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FOLIA VETERINARIA, 46, 2: 57—60, 2002

THE DISTRIBUTION OF S-l00 PROTEIN IN THE PIGEON TESTIS

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SUMMARY

The expression of the S-l00 protein in the pigeon testis using an immnunohistochemical method was studied. The immunopositivity for S-l00 protein was localized in the Sertoli cells and nerve fibres distributed in the intertubular connective tissue either alone in close contact with seminiferous tubules or accompanying blood vessels. Positive-reacting fine nerve fibres were localized in the *tunica albuginea* with a higher concentration of the nerve fibres in the area separating the testicular and epidydimal region. In the interstitial tissue of the epidydimis positive nerve fibres stained for S-l00 protein were seen in close contact with the epidydimal lining epithelium. Squamous and cuboidal epithelial cells of the *rete testis* displayed strong positivity for S-100 protein.

Key words: immunohistochemistry; pigeon; S-l00 protein; testes

INTRODUCTION

S-l00 protein has described in a variety of cellular types of both neuroectodermal and nonectodermal origin. In the testis, it has been observed in the cytoplasm of the interstitial Leydig cells of the human and rat testes (5, 14). The Leydig cells are not the only cells to be stained positively. Holash *et al*. (8) reported the presence of S-l00 protein in the lymphatic endothelial cells of the rat testis. Amselgruber *et al*. (2) studied the distribution of S-l00 protein in various mammals. In the bull, ram, boar and cat testes, S-l00 protein was localized in the Sertoli cells, whereas the endothelial cells of capillaries, veins, and lymphatic vessels reacted S-l00-positive in ruminant. In the Leydig cells, the authors found S-l00 protein to be strongly positive in the cat and rat testes but to a lesser degree, in the pig and horse testes. Sugimura *et al.* (l8) demonstrated pronounced species-specific differences in the labelling of S-l00 protein types in vertebrates. In the fowl, such studies are absent. The aim of the present study was to localize S-l00 protein in the pigeon testes.

MATERIAL AND METHODS

Tissue samples were obtained from 6 adult pigeon testes by cervical dislocation and fixed in 4 % formalin in 0.1 mol phosphate buffer, pH 7.2 for l2 h. After washing, tissue samples were dehydrated in ethanol, and embedded in paraffin. Sections 5 mm thick, were deparafinized in xylene and treated with l.5 % hydrogen peroxyde in methanol for 30 min to eliminate endogenous peroxidase reaction. To demonstrate the S-l00 protein, the avidin-biotin-peroxidase complex (ABC) method (9) was used. A commercial antiserum containing a polyclonal rabbit anti-S-l00 protein antibody was used. After washing in phosphate-buffered saline (PBS), the sections were immersed in non-immune goat serum and incubated with the primary antibody diluted l : l00 in PBS overnight in a humidity chamber maintained at 4 °C. The sections were then washed in PBS and incubated for 45 min successively with goat anti-rabbit biotinylated secondary antibody. After 1 h of incubation, the sections were incubated with ABC and developed with 0.05 % 3.3´-diaminobenzidine and 0.03 % v/v hydrogen peroxide for l0 min. Some sections were conter-stained with Meyer's haematoxylin. The negative controls were performed by omiting the primary antibody.

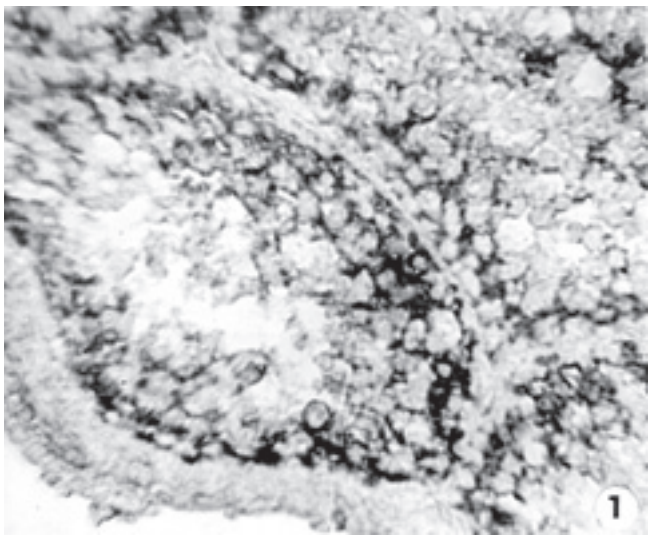

Fig. 1. Immunoreactivity to S-100 protein is seen inside the seminiferous tubules in the Sertoli cells (× 570)

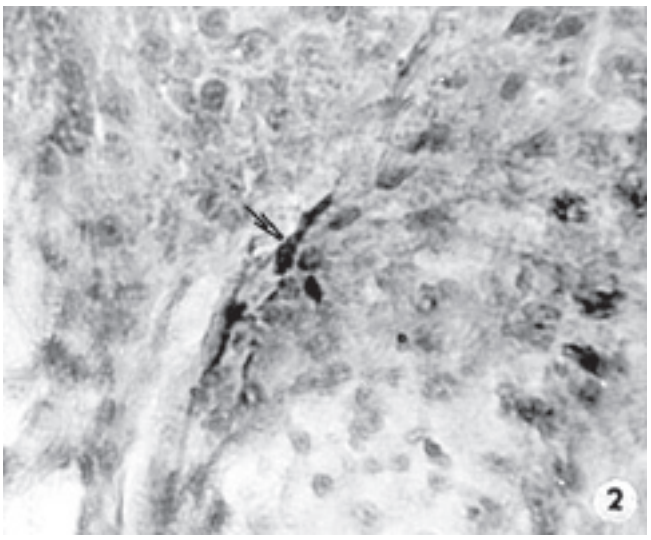

Fig. 2. Positive reaction to S-100 protein is seen in the nerve fibres among the seminiferous tubules (arrow) (× 570)

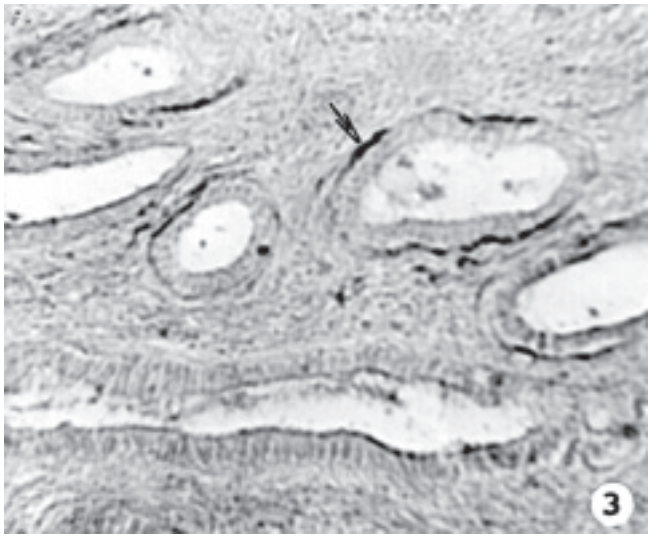

Fig. 3. Positive reaction to S-100 protein on the nerve fibres next to lininig epithelium of the *epidydimis* is seen (arrow) (× 540)

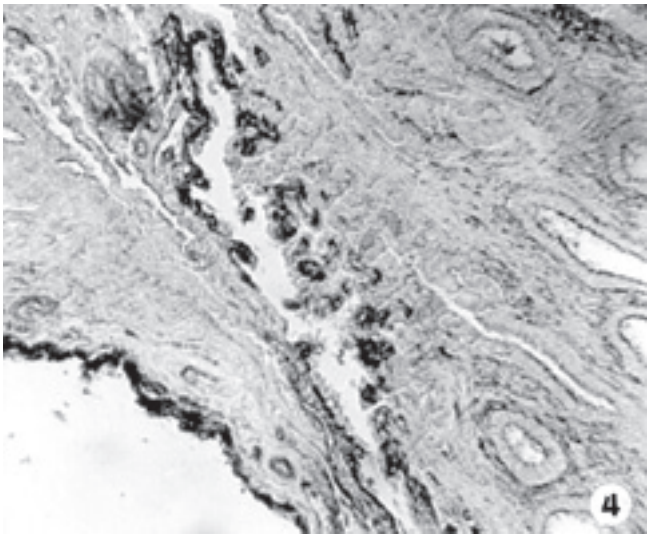

Fig. 4. Positive reaction to S-100 protein is seen in the epithelial cells of the *rete testis* (× 180)

RESULTS

The immunoreaction to S-l00 protein was found inside the seminiferous tubules in the Sertoli cells, where the cytoplasm and the nucleus gave strong positive reaction (Fig.1). In some cases, the cytoplasmic processes of the Sertoli cells form a dense red inside the seminiferous tubules. Most of positive the Sertoli cells lie among the spermatogonia and only a small amount of the positive cytoplasmic processes connect the basal layer of the *lamina propria*. Other S-l00 protein positive structures, the nerve fibres, were localized at the periphery of blood vessels and among the seminiferous tubules (Fig. 2). Positively reacting fine nerve fibres were also localized in the *tunica albuginea* with a higher concentration of the nerve fibres in the area separating the testicular and epidydimal region. In the interstitial tissue of the *epidydimis,* positive nerve fibres stained for S-l00 protein were seen in close contact with the epidydimal lining epithelium (Fig. 3). Squamous and cuboidal epithelial cells of the *rete testis* displayed strong positivity for S-100 protein (Fig. 4).

DISCUSSION

The Sertoli cells of the fowl testis positive to S-l00 protein exhibit morphological characteristics similar to those described in some mammals. The immunoreaction to this substance was, with the exception of the dog and rat, also found in the Sertoli cells in the majority of mammals (2). The study in mammals demonstrates that in the testicular tissue S-l00 protein is expressed in

a species-specific manner. In the tubular compartment of the pigeon testis, the S-l00 protein has been found only in Sertoli cells. All the developmental stages of germ cells were consistently negative for S-l00 protein as it was stated in mammals. Amselgruber *et al.* (2) have found differences in the staining intensity of the immunoreaction in the Sertoli cells which depend upon cyclic variations of the seminiferous epithelium, being the strongest in the stages associated with the elongation of spermatids. No such relation was seen in the pigeon testis. The protein is not restricted to Leydig cells as described for human and rat testis (l1, 14) but is restricted to Sertoli cells, as shown in the bull (1).

Positive staining of peritubular cells was found in rodents and carnivores whereas positive immunoreaction for S-l00 protein in the endothelial cells of capillaries, veins, and lymphatic vessels was restricted to the boar and ruminants (2). It is worth mentioning that the cells in the pigeon testes stained positively for S-l00, exibit morphological features, such as fine cytoplasmic processes, characteristic for the astrocytes, Schwann cells, pituicytes, pituitary folliculo-stellate cells, satellite cells of autonomic ganglia and the adrenal medulla, melanocytes, Langerhans' cells, reticular cells of lymphoid organs and Leydig cells (l4). The Sertoli cells belong to this group of cells morphologically.

Ferrer *et al.* (7), found a positive staining of myoepithelial cells of the apocrine sweat gland in the dog and also in the endothelial cells of the capillaries, veins and lymphatic vessels of the pig, sheep and bovine testes (2). Similarly, S-l00 immunoreaction in the endothelial cells of blood capillaries was reported by Iwanaga *et al.* (10) in pigs and cattle, and by Sugimura *et al.* (l8) who also found selective immunoreactivity for S-l00 protein in the endothelial cells in these animals. Michetti *et al.* (l4) have found S-l00 protein in lymphatic endothelial cells in the rat and human. Other interstitial cell types, in this species, including blood vessels, peritubular contractile cells, and germinal cells, appeared to be free from immunoreaction. Lymphatic endothelial cells exibited a subcellular distribution of the antigen similar to that observed in Leydig cells.

In testicular physiology the S-l00 protein could be involved in establishing the blood-testis barrier and may play a role in secretory and absorptive functions in the intratesticular excurrent duct system. The testis endothelium, has been suggested, contributes to the blood-testis barrier and these endothelium-barrier features are influenced by Leydig cells. The observations carried out *in vivo* (4, l3) and *in vitro* (6) revealed a relation between S-l00 and microtubules. It suggests a possible involvement of this protein with the cytoskeleton in a variety of definite cellular types. In mammals, different cellular types of the testis, positive to S-l00 protein, support the hypothesis that this is a multifunctional protein and may have different functions in testicular physiology. The presence of S-100 protein in the *rete testis*, which was also observed in mammals (2), may play a role in the secretory and absorptive functions in the excurrent duct system.

In the pigeon, the immunoreaction to S-l00 protein is not so widely distributed as in mammals. Its presence within the Sertoli cells indicates that S-l00 protein plays a role in the microtubule system in these cells. The presence of the nerve fibres inside the interstitial tissue among the seminiferous tubules and inside the *tunica albuginea* has been described in the rat testis (15, 16, 17) and is localized between the smooth muscle cells and fibroblasts. The presence of S-100 protein among the seminiferous tubules and in the *epididymis* is related to Schwann cells coating the nerve fibres (3) and confirms the results obtained with acetylated a-tubulin (12) in the same species and in Japanese quail testes.

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THE EFFECT OF CHRONIC CADMIUM AND MERCURY EXPOSURE ON THE PLASMA TOTAL ANTIOXIDANT STATUS IN RATS

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ABSTRACT

The effect of chronic exposure of cadmium (cadmium chloride – $CdCl_2$) and mercury (mercury chloride – $HgCl_2$) on the plasma total antioxidant status (TAS) was investigated in rats. A daily administration of cadmium in 5 different doses from 1.83 to 29.27 mg $CdCl_2.kg^{-1}.day^{-1}$ for 30 days led to a significant decrease in the plasma TAS in all the experimental groups. Mercury intoxication (0.15—2.47 mg $HgCl_2.kg^{-1}.day^{-1}$ for 30 days) led to a decrease in the TAS as well, most significantly in the groups with the highest doses of $HgCl_2$. The results show, that chronic cadmium and mercury intoxication causes dose-dependent consumption of extracellular antioxidants.

Key words: cadmium; chronic intoxication; mercury; plasma; rat; total antioxidant status

INTRODUCTION

Transition metals and their compounds are known to cause serious problems due to their accumulation and toxicity in living organisms.

Cadmium (Cd) is one of the most widespread environmental pollutants. It is released during the combustion of coal and mineral oils, smelting and alloy processing. Cd enters the organism via inhalation in the form of dust, but also by the digestive system. In cells, Cd binds to the sulphhydryl (-SH) groups of proteins. It can affect various metabolic processes, especially energy metabolism, membrane transport and protein synthesis (12). Cd may cause DNA fragmentation (10) and leads to apoptosis in cell (6). Cd is considered to be a carcinogen (7) and due to its negative influence on immune response it increases the intensity of infection (3). The production of reactive oxygen species (ROS) is also associated with Cd toxicity. Chronic Cd intoxication leads to lipid peroxidation in the liver, kidneys, and testes (12). Cd ions are strong inducers of superoxide radical ($O_2^{\cdot -}$) production in cell cultures (18).

Mercury (Hg) and its compounds are considered to be one of the most important pollutants. Hg is used in the manufacture of batteries, thermometers, paints and electrical and measuring apparatuses (2). The main targets of mercury are the -SH groups of the proteins. It leads to changes in protein shape and activity. Hg intoxication leads to oxidative stress in cells and to subsequent tissue damage. This may occur through multiple mechanisms: the rapid exhaustion of the free -SH groups (as a consequence of the reaction of mercury with thiols), a decreased activity of antioxidant enzymes or participation in the Fenton reaction (20). Hg also has influence on DNA and RNA structure and function (19) and affects immunological processes too (14, 15). The potential mutagen effect of Hg is probably connected with the excessive production of H_2O_2 (1).

In our study we observed the influence of chronic cadmium and mercury intoxication on the plasma TAS, an integrated marker of all plasma antioxidant effects in rats.

MATERIALS AND METHODS

One hundred and thirteen albino rats *(Rattus norvegicus alb.),* aged 3 months (91 ± 2 days), of average weigh 308 ± 34 g, were used for the experiment. The animals were housed in conventional conditions and they were fed *ad libitum* with a commercial laboratory rat fodder (Larsen diet pellets, Velaz Prague). The experiment was organized in two series. The first one was carried out in the summertime and the second one in the wintertime.

Fifty-three rats (age 91 ± 2 days, weight 318 ± 29 g) were used for the first series of the experiment. The animals were

divided into 6 groups each of 8 to 10 animals. Cadmium was given to the experimental groups as a cadmium chloride compound ($CdCl_2.2H_2O$, Sigma) in the tap water. The Cd content in $CdCl_2$ was calculated on the basis of the molecular weights of Cd and $CdCl_2.2H_2O$. The LD_{50} value of cadmium (acute intoxication) as a $CdCl_2$ compound *per os* for rat is 225 $mg.kg^{-1}$ (9). Doses ranging from 1/8 LD_{50} to 2 LD_{50} (groups Cd1 – Cd5) were divided into 30 daily doses (Tab. 1). Each daily dose was dissolved in 40 ml of water, that is an average daily intake of water for a rat weighing 300 g according to our experience. After 30 days the actual average intake of $CdCl_2$ was calculated. A control group (CdC) drank clear tap water.

