectively (1).

The females had lighter eyeballs than the males despite their heavier weight. The right eyeball was lighter than the left in agreement with an earlier observation (14). This lighter right eyeball was however, expressed through a longer palpebral fissure. The mean orbit circumference of the eye was 10.92 ± 0.83 centimetres; the orbit circumference of the left eye was wider than the right in correspondence with the differences in eyeball weight. The heavier left eyeball obviously had a larger surface area requiring in the process wider orbits for rotation.

In conclusion, the results obtained in this study could be used as baseline research data in bioengineering and optometric studies as eyelid measurements and eyeball movements have been used to determine the level of a resting animal's awareness (7), and in comparative and regional anatomy of the goat head (2)

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BLOOD GAS AND ACID-BASE VALUES IN FOALS DURING THE FIRST WEEK OF LIFE

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ABSTRACT

In foals, the neonatal period represents a critical stage, as the newly born animals have to adapt to extra uterine life. During this early stage, all the specific homeostases are fully established and the subjects must be thoroughly monitored to detect early signs of respiratory or metabolic disorders. Therefore, our purpose is to study their assessment, with a view to obtaining several physiological reference values as indicators of dynamic homeostatic processes occurring during the first week of life in foals. We used six English Thoroughbred foals with an average body weight of 39 ± 1 kilograms, one day old and full-term born. The animals were kept in individual boxes with their mothers. With all the subjects, starting from the first day of birth and for the following five days, every day and at the same hour (08.00 a.m.), blood samples were taking from the brachial artery. pH, carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), bicarbonates concentration (HCO_3^-), haemoglobin (Hb) and haematocritic value (Hct) were determined by means of a selective ions haemogasanalyzer. The recorded values were processed by means of ANOVA for repeated measurements and Bonferroni's multiple comparison test was applied in order to determine the statistical significance between the mean values of the studied parameters from the first to the sixth day of the study. From the analysis of the obtained results, we observed statistically significant differences for pH (HCO_3^-), carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), haemoglobin (Hb), haematocritic value (Hct), but no statistically significant differences for rectal temperature. The obtained results contribute to the evaluation of acid-base homeostasis mechanisms and the physiological evolution of parameters and organs involved during the first week of life in the foal.

Key words: acid-base balance; blood gas parameters; foal; neonatal physiology.

INTRODUCTION

For the foal, the first week after birth (neonatal period) represents a critical moment during which all organic functions must adapt to extra-uterine life. Delivery and the following twenty-four hours therefore represent the critical stage for the detection of foal health problems. This period represents a transition phase between the foetal functions and those of the newborn in which all the specific homeostases (cardiovascular, respiratory, metabolic and thermoregulatory) are fully established. Intensive care must be functional to support animal homeostasis and has to be specific as to avoid any iatrogenic side effects. Among the techniques useful in making a diagnosis of pulmonary and metabolic dysfunctions, haemogasanalytical examination is critical since it allows metabolic and qualitative

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Running title: Acid-base balance in the foal.

assessment of gas exchanges by means of O_2 and CO_2 analysis.

Studies on acid-base balance and blood gases pressures have showed that there is a modification of the moderate metabolic and respiratory acidosis present at the birth and during the first two days of life (1). Specific literature has investigated the postnatal development of respiratory function and of acid-base balance in lambs during experimental hypoxia or normoxic and/or hypoxic hypercapnia (2, 5, 6, 7, 19), in calves (8) and both in premature and normal foals at different periods of neonatal life (12, 13, 17, 18, 20).

Given the importance of holding physiological reference values as indicators of dynamic homeostatic processes taking place during the twenty-four hours following birth and in the first week of life, with regard to respiratory function and acid-base balance regulation, our goal is the assessment of several haemogasanalytical parameters during the first week of life in the foal.

MATERIAL AND METHODS

We used six English Thoroughbred foals, average body weight 39 ± 1 kilograms, one day old and full-term born. The animals were kept in individual boxes with their mothers. With all the subjects, starting from the first day of birth and for the following five days, every day and at the same hour (08.00 a.m.), blood samples were taking from the brachial artery, as described by Fisher *et al.* (10), using preheparinized ventilated syringes for haemogasanalysis (frozen-dry lithium-heparin), with a 23 G needle. Samples were analyzed within fifteen minutes from the collection using 40 ml of blood. With the obtained samples, using a selective ions haemogasanalyzer (Stat Profile Phox, Nova Biomedical), we determined the following parameters: pH, carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), bicarbonates concentration (HCO_3^-), haemoglobin (Hb) and haematocritic value (Hct). Because of temperature influences on blood gases' partial pressure (15), for each assessment the analyzer reference temperature was individually set on the basis of the subject rectal temperature. Temperature was recorded by means of a digital thermometer whose probe was inserted to a depth of five centimetres into the *rectum.*

On the obtained values, ANOVA for repeated measurements and Bonferroni's multiple comparison test were applied in order to determine the statistical significances between the mean values of the studied parameters from the first to the sixth day of the study.

RESULTS

Table 1 shows the mean values of pH, bicarbonates concentration (HCO_3^-), carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), haemoglobin (Hb), haematocritic value (Hct) and rectal temperature, expressed in their conventional units together with the relative standard deviations, obtained during the first week of life in six foals.

Figures 1—7 show the graphs of the considered parameters (pH, HCO_3^-, pCO_2, pO_2, Hb, Hct and rectal temperature), expressed in their conventional units, obtained during the first week of life in six foals.

From the analysis of the results, we observed statistically significant differences for pH ($F_{(5,25)} = 77.26$, $P < 0.0001$; ANOVA for repeated measurements), bicarbonates concentration (HCO_3^-) ($F_{(5,25)} = 133.55$, $P < 0.0001$; ANOVA for repeated measurements), carbon dioxide partial pressure (pCO_2) ($F_{(5,25)} = 10.27$, $P < 0.0001$; ANOVA for repeated measurements), oxygen partial pressure (pO_2) ($F_{(5,25)} = 10.27$, $P < 0.0001$; ANOVA for repeated measurements), Haemoglobin (Hb) ($F_{(5,25)} = 29.25$, $P < 0.0001$; ANOVA for repeated measurements) and Hematocritic value (Hct) ($F_{(5,25)} = 137.2$, $P < 0.0001$; ANOVA for repeated measurements), while rectal temperature did not show statistically significant differences during the experimental period.