For the second series of experiment 60 rats were used (age 91 ± 2 days, weight 298 ± 35 g). The rats were divided into 6 groups each of 10 animals. Mercury was administered to the experimental groups as a mercury chloride compound ($HgCl_2$, Lachema, Brno) in tap water. The LD_{50} of $HgCl_2$ compound (*per os* acute intoxication) for a rat is 37 $mg.kg^{-1}$ (2). Five different doses from 1/8 LD_{50} to 2 LD_{50} (groups Hg1 – Hg5) were divided into 30 daily doses as well as in the first series of experiment (Tab. 2). The control group (HgC) drank tap water.

After 30 days of treatment, all the groups were anaesthetized (Sodium pentobarbital, Pentobarbital Spofa, 50 $mg.kg^{-1}$ *i.p.*). After thoracotomy blood was collected from the heart using heparin (Heparinum natricum, Heparin Léčiva inj., 5000 $IU.l^{-1}$) as anticoagulant. Samples were centrifuged at 1500 *g* for 10 min and the blood plasma was used for the TAS assay.

The plasma total antioxidant status was determined by a spectrophotometric method (11) with a RANDOX-Kit (Total antioxidant status, RANDOX laboratories, UK). The measurement was carried out on an automatic spectrophotometric analyser Cobas Mira S (Roche, Switzerland).

The statistical significance of the differences between the groups was determined by using an unpaired Student´s *t*-test, p values of less than 0.05 were considered significant.

RESULTS

Data presented in Tab. 1 and Fig. 1 show, that daily administration of cadmium chloride for 30 days led to significant reduction of plasma TAS in all cadmium-treated groups compared to the control group (CdC: 1.17 ± 0.09 $mmol.l^{-1}$; Cd1: 1.03 ± 0.11 $mmol.l^{-1}$, $p < 0.01$; Cd2: 0.97 ± 0.02 $mmol.l^{-1}$, $p < 0.001$; Cd3: 0.97 ± 0.17 $mmol.l^{-1}$, $p < 0.01$; Cd4: 0.91 ± 0.17 $mmol.l^{-1}$, $p < 0.01$; Cd5: 1.05 ± 0.11 $mmol.l^{-1}$, $p < 0.05$). There was also significant difference between groups Cd4 and Cd5 ($p < 0.05$).

In mercury chloride-treated rats (Tab. 2 and Fig. 2) TAS also decrease in experimental groups compared to the control group (1.05 ± 0.08 $mmol.l^{-1}$). Highly significant was the reduction of the TAS value in group Hg1 (0.94 ± 0.06 $mmol.l^{-1}$, $p < 0.001$), group Hg4 (0.81 ± 0.04 $mmol.l^{-1}$, $p < 0.001$) and group Hg5 (0.85 ± 0.05 $mmol.l^{-1}$, $p < 0.001$). Significant lower was the TAS in group Hg3 (0.96 ± 0.10 $mmol.l^{-1}$, $p < 0.05$). Significant differences were

Tab. 1. Dose of $CdCl_2$ and TAS in the first series of the experiment

Exp. group	n	Dose of $CdCl_2$ $mg.kg^{-1}.day^{-1}$	$mg.kg^{-1}.30\ days^{-1}$	multiple of LD_{50}	TAS $mmol.l^{-1}$
CdC	8	–	–	–	1.17 ± 0.09
Cd1	10	1.83	54.88	1/8 LD_{50}	1.03 ± 0.11
Cd2	8	3.66	109.75	1/4 LD_{50}	0.97 ± 0.02
Cd3	9	7.32	219.50	1/2 LD_{50}	0.97 ± 0.17
Cd4	9	14.63	439.00	LD_{50}	0.91 ± 0.17
Cd5	9	29.27	878.00	2 LD_{50}	1.05 ± 0.11

Tab. 2. Dose of $HgCl_2$ and TAS in the second series of the experiment

Exp. group	n	Dose of $HgCl_2$ $mg.kg^{-1}.day^{-1}$	$mg.kg^{-1}.30\ days^{-1}$	multiple of LD_{50}	TAS $mmol.l^{-1}$
HgC	10	–	–	–	1.05 ± 0.08
Hg1	10	0.15	4.63	1/8 LD_{50}	0.94 ± 0.06
Hg2	10	0.31	9.25	1/4 LD_{50}	1.01 ± 0.12
Hg3	10	0.62	18.50	1/2 LD_{50}	0.96 ± 0.10
Hg4	10	1.23	37.00	LD_{50}	0.81 ± 0.04
Hg5	10	2.47	74.00	2 LD_{50}	0.85 ± 0.05

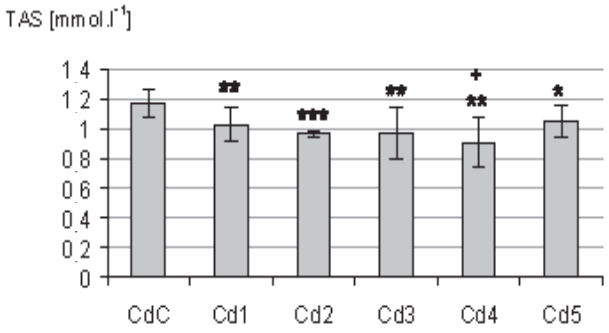

Fig. 1. The TAS changes after $CdCl_2$ administration in rats

* — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$ compared with control group
\+ — $p < 0.05$ compared with group Cd5

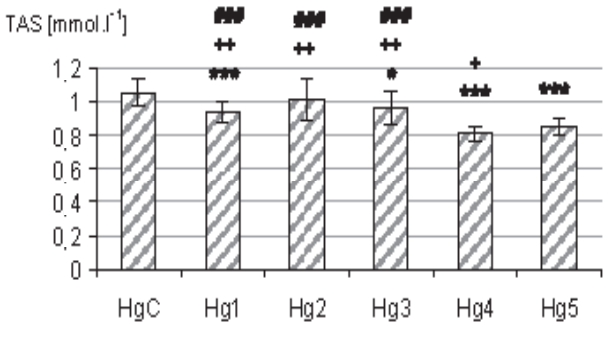

Fig. 2. The TAS changes after $HgCl_2$ administration in rats

* — $p < 0.05$; *** — $p < 0.001$ compared with control group
\+ — $p < 0.05$; ++ — $p < 0.01$ compared with group Hg5
— $p < 0.001$ compared with group Hg4

between group Hg5 and other experimental groups (Hg1: $p < 0.01$; Hg2: $p < 0.01$; Hg3: $p < 0.01$; Hg4: $p < 0.05$) and also between group Hg4 and groups Hg1 ($p < 0.001$), Hg2 ($p < 0.001$) and Hg3 ($p < 0.001$).

The average plasma TAS was significantly higher in the first series of the experiment, than in the second one (1.03 ± 0.13 mmol/l *vs.* 0.93 ± 0.10 mmol/l, $p < 0.005$). There were also significant differences between individual groups (CdC *vs.* HgC: $p < 0.01$; Cd1 *vs.* Hg1: $p < 0.05$ and Cd5 *vs. Hg5: $p < 0.001$).*

DISCUSSION

Various possible mechanisms have been suggested to explain the damage induced by heavy metals. Proteins are major targets of damage by metals and the loss of protein function is usually a consequence of protein modification by metals. Metals have a special affinity toward -SH groups of proteins. By covalent binding to -SH groups, metals can block the functional sites of the catalytic or binding domains of enzymes, or modify protein conformation.

The second possible mechanism may be the displacement of metal, which is essential for biological activity of one molecule by another one. Most frequently, zinc-requiring enzymes are inactivated through direct displacement of Zn by another metal ion from the binding site (16). Possibly, cell damage may be the result of the generation of ROS by metals. Transition metals (primarily Fe^{2+} and Cu^{+}) are known to be able to generate extremely reactive oxygen species (hydroxyl radical ·OH) by the Fenton reaction (4). We may assume an analogous catalytic effect of other transition metals.

A number of items have focused their attention on the influence of chronic cadmium or mercury intoxication on the activity of intracellular antioxidants. In tissues with the highest accumulation of cadmium or mercury, chronic intoxication leads to lipid peroxidation and generally to the elevation of antioxidant enzyme activities (21, 5).

Kostić *et al*. (8) have observed increased red blood-cell superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase activity and reduced and oxidized glutathione concentration, as a biological response to chronic cadmium chloride intoxication ($15\ mg.kg^{-1}.day^{-1}$ for 30 days). They considered that the elevation of antioxidant activity is an adaptive process, which needs a certain amount of time to be fully developed and represents the main defence system against cadmium toxicity. Moreover, they found an elevated plasma concentration of ascorbate and tocopherol.

Contrary to their results Shukla and Chandra (17) showed that *i.p.* administered cadmium ($0.4\ mg.kg^{-1}.day^{-1}$) induces a significant decline in plasma tocopherol after 30 days. Perrin *et al.* (13) investigated the TAS in workers exposed to mercury vapours, but they found no significant differences between the exposed and control groups.

In the present study, we analysed the influence of two heavy metals – cadmium and mercury – on the plasma total antioxidant status. We observed the influence of various doses of metals on the intensity of response.

In our experiment an average plasma TAS was significantly higher after cadmium administration than after mercury administration. We cannot consider it dependent on the type of heavy metal, because there was a significant difference between the control groups as well. The animals were housed in conventional conditions, without any control of temperature and light, therefore the lower TAS in the second series of the experiment may be related to the elevated needs for antioxidants in wintertime.

In the cadmium chloride-treated rats, the TAS was significantly lower in comparison to the control group. In groups Cd1 – Cd4, the TAS value declined depending on the elevation of Cd dose.

In the mercury chloride-treated rats, the TAS decrease also depended on the dose of Hg. The decrease was most evident in two highest doses of Hg (groups Hg4 and Hg5). The TAS values in these two groups were significantly lower also in comparison to groups Hg1, Hg2, and Hg3.

The decrease of TAS could be an answer of plasma antioxidants to an elevated production of reactive oxygen species. As already mentioned, Kostić et al. (8) showed that chronic cadmium intoxication increased plasma levels of tocopherol and ascorbate. However, vitamin E and vitamin C together constitute only 12 % of the TAS in comparison with albumin, which constitutes 43 % of the TAS (11). In particular exhaustion of -SH groups of proteins (albumin) and peptids (glutathione) could be at the bottom of the reduction of the TAS.

From the present results it can be concluded, that chronic cadmium and mercury intoxication caused a significant reduction of the plasma total antioxidant status in rats. The decrease of the TAS was related (especially in mercury intoxication) to the dose.

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THE CONCENTRATION OF CADMIUM AND LEAD IN LIVER AND KIDNEYS IN *Apodemus flavicollis* AND *Cleithrionomys glareolus*

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ABSTRACT

In this study, the concentration of cadmium and lead in liver and kidneys in two rodent species — *Apodemus flavicollis* and *Cleithrionomys glareolus* — was studied. The mean concentration of cadmium is significantly higher in the kidneys (0.213 and 0.521 mg.kg^{-1}) of both species than in the liver (0.032 and 0.073 mg.kg^{-1}). Moreover, the level of cadmium is significantly higher in the kidneys and in the liver of *Cleithrionomys glareolus* compared with *Apodemus flavicollis*. When evaluating the contamination of *Apodemus flavicollis* and *Cleithrionomys glareolus* with lead, the higher concentrations of this element in *Cleithrionomys glareolus*, both in the kidneys (0.780 mg.kg^{-1}) and the liver (0.268 mg. kg^{-1}) were detected. The level of lead in *Apodemus flavicollis* is lower in the kidneys (0.503 mg.kg^{-1}) and in liver (0.177 mg.kg^{-1}). The differences between the species were not significant.

Key words: *Apodemus flavicollis*; cadmium; *Cleithrionomys glareolus;* kidneys; lead; liver

INTRODUCTION

Developments in industry and agriculture produce a reorganization of the elements in the food chain. Some metals are essential for life, others have unknown biological functions, either favourable or toxic, and some others have the power to produce disease. Those causing toxicity are the ones, which accumulate in the body through the food chain, water and air (2, 3, 5, 7, 13).

Cadmium and lead are not ubiquitous in the environment, but have been extensively used in industry. They are persistent in the environment once discharged, and they stay in the animal and human body with long-half-lives when absorbed. These behavioural characteristics make them good long-term polluters of the environment. Further, they are insidious intoxicants for animals (8, 14) as well as humans (4).

The yellow-necked field mouse *(Apodemus flavicollis)* is slightly larger and more brightly coloured than the wood mouse. It eats mainly seeds, especially acorns, beech mast, and hazel nuts, but also takes some insects and other invertebrate food. The bank vole (*Cleithrionomys glareolus*) eats a wide range of vegetable matter and some insects.

The purpose of this study was to determine cadmium and lead concentration in the liver and kidneys in small rodent species *Apodemus flavicollis* and *Cleithrionomys glareolus.*

MATERIAL AND METHODS

The samples of liver and kidney were taken from adult animals of the species *Apodemus flavicollis* (n = 15) and *Cleithrionomys glareolus* (n = 8) which were caught in the surroundings of the town Prievidza, Middle Slovakia in the spring period (April).

Samples of liver and kidney were collected at the hunting place. The tissue samples were kept at –18 °C until analysis. In the laboratory, the samples (liver, kidney cortex) were weighed (2 g) and ashed with diluted nitric acid p.a. (HNO_3: H_2O = 2:1) at 130 °C for 2 hours. Undissolved particles were filtered off and the solution diluted to 25 ml. The concentrations of lead and cadmium were analysed by AAS (Perkin-Elmer 4100 ZL) in a graphite furnace.

From the final data, basic statistical characteristics were calculated (mean, standard deviation, median, minimum, maximum), and an analysis of variance by *t*-test was completed for each variable.

RESULTS

The average concentrations of cadmium and lead in the liver and kidneys of wild rodents in *Apodemus flavicollis* and *Cleithrionomys glareolus* are listed in Table 1.

The mean concentration of cadmium is significantly higher ($P < 0.05$) in the kidneys (0.213 and 0.521 mg.kg^{-1}) of both species in comparison with the liver level (0.032 and 0.073 mg.kg^{-1}). Furthermore, the level of cadmium is significantly ($P < 0.05$) higher in the kidneys and in the liver of *Cleithrionomys glareolus* when compared with *Apodemus flavicollis* (Fig. 1). The kidney : liver ratio of the concentration of cadmium is in *Cleithrionomys glareolus* 1 : 7.2, and in *Apodemus flavicollis* 1 : 7.1.

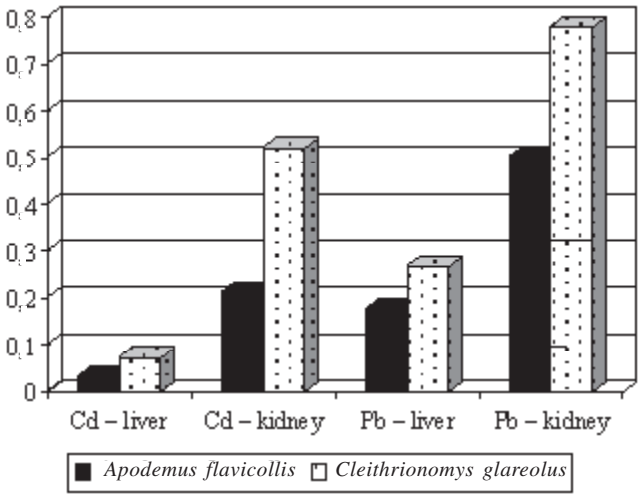

Fig. 1. The level of cadmium and lead (mg.kg^{-1}) in the kidneys and liver of *Apodemus flavicollis* and *Cleithrionomys glareolus*

In evaluating the contamination of *Apodemus flavicollis* and *Cleithrionomys glareolus* with lead we found a higher concentration of this element in *Cleithrionomys glareolus*, both in the kidneys (0.780 mg.kg^{-1}) and in the liver (0.268 mg.kg^{-1}). The level of lead is in *Apodemus flavicollis* lower in the kidneys (0.503 mg.kg^{-1}) as well as in liver (0.177 mg.kg^{-1}). Differences between the species were not significant. The kidney : liver ratio of the concentration of lead is in *Cleithrionomys glareolus* 1 : 2.9, and in *Apodemus flavicollis 1 : 2.8.*

DISCUSSION

Lead poisoning has been a part of history since 4,000 years before Christ. Yet, even today with increasing awareness of the toxicity associated with lead, it is one of the most common toxicants in large and small animals (11). In our study we report the high accumulation of lead in the liver and in the kidneys in both rodent species.

Oral consumption is the major route of lead exposure for animals. Lead accumulates in the body so that chronic exposure to small amounts may lead to toxicosis. In the kidney, lead causes degeneration and necrosis of renal tubule cells. Liver degeneration and necrosis can follow both acute and chronic exposure. In young animals it affects the metabolically active growth centres of long bones (11).

A comparative of study of heavy metal concentrations in the tissues of red foxes from adjacent urban, suburban, and rural areas has been published (4). The kidney and liver of suburban and rural foxes contained the highest cadmium concentrations, whereas urban foxes contained the highest lead levels. Foxes from the urban centre were characterized by elevated lead concentrations during the first 2 years of life, but this transient lead accumulation was absent in suburban or rural animals. The liver of juvenile foxes contained a median lead concentration of 0.99 mg.kg^{-1} in the city compared to only 0.47 and 0.37 mg.kg^{-1} in the suburban and rural area, respectively. All these values are higher than we have found in *Apodemus flavicollis* and *Cleithrionomys glareolus* (0.177 and 0.268 mg.kg^{-1}).

Cadmium accumulates mainly in the kidneys and the liver of farm animals (9, 10) as well as in wild animals (16, 15). In cadmium we report significant age-dependent accumulation.

Table 1. The concentration of cadmium and lead (mg.kg^{-1}) in the kidneys and liver of *Apodemus flavicollis* and *Cleithrionomys glareolus*

	Liver				Kindey			
	Cd (mg. kg^{-1})		Pb (mg. kg^{-1})		Cd (mg. kg^{-1})		Pb (mg. kg^{-1})	
	A. f.	*C. g.*	*A. f.*	*C. g.*	*A. f.*	*C. g.*	*A. f.*	*C. g.*
$\bar{x}$	0.032	0.073	0.177	0.268	0.213	0.521	0.503	0.780
± s	0.016	0.018	0.131	0.141	0.106	0.255	0.305	0.508
Median	0.040	0.080	0.165	0.195	0.210	0.520	0.480	0.570
Minimum	0.010	0.030	0.060	0.130	0.070	0.150	0.180	0.410
Maximum	0.060	0.090	0.570	0.500	0.420	0.970	1.310	1.770

**A. f. — A. flavicollis; ** — C. g. — C. glareolus*

In the kidneys of suburban foxes, cadmium concentrations increased from a median value of 0.73 $mg.kg^{-1}$ in juvenile animals to 1.82 $mg.kg^{-1}$ in adults. Similarly, the liver of suburban foxes contained increasing cadmium levels from a median of 0.21 $mg.kg^{-1}$ in juvenile animals to 0.94 $mg.kg^{-1}$ in adults. An age-dependent storage of cadmium was also found in foxes from the rural surroundings, but no such accumulation occurred in urban foxes from the city centre, where even adult animals contained very low levels (4). In a study describing arsenic, cadmium, lead, copper and zinc in cattle from Galicia, Spain (1), it was reported that age did influence accumulation. Moreover, Komarnicki (6) in the study of heavy metal accumulation in urban populations of moles, *Talpa europaea* L. (Insectivora) at three sites along the "Donaukanal" in Vienna found a significant increase in cadmium concentration with age in the kidneys, liver, heart, lung, skull and the femur.

From our data we can suggest that a decline in numbers of small rodents recorded in recent decades may result from the extensive use of fertilizers and pesticides in agriculture, increased industrial production as well as heavy agricultural mechanization. On the other hand, small rodent species, in relation to their food chain, may serve as a bioindicator to detect certain toxic hazards as heavy metals in the game.

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THE MORPHOMETRY OF THE SKULL IN GUINEA PIGS AND ITS CORRELATION WITH BODY MASS

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ABSTRACT

The present study was undertaken to elucidate the relationships between body mass and skull parameters in female and male guinea pigs. Ten skulls of adult male guinea pigs weighing 790.10 ± 31.50 g, and 10 adult female guinea pigs with weights 593.50 ± 51.54 g were used in this study. Of the skull, the *viscerocranial, frontal, parietal, basal* and *dental* lengths, the *maximum zygomatic* and *neurocranium* widths, the skull and *facial* indices were determined in the present study. A negative correlation was found between the body mass and *facial* and skull indices in male guinea pigs, and this correlation was revealed to be significantly positive in female guinea pigs. There was a positive correlation between the body mass and skull, *viscerocranial*, *frontal, basal* and dental lengths and *maximum zygomatic* width in male and female guinea pigs.

Key words: body mass; correlation; guinea pig; measurements; skull

INTRODUCTION

There are various opinions about the body mass growth and *cranial* growth in the literature. Some authors reported that postnatal skeletal and body weight were evaluated using the bone measures in dogs (14, 13). By others (4) sutural growth and calvarial volumetric changes in rabbits were computed and correlated to weight increase. Therefore, high correlations were found between growth at the sutures of the rabbit *calvarium*.

The relationship between skull measurements and body mass was investigated in female miniature pigs, and was found to have low correlation coefficients (15).

Cranial growth is usually estimated by *roentgen cephalometry* or, if possible, by direct *osteometry* in rabbits (9, 2, 5, 3, 16).

Recent studies have shown a clear correlation between craniofacial morphology and the masticatory function in rats. However, the correlation between skull parameters and body mass has not been specifically discussed in guinea pigs.

The aim of this study was to determine the relationship between body mass and skull parameters in adult guinea pigs by using direct *craniometric* measurements. Thus, the correlation coefficients were computed between skull parameters and body mass.

MATERIALS AND METHODS

A total of 20 skulls of adult guinea pigs were used in this study. The animals were divided in two groups. Group 1 consisted of 10 male guinea pigs weighing 790.10 ± 31.50 g, and group 2 consisted of 10 female guinea pigs weighing 593.50 ± 51.54 g.

The guinea pigs were killed by means of an overdose of sodium pentobarbital.

The skulls were macerated in accordance with the method described by the first author (11). Direct skull measurements were made on the skull after gentle removal of soft tissues and muscles by boiling and dissection.

The position of certain points and markers on the skull were determined in making linear measurements with vernier callipers. Eight different craniometric measures of the skull (Figs. 1 and 2) were made according to a group of authors (6, 16, 10). The skull and facial indices were calculated as described by Onar (11).

The reference points were defined as follows (Figs. 1 and 2)

Akrokranion (A): a point marking the most posterior point of the *external occipital protuberance.*

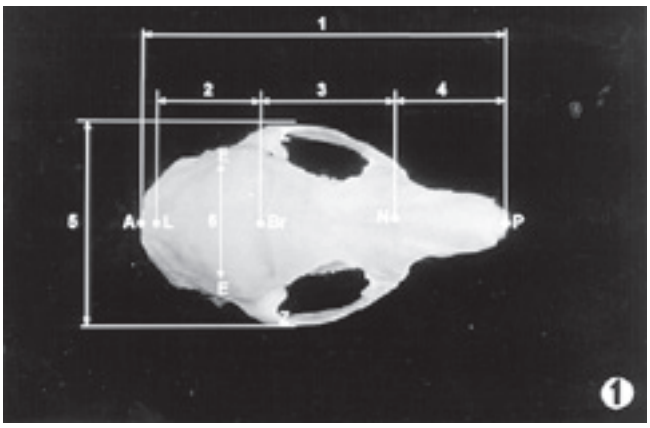

Fig 1. A — Akrokranion, Br — Bregma, E — Euryon, L — Lambda, N — Nasion, P — Prosthion, Z — Zygion, 1 — Skull length, 2 — Parietal length, 3 — Frontal length, 4 — Viscerocranial length, 5 — Maximum zygomatic width, 6 — Maximum width of neurocranium

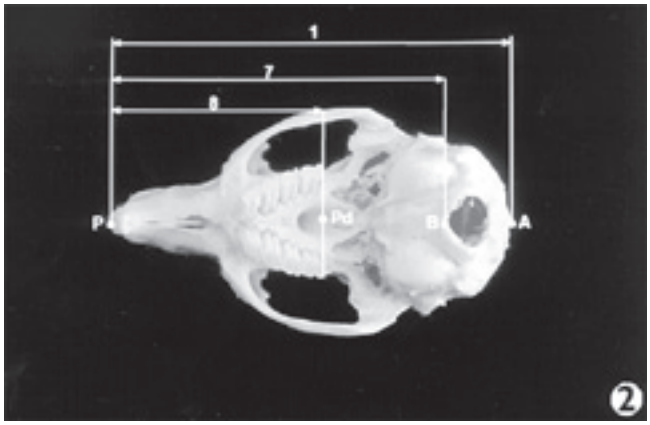

Fig 2. A — Akrokranion, B — Basion, P — Prosthion, Pd — Postdentale, 1 — Skull length, 7 — Basal length, 8 — Dental length

Nasion (N): a point in the junction on the median plane of the right and left *nasofrontal sutures.*

Prosthion (P): the oral end of the *interincisive suture,* located between the roots of the upper central incisor teeth.

Postdentale (Pd): the median point of the line joining the aboral points of the alveoli of the hindmost cheekteeth.

Euryon (E) : the most lateral point of the *braincase.*

Zygion (Z) : the most lateral point of the *zygomatic arch.*

Bregma (Br): a point in the junction of the *interparietal* and paired *parietal and frontal* bones.

Lambda (L) : a point marking the junction of the *interparietal* and paired *parietal* bones.

Basion (B): the middle of the ventral margin of the *foramen magnum.*

The following craniometric measures were used in this study (Figs. 1 and 2):

Skull length (1): A-P

Parietal length (2): A-Br

Frontal length (3): Br-N

Viscerocranial length (4): N-P

Maximum zygomatic width (5): Z-Z

Maximum width of neurocranium (6): E-E

Basal length (7): B-P

Dental length (8): Pd-P

Skull index: Z-Z × 100/A-P

Facial index: Z-Z × 100/N-P

In order to determine the likely relationship, if any, between the body weight and the skull parameters, the correlation coefficients were computed (15, 8). To analyse the data derived from the two groups we used the test devised by Duncan (7).

RESULTS

The mean ($\bar{x}$ and standard deviation (SD) values of the investigated features are presented in Table 1.

Except the facial index, there was a level of $p < 0.001$ relationship between the body mass and skull parameters, and there was a level of $p < 0.05$ relationship between body mass and skull index.

Table 1. Skull measurements of male and female guinea pigs

*** — $p < 0.001$, ** — $p < 0.01$, ^{NS} — not significant
numbers in parameters refer to Figs. 1 and 2

	Sex	n	X	SD	t
Body mass (g)	M	10	790.10	31.501	10.2931***
	F	10	593.50	51.535	
Skull length (1)	M	10	68.65	0.958	18.5984***
	F	10	59.63	1.119	
Parietal length (2)	M	10	20.53	0.354	15.6150***
	F	10	17.79	0.426	
Frontal length (3)	M	10	22.64	0.465	7.1409***
	F	10	20.43	0.861	
Viscerocranial length (4)	M	10	28.75	0.570	18.0812***
	F	10	24.44	0.494	
Maximum zygomatic width (5)	M	10	38.19	0.413	18.3533***
	F	10	32.62	0.865	
Maximum width of neurocranium (6)	M	10	24.37	0.191	12.3695***
	F	10	22.59	0.412	
Basal length (7)	M	10	59.34	0.921	14.6814***
	F	10	50.67	1.631	
Dental length (8)	M	10	37.98	0.631	19.0306***
	F	10	32.15	0.736	
Skull index	M	10	55.63	0.709	3.4958**
	F	10	54.71	0.441	
Facial index	M	10	132.84	1.874	0.8270NS
	F	10	133.48	1.573	

Abbreviations:

Max. zyg. width: Maximum zygomatic width
Max. width of *neurocranium*: Maximum width of *neurocranium*
(1) — (8) — in mm; *t* – test by Duncan

The skull, viscerocranial, frontal, parietal, basal and dental lengths, and the maximum zygomatic and neurocranium widths were measured (Table 1). The correlation analyses of the investigated features in male and female guinea pigs are presented in Table 2.

A negative correlation was found between the body mass and facial and skull indices in male guinea pigs, and this correlation was determined to have a significant positive correlation in female guinea pigs.

A positive correlation between the body mass and skull index in female guinea pigs was significant at a level of $p < 0.001$, whereas this correlation in male guinea pigs was insignificant.

While the correlation between the body mass and parietal length and maximum width of the neurocranium was significant at a level of $p < 0.05$ in male guinea pigs, but this correlation in female guinea pigs was insignificant.

There was a positive correlation between the body mass and skull, viscerocranial, frontal, basal and dental lengths and maximum zygomatic width in male and female Guinea pigs.

Table 2. Correlation analyses of the investigated features of male and female guinea pigs

*** — $p < 0.001$, ** — $p < 0.01$, * — $p < 0.05$, ^{NS} — not significant

Male ⇓ / Female ⇒	Body weight	Skull Length length	Viscero-cranial	Frontal length	Parietal length width	Max. Zygo. neuro.	Max. width of	Basal length	Dental length	Skull index	Facial index
Body weight		**0.7508*****	**0.7639*****	**0.8361*****	**0.2883**^{NS}	**0.8075*****	**0.3665**^{NS}	**0.7433*****	**0.7123*****	**0.7664*****	**0.4931***
Skull length	**0.7618*****		0.9607***	0.9454***	0.6204**	0.9755***	0.0783^{NS}	0.9171***	0.7731***	0.6833***	0.5235*
Viscerocranial length	**0.6629****	0.5507*		0.9742***	0.5693**	0.9046***	0.0488^{NS}	0.9055***	0.8115***	0.5501*	0.2979^{NS}
Frontal length	**0.5781****	0.6941***	0.2741^{NS}		0.4376^{NS}	0.9118***	0.0198^{NS}	0.9367***	0.8628***	0.6127**	0.3591^{NS}
Parietal length	**0.6432****	0.8036***	0.4637*	0.5748**		0.5831**	0.4758*	0.4553*	0.1774^{NS}	0.3529^{NS}	0.3215^{NS}
Maximum zygomatic width	**0.5255***	0.4942*	0.7342***	-0.0243^{NS}	0.3441^{NS}		0.1789^{NS}	0.8773***	0.7083***	0.8273***	0.6765**
Maximum width of neurocranium	**0.4804***	0.6865***	0.4133^{NS}	0.6866***	0.3391^{NS}	0.3574^{NS}		-0.0848^{NS}	-0.2536^{NS}	0.4009^{NS}	0.3239^{NS}
Basal length	**0.7045*****	0.7397***	0.6180**	0.3835^{NS}	0.9331***	0.4476*	0.2546^{NS}		0.9259***	0.5679**	0.3976^{NS}
Dental length	**0.6249****	0.7382***	0.4766*	0.2502^{NS}	0.7319***	0.6221**	0.4348^{NS}	0.7820***		0.3757^{NS}	0.1817^{NS}
Skull index	**-0.3947**^{NS}	-0.6807***	0.0156^{NS}	-0.7794***	-0.5911**	0.3004^{NS}	-0.4495*	-0.4349^{NS}	-0.3954^{NS}		0.9051***
Facial index	**-0.5375***	-0.4013^{NS}	-0.8482***	-0.4156^{NS}	-0.3945^{NS}	-0.2634^{NS}	-0.3158^{NS}	-0.5324*	-0.1975^{NS}	0.2167^{NS}	

DISCUSSION

While there is information on *craniofacial* morphology and its correlation with body mass of the dog and rabbit in literatures, there are no records available on the guinea pigs' skull. For this purpose, the variations in the skull measurements and correlations between the body mass and craniometric measurements obtained for the male and female guinea pigs' skulls in the study were found.

The variability of measurements of *cranial* growth in the rabbit (2, 3, 5, 1) has been reported. However, the correlations between direct skull measurements were not showen in rabbits.

We observed that the correlations between the body mass and *craniometric* measurements in this study, and have presented them in Table 2.

The mean ($\bar{x}$) and standard deviation (SD) values of the features investigated are present in Table 1, and there was significance at the level of $p < 0.001$ in the differences between features investigated in male and female guinea pigs.

An increase in the skull, *viscerocranial, frontal, basal* and dental lengths, the maximum *zygomatic* and *neurocranium* widths, and skull and *facial* indices related to the body mass, and a significant level of this increase was observed in female guinea pigs.

However, a negative correlation was found between the body mass and skull and *facial* indices in male guinea pigs. This showed that the skull of the male guinea pigs took on a long-nosed shape.

While the correlation between the body mass and parietal length and maximum width of *neurocranium* was significant at a level of $p < 0.05$ in male guinea

pigs, but this correlation in female guinea pigs was in significant. This was supported by the fact that there was a long-nose shape in the male guinea pigs.