By applying Bonferroni's multiple comparison test, pH shows a statistically significant increase on the 3rd, 4th, 5th and 6th day compared to the 1st and 2nd day ($P < 0.001$) and a statistically significant decrease on the 5th and 6th day compared to the 3rd day ($P < 0.05$ and $P < 0.01$) and on the 6th day compared to the 4th day ($P < 0.001$); bicarbonates concentration (HCO_3^-) shows a statistically significant decrease on the 3rd, 4th, 5th and 6th day compared to the 1st and 2rd day ($P < 0.001$), on the 4th, 5th and 6th day compared to the 3rd day ($P < 0.001$) and on the 6th day compared to the 4th and 5th day ($P < 0.001$ and $P < 0.01$); carbon dioxide partial pressure (pCO_2) shows a statistically significant increase on the 3rd day compared to the 1st day ($P < 0.01$) and a statistically significant decrease on the 6th day compared to the 2rd day ($P < 0.05$), on the 5th and 6th day compared to the 3rd day ($P < 0.001$) and on the 6th day compared to the 4th day ($P < 0.05$); oxygen partial pressure (pO_2) shows a statistically significant increase on the 2rd, 3rd, 4th, 5th and 6th day compared to the 1st day ($P < 0.001$), on the 3rd day compared to the 2rd day ($P < 0.001$) and a statistically significant decrease on the 6th day compared to the 2rd day ($P < 0.05$), on the 4th, 5th and 6th day compared to the 3rd day ($P < 0.05$ and $P < 0.001$) and on the 6th day compared to the 4th day ($P < 0.001$); haemoglobin (Hb) shows a statistically significant decrease on the 3rd, 4th, 5th and 6th day compared to the 1st ($P < 0.001$) and 2rd day ($P < 0.05$, $P < 0.01$ and $P < 0.001$) and a statistically significant decrease on the 6th day compared to the 3rd, 4th and 5th ($P < 0.001$ e $P < 0.01$); haematocritic value (Hct) shows a statistically significant decrease on the 2rd, 3rd, 4th, 5th and 6th day compared to the 1st day ($P < 0.001$), on the 3rd, 4th, 5th and 6th day compared to the 2rd day ($P < 0.001$), 4th, 5th and 6th day compared to the 3rd day ($P < 0.01$ and $P < 0.001$) and on the 6th day compared to the 4th and 5th day ($P < 0.001$).

DISCUSSION

From the analysis of the obtained results, we observed that the studied parameters are different with respect

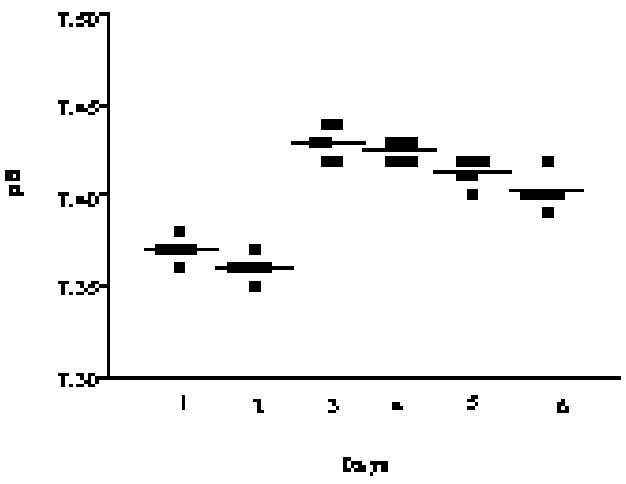

Fig. 1. Scatter graph of pH mean values obtained during the first week of life in six foals

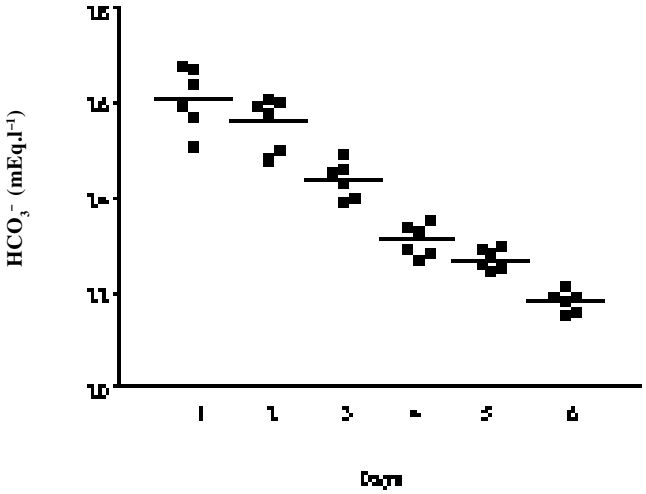

Fig. 2. Scatter graph of HCO_3^- (mEq.l^{-1}) mean values obtained during the first week of life in six foals

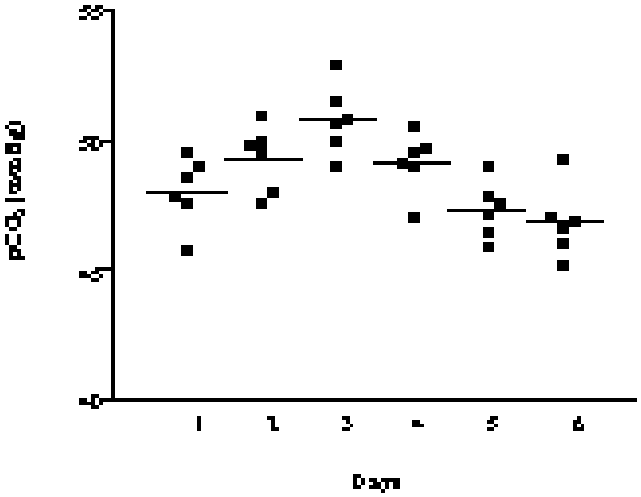

Fig. 3. Scatter graph of pCO_2 (mmHg) mean values obtained during the first week of life in six foals

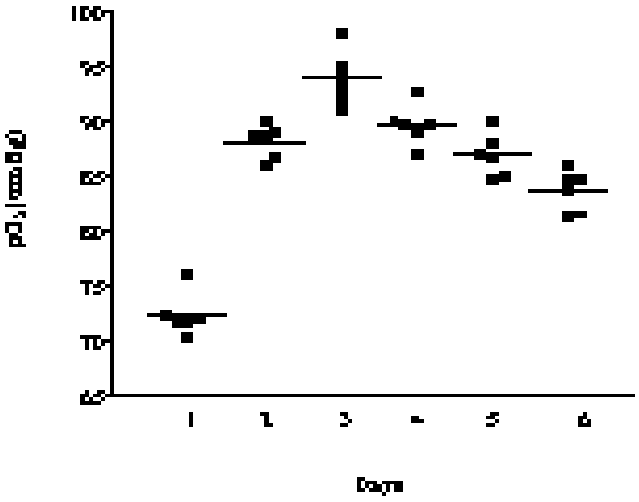

Fig. 4. Scatter graph of pO_2 (mmHg) mean values obtained during the first week of life in six foals

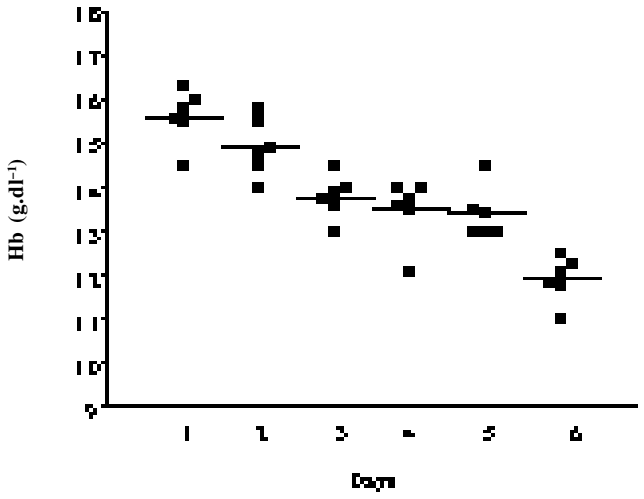

Fig. 5. Scatter graph of Hb (g.dl^{-1}) mean values obtained during the first week of life in six foals