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ISO-ENERGETIC REPLACEMEMT OF ARTIFICIALLY DRIED GRASS BY CONCENTRATE INCREASES MAGNESIUM ABSORPTION IN COWS (A SHORT COMMUNICATION)

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ABSTRACT

In a feeding trial with cows fed a fixed level of energy intake sufficient for maintenance and the production of 12 kg of milk, the expectation was tested that a decrease in the dietary roughage to concentrate ratio would stimulate magnesium (Mg) absorption. Six cows were fed either a ration containing 20 or 60 % of total energy intake as concentrate according to a 28 × 28-day cross-over design. It was found that increasing the amount of concentrate from 20 to 60% raised Mg absorption from 12.0 to 19.7 % of intake. The increase in Mg absorption is explained by the associated decrease in dietary potassium (K) concentration from 39.4 to 25.2 g.kg^{-1} dry matter (dm). When the results of the present study are compared with those of a study with dry cows fed the same rations, it is concluded that the plane of nutrition may not affect the % of Mg absorption.

Key words: absorption; concentrate; magnesium; potassium; roughage

INTRODUCTION

Grass and grass silage are generally rich in potassium (K) in areas with intensive livestock production (2, 4). High K intake inhibits magnesium (Mg) absorption in cows (5), which enhances the risk of hypomagnaesemia (6). To prevent a hypomagnaesemic tetany in dairy cows, it is common practice to supplement commercial concentrates with MgO (3). However, concentrates are generally low in K, thus, the K concentrations of rations composed of forage and concentrates are lower than of those in which K-rich forage is the sole source of nutrition. Consequently, Mg absorption may be increased after decreasing the roughage: concentrate ratio, which was indeed shown in dry, non-pregnant cows (7). The observation implies that the supplementation of concentrates with Mg could be unnecessary. It is not known whether our observation in dry cows with a low level of dry-matter intake (7) extends to cows with high-feed intake such as high-producing animals. In practice, high-producing dairy cows may be fed with rations containing up to 60 % of the energy as concentrates. In an attempt to obtain information relevant for high-producing cows, we measured Mg absorption in cows fed at two times the maintenance requirement in the form of diets containing artificially dried grass and either 20 or 60 % concentrates while Mg intake was kept constant. It is appreciated that exchanging dried grass with concentrate is associated with multiple changes in the composition of the whole ration that might affect Mg absorption, but it is well known that K intake is the major determinant (3).

MATERIALS AND METHODS

Six cows were used. They were at least 200 days in milk and had an initial milk production of 16 kg.day^{-1}. However, during the two-week transition period during which the cows were adapted to the experimental rations, milk production dropped to 12 kg.day^{-1}. Therefore, energy (NE_l) intake was fixed at a level sufficient for maintenance and production of 12 kg milk containing 4.75 % fat.

The trial had a 28 × 28-day cross-over design. The cows were randomly assigned to the order of the two treatments. The experimental rations consisted of artifically dried grass and a pelleted concentrate (Table 1). The exchange of the artificially dried grass and concentrate was iso-energetic and based on their NE_l values (8). Since the Mg content per unit of energy differed between the artificially dried grass and the concentrate, the contents being 0.24 and 0.46 g Mg/MJ NE_l

Table 1. Ingredient composition and analyzed Mg and K content of the experimental rations

	Experimental ration[1]	
Grass:	**80 %**	**40 %**
Concentrate:	**20 %**	**60 %**
Ingredients:	kg.day^{-1}	
Artificially dried grass, DM[2]	11.1	5.5
Experimental concentrate, DM	2.1	6.4
Mg-rich concentrate, DM	0.2	0.1
Total DM intake	13.4	
12.0		
Chemical analysis of the whole ration:	g.kg^{-1} DM	
Mg	2.72	2.97
K	34.9	25.2

[1] — Percentage refers to the amount of artificially dried grass or concentrate fed as a % of total energy intake (NE_l);
[2] — DM — dry matter

respectively, the artificially dried grass was supplemented with appropriate amounts of a Mg-rich concentrate (Table 1) so as to avoid differences in Mg intakes between the two treatments.

During the last two weeks of each experimental period, faeces and urine were collected quantitatively from each cow. Urine was collected using a urine collector attached to the cows by a leather harness. Proportional samples from faeces and urine were taken and analysed for their Mg content (5). Milk production was recorded and sampled twice daily during the last week of each experimental period.

RESULTS

The cows consumed all feed supplied, the energy intake being about two times the maintenance requirement. Mg balances are shown in Table 2. A decrease in the dietary grass : concentrate ratio was associated with a significant increase in Mg absorption. Increasing the amount of concentrate from 20 to 60 % raised Mg absorption from 4.4 to 7.0 g.day^{-1} at a similar Mg intake. Magnesium excretion with the milk was very low due to a low milk production. The initial drop in milk production during the pre-experimental period was continued during the first experimental period. Average milk production for both dietary treatments during the last week of the first experimental period was only 4.0 kg.day^{-1} (SE 1.22; n = 6), which was similar (p = 0.700) to the average milk production during the last week of the second experimental period; i.e 3.3 kg.day^{-1} (SE 1.18, n = 6). Milk production was not affected by dietary treatment (p = 0.228). The reason for the drop in milk production is not known, but it could be related to aversion to the leather harnasses. Apart from the above mentioned fall in milk production, the experiment ran without complications.

Table 2. Mg balance (g.day^{-1}) in cows (n = 6) after the feeding of the experimental rations

	Experimental treatment				
	80 % grass 20 % concentrate		**40 % grass 60 % concentrate**		
	Mean	**SE**	**Mean**	**SE**	***P* value[2]**
Intake	36.3	nd[1]	35.6	nd[1]	
Faeces	31.9	0.56	28.6	0.52	0.001
Absorption	4.4	0.56	7.0	0.52	0.003
Urine	2.5	0.44	4.8	0.49	0.003
Milk	0.5	0.19	0.6	0.22	0.205
Balance	1.4	0.21	1.6	0.23	0.317

nd[1] — not determined because the cows were fed a restricted amount of feed
[2] — Student's paired *t*-test

DISCUSSION

This study confirms our earlier work in dry cows (7) that, at constant Mg intakes, the replacement of artificially dried grass by an iso-energetic amount of concentrate increases Mg absorption. The increase in Mg absorption, from 12.0 to 19.7 % of intake, after raising the amount of concentrate from 20 to 60 % was associated with a decrease of the dietary K concentration from 34.9 to 25.2 g K kg^{-1} dry matter. Indeed, many controlled experiments have shown a cause-and-effect relationship between K intake and Mg absorption (3).

The rations used in this study were also fed to non-pregnant, rumen fistuled, dry cows, but at a level sufficient to maintain their energy balance (7). An increase in the amount of concentrate from 20 to 60 % raised the apparent Mg absorption in the dry cows from 13.7 to 21.5 % of intake. On the basis of stepwise regression analysis it was concluded that the differences in ruminal K concentrations between the dietary treatments, were responsible for the variance in Mg absorption (7). When Mg absorption (% of intake) of the current experiment is compared with that of the experiment with the dry cows, it appears that the percentage of apparent Mg absorption primarily is determined by the ration fed. In other words, it seems that the plane of nutrition does not have a major influence on the percentage of apparent Mg absorbed.

In earlier work with non-pregnant, dry cows (5) we have shown that Mg absorption, expressed as % of Mg intake, may rise by about 0.52 % units when the dietary K content falls by 1 g.kg^{-1} dm. The requirements of apparently absorbable Mg, expressed in g.day^{-1}, of lactating cows are well known (1). The practical relevance of this study lies in the implication that it can be substantiated whether or not concentrate-rich rations fed to high-producing dairy cows have to be supplemented with Mg. The amount of Mg to be added to the concentrate can be calculated on the basis of the Mg requirement of the cows, the intakes of K and Mg with the roughage and the unsupplemented concentrate, and the predicted % of apparent Mg absorption.

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PRODUCTION AND UTILIZATION OF CAMEL MILK IN EASTERN ETHIOPIA: THE CASE OF JIJIGA AND SHINNILE ZONES (A SURVEY)

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ABSTRACT

The production and utilization of camel milk in Jijiga and Shinile zones of the Somali National Regional State, Eastern Ethiopia were studied. The main objective of this study was to describe camel milk production and utilization by selected herders in the study areas. Eighty-four households from four districts of Jijiga and Shinile zones were interviewed using a structured questionnaire. The results of the study show that the mean lactation milk off-take per dam in the Jijiga site was 2009 kg with a mean lactation length of 15 months. Whereas in the Shinile site the average milk off-take was 1244 kg with an average lactation length of 13 months. The results of the study reveal also that camel milk is an important food item to the sample respondents. It is consumed either in fresh or sour form without processing. In fact, the camel herders did not practice milk processing, and milk souring is the only technique they used to extend the shelf life of camel milk. The Jijiga herders were found to be market-oriented in that they sold part of their camel milk production and generated cash income. Whereas the Shinile respondents used the almost all their milk for household consumption.

Key words: camel; consumption; herd diversification; marketing; milk off-take; souring

INTRODUCTION

In Ethiopia, as in most dry lands of Africa and Asia, camels are the principal source of income and food for millions of pastoralists. In addition, camels play a central role in providing draught power and determining the wealth and social status of pastoralists. Ethiopia's camel population is estimated to be one million (9). This number ranks the country third in Africa after Somalia and the Sudan, and fourth in the world (India included).

The dromedary camel is well-suited to harsh and inhospitable dry environments because it is gifted with special anatomical, physiological, and behavioral characteristics. These adaptive characteristics are tolerance of heat up to 45 °C and water deprivation for 1—2 months (29). These peculiar characteristics of the camel surpass every other domestic animal. In addition, its higher efficiency in utilizing low quality sparse and thorny vegetation enables the camel to thrive better and produce reliable and sustainable food sources. It is not uncommon to continue to milk camels when other livestock species have stopped giving milk during prolonged drought periods (36). Camel milk production offsets the deficit in cow and small ruminant milk in many areas and makes a major contribution to the protein and calorie intakes of the nomadic population

Camels inhabit almost all-peripheral drier lowlands generally below 1500 metres above sea level (m a.s.l) in Ethiopia, except the western areas where high humidity and the incidence of trypanosomiasis are common (28). These areas include the major parts of the Somali and Afar National Regional States and some parts of the Oromia National Regional State[1]. Afar camels are small but extremely hardy and, except for some used for salt transport, are kept almost entirely as milk producers. Ethiopia's largest camels are of the Ogaden type, owned by the Somali in the east of the country. These are also almost entirely used for milk production and rarely for transport except to move the household and its goods and chattels when changing camps. Borana camels (found in the Oromia National Regional State) are of intermediate size and intermediate use and are employed as draught and dairy animals.

The eastern part of Ethiopia is considered the heartland of the camel population because it is the home for two-thirds of the nation's camel population (4). Although the contribution of camels to the national economy is not fully quantified,

[1]According to the Ethiopian Federal Democratic Republic administrative hierarchy, the country is divided into nine ethnically-based National Regional States, which are in turn divided into zones, districts and villages (locally called *Kebeles* in urban areas and Peasant Associations in rural areas) in that order. In addition to the nine regional states, the two major cities of the country, namely Addis Ababa and Dire Dawa are organized as chartered cities.

Schwartz (27) estimated the production of 20,000 metric tons of camel meat and 174,000 metric tons of camel milk per year in Ethiopia.

Although Ethiopia possesses a large number of camels, little is known about camel husbandry practices or the productive and reproductive performance of camels (28). Given this state of affairs, this study was designed and executed as one component of the European Camel Project (ECP)[2]. Therefore, the results of this study are expected to provide basic information on camel herder households' characteristics, herd demography, camel milk production, consumption, processing and marketing in eastern Ethiopia.

MATERIAL AND METHOD

Description of the Study Areas

This study was conducted in the Somali National Regional State (SNRS). The SNRS is located in Eastern Ethiopia. It is one of the nine ethnically-based regional states of the country and it is divided into nine zones. The total population of the SNRS in 1999 was estimated to be 3.6 million (5.84 percent of the country's population). The overwhelming majority of the population of the SNRS (85 percent) are rural (23). The same source shows that the SNRS has a population density of 9.6 persons per square kilometre as compared to the national population density of 49.3 persons per square kilometre.

Data for this study were collected from two zones of the SNRS, namely Jijiga and Shinile. These two Zones are further divided into five districts each. Geographically, the study areas lie between 8° 44´ N and 11° 00´ N latitude and between 40° 22´ E and 44° 00´ E longitude. The altitude ranges from 1760 to 2300 m a.s.l. in Jijiga, and from < 500 to 1100 m a.s.l. in Shinile. The climate of the study areas is largely influenced by their proximity to the Gulf of Aden in the north and the Indian Ocean in the east. The distribution of rainfall is very erratic and bi-modal in nature. The average annual rainfall for Jijiga and Shinile was 457 mm and 686 mm, respectively. Mixed agriculture and nomadic pastoralism are the dominant production systems in Jijiga, while nomadic pastoralism is the principal activity in the Shinile zone (14).

According to CSA (5), with 23.6 percent of the regional population in 1997, the Jijiga zone was the most populous zone of the SNRS whereas the Shinile zone was the third populous zone in the region (in 1997, it accounted for 10.4 percent of the regional population). In terms of the camel population, the two zones accounted for about 19 percent of the total camel population in the SNRS in 1999 (6).

Sampling Procedure

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

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

Data Collection and Analysis

Single-Visit-Formal-Survey Methods (16) were adopted to collect data on camel herders' households characteristics, herd demographic characteristics, and milk production performance. The survey was carried out between February and December 1996. Various data collection techniques and tools such as questionnaire, record sheets (measurements), and progeny history examinations were used during information gathering. Moreover, discussions with elders, ethnic leaders and development agents were made in all the selected sites. The discussions were particularly useful to gain more insight into community beliefs and attitudes towards camel keeping.

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

Calibrating the traditional milk equipment which are used for milking, fermenting, and selling purposes was the technique used to estimate the herd milk off-take and the amount of milk consumed and sold. Information on retail prices of camel milk was collected by conducting rapid market appraisal surveys in seven principal towns in the study areas. More specifically, 10 key informants (milk retailers and consumers) were interviewed from each town. The key informants were selected randomly and interviews were held in the market places by employing a semi-structured interview method.

[2]European Camel Project (ECP) under the title 'use of camels in arid regions as potential source for milk and meat products' was launched in Ethiopia in 1995. The ECP had been coordinated jointly by the Hohenheim University (Germany) and by the Alemaya University (Ethiopia) until it was phased out in February 1999.

RESULTS AND DISCUSSION

Throughout this paper, the term camel milk utilization is used in the sense that it encompasses camel milk consumption, processing, and marketing.

Household Characteristics

The characteristics of the households studied are shown in Table 1. Both in Jijiga and Shinile societies, the basic productive and decision-making unit is the household. In this study, a household is understood as an economic unit whose members include a married man, his wife or wives, unmarried children and other dependent family members who share the family resources (10). Though most of the sample respondents reported to be in a monogamous type of marital relationship, polygamy was observed in both sites. The proportion of polygamous households was 45.3 % and 22.6 % in Jijiga and Shinile, respectively.

The average family size per household and the degree of polygamy were higher in Jijiga than in Shinile. This was perhaps due to differences in wealth status among the households studied in the two zones. According to Wilson (30), on the basis of the degree of mobility of the herders, the livestock production systems in the tropics could be classified into nomadic systems (irregular movement in search of feed and water and no permanent base), transhumant systems (regular seasonal movement with returns to a permanent base for at least part of the year), and sedentary systems (where animal movement is restricted to a very short radius from a permanent fixed base). In this respect, the survey results reveal that the transhumant type of livestock management was the dominant system in both sites, although crop production has been practised for quite some time in the Jijiga area. In fact, about 42 % of the respondents in Jijiga reported that they had been engaged in crop production for more than 10 years, whereas the corresponding figure for Shinile was only 3.2 percent.

The average number of camels per household was 35.2 and 22.7 in Jijiga and Shinile, respectively (Table 1). Whereas the average livestock holding per household was 50.1 TLU and 40.5 TLU in Jijiga and Shinile, respectively[3]. The more settled herders in the Jijiga area are accustomed to rear various livestock species, in a larger number, than the mobile Shinile herders. The higher livestock holding in Jijiga can be explained partly by the availability of a relatively better feed resource from the natural vegetation and crop residues as well as the availability of water from ground cisterns and ponds.

[3]One Tropical Livestock Unit (TLU) is equal to 250 kg which is equivalent to 1 camel; 0.7 cattle; 0.8 horse/mule; 0.5 donkey; 0.1 goat/sheep — ILCA (16).

Table 1. General characteristics of the sample households

Features	Shinile (n = 31)	Jijiga (n = 53)
Number of wife per household		
Only one (%)	54.7	77.4
More than one (%)	45.3	22.6
Average Family size (number)	10.6	9.8
Livestock production system		
Sedentary (%)	24.5	3.2
Transhumant (%)	73.6	67.8
Nomadic (%)	1.9	29.0
Average Herd size (camel heads)	35.2	22.7
Average livestock holding (TLU)	50.1	40.5
TLUs/ capita	5.2	4.3
Crop production activities		
Not practising	22.6	87.1
Practising for less than 10 years	35.4	9.7
Practising for more than 10 years	42.0	3.2
Crop culture	maize, sorghum, chat	maize
Camel types	Ogaden type	Afar type

The per capita livestock holding varied between the two sites. The average per capita livestock holding (in TLU) was 5.2 and 4.3 for Jijiga and Shinile, respectively. Obviously, the result obtained in Jijiga is in complete agreement with the findings of Pratt and Gwy- nne (1977): cited by Coppock (3) who claimed that the threshold for the subsistence economy, in terms of livestock holding for the pastoral system, is at least 5 TLUs per person. In this respect, the per capita livestock holding in Shinile was below this figure. The report by Coppock (3) and the results of this study challenge the commonly held view that the lowlands have a large and marketable surplus of animals for sale. The average camel herd size per household, for both sites, is far greater than what was reported for similar environments (13).

Moreover, the average livestock holding (in TLU) in Jijiga was greater than the one reported by Herren (13) for nomadic pastoralists of southern Somalia. This indicates that the sample households kept camels along with other livestock species. In fact, the survey results reveal that all of the sample respondents in the study areas kept camels. Cattle factor as the second most important livestock species kept by 79.2 % and 71 % of the sample households in Jijiga and Shinile, respectively. The other important livestock species kept in the study areas include goats (kept by 73.6 % and 61.3 % of the sample households in Jijiga and Shinile, respectively), sheep (kept by 54.7 % and 58.1 % of the sample households in Jijiga and Shinile, respectively) and donkeys (kept by 18.3 % and 38.7 % of the sample households in Jijiga and Shinile, respectively) in that order.

The survey results also show that herd diversification is a strategy adopted by the pastoralists in the study

areas. In other words, the proportions of sample households in Jijiga area with four, three and two livestock species were 47.2 %, 22.6 %, and 28.3 %, respectively. The corresponding figures for Shinile area were 41.9 %, 16.1 %, and 32.3 %, respectively. Herders usually keep such a diversified livestock species in order to secure their livelihood. This strategy of rearing different species of animals has ecological and economic advantages. Different species (grazers and browsers) can utilize different ecological niches of a given grazing area much more efficiently than a single species. Moreover, herd owners with different types of livestock are less vulnerable to calamities than those with only one species. For instance, an outbreak of certain diseases in a particular area has less chance of infesting all species at the same time.

Camel herd Demography

The distribution of the family herd by age and sex is shown in Table 2. In the Jijiga site, the calves, immature camels and adult camels accounted, on the average, for 17.7 %, 21.1 %, and 61.2 % of the herd, respectively. Whereas in the Shinile site they accounted for 15.7 %, 26.1 %, and 58.2 %, respectively. In Jijiga, female camels represented 75.4 % of the herd of which 68.7 % were females of breeding age. The corresponding figures for the Shinile site were 73.6 % and 67.1 %, respectively. The higher proportion of younger and breeding age camels indicates the growth potentials of the camel herds studied.

The proportion of female calves was slightly higher than that of the male calves in both sites. The respondents reported that the relatively higher proportion of female calves was due to the rigorous process of selecting bulls from more female-bearing ancestors. A similar reason was reported from Sudan (21). On the other hand, the variation in the proportion of immature males and females is attributed to the culling of more young males with a view to building up a female-dominated herd.

Table 2. Average Camel Ownership by Age and Sex*

Age and Sex Group	Jijiga		Shinile	
	Heads	Percentage	Heads	Percentage
Calves				
Male	2.6	7.5	1.6	6.9
Female	3.6	10.2	2.0	8.8
Immature				
Male	2.7	7.7	2.5	10.8
Female	4.7	13.4	3.5	15.3
Adult				
Male	3.3	9.4	2.0	8.3
Female	18.2	51.8	11.2	49.5
Total	35.2	100	22.7	100

* — Those animals less than two years of age were considered as calves, between two and four years as immature and above four years as adults

The larger proportion of adult females (breeding females) in the herd indicates the herders' strategy to easily restore their herd after drought and other natural calamities, which usually decimate the animals. Similarly, the relatively large number of females in the herd could also indicate the herders' strategy to ensure a continuous supply of milk for family consumption. Though camels, in the areas studied, as elsewhere in the pastoral areas of east Africa, are mainly kept for milk production, in countries like India the proportion of male camels is higher mainly because camels are kept for traction purpose (22).

Milk Production

The potential of camels as milk animals has been supported by many researchers (12, 35, 19, 28, 27). Camel milk contains proteins, vitamins, energy and minerals in forms suitable for both man and the calf. It is a good source of vitamin C, and the high vitamin C content is extremely useful in areas where vegetables and fruit are in scarce supply or completely unavailable. An important adaptation feature of the camel to desert conditions is its ability to dilute its milk. This involves the production of milk with a higher water content when the animal is dehydrated than when it is fully watered (35, 31). The dilution of milk under dehydration could be a physiological adaptation to ensure an adequate supply of water to young animals with access to no other source while at the same time continuing to provide them with an adequate supply of other nutrients.

The literature on camel milk production is controversial and often muddled by a failure to distinguish between two different issues: *total yield* (milked-out) and the *actual off-take* for human consumption that still allows the calf to survive and grow (13). The discussion in this section therefore deals only with the actual off-take for human consumption.

The proportion of lactating camels per family during the wet season was 17 % and 17.6 % of the total number of camels per family in Jijiga and Shinile, respectively. The proportions of lactating camels in the study areas are similar to the findings of Herren (13), who reported proportions in the range of 15 to 22 % of total herd size.

The mean lactation milk off-take per dam in the Jijiga site was computed to be 2009 kg with a mean lactation length of 15 months. Whereas in the Shinile site the average milk off-take was 1244 kg with an average lactation length of 13 months. Several studies had estimated the average milk off-take at 1000—2400 kg per camel and per lactation for different areas of Ethiopia (20, 2, 11, 26, 34, 32, 18). The national average milk production per cow and per lactation was estimated at 210 kg (1). As a result, one can safely say that camel is superior in its milk yield and gives more sustainable and reliable milk than dairy cows.

The average daily milk off-take for Jijiga site was 4 and 5 kg per dam in both the dry and wet seasons,

respectively. Whereas in the Shinile site it was 3 kg per dam in both the dry and wet seasons. These results are in complete agreement with the findings of other researchers which reported an average daily milk off-take in the range of 3 to 7 kg per dam for camels in the Afar, Awash, Borana and Somali regions of Ethiopia (20, 2, 26, 18). For comparison purposes, estimates of mean daily milk off-take were about 1.3 kg from local cows and about 2.8 kg from crossbred cows (33). The milk off-take appears relatively consistent between wet and dry seasons in both sites. The milking frequencies were, on the average, three times per day in the wet season and two times per day during the dry season in both sites.

In general, the sample respondents in Jijiga produced more milk than their counterparts in Shinile. The reasons for this variation may be breed difference as camels in Jijiga are the milk type (28) and/or higher degree of milk commercialization in Jijiga (Tab. 3).

Table 3. Milk Production Parameters of Camel Herds*

Particular	Average values Jijiga	Shinile
Number of lactating camels		
Wet season	6	4
Dry season	5	3
Daily off-take (kg/dam)		
Wet season	5	3
Dry season	4	3
Lactation off-take (kg)	2009	1244
Lactation length (months)	15	13
Milking frequency (days)		
Wet season	3	3
Dry season	2	2
Milk shelf life (days)	12	8

* — The estimation of particular variables like off-take was made only with the households having lactating camels during the study period

Milk Consumption and Processing

The sample respondents reported that camel milk was consumed in fresh or fermented form. They also reported that it was mostly consumed with tea or was added to grain foods or porridges. More specifically, it was reported that quite often fresh camel milk was consumed with tea and sour milk with boiled sorghum, rice, or maize (sorghum) porridges. The habit of milk consumption appears to be similar across camel-herding societies of Ethiopia (11, 13).

All of the sample respondents reported that they strongly believed that camel milk had a very good medicinal value and they used it for treating malaria or jaundice, gastro-intestinal disorders and strong cough (pneumonia). The medicinal value of camel milk has been reported by some researchers (19, 21).

Camel-milk consumption patterns by season are presented in Table 4. The majority of households in Jijiga reported that they divided their production into two parts (for consumption and sale). In fact, only about 24 % and 40 % of the sample households in Jijiga reported that they consumed their total milk production in the wet and dry seasons, respectively. Whereas almost all of the sample respondents in Shinile reported that they consumed nearly all of their milk production. The mobile nature of the Shinile households and the relatively low milk production per dam could be the reasons for the low level of milk sold by the sample respondents. A closer look at Table 4 shows that in Jijiga area, on average, about 57 % and 60 % of the sample households sold at least one-third of their daily total milk production in wet and dry seasons, respectively.

Table 4. Percentage Distribution of Respondents by the Proportion of Camel Milk Consumed

Amount consumed per day*	Jijiga (n = 53)	Shinile (n = 31)
Wet season		
One-third	32.1	0.0
Two-third	43.4	3.2
All	24.5	97.8
Dry season		
One-third	20.8	0.0
Two-third	39.6	0.0
All	39.6	100

* — Out of total herd milk off-take per day

The majority of the respondents in both sites reported that they practised milk souring (Table 5). The sample respondents who reported to have practised milk souring stated that they added a small quantity of previously fermented milk into fresh camel milk so as to accelerate the fermentation process. However, none of the respondents reported that they used additives like green pepper (*Capsicum frutescens*) for milk souring purpose as reported by Gebremariam (11) for Afar (Ethiopia) pastoralists and by Holter (15) for pastoralists in Northern Sudan.

Soured milk is principally used for household consumption. None of the sample respondents who reported to have practising milk souring produced butter and/or cheese. This was reported to be mainly due to the very low churning yield of camel milk. However, some authors (24, 17, 25) reported the possibilities of getting different products from camel milk if either rennet or chlorate is added to soured milk.

Table 5. Percentage Distribution of Respondents on the Basis of their Camel Milk Souring Practices

Particulars	Jijiga (n = 53)	Shinile (n = 31)
Not sour milk at all	22.6	19.4
Sour milk when forced*	24.5	6.5
Sour milk purposefully	58.8	74.2

*The respondents reported that they soured milk only when they had surplus milk, over and above the family consumption requirement, which they could not sell

The survey results reveal that the sample respondents in both sites did not practise milk processing. As a result, they consumed camel milk either fresh or soured without any processing. The respondents reported that camel milk could be safely consumed, on the average, after keeping it for 12 days in Jijiga and 8 days in Shinile (Table 3). The longer shelf life of camel milk as compared to other milk types is supported by research findings (12, 29, 17). The longer shelf-life of camel milk may be attributed to its bacteriostatic and bactericidal effects against pathogenic microbes (17, 7). This longer storage quality can be used as an advantage in camel milk preservation. Camel milk with its longer shelf life remains an important and reliable food source for the pastoralists during long journeys in search of feed and water.

The sample respondents pointed out that smoking of milk containers was their milk preservation technique. The principal effect of smoking of milk containers is to suppress the multiplication of pathogenic micro-organisms thereby serving as a method of disinfection. Smoking of milk containers was reported to be a common practice in Somalia (24).

Milk Marketing

Almost all the sample respondents who reported selling camel milk indicated that milk selling is the responsibility of women. They also reported that they usually sold their marketable milk in the morning and the milk they sold was reported to be a mixture of the milk from the previous evening and the following morning. The sample households stated that they kept the milk destined for market in plastic containers and Jerry cans and transported it to the market centres. The survey revealed that the marketing centres could be divided into milk collection centres (primary markets) and principal (terminal) markets. The milk collection centres include those markets that are small in size and located in close proximity or not very far away from settlement points. The terminal markets are the major urban centres (in the case of this study Dire Dawa city is the terminal market for the Shinile Zone and Jijiga city is the terminal market for the Jijiga zone) where milk collected from the milk collection centres will be sold to retailers and consumers.

At the milk collection centres the herders sell their milk to milk collectors, retailers and consumers. Milk collectors are traders who buy milk from rural areas and transport it to urban areas and sell it to retailers and/or consumers at a profit. In the context of this study, retailers include market intermediaries (retailers in the strict sense of the term), owners of tea/coffee shops, bars, restaurants and hotels who buy milk either from milk collectors or camel herders and sell it to their customers.

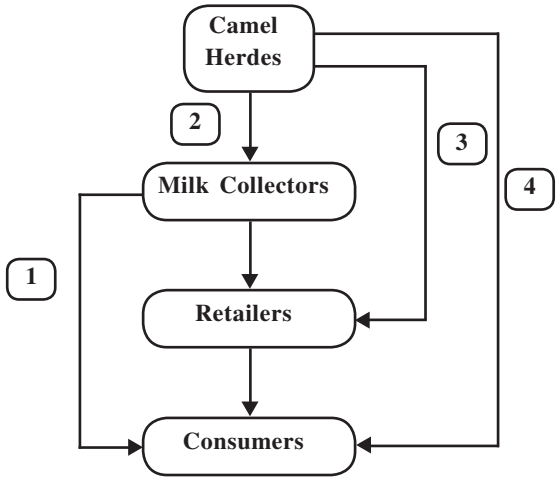
With regard to the means of transportation used in milk marketing, the survey results show that it varied depending on the distance between the settlements and the market centres. In this respect, except those respondents whose settlements were not very far from the milk collection centres, the sample respondents reported that they used male camels to transport milk from the settlements to the milk collection centres. In fact, as a great majority of the sampled settlements are located far away from the milk collection centres, almost all the respondents who reported selling sold milk had to travel long distances to sell their milk.

Because of the poor marketing infrastructure (poor transport and communication systems) it takes a long time to get the camel milk to markets and the quality suffers in the process. In fact, some respondents reported that they did not take milk to the market during some months, especially when they settled in areas far away from the milk collection centers. Given that milk marketing is a time-consuming process for herders located far away from urban areas and herders are responsible for their own marketing, women took turns in transporting and selling milk. The milk collectors reported that they used pick-up vehicles to transport milk from the milk collection centres to the terminal markets.

The marketing system involves different types of marketing agents who have important roles to play. The agents constitute the marketing channels through which produces reach the ultimate consumers. This study revealed that milk is marketed through four channels. The milk marketing channels in the study areas vary greatly, from direct herder – consumer sales to multiple intermediary channels involving milk collectors and retailers. For ease of understanding, the milk marketing channels in the study areas are sketched hereunder:

As already noted, milk is sold to milk collectors, retailers, or directly to consumers at the nearby local markets. In this respect, more about 80 percent of respondents in Jijiga who sold camel milk reported that they delivered their milk to milk collectors and the remaining (20 %) indicated that they sold their milk to retailers and/or consumers. It is important to note that camel herders located near urban areas have a spatial advantage over those located in distant places in that they sell fresh milk directly to consumers and save the commision which would otherwise be earned by milk collectors.

Fig. 1. Milk Marketing Channels in the Study Areas



More specifically, the marketing channels include:

— Camel herders → Milk Collectors → Consumers
— Camel herders → Milk Collectors → Retailers → Consumers
— Camel herders → Retailers → Consumers
— Camel herders → Consumers

Retail prices of camel milk, during dry and wet seasons, in different localities of Eastern Ethiopia are presented in Table 6. The data presented in Table 6 are average prices computed from information collected through rapid market appraisal surveys. The table shows clearly that the prices of camel milk were stable in Jijiga town across the seasons. The market survey revealed also that milk collectors made, on the average, a cut of 0.35 Birr per litre of milk.[4]

Table 6. Retail Prices of Camel Milk in Major Towns of Eastern Ethiopia

Towns/Season	Price (Birr/ litre) Wet season	Dry season
Jijiga	2.0	2.0
Kebribeyah	1.5	1.8
Hartisheik	1.5	3.0
Fafen	1.5	2.0
Dire Dawa	3.0	3.5
Shinile	3.0	4.0
Errer	4.0	4.5

Table 6 shows also that camel milk fetches relatively higher prices in the Shinile zone (see the market prices in Dire Dawa, Shinile and Errer markets in Table 6). This is explained by the fact that the proportion of marketable milk is relatively smaller in the Shinile zone as compared with that of the Jijiga zone (see Table 4).

CONCLUSION

This study reveals that, in both the Jijiga and Shinile areas, respondents keep different species of animals. This fact indicates their production strategy, which is focused on risk aversion and better utilization of available feed and water resources. In fact, all of the sample respondents reported that they kept camels. However, the number and composition of other livestock species owned by the respondents varied between sites and among households within each site. The results of the study show that female camels were dominant in the family herd structure. Moreover, the proportion of breeding females out of total females was more than 67 %, implying the good breeding status and rapid expansion potential of the herd in the future.