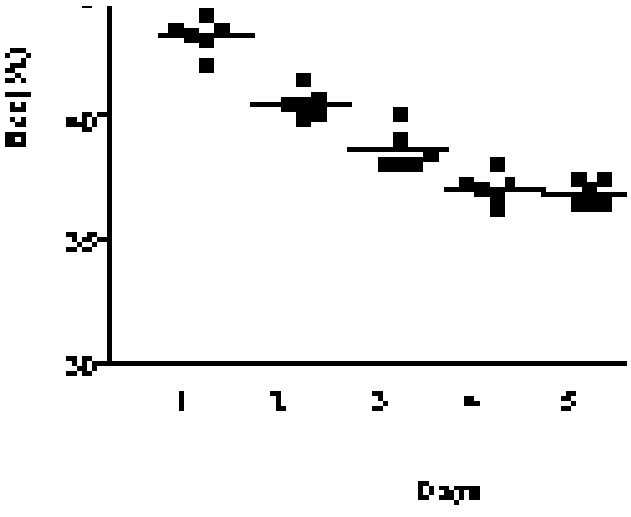

Fig. 6. Scatter graph of Hct (%) mean values obtained during the first week of life in six foals

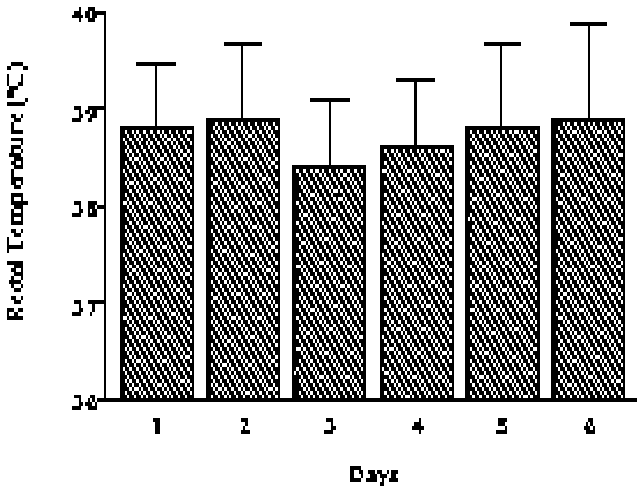

Fig. 7. Column bar graph of rectal temperatur (°C) mean values obtained during the first week of life in six goal

to those recorded in adults. This confirms that organic functions in new born foals are in a transient phase and organism immaturity is compensated from a wide range of tolerance in several apparatuses. Among these, pO_2 shows the lowest values only on the 1st day, while during subsequent days it showed a statistically significant increase compared to the 1st day, which confirm a gradual improvement in pulmonary function. Delivery, as is well-known, imparts great stress to the foetus, since uterine contractions affect the uterus-placental blood flow, causing hypoxia and neonatal hypercapnia (4) with low O_2 and high CO_2 tension in the blood. Such a condition is worsened by a neonatal reduced respiratory clearance characterized by hypoventilation that cannot guarantee CO_2 removal at the same rate of production. This results in an increase of carbon dioxide alveolar percentage with an increase of blood pCO_2 levels in the first three days of experimental period.

CO_2 retention would cause an increase in carbonic acid concentration, therefore lowering pH (16). In newly born animals, hypoxia and hypercapnia induce a redistribution of blood circulating volume in those tissues and organs, whose activation priority is not immediately indispensable for life (digestion, muscular and cutaneous tissues). Accordingly a sufficient supply of O_2 in heart and brain is maintained (21). Anaerobic glycolysis is therefore activated reaching values 69—96 % higher than in the adult subject (11). This results in the storage of lactic acid in inadequately vascularised tissues and a dysfunction on CO_2 release at pulmonary level so that in newly born foals a mixed respiratory-metabolic acidosis is established. Metabolic acidosis component is more substantial during the first ten to fifteen minutes after birth since O_2 availability at pulmonary level puts an end to the mechanism of O_2 intentional saving and the tissues lactic acid flows into the circulation (21).

Carbon dioxide partial pressure (pCO_2) and oxygen partial pressure (pO_2) show increased values from the 1st to the 3rd day and decreased values during subsequent days. During the first days of life the respiratory center is stimulated from the increased CO_2 and pH (16) resulting in increased pulmonary ventilation. The carotid chemoreceptors, which are perfectly developed and susceptible to blood gas changes at birth, show an increase of O_2 sensitivity (14) and act on the respiratory center inducing a rise in tidalic volume and in respiratory rate (22). This could be held responsible for the statistically significant rise of pO_2 recorded during the days following birth. This rise could indicate the improvement of respiratory function due to the increase of gases (O_2 and CO_2) diffusion capacity through the alveolar capillary membrane and to lung capillary vascularization (9).

Haemoglobin (Hb) and haematocritic value (Hct) show the highest values at birth, in order to oppose the hypoxic status of the newborn foal and decrease progressively over subsequent days. Haemoglobin varies from foetal to adult form and, as metabolic response to acidosis, a remarkable release of tissue oxygen shifts O_2 the dissociation curve to the right preventing hypoxia due to acidosis (3).

On the basis of the obtained results, we are able to confirm an improvement of respiratory function, day by day, because of the increase of pulmonary diffusion of respiratory gases and haemoglobin biochemical profile. In fact, blood gases, which are in equilibrium with alveolar gases, are an obvious indicator of gas exchanges, and therefore of the relation between metabolic rate and ventilation.

Table 1. Mean values of pH, bicarbonates concentration (HCO_3^-), carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), haemoglobin (Hb), haematocritic value (Hct) and rectal temperature, expressed in their conventional units together with the relative standard deviations, obtained during the first week of life in six foals

PARAMETER	DAYS					
	1	2	3	4	5	6
pH	7.37 ± 0.01	7.36 ± 0.01	7.43 ± 0.01	7.42 ± 0.01	7.41 ± 0.01	7.40 ± 0.01
HCO_3^- ($mEq.l^{-1}$)	26.10 ± 0.65	25.60 ± 0.55	24.37 ± 0.40	23.10 ± 0.34	22.70 ± 0.24	21.80 ± 0.22
pCO_2 (mmHg)	48.03 ± 1.32	49.30 ± 1.26	50.80 ± 1.33	49.10 ± 1.17	47.32 ± 1.08	46.82 ± 1.34
pO_2 (mmHg)	72.40 ± 1.88	88.09 ± 1.54	92.83 ± 2.48	89.60 ± 1.77	86.83 ± 2.01	83.70 ± 1.91
Hb ($g.dl^{-1}$)	15.62 ± 0.62	14.92 ± 0.65	13.80 ± 0.49	13.50 ± 0.71	13.40 ± 0.58	11.93 ± 0.52
Hct (%)	43.20 ± 0.68	40.50 ± 0.57	38.60 ± 0.78	37.10 ± 0.61	36.90 ± 0.51	34.10 ± 0.52
Rectal temperature (°C)	38.80 ± 0.67	38.90 ± 0.76	38.40 ± 0.71	38.60 ± 0.70	38.80 ± 0.86	38.90 ± 0.97

The results we obtained of physiological conditions in foals, outline the occurrence of acid-base homeostatic mechanisms (already available at birth, though not completely functional), and the physiologic evolution of the organs and parameters studied. Our future investigations will converge in this direction, since variations of age-dependent data of respiratory function and of acid-base balance regulation could represent a point of reference to better interpret, also from a clinical point of view, phenomena occurring during the first week of life in the foal.