The survey results reveal also that, in the study areas, camels are kept mainly for milk production and to a much more limited extent for meat and transportation. Camel milk was found to be an important food item and source of cash income to the sample respondents. In fact, the Shinile households consumed almoust all their milk production. Whereas the Jijiga sample respondents sold part of their production and earned cash income which could be used for different purposes. Most of the respondents who reported selling camel milk indicated that they sold their milk to milk collectors, who in turn, sold it to retailers and/or consumers with a certain margin.

One surprising finding of this study is that sample respondents did not practise milk processing. It is, however, evident that milk processing techniques can help produce different products which could be kept for long time, transported over long distances and sold at good prices. Therefore, milk processing can be used as a mechanism for adding value to surplus milk, especially during the wet season, and increasing the shelf life of camel milk. In this respect, it is advisable that a study be made to identify appropriate milk processing methods from the neighboring countries and adapt them to the local situations.

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[4] 1 Birr is the Ethiopian national currency. Between February 15, 1973 and September 30, 1992, the Birr had been pegged to the US dollar at the rate of 1 US $ = 2.07 Birr. The Transitional Government of Ethiopia devalued the Birr in September 1992 and fixed the exchange rate at 1 US $ = 5 Birr. Currently, the exchange rate is determined by auctions held weekly in Addis Ababa and it is now around 1 US $ = 8.51 Birr.

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VITAMIN A LEVELS IN THE LIVER OF BROILER CHICKS

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ABSTRACT

Levels of vitamin A were measured in the liver of broiler chicken HYBRO G (n = 72) from three different farms. All broilers were fed KZ HYB 1, 2, 3 rations. The broilers from farm A, harvested at the age of 38 days, supplied feed containing 11 457 IU of vitamin A.kg^{-1}, had mean carcass weight 1.98 kg. The mean concentration of vitamin A in healthy livers of this group was 677, in livers with pathological changes 617 IU of vitamin A.g^{-1}. The broilers from farm B were harvested at the age of 42 days with mean carcass weight 1.87 kg. During the fattening, they were supplied rations containing 10 463 IU of vitamin A.kg^{-1}. In this group, healthy livers contained on average 603 IU of vitamin A.g^{-1} and the pathological ones 515 IU.g^{-1}. The broilers from farm C were harvested at the age of 42 days and mean carcass weight of 1.75 kg. The concentration of vitamin A in the feed is unknown. Healthy and pathological livers contained on average 557 and 295 IU of vitamin A.g^{-1}, resp. The highest harvested weigh and highest level of vitamin A in the liver was found in broilers fed rations with the highest content of vitamin A.

Key words: broiler chickens; liver; vitamin A

INTRODUCTION

Vitamins are an important part of the metabolic complex of animal organisms. Although the available knowledge about them is extensive and included information about their various interactive metabolic activities still new knowledge and qualitative characteristics of these essential, biologically active compounds are discovered and presented. This applies also to vitamin A in relation to its level in various organs of animals including poultry. Menkin *et al.* (9) observed that supplementation of rations for broiler chickens with choline amounting to 1000—1500 g.t^{-1} of feed increased significantly the level of vitamin A in the serum and liver, increased synthesis of serum proteins by 9—14.6 % and had no effect on organoleptic properties of broiler meat.

Hamdoon and Rahman (2) found that administration of various doses of vitamin A added to the feed of chicks from day 6 to day 46 of age produced no toxicity symptoms. Values of vitamin A in plasma and liver of chicks were higher than those in the control groups and plasma ß-carotene levels were suppressed. Koreleski and Swiatkevicz (7) proved experimentally that broiler chicks fed the starter rations from one day of age contained higher levels of vitamin A in the liver on days two and five in comparison with the control that received different feed.

Hanan *et al.* (9) added yoyoba oil to feed rations of broiler chicks and observed its effect on the growth, antibacterial activity and some biochemical parameters of their carcasses. Chickens fed rations supplemented with maize oil served as a control. The authors observed that the chickens fed yoyoba oil reached higher final weight, muscle yield, higher weight of internal organs and also higher levels of vitamins A and E in the liver in comparison with the control.

Concentrations of ß-carotene and vitamin A in the liver of broiler chicks fed rations supplemented with various components were observed by Johanssen *et al.* (5). Huan *et al.* (4) stressed the effect of vitamin A against pyrolizidine alkaloids present in *Senecio jakobaea,* the plant found in the mixed feed supplied to broiler chickens. The level of vitamin A in these chicks was decreased in the serum and increased in the liver which indicated that the respective alkaloids disturbed mobilisation of vitamin A from the liver although reversibly. Histopathological examination proved that the effect of these toxins, affecting particularly the liver, was alleviated by high

concentrations of vitamin A that protected hepatic cells against the toxic action.

The presence of *Senecia* plant in the feed causes decreased weight gains at its long-term feeding. Košutszký and Škrobánek (8) studied the influence of PCB contained in the feed for broiler chicks on biochemical parameters of blood including vitamin A and observed increased values of AF, cholesterol and triglycerides and decreased values of vitamin A, haemoglobin, haematocrit and erythrocytes.

Richter *et al*. (10) experimented with broiler chicks using different feed (with or without maize) with addition of ß-carotene and vitamin A and observed a positive correlation between supplementation with ß-carotene and vitamin A and the level of vitamin A in the liver. In another study Richter *et al.* (11) reported their results focusing on the requirements of chicks, pullets, and hens on vitamin A. They observed that up to 8 weeks of age broilers and layers required for their optimum growth 1500 IU of vitamin A.kg^{-1} feed. During laying they need 4000 IU of vitamin A.kg^{-1} mixed feed. To ensure reserve of vitamin A in the liver on the level of 100 IU.g^{-1} the rations should be supplemented with 2000 IU.kg^{-1}. The optimum function of the liver and its protection is ensured by adding 4000–6000 IU vitamin A.kg^{-1} feed. Konopatov *et al.* (6) observed cobalt in feed and liver and vitamin A in the liver of chicks from 7 up to 95 days of age. They observed that concentration of Co in the liver was directly related to the level of Co in the feed. Co in the feed was directly related to vitamin A in chicken liver.

Bikomskaya *et al.* (1) fed various quantities of spiruline biomass to chickens and observed its influence on vitamin A levels in the liver. They observed that 49 day old chickens supplied feed supplemented with spiruline had significantly higher level of vitamin A in the liver in comparison with the control. The more spiruline was in the feed the higher level of vitamin A was detected in the liver. Even this short review proves that the role of vitamin A is still topical, its research is in progress and focuses on its expanding and new interaction activities and relations. Vitamin A has been assessed from different points of view stressing particularly its action and importance in an organism that comes into contact with new, particularly synthetic substances, penetrating into the food chain from the outer environment or due to adjustments in feed ration formulations.

We focused on the sphere that has attracted little attention — the level of vitamin A in the liver of broiler chicks — as food for human consumption.

MATERIAL AND METHODS

The values of vitamin A in the liver of broiler chicks were observed on 3 farms in the eastern and central Slovakian regions producing broiler chickens. All three farms used for fattening broiler hybrids HYBRO G and KZ HYB 1, 2, 3.

The age of chickens when harvested for slaughter was 38 days on farm A and 42 days on farms B and C. Mean weight of carcasses from farms A, B and C reached 1.98 kg, 1.87 kg and 1.75 kg, resp. The level of vitamin A in the feed was 11 457 IU.kg^{-1} on the farm A, 10 463 IU.kg^{-1} on the farm B and was not specified for the farm C.

In all the cases, livers samples for determination of vitamin A were taken after slaughter. Examinations were carried out on healthy livers and livers with pathological changes that were sent for examination to the State Veterinary Institute where, besides determination of vitamin A, pathological changes were diagnosed.

RESULTS AND DISCUSSION

Results obtained in our study are presented in Table 1.

The mean carcass weight of slaughtered chickens from farm A, slaughtered at the age of 28 days was 1.98 kg. Feed rations supplied to them during the fattening contained 11 457 IU vitamin A.kg^{-1}. The level of vitamin A in the healthy chickens ranged from 475 to 935 IU.g^{-1}liver and its mean value reached 677 IU.g^{-1}. The content of vitamin A in the livers with pathological changes ranged from 360 to 835 IU.g^{-1} liver with the mean of 617 IU.

The broilers from farm B were 42 days old. Their mean weight at slaughter was 1.87 kg. During the fattening, the broilers received feed containing 10 463 IU vitamin A.kg^{-1}. Livers from healthy broilers contained 430–870 IU vitamin A.g^{-1} with the mean value of 603 IU vitamin A.g^{-1}. Vitamin A levels in pathologically changed livers ranged from 343 to 635 IU.g^{-1} with the mean value of 515 IU.g^{-1}.

Table 1. Values of vitamin A in the liver of broiler chicks and in the rations used

Farm	Age of broiler in days at the time of harvesting	Mean weight at harvesting in kg	Values of vitamin A in the liver in IU.g^{-1} liver: Healthy liver min. – max. $\bar{x}$	Pathological liver min. – max. $\bar{x}$	Content of vitamin A in the mixed feed in IU.kg^{-1}
A	38	1.98	475–935 677	360–835 617	11.457
B	42	1.87	430–870 603	343–635 515	10.463
C	42	1.75	442–695 557	160–502 295	unstated

The broilers from farm C were harvested at 42 days of age. Their mean weight after the slaughter was 1.75 kg. The content of vitamin A in the mixed feed was not stated. The livers of healthy broilers contained 442—695 IU.g^{-1}. Vitamin A levels in pathologically changed livers were between 160 and 502 IU.g^{-1} with the mean reaching 557 IU.g^{-1}. Pathologically changed livers contained 160–502 IU vitamin A.g^{-1} and its mean value was 295 IU.g^{-1}.

Although papers of similar orientation and character are not available in the literature, our results allow us to formulate some conclusion that are valid on a wider scale and may become a subject of further research focusing on quantitative observations of vitamin A in the liver as a commodity and donor of vitamin A to human nutrition.

Our results indicate that vitamin A contained in the diet affects the weight of broilers. This is confirmed by different mean carcass weight of broilers from farms A and B. We may assume that the diet of broilers on farm C contained less vitamin A than that fed on farm B.

Similar results were obtained for vitamin A levels in the healthy livers. The mean value of vitamin A in the liver was the highest in broilers from farm A which were supplied feed with the highest content of vitamin A, i.e. the respective livers are most valuable from the point of view of nutrition.

Lower levels of vitamin A in pathologically changed livers were probably the consequence of decreased quantity of the active hepatic parenchyma.

CONCLUSION

Values of vitamin A were observed in the liver of broilers HYBRO G from 3 different farms fed commercial feed KZ HYB 1, 2, 3.

The slaughter age of broilers from farm A was 38 days and their mean weight after slaughter 1.98 kg. Their feed rations were supplemented with 11 457 IU of vitamin A.g^{-1}. Healthy livers contained on average 677 IU vitamin A.g^{-1} while pathologically changed ones only 617 IU.g^{-1}.

Broiler halls on farm B were emptied when the broilers were 42 days old and their mean weight after slaughter was 1.87 kg. Their diet was supplemented with 10 463 IU of vitamin A.g^{-1}. Healthy livers of these birds contained on average 603 IU vitamin A.g^{-1} and those with pathological changes only 515 IU.g^{-1}.

Broilers from farm C were slaughtered at the age of 42 days and their mean weight after slaughter was 1.75 kg. The content of vitamin A in their feed was not reported. Healthy livers of these broilers contained on average 557 IU vitamin A.g^{-1} and livers with pathological changes 295 IU.g^{-1}.

It has been observed that the highest slaughter weight and levels of vitamin A in the liver were reached in broilers supplied feed with the highest content of vitamin A.

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SUBCHRONIC TOXICITY TO CHICKS OF SODIUM SALINOMYCIN IN THE PREPARATION SYNVERTAS plv. a.u.v.

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ABSTRACT

From day 3 to 45 of age chicks of Ross hybrid were fed complete mixed feed Hyd–01 and Hyd–03 with salinomycin in the form of preparation Synvertas plv. a.u.v. (Biotika a.s., Slovenská Ľupča, SR) at doses 60 mg (600 mg Synvertas), 90 mg (900 mg Synvertas), and 180 mg (1800 mg Synvertas) per 1 kg feed. Group 4 was fed salinomycin in the form of preparation Sacox 120 at a dose of 60 mg (500 mg Sacox) per 1 kg feed. Group 5, which served as a control, was fed identical feed without any anticoccidial drugs.

Synvertas preparation, supplied at preventive dosage of 60 mg salinomycin. kg^{-1} feed, increased significantly the final body weight of chicks in comparison with the control and other experimental groups.

Feeding salinomycin at a dose of 90 mg.kg^{-1} feed resulted in a decreased body weight and an additional increase in salinomycin dose to 180 mg.kg^{-1} only intensified its negative effect.

The feed consumption per kg weight gain increased with time. Clinical observations detected no changes in the health status of chicks throughout the 42-day experiment even at a dose of 180 mg.kg^{-1} feed. However, chickens receiving this dose showed significant decrease in the number of erythrocytes and haemoglobin, decrease in calcium level, and increase in blood bilirubin. Post mortem examination showed mild hyperaemia and splenomegaly in some chicks.

Key words: chicks; salinomycin; subchronic toxicity; Synvertas plv. a.u.v.

INTRODUCTION

Anticoccidial effect of salinomycin is based on its ability to form hypophilic complexes with basic cations and transfer them across biological membranes In this way it increases the intracellular concentration of sodium and decreases the concentration of potassium. These changes are associated with increased levels of calcium in mitochondria and the reticulum (5, 10, 4). Ionophores also cause changes in the release of acetylcholine which affects the transfer of nerve impulses in synapses (8).

Toxic doses of ionophore anticoccidials cause necrosios of cells, particularly of monocytes (4). However, their toxicity is affected by various factors including their forms in respective preparations (5), which can result in different tolerance of various preparations having identical content of the active ingredient. Because of that we investigated the subchronic toxicity of sodium salinomycin to chicks when supplied in the form of newly developed preparation Synvertas plv.a.u.v. (Biotika a.s., Slovenská Ľupča, Slovakia) and compared it to that of the preparation Sacox 120.

MATERIAL AND METHODS

The subchronic toxicity of the preparation Synvertas plv. a.u.v. was tested on 200 chickens of Ross hybrid, of both sexes, starting from 3 days of age. The chickens were fed complete mixed feed Hyd-01 up to day 28 of age and HYD-03 from day 29 to 42 days of age. The first three experimental groups of chicks received feed supplemented with Synvertas plv. a.u.v., the first group at a dose of 600 mg, i.e. 60 mg salinomycin per kg feed, the second group at a dose of 900 mg.kg^{-1} (90 mg salinomycin) and the third group at a dose of 1800 mg.kg^{-1} feed (180 mg salinomycin), i. e. treble the preventive dose.

The fourth experimental group received feed supplemented with preparation Sacox 120 (producer Hoechst Roussel Vet., Germany) at a dose of 500 mg, i.e. 60 mg salinomycin in 1 kg feed. The fifth group of chicks, the control, was fed identical feed free of anticoccidials. The mixed feed used contained no other anticoccidials. All the chickens were kept under identical microclimate on deep bedding, fed *ad libitum and had free access to drinking water.*

Before the experiment, the chickens were weighed and blood samples withdrawn from v. cutanea ulnaris of 10 chicks from each group were subjected to haematological examination. Weight of all chickens was determined on day 14, 28, and 42 of the experiment. The chicks were observed daily for any clinical changes. On day 42 of the experiment blood sera of 20 chicks were examined for the level of calcium, phosphorus, urea, bilirubin, total proteins, cholesterol, and the activity of alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Blood samples for biochemical examination were obtained by decapitation and those intended for haematological examination were taken from *v. cutanea ulnaris* of 10 chicks from each group at the end of the experiment. Pathological-anatomical examinations were also performed at that time.

Haematological examinations were carried out by the methods of Slanina *et al.* (11).

Biochemical examinations were conducted photocolorimetrically using Bio-Lachema tests, Brno, CzR. Statistical significance of the results was determined by Student's *t-test.*

RESULTS

Throughout the experiment no clinical changes were observed in the investigated groups of chickens. The body weight of chickens from the first experimental group that received feed supplemented with Synvertas at a preventive dose of 60 mg salinomycin per kg feed reached 310.83 g, 718.67 g and 1335.7 g on days 14, 28, and 42 of the experiment, resp., which was significantly higher in comparison with the second (217.67 g, 563.6 g and 1033.5 g, resp.), third (226.1 g, 513.6 g and 958.2 g, resp.) and fourth experimental group (221 g, 539.9 g and 1125.3 g, resp.) and the control group of chicks (197.8 g, 484.5 g and 990.01 g, resp.). Chickens from the 2nd experimental group, receiving 90 mg salinomycin per kg feed, showed a significant decrease in body weight in comparison with the 1st group, however, in comparison with the control the weight of these chicks was significantly higher on day 28 and insignificantly higher at the end of the experiment. The lowest final mean weight was recorded in chickens from the 3rd experimental group. The final weight of chickens from the fourth group was significantly higher than that of chicks from the third group and the control chicks.

The consumption of feed per kg weight gain in groups 1 to 4 reached 2.1, 2.31, 2.93, and 2.24 kg, resp., while the control chicks consumed 2.36 kg feed per kg weight gain.

Table 1. Biochemical examination of blood of subchronically intoxicated chickens

Parameter		Group 1	2	3	4	5
Calcium	$\bar{x}$	2.35	2.13	1.88	2.55	2.52
(mmol.l^{-1})	s	± 0.235	0.27	0.342	0.156	0.135
Phosphorus	$\bar{x}$	1.82	1.84	2.14	1.81	1.94
(mmol.l^{-1})	s	± 0.384	0.38	0.263	0.173	0.42
Alkaline phosphatase	$\bar{x}$	93.47	67.18	132.16	62.93	142.26
(μkat.l^{-1})	s	± 52.24	51.51	88.22	33.58	95.85
AST	$\bar{x}$	2.02	1.82	1.77	1.95	1.65
(μkat.l^{-1})	s	± 0.467	0.315	0.213	0.400	0.283
ALT	$\bar{x}$	0.073	0.076	0.073	0.110	0.160
(μkat.l^{-1})	s	± 0.039	0.022	0.032	0.027	0.165
Urea	$\bar{x}$	250.01	230.26	237.4	213.08	238.05
(g.l^{-1})	s	± 34.78	60.72	49.3	52.55	36.18
Bilirubin	$\bar{x}$	4.56	3.60	8.62	5.069	6.91
(mmol.l^{-1})	s	± 0.867	0.557	5.03	1.099	2.633
Total proteins	$\bar{x}$	27.76	27.08	25.63	26.08	27.63
(mmol.l^{-1})	s	± 1.846	1.73	3.03	2.56	1.196
Cholesterol	$\bar{x}$	2.11	1.87	1.87	1.899	2.20
(mmol.l^{-1})	s	± 0.445	0.424	0.392	054	0.318

Significance of differences:

Calcium:	2 : 4 and 5	$P < 0.005$	Bilirubin:	1:3	$P < 0.05$
	3 : 1, 4, 5	$P < 0.005$		2:3	$P < 0.025$
Phosphorus:	3 : 4	$P < 0.01$		4:3	$P < 0.05$

The haematological examination performed at the end of the experiment showed a significant decrease in erythrocyte count (1.82 $T.l^{-1}$) and haemoglobin level (67.52 $g.l^{-1}$) in the chickens from the 3rd group in comparison with those from the 1st group (2.89 $T.l^{-1}$ and 83.68 $g.l^{-1}$, resp.). When compared to the chicks from the 4th group, the decrease in respective blood parameters (2.59 $T.l^{-1}$, 81.92 $g.l^{-1}$, resp.) bordered on significance. The decrease in erythrocyte count (2.16 $T.l^{-1}$) and haemoglobin level (73.44 $g.l^{-1}$) was insignificant in comparison with the control (2.23 $T.l^{-1}$, 74.9 $g.l^{-1}$, resp.). No significant intergroup differences were observed in the number of leukocytes and leukogram values.

Biochemical examination of blood at the end of the experiment showed that blood calcium level of chickens from the 3rd group (1.88 $mmol.l^{-1}$) was significantly lower in comparison with chickens from groups 1, 4 and 5 (2.35 $mmol.l^{-1}$, 2.55 $mmol.l^{-1}$ and 2.52 $mmol.l^{-1}$, resp.). The blood calcium content of the chickens from the 2nd group (2.3 $mmol.l^{-1}$) was significantly lower than that in the chickens of groups 4 and 5. Blood phosphorus levels of chicks from the 3rd group (2.14 $mmol.l^{-1}$), determined at the end of the experiment, differed significantly from those of the 4th group (1.81 $mmol.l^{-1}$). Blood phosphorus content in groups 1 and 2 and in the control reached 1.82 mmol. l^{-1}, 1.84 $mmol.l^{-1}$, and 1.94 $mmol.l^{-1}$, resp. The differences between these three groups were insignificant. Determination of blood bilirubin showed statistically significant differences between chickens from groups 3 (8.62 $mmol.l^{-1}$) and 1 (4.56 $mmol.l^{-1}$) and groups 2 (3.60 $mmol.l^{-1}$) and 4 (5.07 $mmol.l^{-1}$). The blood bilirubin level in the control chicks reached 6.91 $mmol.l^{-1}$. No significant intergroup differences were observed neither in the level of urea, total proteins, and cholesterol nor in the activity of alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase.

Pathological-anatomical examination of chickens at the end of the experiment revealed moderate anaemia that was more pronounced in the chickens from the third group. Four chickens from this group had enlarged and hyperanaemic spleen. No other symptoms suggesting unfavourable action of salinomycin were observed in the chickens.

DISCUSSION

The salinomycin dose recommended for chickens ranges from 40 to 60 $mg.kg^{-1}$ feed (2, 1). The highest dose tolerated by chickens without any side effects is 90 $mg.kg^{-1}$ feed. This dose decreases weigh gains (6). In our experiment the dose of 90 mg salinomycin per kg feed (group 2) administered in the form of preparation Synvertas resulted in significantly lower weight of 42 day old chickens in comparison with the dose of 60 mg per kg feed (group 1). However, the chickens from the second group had higher weight than the control ones and insignificantly lower than those from the fourth group that received 60 mg salinomycin.kg^{-1} feed in the form of preparation Sacox 120. The dose of 180 mg of salinomycin caused marked decrease in the weight of chickens. The consumption of feed per kg weight gain was the lowest with the 60 mg dose followed by the 90 mg dose of salinomycin in the preparation Synvertas. This indicates lower unfavourable effects on weight gains of preparation Synvertas in comparison with Sacox.

Some authors (3, 10) reported increased activity of blood serum aspartate aminotransferase related to damage to muscles by salinomycin. The doses of salinomycin used in our study had no effect on the activity of this enzyme. An increase in salinomycin dose decreased blood calcium levels which may be related to the influence of ionophore anticoccidial preparations on the transfer of ions across biological membranes (7).

Clinical symptoms of salinomycin intoxication include anorexia, apathy, dyspnoe, diarrhoea and muscle weakness. Birds sit for most of the time, get up with difficulties, stagger, become even unable to stand up and remain lying in dorsal or lateral positions. Cyanosis may appear in the advanced stage (9, 4, 12). In our experiment, lasting for 42 days, we observed no clinical symptoms suggesting unfavourable health influence even after administration of 180 mg salinomycin.kg^{-1} feed. Similarly, post mortem examination failed to detect visible damage to internal organs.

CONCLUSION

The clinical, haematological, biochemical and pathological-histological examinations performed in our study showed that the preparation Synvertas (Biotika a. s., Slovenská Ľupča, SR), administered at preventive dose of 60 mg salinomycin (600 mg Synvertas) per kg feed, had no negative influence on the health of chickens after 42-day continuous application to the feed. The latter dose improved weight gain and utilisation of feed. The dose of 90 mg salinomycin (900 mg Synvertas) caused moderate decrease in the parameters mentioned.

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THE DETECTION OF MYELOPROLIFERATIVE DISEASES IN CHICKENS, USING BONE-MARROW SAMPLING FOR THE EVALUATION OF MYELOGRAMS AND MITOTIC INDEX

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ABSTRACT

The present work deals with the detection of myeloproliferative diseases in chickens using *intra vitam* and *post mortem* bone-marrow sampling. Intra vitam (the first trial), samples of bone marrow were taken from 40-day-old broiler chickens (n = 32) after fasting. Myelograms are summarized. The effect of X-ray irradiation by a single total dose of 3.8 Gy on the mitotic activity of the bone-marrow was compared in both experimental and control chickens in the second trial in 100 cross-breed chickens (20 chicken for pilot experiment, 30 controls and 50 experimental chicken). Using total body single dose irradiation of 3.8 Gy in 58-day-old chickens, five experimental and three control chickens were decapitated at each interval at 1, 2, 3, 6, 8, 10, 14, 18, 21, 25 days post irradiation. A decrease in mitotic activity on the 25th day after irradiation ($\alpha = 10\,\%$) was recorded. In the third trial in 37-day-old broiler chickens (n = 24) were irradiated with gamma rays, using a single total body dose of 4.5 Gy. The chickens were decapitated at 1, 6, and 24 h intervals post-irradiation. In each interval 5 experimental and three control chickens were used The evaluation of the myelograms after irradiation by a single total gamma-ray dose of 4.5 Gy showed the inhibition of erythropoiesis and myelopoiesis at one, six, and twenty-four hours after irradiation. The decrease in erythropoiesis and myelopoiesis, morphological changes such as karyorrhexis, karyolysis, cellular disintegration, and hypocellularity were characteristic findings of aplastic anaemia.

Key words: anaemia, bone marrow; chickens; mitotic index; myeloproliferative diseases

INTRODUCTION

Bone marrow has a high turnover even under physiological conditions and responds to many disorders and myeloproliferative diseases by changes in mitotic activity to mitotic disorders, development of chromosomal aberrations, mutations, frequently by total disintegration of cells.

As in mammals erythropoiesis, rubricytes, prorubricytes, basophilic rubricytes and polychromatophilic rubricytes are found similarly in birds. Nuclear condensation, which is considered the proof of maturation of erythroid precursors, is not as pronounced as in mammals. Granulocyte series in birds are similar to those in mammals. The presence of metagranuloblasts in bone marrow of birds is an indication of the stage between myeloblasts and promyelocytes. Theses cells resemble myeloblasts (granuloblasts) but their cytoplasm also contains small vacuolized spaces. No monoblasts were found in the bone marrow of birds. It seems that precursors of monocytes and heterophils resemble each other in the early stages of haemopoiesis (1, 2).

From the point of view of the damage to bone marrow, the mitotic index, as a parameter indicating the activity and function of bone marrow, is justifiably used in the cytogenetics of poultry. The impaired bone marrow appears frequently pale, atrophic, hypoplastic, with necrotic foci and frequently only with a signs of haemopoietic tissue. It produces less valuable elements. Massive diseases cause the depletion of bone marrow. Different environmental factors can develop erythropoiesis and myelopoiesis suppression including pathogens, etc. The cytology of some tumours enables their differentiation is manifested by

pleomorphic lymphoid cells including lymphoblasts in addition to small and large lymphocytes and reticular cells (2, 5).

Several authors were involved in sampling bone marrow from birds (5, 1, 3) from various locations (femur, tibia, metatarsus, and others) both *intra vitam* and *post mortem.*

Our study presents sampling of bone marrow using both *intra vitam* and *post mortem* methods in chickens. This indicates the importance of a myelogram in the diagnosis and differential diagnosis of bone marrow damage in avian medicine (including, the influence of different pathogens, myeloproliferative diseases, and nutritional and other environmental factors).

MATERIAL AND METHODS

Bone marrow was sampled *intra vitam* (first trial) and *post mortem* (second and third trials).

Intra vitam aspiration of bone marrow was undertaken from the proximal epiphysis of the dorsal femur using a sterile puncture needle using a modification method (2, 3). The chickens were fixed in a lateral position using local anaesthesia (1 % lidocain).

In the first trial, the samples were taken from 40-day old broiler chickens (n = 32) after fasting. The bone marrow was withdrawn by aspiration into a syringe fixed to the puncture needle. After obtaining 0.3 ml of the bone marrow, the aspiration was discontinued to prevent dilution of the bone marrow with blood (3, 4, 5). The bone-marrow smears were prepared immediately after sampling, using a panoptic Pappenheim method of staining after 24 h drying. Before the sampling, the aspiration syringe was rinsed with an anti-coagulant solution (0.25 ml EDTA, heparin, or others) to prevent bone-marrow coagulation.

The mitotic index (MI) was determined in the bone marrow. The Feulgen method (4), by fixing the bone marrow with acetyl alcohol, was used for the evaluation of the MI by counting individual elements of bone-marrow per 10 000 cells. Together with MI the chromosomal aberrations was followed in the anaphasis.

The myelogram was evaluated under immersion counting 500 cells (2, 5) in both experimental and control chickens. The correlation *t*-test was used to determine statistical significance.

In the *second trial* the bone marrow was sampled *post mortem* from the proximal epiphysis of the femur. in 100 cross-breed chickens (20 chicken for pilot experiment, 30 controls and 50 experimental chicken). Using total body single dose irradiation of 3.8 Gy in 58-day-old chickens, five experimental and three control chickens were decapitated at each interval at 1, 2, 3, 6, 8, 10, 14, 18, 21, 25 days post irradiation.

In the *third trial* the bone marrow was sampled *post mortem* from the proximal epiphysis of the femur in 37-day-old broiler chickens (n = 24) irradiated with gamma rays, using a single total body dose of 4.5 Gy. The chickens were decapitated at 1, 6, and 24 h intervals post-irradiation. In each interval 5 experimental and three control chickens were used.

The procedures for evaluation of the bone marrow was the same as for *intra vitam* assessment.

RESULTS AND DISCUSSION

In the *first trial* (n = 32) the bone marrow was sampled *intra vitam* as described in the material and methods. The average values of the myelogram in this trial were the following: proerythroblasts 6.78 %, erythroblasts 51.28 %, myeloblasts 1.90 %, promyelocytes 3.45 %, heterophilic myelocytes 7.03 %, basophilic myelocytes 0.43 %, eosinophilic myelocytes 1.50 %, heterophilic granulocytes 9.83 %, basophilic granulocytes 0.28 %, eosinophilic granulocytes 1.43 %, lymphocytes 7.40 %, thromboblasts 0.73 %, thrombocytes 7.73 %, plasmatic cells 0.15 %, and reticulocytes 0.08 %.

In the *second trial* the bone marrow was sampled *post mortem* from the proximal epiphysis of the femur in 100 cross-breed chickens (20 chicken for pilot experiment, 30 controls and 50 experimental chicken). Using total body single dose irradiation of 3.8 Gy in 58-day-old chickens, five experimental and three control chickens were decapitated at each interval at 1, 2, 3, 6, 8, 10, 14, 18, 21, 25 days post irradiation. The mitotic index (MI) in control chickens ranged from 4.5 to 5.8 % in the whole experimental period. The mitotic index was significantly decreased on 25th day after irradiation at the dose of 3.8 Gy ($\alpha = 10$ %) (Fig. 1).

The frequency of chromosomal aberrations varied with the time and dose of irradiation and occasionally reached 13 % (3). The occurrence of chromosomal aberrations in control chickens were between 1—2 %.

Similar levels of chromosomal aberrations as observed in experimental group can be present in IBD (infectious bursal disease) and osteopetrosis, and the bone marrow in many cases can be aplastic of jellied, even gelatinous, consistency, brittle structure with only a hint of haemopoietic tissue (2, 3, 5).