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THE INFLUENCE OF INCREASED CONCENTRATIONS OF VOLATILE GASEOUS SUBSTANCES ON SELECTED LIVER PROFILE PARAMETERS IN THE BLOOD SERUM OF THE POLAR FOX (*Alopex lagotus*)

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ABSTRACT

Investigations were conducted to determine the impact of gaseous pollutants on the liver profile parameters in the blood serum of the polar fox. The animals which formed the control group were kept on a farm while the treatment group was kept in an air conditioned chamber. The biochemical examination of fox blood serum showed a slight increase in ALP, AST, ALT, GGT and bilirubin level and a decrease in LDH in the animals from the treatment group compared to the control.

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

INTRODUCTION

Currently fox rearing and breeding have been based on selection and breeding efforts where, apart from the improvement in productive qualities and reproduction indices, the tendency to obtain the best possible skins predominates. To make progress in this area it is important to meet the animal nutrition requirements, modify rearing conditions according to the latest knowledge and keep the animals in healthy physical shape. In order to reach these goals one must also pay attention to the animals' environment. Optimum microclimate conditions, i.e. appropriate temperature, moisture, relatively clean air, etc. (5, 6, 7), should be maintained to ensure the full physiological and productive efficiency of the animals. However, fox farms frequently face problems of environment pollution. At present, no reports on the impact of gaseous volatile substances on the health of foxes kept on farms are available in the literature.

The aim of our study was to investigate the influence of increased levels of air pollutants on selected liver profile parameters in the blood serum of mature polar foxes.

MATERIALS AND METHODS

The investigations were conducted on a farm situated in the south-eastern part of Poland. During the experiment about fifty polar foxes *(Alopex lagotus)* were kept on this farm. The animals (n = 12) caged in a pavilion system made up the control group (six females and six males). In order to investigate the effect of volatile gases on foxes a group of animals (the treatment group) was selected at random (six females) and placed in an air-conditioned chamber with constant air flow. The air was monitored continuously both in the chamber and outside on the farm (6, 7).

During the experiment the animals were supplied the same diet according to the feeding standards for fur animals in relation to their age. The animals were under veterinary and animal husbandry supervision throughout the experiment.

Blood samples were collected from the foot vein *(v. saphena lateralis)* three times (I—III). The blood serum was examined for the activity of alkaline phosphatase (ALP), asparagine aminotransferase (AST) and alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), and lactate dehydrogenase (LDH) using Cormay diagnostic kits and the total bilirubin (Bil) was determined by a reflectometer.

The data were evaluated statistically with a variance analysis for restrictive weighted nonorthogonal triple cross classification.

RESULTS AND DISCUSSION

Liver is an organ exposed especially to all harmful substances. The general condition of this organ is reflected in parameters constituting the so-called liver profile which include, among others, ALP, AST, ALT, GGT, LDH and bilirubin levels. The liver adapts to the presence of harmful compounds through the intensified production of enzymes participating in the metabolic degradation of their components. This phenomenon is commonly known as enzymatic induction and correlates to the changes observed in slaughtered animals (2, 3, 5).

The results of the biochemical examinations of the blood of the experimental and control foxes are summarised in Figs. 1—4.

The mean ALP levels reached 46.35 U.l^{-1} in females and 48.84 U.l^{-1} in males from the control group and 51.38 U.l^{-1} in females from the treatment group (Fig. 1). The slight increase in the activity of ALP observed in the experimental animals was insignificant. The ALP activity of both examined groups proved to be higher than the reference values of Berestov (2), however, they were in the standard range for canine (3, 5, 12). The ALP values of control foxes did not correspond fully to those obtained by other authors (4, 10, 11).

An increase in blood serum AST and ALT levels is considered a sensitive index of pathological changes in the liver which precedes the morphological manifestations of hepatocyte damage (2). In the examinations performed the mean AST values were 35.62 U.l^{-1} in females and 39.55 U.l^{-1} in males maintained on the farm and 36.60 U/l in those kept in the chamber (Fig. 1). The AST activity slightly surpassed the reference values mentioned by Berestov (2) yet corresponded to the standards presented by Boyd (3) and Meyer (5). The AST level in the examined groups did not indicate any statistical differences. In the treatment group statistically significant differences ($P < 0.001$) were observed between individual samplings (I to III).

The mean ALT activity in the females from the treatment group was a little higher (55.15 U.l^{-1}) compared to that in the control (54.84 U.l^{-1}) (Fig. 1). The highest levels of this enzyme were reported for the control males (57.29 U.l^{-1}) but did not exceed the reference values (3, 5). No statistically significant differences between the groups were detected. In the foxes kept in the chamber statistical differences ($P < 0.005$) were found between the three samplings (I to III).

The slightly elevated activity of AST and ALT in the treatment group may imply the activation of mechanisms involved in the metabolism of gas pollutants. The enzyme levels reached in successive samplings in the treatment group indicated that some balance and adaptation to

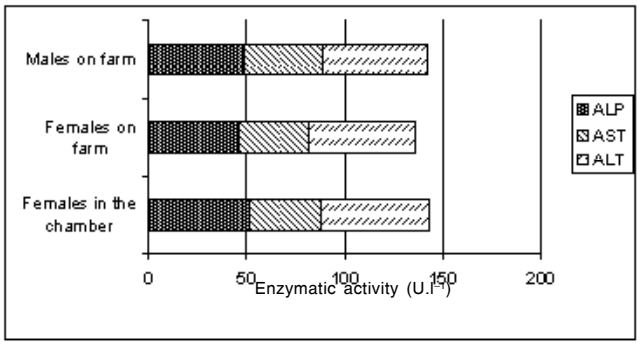

Fig. 1. The mean activity of ALP, AST, ALT in foxes kept on the farm and in the climatic chamber

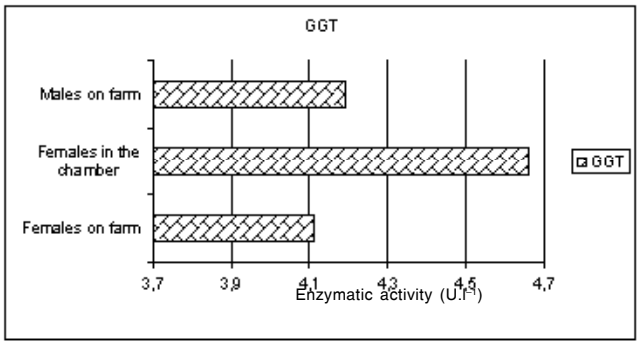

Fig. 2. The mean GGT levels in foxes kept under different environmental conditions (U.l^{-1})

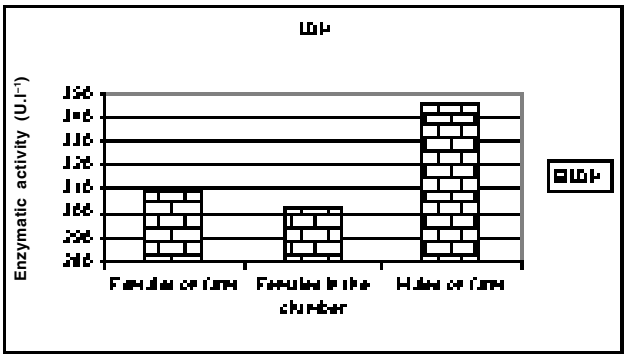

Fig. 3. The mean LDH levels in foxes from the control and treatment group

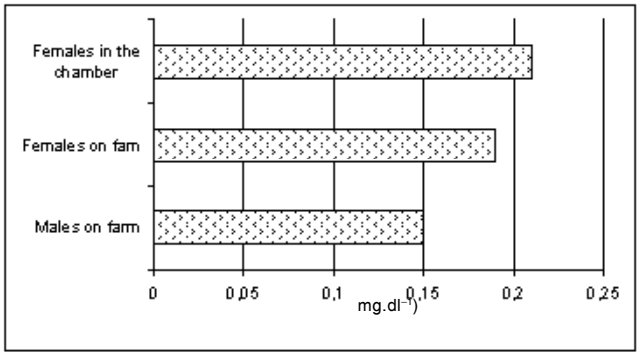

Fig. 4. The bilirubin level in foxes in successive samples (mg.dl^{-1})

different maintenance conditions may have developed in the organisms. The increase in ALT and AST activity in the IIIrd sampling may reflect the response of foxes to gaseous pollutants.

The mean GGT activity in the foxes from the control group indicated a similarity in results (Fig. 2). The highest concentrations of GGT (4.66 $U.l^{-1}$) were recorded in the foxes kept in the chamber and were within the reference range (3, 5).

The highest GGT activity in both animals and men is detected in the kidneys. Its increase is likely the consequence of liver infection or inflammation. So far it has been shown that serum activity increase usually follows some physical effort. Therefore, when analysing the impact of gas pollutants it is advisable to consider the response of enzymes to each individual substance (2, 9).

Lactate dehydrogenase is an enzyme detected in all body cells. The studies showed that its highest activity was recorded in the foxes from the control group (males 345.93 $U.l^{-1}$, females 309.78 $U.l^{-1}$) (Fig. 3). In the successive samples the level of this enzyme showed a tendency to decrease in males and slight growth in females. All the values were in the broad reference range given by Boyd (3) and Meyer (5).

The increased LDH activity is associated, among others, with cell necrobiosis, the state of elevated cellular membrane permeability resulting from ischaemia or the action of toxins. The activity of this enzyme may be inhibited by the activity of free radicals due to the high affinity of LDH to hydroxyl radicals (8).

The mean bilirubin level in fox blood serum exceeded the reference value of Berestov (2), yet it was consistent with the standards of Boyd (3), Meyer (5) and Winnicka (12).

The bilirubin level in the control group ranged between 0.12—0.17 $mg.dl^{-1}$ for males and 0.16—0.20 mg. dl^{-1} for females (Fig. 4). It turned out to be the highest in the treatment group varying from 0.17 to 0.25 $mg.dl^{-1}$. Considering all three samples, a tendency of bilirubin to increase was noted. However, the differences were insignificant.

The liver is an organ in which harmful toxic substances are detoxified but also compounds absorbed earlier potentially accumulate. This may lead to various changes in the liver (hypersensitivity, hepatocyte damage). Because of the accumulation of endotoxins, for example, ammonia, mercaptans, and phenols, its metabolic functions may be impaired and protein biosynthesis processes may become affected. Along with the agents damaging the liver parenchyma scars may appear with progressive cellular necrosis. This is reflected in an increased concentration of bilirubin and a slight rise in ALT, AST and LDH activity (3, 4, 5).

The gaseous pollutants entering the body act as xenobiotics and induce various changes. Intensification of these disturbances depends on many factors, among others self defense processes and characteristics like species, age and gender and intensity of exposure (concentration and properties of compounds). Our investigations on the fox farm showed that the level of gaseous pollutant in the conditioned chamber was higher than on the farm. In the air in the chamber ammonia was increased. High levels of other gases were also detected, for example 2-methyl 1-propanol, phenol, ethylbenzene, naphthalene, 1-penthanol, indoles, dodecane, cyclobutanole, and benzene. Consequently, the study focused on the investigation of the body response to the action of harmful compounds (6, 7).

In conclusion it should be noted that the investigation of the exposure of foxes to volatile gaseous pollutants has not provided an explicit answer with regard to liver profile parameters. Prolonged exposure to air pollutants under different maintenance conditions will be the subject of further investigations.

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THE INFLUENCE OF AIR POLLUTION ON LYSOZYME ACTIVITY IN THE POLAR FOX *(Alopex lagotus)*

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ABSTRACT

The study was conducted to investigate the influence of gaseous air pollutants on lysozyme activity in foxes as one of the non-specific immunity elements. The material for analysis was collected from adult foxes and their offspring, kept on a farm in cages in the pavilion system (control group A) and in a confined space (group B). Lysozyme activity in the adult females from group A and B ranged between 1.40—18.8 µg.ml^{-1} and 1.57–1.93 µg.ml^{-1}, respectively, while that in the adult males kept in the pavilion system (group A) was between 1.30—1.65 µg.ml^{-1}. The lysozyme values in the young foxes ranged between 0.83—1.05 µg.ml^{-1} and 0.85—1.13 µg.ml^{-1} in females and males of the control group (A1), resp. In the treatment group (B1) the respective ranges were 0.87—1.17 µg.ml^{-1} and 1.18—1.30 µg.ml^{-1} for females and males, resp. The animals exposed to more polluted air showed an increase in lysozyme activity.

Key words: air pollution; lysozyme activity; polar fox *(Alopex lagotus)*

INTRODUCTION

Lysozyme is considered a natural inhibitor of the development of micro-organisms. It was discovered by Fleming in 1929 while studying the biological bactericides. Lysozyme (muramidase) is an enzyme detected in systemic bodily fluids, among others in blood, saliva, tears, gastric and intestinal juice, milk, semen, urine, and organ tissues. Its activity results in damage to some bacterial cellular walls under both aerobic and anaerobic conditions and is based on non-specific immunity mechanisms. The alkaline properties of lysozyme are strong enough to neutralize the acid mediators of inflammatory processes. Pathologically changed organs and tissues exhibit an increased activity of lysozyme which stimulates the organism's self defence system. The literature dealing with lysozyme activity is scarce. It usually involves horses, as the level of this enzyme in mare's milk is high, but it has also been investigated in poultry including eggs and in fish (1, 2, 3, 4, 10).

The objective of the present study was to investigate the influence of gaseous pollutants on lysozyme activity in the polar fox as one of the non-specific immunity elements.

MATERIALS AND METHODS

The investigations were conducted on farm "C" situated in the south-eastern part of Poland housing about 50 polar foxes *(Alopex lagotus)*. The animals were kept in cages in a pavilion system (group A: females n = 6, males n = 6). To identify the effect of gaseous pollutants in the animal environment, a group of animals was selected and placed in a confined space with restrained air movement (group B – 6 females). After parturition the adult females were transferred from the confined space to the pavilion, while their offspring remained under the same conditions throughout the growth and development stages till slaughter (group B1: 8 females, 5 males). The young of the foxes from the pavilion, i.e. group A, made up the treatment group – A1 (6 females, 5 males). Throughout the investigation period the animals were under veterinary and animal husbandry supervision.

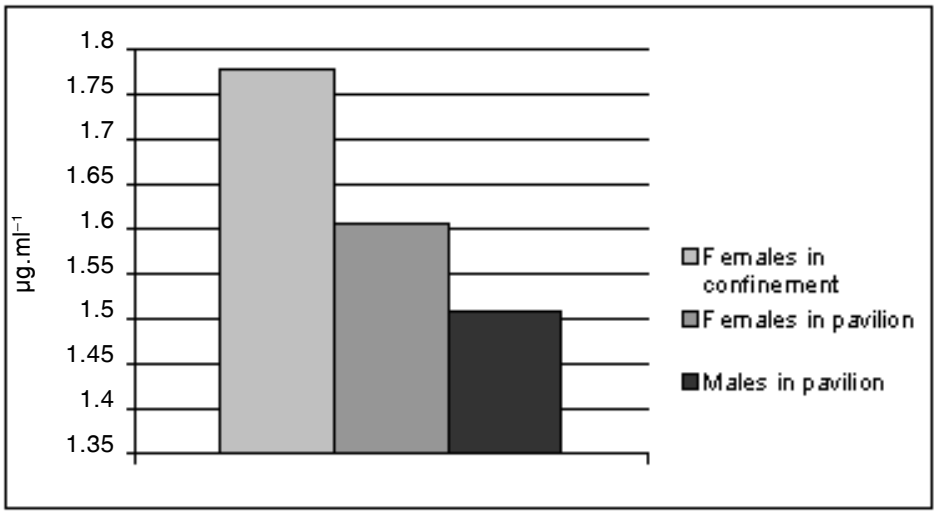

Fig.1. Lysozyme activity level in adult foxes related to maintenance conditions (µg.ml^{-1})

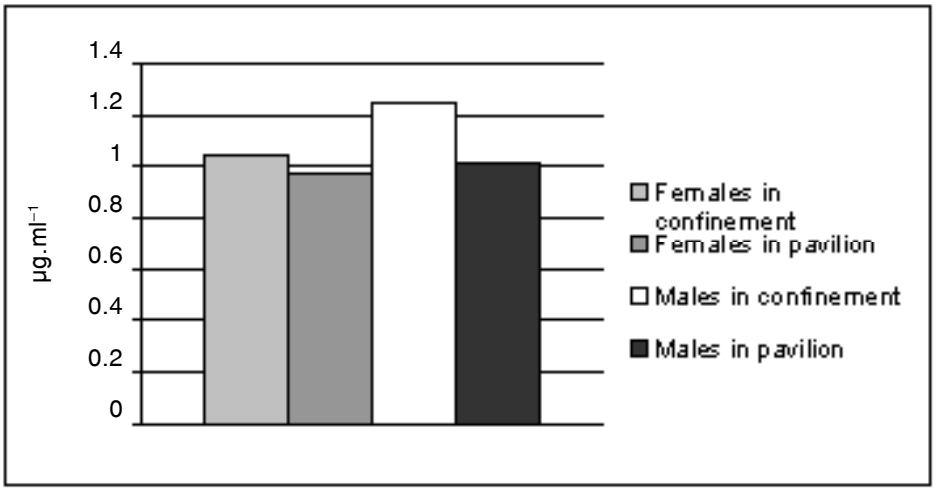

Fig. 2. Lysozyme activity level in young foxes related to maintenance conditions (µg.ml^{-1})

Table 1. Lysozyme activity in adult polar foxes kept under different environmental conditions (µg.ml^{-1}; $\bar{x} \pm SD$)

No.	Females in confinement (group B)		Females in pavilion (group A)		Males in pavilion (group A)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1	1.88	0.38	1.88	0.17	1.50	0.10
2	1.68	0.03	1.63	0.12	1.50	0.02
3	1.77	0.21	1.58	0.08	1.60	0.05
4	1.83	0.13	1.40	0.26	1.65	0.20
5	1.93	0.06	1.65	0.22	1.30	0.10
6	1.57	0.25	1.50	0.15	1.51	0.01

Blood samples for examination were collected from the foot vein *(v. saphena lateralis)* three times. Lysozyme concentration was determined using the modified Hankiewicz plate method (5).

RESULTS AND DISCUSSION

The health parameters and animal productivity depend mainly on feeding and maintenance conditions. The immune system is also affected by the above mentioned factors and by the animal habitat.

Air pollution is one of the local climate factors which can disturb animal body homeostasis. In this respect the animal immune system and non-specific immunity mechanisms, among others the lysozyme levels, are the elements reflecting the changes.

The lysozyme values in the polar fox related to the maintenance conditions are presented in Tables 1 and 2. The lysozyme activity in the adult female foxes included in the experiment was in the following intervals: 1.40—18.8 µg.ml^{-1} – control (A), 1.57—1.93 µg.ml^{-1} – group B (in confinement), 1.30—1.65 µg.ml^{-1} – (group A as well).

Figure 1 shows that the females in the enclosed space exhibited a substantial increase in lysozyme activity as compared to other foxes kept on the farm (group A). A difference in lysozyme levels in the examined groups indicate the impact of the environment, i.e. gaseous pollutants. In the confined house only minimal (although constant) airflow was ensured to remove volatile gaseous substances released from excrement or feed residua (7, 8).

Table 2. Lysozyme activity in young foxes kept under different environmental conditions (μg.ml^{-1}; $\bar{x} \pm SD$)

No.	Foxes in confinement (group B1) $\bar{x}$	SD	Foxes in pavilion (group A1) $\bar{x}$	SD
		females		
1	1.05	0.13	1.00	0.44
2	1.05	0.18	0.98	0.22
3	0.87	0.67	1.02	0.27
4	1.17	0.37	1.05	0.15
5	1.02	0.35	0.83	0.72
6	1.12	0.16	0.98	0.36
7	1.00	0.36	–	–
8	1.08	0.40	–	–
		males		
9	1.30	0.30	1.13	0.21
10	1.27	0.35	0.97	0.76
11	1.23	0.17	1.13	0.58
12	1.18	0.25	0.85	0.39
13	1.24	0.27	0.98	0.20

The lysozyme values in young foxes ranged between 0.83—1.05 μg.ml^{-1} for the control group (females – A1) and 0.85—1.13 μg.ml^{-1} for males. The values reached in the treatment group were 0.87—1.17 μg.ml^{-1} (group B1 – females) and – 1.18—1.30 μg.ml^{-1} (males). The lower lysozyme values found in young foxes indicate that the young organisms and also their immunity is still in the stage of development. The comparison of lysozyme values of males and females from the two experimental groups (A1 and B1) showed that higher lysozyme activity was found in the animals exposed to increased air pollution (Fig. 2). This fact suggests stimulation of adaptative mechanisms in animals from group B1 to the respective environment reflected particularly in activation of non-specific immunity.

Lysozyme represents one of many barriers that protect animals against hazardous environmental agents. Besides the acute phase proteins, it reflects also the immune system activation status and allows us to detect the animals, which under go harsh immunological stress phases (1, 6). The increase in lysozyme activity as a non-specific humoral immunity indicator related to the experimental agent is considered relevant with regard to the activation of the defence response of the organisms of foxes. The volatile gaseous substances released into the environment penetrate the animals as xenobiotics and change their immune system mechanism. Affected by the xenobiotics the organism's tissues cease to be tolerated by the immunity system. Świerczewska *et al*. (9) claim that the animal maintenance system exerts a significant influence on the level of all enzymes, i.e. enzymatic homeostasis. In extensive maintenance the microclimate conditions go out of control.

The study performed was only a part of the fox health monitoring intended to detect the influence of volatile gaseous substances. This issue should be addressed in the future using appropriate examination methods.

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THE WHEAT PROTEIN, SEITAN, COLORED BY RED FERMENTED RICE IN THE INNOVATION OF POULTRY MEAT PRODUCTS

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ABSTRACT

The steadily increasing critical consciousness of consumers of healthy products and foodstuffs for everyday essentials regards the industry as unprepared to take this into consideration. In an effort to offer a new and healthy product to consumer, we used wheat protein – seitan, for the production of mosaic poultry salami. Seitan, colored by red fermented rice is rich in proteins, does not contain fat and cholesterol and can replace part of the meat in meat products. The consumer values a color that is reminiscent of beef. The organoleptic characteristics of the meat products were evaluated by professionals. Apart from an attractive appearance, seitan also imparted a special taste to the products and a firm consistency. Seitan colored by red fermented rice did not influence the color stability of the meat products.

Key words: mosaic poultry salami; seitan; red fermentenented rice

INTRODUCTION

Colorants are considered to be essential in the foodstuff industry manufacturing process. Food coloration improves food preservation and has thus become an important goal in the food industry. Food colorants may be natural or synthetic and often devoid of nutritional value. Most of the natural dyes are extracts from plants and plant products or are produced by microorganisms, which provide benefits to production. Since the number of permitted synthetic colorants has decreased because of undesirable toxic effects including mutagenicity and potential cancerogenicity, interest has focused on the development of food pigments from natural sources (10).

Microbial colorants that occur only in certain microorganisms are beneficial because of their thermal stability in a wide pH range and because of their ability to react with the substratum (mostly proteins) creating a stable complex (4). Furthermore, these colorants have some antibacterial effect as has been demonstrated by a number of authors (5, 8, 14).

A typical representative of these colorants is the mixture of the pigments produced by the mould *Monascus* spp., belonging to the family Aspergillaceae, the genus *Monascus* (13). Red fermented rice, also known as red-koji, or hongqu is produced on the rice substratum by the fermentation of the fungus *Monascus purpureus*. It contains mainly of nonglutinous rice, red yeast and byproducts of the fermentation. The color shade of the mixture and ratio of the pigments can be changed by fermentation. Active side chain binding on the food component, prevents colorant diffusion to the other parts of the product and that way the attractive look of the final meat or vegetable product is kept (6).

The organoleptic evaluation of the microbial colorant – a very fine spicy flavour, was the reason why the fermentation of various products has been spread in the food industry. It has been used mostly in Far Eastern cuisine and its antibacterial effect is considered to be a positive attendant phenomenon (11).

The maintained color of the meat and sausages is due to toxic nitrites. Although substances added to the product in residual quantity do not induce acute toxicity, there is a latent health hazard from the angle of the eventual production of nitrosamines after their consumption. This fact has prompted the reduced use of nitrites in meat products and their replacement by other additives, such as *Monascus*. *Monascus* fulfils all the functions of nitrites and its effect is even better. Due to the

use of this microbial colorant we can achieve a red color in meat products requested by consumers and also conservation of the products by reducing the activity of the toxins produced by microorganisms (9).

The aim of our study was to prepare an innovatory poultry meat product in which part of the meat was replaced by seitan (wheat protein) colored by red fermented rice.

MATERIAL AND METHODS

To produce a new poultry product we used a meat preparation, that was produced in Hydina ZK Košice and the wheat protein, seitan (Alfa Bio Banská Bystrica), colored by two grams of red fermented rice per 1000 grams of seitan. We prepared three products. The first product (M) was poultry salami with the seitan addition to the amount of 300 grams per 1000 grams in the poultry preparation. Seitan was modified to fine mosaic with grain 0.3–0.5 cm. In the second product (H) the seitan was cut in 0.8–1.0 centimetre cubes and added in the amount of 150 grams per 1000 grams of poultry preparation. The third product (C) was a control sample without the seitan addition. The products were placed into a polyamide cover (∅ 45 mm) and heat treated for ten minutes at 70 °C. The samples were stored at 4 °C for seven days and at -18 °C for thirty days.

All products were organoleptic evaluated and microbiologically examined. The microbiological examination was performed on Days 2, 7 and 30 after production. The total count of microorganisms, the number of coliforms and the presence of *Salmonella* spp. and *Staphylococcus* spp. were determined. The organoleptic evaluation was done on a five-point scale by a six-member commission evaluating the color, taste, consistency, cutting view and inner view on Days 2, 7 and 30 after production and storage.

RESULTS AND DISCUSSION

The use of red fermented rice improves the intensity and color stability in the final products (3). For the production of new poultry products we used a seitan (wheat protein) colored by red fermented rice. This colored seitan, which replaced part of the meat, is rich in proteins and it does not contain fat, either cholesterol in contrast with meat. We compared these three products of mosaic poultry salami 2, 7 and 30 days after production and storage at 7 °C and –18 °C.

There are evaluations of taste, consistency, cutting view and inner view of the new poultry products in Table 1 on the second day after production and thirty days after production in Table 2. These products were evaluated as very good. The product M gained the best results. The seitan colored by red fermented rice fitted well into the whole and did not affect the general look of the product. Furthermore, this colored seitan also provides a special taste and improves the consistency of the product. Similar positive evaluations of products with red fermented rice addition have been demonstrated by several authors (2, 1, 4, 7).

The organoleptic evaluation after seven days of storage at cooled temperature was similar to results from the second day after production. After the thirty days storage in a refrigerator we determined the stability and the influence of the seitan on the organoleptic characteristics. We found out that this colored seitan kept its color even after thirty days, did not influence the color stability and the consistency of the product was evaluated as very good.

The results of the microbiological examination are presented in Table 3. These results correspond with the values prescribed in the *Slovak Codex Alimentarius (12).* The red fermented rice addition in our meat products did not confirm the microbiological stability improvement definitely, as has been affirmed by a number of authors (5, 8, 14).

It is necessary to undertake a lot of examinations and to test many technological procedures in order to achieve the introduction of red fermented rice in large-scale production.

CONCLUSION

Table 1. The results of the organoleptic evaluation of poultry products two days after production

Organoleptic parameters	M	H	C
Color	4.83	4.67	4.17
Taste	4.83	4.67	4.83
Consistency	5.00	4.50	4.67
Cutting view	4.86	4.17	4.33
Inner view	4.50	4.17	4.00
T o t a l	24.02	22.18	22.00

M — product with 300 g seitan addition
H — product with 150 g seitan addition
C — control

Table 2. The results of the organoleptic evaluation of the poultry products thirty days after production

Organoleptic parameters	M	H	C
Color	4.83	4.67	4.00
Taste	4.83	4.67	4.50
Consistency	4.60	4.00	3.83
Cutting view	4.86	3.83	3.83
Inner view	4.12	4.00	4.12
T o t a l	23.24	21.17	20.28

M — product with 300 g seitan addition
H — product with 150 g seitan addition
C — control

Table 3. The results of microbiological examination of the poultry products in one gram of the samples

Samples	Day/temperature of storage	Total count of MOs	Coliforms	*Staphylococcus aureus*	*Salmonella* spp. (in 25 g)
C	2/4 °C	1.2. 10^3	0	0	0
	7/4 °C	2.8. 10^3	0	0	0
	30/-18 °C	5.1. 10^2	0	0	0
H	2/4 °C	5.0.10^2	0	0	0
	7/4 °C	2.4.10^3	0	0	0
	30/-18 °C	4.8.10^2	0	0	0
M	2/4 °C	5.0. 10^2	0	0	0
	7/4 °C	2.3. 10^3	0	0	0
	30/-18 °C	4.6. 10^2	0	0	0

M — product with 300 g seitan addition
H — product with 150 g seitan addition
C — control

We prepared a new poultry meat product, in which the part of meat was replaced by the wheat protein seitan, colored by red fermented rice. The consumer values a color that is reminiscent of beef and with a lower amount of fat and cholesterol in the final product. The results of microbiological examination and organoleptic evaluation of taste, consistency, cutting view and inner view are very good. This colored seitan imparts a special taste to the product, improves its consistency and it also has no influence on the color stability, not even after thirty days of storage.

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CHRONICLE

PROF. JOZEF VODRÁŽKA, MVD, DSc — PIONEER SLOVAK PHARMACOLOGIST CELEBRATES HIS 82nd BIRTHDAY

In connection with the 82nd birthday of Prof. Jozef Vodrážka, DVM, DSc, allow us to inform readers of this journal about the work and life of this important pioneering Slovak pharmacologist, in a concise form. We think that more than 25 years of continual co-working and co-publishing professional and scientific articles and reports with him, requires us to commemorate and demonstrate — at least in a condensed form — his professional and scientific profile and also the milestones in his life.

Fig. 1. Prof. Jozef Vodrážka, DVM, DSc

University teacher Jozef Vodrážka (see Fig. 1)was born on the 5th February 1922 in Málinec-Hámor, in the district of Lučenec, Slovakia.

The first part of his university education was at the Veterinary University in Vienna (Austria) and he finished his studies in Brno (Bohemia) in 1946. In the years 1946—1947 he worked at the Slovak Ministry of Agriculture and Agricultural Reform in Bratislava. Then in the year 1948 he continued his studies in Great Britain. In the period 1949—1950 he was a worker at the State Diagnostic Institute in Bratislava, and from the 1st August, 1950 he worked until his retirement at the Veterinary University in Košice. In 1962 he was promoted to the position of docent, then in 1966 he received his DSc and in 1967 he was made professor. From 1975 he was also active as an external worker in The Institute of Experimental Pharmacology, at The Slovak Academy of Science, in Bratislava. Later on he examined at Makarere University (Uganda) and in 1980 he visited Cairo University.

He laid the foundations of veterinary pharmacology at the university and as a pedagogical subject in Slovakia. He developed and published two critical methods for testing and evaluating chemical agents in sheep for antihelmintic activity and effectiveness (the method of total tracheotomy, see e.g. article in *Veterinary Record,* 72, 1960: 404—405), and liver parasites (collateral tubing method for the gall-bladder, see e.g. *Nature,* 199, 4888, 1963: 96—97). These were widely cited in domestic and foreign professional and scientific literature (e.g. *Proceedings of the 1st international conference of the World association for the advancement of veterinary parasitology,* Hannover, 1963, 34—44, 62—73, 129—136; *Proceedings of the 18th world veterinary congress,* Paris, 1967, 1: 101—107; *Tijdschr. Dirgen.,* 1967, 92: 977—979 etc.).

He read many papers about his methods at various world conferences, symposia, and congresses; and also demonstrated them in many veterinary colleges, universities, and research institutes in Europe and overseas. He introduced several veterinary antihelmintic preparations and tested their effectiveness and tolerance before their industrial production and practical use. He was also one from the first teachers of the University of Veterinary Medicine in Košice (Slovakia) and an organizer of postgraduate studies for veterinary surgeons and pharmacists in Slovakia. He was author of more than 150 profes-

sional and scientific reports and articles at home and abroad. In addition, he was also the principal author of the book *"Veterinary Medicine and Pharmacology for Pharmacists",* (Martin 1974), and its two later editions (1982 and 1986) and an important book *"Veterinary Pharmacology"* (Brno 1980).

Furthermore, he was also one of the co-founders and a member of the *European Association of Veterinary Physiologists, Pharmacologists and Biochemists.* In the period 1967 to 1973 he was vice-chairman of the *Slovak Pharmacological Society* and a member of many *ad hoc* commissions and scientific boards at the ministries (for agriculture; health), as well as at research institutes. In addition, he was a member of committees at our veterinary university and of the *Czechoslovak Pharmacopoeia Commission.*

Fig. 2 Decoration of prof. J. VODRÁŽKA, DVM, D.Sc. at the UVM in Košice

He was decorated with the *Silver Adami Medal* (1969) and with the *Golden Adami Medal* at the University of Veterinary Medicine in Košice (1977) and received other *Commemorative Medals* from our university and from foreign institutions.

His scholars, co-workers, and co-authors of several articles and reports, wish him good health and satisfaction *"ad multos annos"* on the occasion of his eightieth birthday.

Václav Šutiak
UVM Košice

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