The proportion of medullar myeloid and erythroid cells may vary depending on the degree of haemopoietic damage and are accompanied by important morphological changes

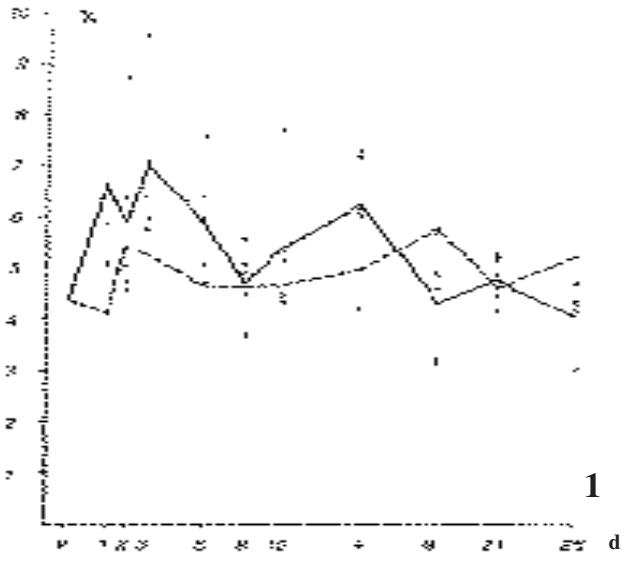

Fig. 1. The mitotic activity, control chickens, compared with bone marrow postirradiation damage (X-ray radiation, total dose 3.8 Gy, acute irradiation: control chickens ---, irradiated chickens —)

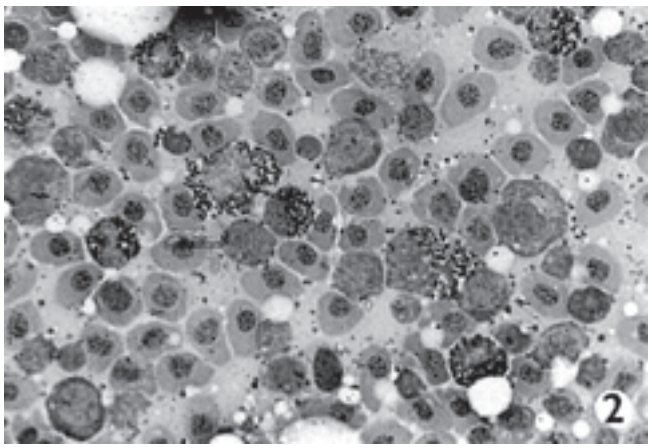

Fig. 2. The bone marrow of control chickens (staining: Pappenheim panoptic method, magnification: 100 ×)

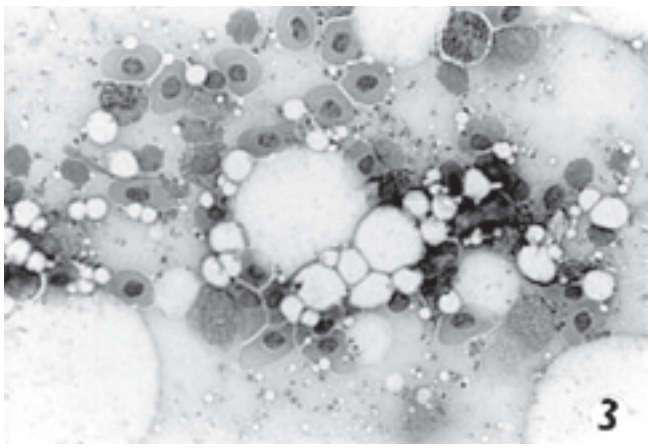

Fig. 3. The bone marrow of chickens after gamma irradiation (total dose: 4.5 Gy), decrease of erythro- and myelopoiesis, multiplication of adipose tissue 24 hours after irradiation (staining: Pappenheim panoptic method, magnification: 100 ×)

also related to the degree of damage. Lymphoid leukosis is characterized by the presence of extensive pyroninophilic lymphoblasts. In the third trial the bone marrow was sampled *post mortem* from the proximal epiphysis of the femur in 37-day-old broiler chickens (n = 24) irradiated with gamma rays, using a single total body dose of 4.5 Gy. The chickens were decapitated at 1, 6, and 24 h intervals post-irradiation. In each interval 5 experimental and three control chickens were used. The myelogram of chickens in this showed a slight decrease of erythro- and myelopoiesis in the bone marrow that leads to the beginning of aplastic anaemia.

During the autopsy of the irradiated chickens (second and third trials) we found out that the chickens was slightly anaemic. The total amount of cells as well as representation of individual cellular groups were determined by microscopic examination of the myelogram (Figs. 1, 2). At 4.5 Gy the active tissue was repleaced by addipose tissue and hypocellularity was observed at all intervals followed (1 h, 6 h, and 24 hours post-irradiation). From morphological lesions karyorrhexis, karyolysis, cellular disintegration and nuclear atypia was observed.

The following changes were observed compared to to the control groups:

– A decrease in the total number of proerythroblasts in all intervals followed ($P < 0.001$).

– Erythroblasts (macroblasts and normoblasts) decreased 1 and 6 hours ($P < 0.01$) and 24 hours ($P < 0.001$) after irradiation.

– A decrease in the myeloblast line in all three intervals followed ($P < 0.001$).

– A significant decrease in the promyelocyte values 24 hours post-irradiation ($P < 0.01$).

Bone marrow monitoring is necessary because of numerous impairments and myeloproliferative diseases (avian leukosis complex, osteopetrosis, primary tumours of other haemopoietic cells) as well as erythroblastosis, myeloblastosis, etc. Myeloblastosis and myelocytomatosis are more or less variants of myeloid neoplasms (which affect various tissues) and other damage may have varying aetiology including immunosuppressive states. Myelopoietic damage, fragmentation of nuclei, and decline in the number of mitoses are associated with bone marrow damage induced by ionizing radiation (Figs. 1, 2, 3).

CONCLUSION

The study presents the bone marrow aspiration cytology, as the diagnostic tool for radiation induced myeloproliferative disorders in chickens. Both *intra vitam* and *post mortem sampling was used in selected trials with cross-breed chickens (n = 156), at the age between 37—58 days.*

In the first trial (n = 32) the bone marrow was sampled *intra vitam.* The average values of the myelogram in this trial were the following: proerythroblasts 6.78 %, erythroblasts 51.28 %, myeloblasts 1.90 %, promyelocytes 3.45 %, heterophilic myelocytes 7.03 %, basophilic myelocytes 0.43 %, eosinophilic myelocytes 1.50 %, heterophilic granulocytes 9.83 %, basophilic granulocytes 0.28 %, eosinophilic granulocytes 1.43 %, lymphocytes 7.40 %, thromboblasts 0.73 %, thrombocytes 7.73 %, plasmatic cells 0.15 %, and reticulocytes 0.08 %.

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THE DIAGNOSIS AND DIFFERENTIATION OF SELECTED TICK-BORNE DISEASES: LYME BORRELIOSIS AND BABESIOSIS

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ABSTRACT

We monitored the possible coincidence of pathogens transmitted by ticks in the development of Lyme borreliosis and babesiosis.

In the present trial we examined and monitored 84 dogs clinically and followed up with laboratory tests. Out of 51 dogs which included pet dogs, guard dogs, and hunting dogs we detected seropositivity to Lyme diseases in 18 dogs. Out of 32 police Alsatians, 18 case were seropositive using the ELISA method. One case, a Filla Bras. female, 6 months old, which we present as a case report, was seropositive to Lyme disease and was infected by *Babesia* spp. which were detected by Wright-Giemsa stained blood smear. Of 30 *Borrelia* seropositive dogs, 15 developed gastro-intestinal (GIT) complication with *Giardia* spp. Laboratory tests and clinical signs showed the severity of Lyme disease in the case report, when combined with other pathogens.

Key words: *Babesia canis*; *Borrelia burgdorferi*; clinical signs; diagnosis; dogs; ELISA; *Giardia* spp.; Lyme disease; ticks

INTRODUCTION

Recent clinical experience and literature sources have shown the co-incidence of multiple tick-borne pathogens which result in Lyme disease, babesiosis, ehrlichiosis, and/or other diseases. These studies discuss the progress and complications in the clinical manifestations. The changes in clinical signs and complications make standard diagnostic tests less reliable, although the persistent chronic form can be present in each of them. Therefore in the diagnosis and differential diagnosis of Lyme disease, babesiosis, and other tick-borne diseases, it is important to consider the possibility of a combined infection. This requires precise methods in the protocol used and differential diagnosis, which is of great importance because of the closed contact of man and animals in the environment.

According to the literature and from our knowledge of the occurrence and clinical manifestation of Lyme disease, tick infestation in Slovakia and the serological prevalence of *B. burgdorferi*, patients with Lyme borreliosis can suffer from a coincidence of *B. burgdorferi* with other tick-borne pathogens. In this way infected ticks transmit Lyme disease and can assist the development of complications resulting from the presence of other tick-borne pathogens (2, 8, 9, 11, 12, 13).

Ixodidae are the most important vectors of several diseases in humans and small animals. In Europe, these are mainly Lyme disease, caused by *Borrelia burgdorferi* sensu lato, granulocytic ehrlichiosis caused by *Ehrlichia canis* (in Southern Europe), *Babesia canis,* and *Hepatozoon canis.* These infections are often transmitted in North-Western Europe by the ticks genera *Dermacentor* or *Rhipicephalus* sucking on dogs while they are in endemic area in Southern Europe.

Ehrlichiosis is caused by *Ehrlichia canis.* It is an immunosuppressive, chronic disease of domestic and wild animals, and the disease develops as a result of the damage to the mononuclear blood cells and bone marrow depression. It is also known as tropical pancytopenia of dogs. Some species like *E. platys* can affect platelets in dogs and can give rise to slight or unnoticed clinical signs.

The clinical signs of babesiosis, the causative agents of which are *Babesia* spp. *(Babesia canis, B. gibsoni)* can show temporary slight manifestation which can result in an acute manifestation with sudden death. The chronic form of babesiosis is characterized by regenerative macrocytic, hypochromic anaemia due to the destruction of erythrocytes with the presence of intravascular and extravascular haemolysis, haemoglobinuria, and icterus. The severity of these signs is determined by the

degree of parasitaemia. The occurrence of babesiosis has been described in many countries, including Slovakia, in various ways by practising veterinarians and in diagnostic centres (10, 11, 12, 13, 5). From the epidemiological point of view, ticks *Rhipicephalus sanguineus, Dermacentor reticulatus,* and also ticks *Hyalomma* and *Haemophysalis,* fleas, blood transfusion, and other causes are important in the transmission of babesiosis (1, 6, 4, 17).

Lyme borreliosis is a multisystemic disease caused by *Borrelia burgdorferi* sensu lato complex, which can show dermatological, neurological, cardiovascular and arthropathic signs and other complications. The ticks are the vectors of *Borrelia burgdorferi* (in Europe, *Ixodes ricinus*, in North America, *I. scapularis* and *I. pacificus*).

Diagnosis and the evaluation of both the progress and the signs of the disease are very demanding. The signs vary and the disease may recur. The complications which the patient undergoes with Lyme disease can vary. DNA/DNA hybridization, ribotyping, and 16 S ribosomal or RNA sequestration of isolates from different geographical areas (North America, Europe) indicate the occurrence of three distinct strains of *Borrelia burgdorferi* important in the development of Lyme borreliosis *(Borrelia burgdorferi* sensu stricto, *B. afzelii,* and *B. garinii).*

Indirect immunofluorescence assay (IFA), enzyme–linked immunosorbent assay (ELISA) and Western blotting are commonly used for the diagnosis of Lyme disease. They enable the detection of spirochaetal antigens, DNA, or antibodies present in the biological material and sera of infected or vaccinated animals. Recently, a lot of work has been done to develop a fast, sensitive method for diagnosing Lyme disease based on a polymerase chain reaction (PCR).

MATERIAL AND METHODS

84 dogs — 51 pet dogs, guard dogs, and hunting dogs, 32 police Alsatians and one Filla Bras. 6 months old female underwent a general physical examination and laboratory testing (haematology, serum biochemistry, and urine analysis) used in monitoring the patients at the 1st Internal clinic at the UVM, Košice. ELISA (USOL Prague, CzR) as modified by Stefančíková *et al.* (14, 15) was used for the detection of seropositivity to Lyme borreliosis.

A blood smear with Wright-Giemsa staining (Pappenheim panoptic method) was used to evaluate the leukogram and monitor *Babesia* spp. (12).

A faecal examination was carried out in the Institute of Parasitology SAC to determine the presence of *Giardia spp.*

A necropsy was carried out in the Department of Pathology at the UVM, Košice in the case of 6 months old Filla Bras. female which died a few hours after the beginning of treatment.

RESULTS AND DISCUSSION

The work demonstrates diagnostic and differential diagnostic procedures for selected tick-borne disease as outlined by others (3, 16, 7) and describes a case in which *Borrelia burgdorferi* sensu stricto and *Babesia canis* combined to cause disease. (A 6 month old Filla Bras.).

In our clinic, we monitored 84 dogs which had been infected with ticks according to the anamnesis and followed them up with laboratory tests.

Out of 51 dogs (pet dogs, guard dogs, and hunting dogs) 18 were seropositive. Out of 32 police Alsatians, 11 were seropositive. The clinical signs in the seropositive dogs varied. Anorexia, GIT (gastro-intestinal) disturbances, arthropathy, and neuropathy were often diagnosed. Some of the seropositive dogs showed no clinical changes during the monitoring period.

Out of 30 *Borrelia*-seropositive dogs, 15 developed GIT complications with *Giardia* spp. In these cases, the presence of *Giardia* spp. and immunosuppression played an important role in the progress of the Lyme disease.

A case report

Introduction to patient

A 6-month-old, female, Filla Bras., from the region of East Slovakia (Veľké Kapušany village) was without clinical pathological signs at the end of April 2000. The dog was vaccinated against distemper, CPV, rabies, hepatitis and leptospirosis. At the beginning of May 2000 the dog developed slight clinical signs of depression, anorexia, and weakness. From its history and the epidemiological point of view, the private veterinarian removed ticks more often from the different parts of the body and referred to the high prevalence of tick infestation in the area of the dog's environment, and the dog underwent a repeated antibiotic treatment using different antibiotics (these were not specified in the anamnestic data).

On the 4th of May, the dog had worsened, its depression was deeper, the mucous membrane was pale, and the owner found the dog with the signs of apathy, somnolence, anorexia and general weakness, movement limitation, and fever. Two days later, on 6th May, the dog was referred with the owner and with the private veterinarian to the University's Veterinary Clinic of Internal Disease I for diagnosis and treatment. The clinical status by this time required emergency treatment and 6 hours later on the 7th May, the dog died.

Clinical and laboratory examinations

Clinical and laboratory tests were followed up as described in material and methods.

Clinical signs and laboratory findings

The clinical examination showed tachypnoea and tachycardia, marked palpation sensitivity of the abdomen, general signs of severe dehydration and depression, prolonged capillary refill time, hallitosis, signs of splenomegaly and hepatomegaly, lymphadenopathy, *icterus gravis* (icterus of the mucous membrane, tissues, and skin), lung oedema, fibrinous pericarditis and signs

Fig. 1. ***Icterus gravis*** of all **mucous membranes, icterus of conjunctiva**

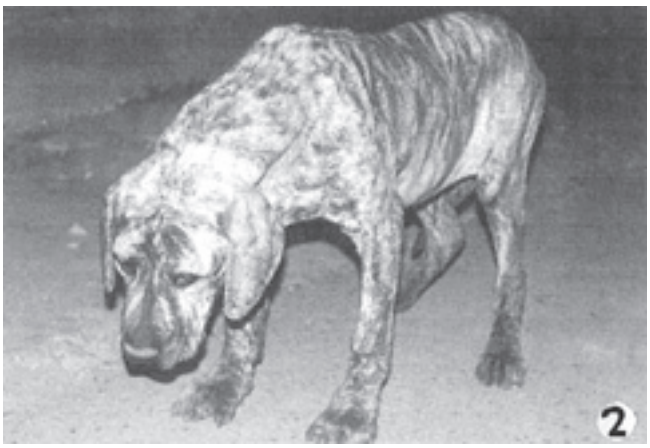
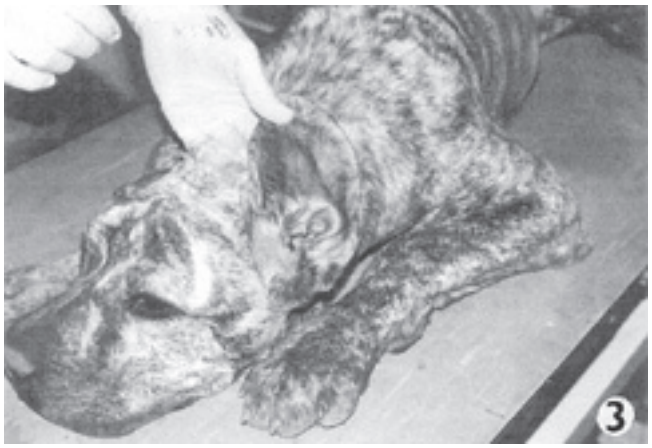
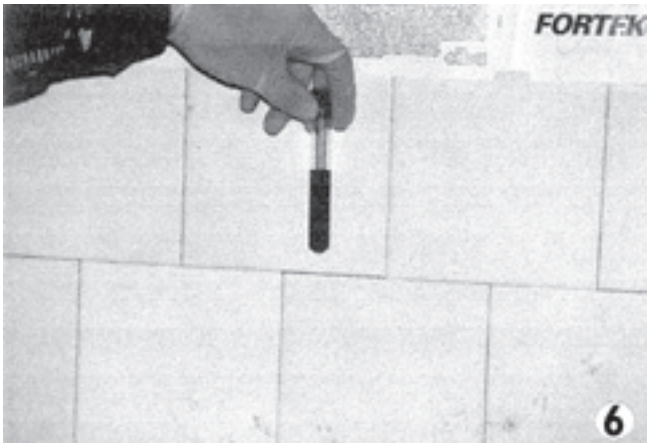


Fig. 2. Clinical signs of severe dehydration, depression, cachexia, lymphadenopathy, muscle degeneration, polyarthritis





Figs. 3, 4, 5. Clinical sign of icterus gravis

Fig. 6. Icteric blood sera, blood haemolysis

of acute renal failure and polyarthritis (Figs. 1—5). The laboratory panel haemogram, serum biochemistry, and urine analysis showed the severe progress of the disease (Tab. 1).

The serum detection for the presence of anti-Borrelia IgG antibodies showed sero-positivity by ELISA.

The leukogram evaluation showed the presence of a single distribution of a few *Babesia canis* in a repeated blood smear a few hours before death. Erythrocytes showed polychromasia and poikilocytosis.

Necropsy findings showed *icterus gravis* of all mucus membrane and tissues, generalized subacute and acute lymphadenitis, hepatomegaly, and slightly enlarged spleen, a degenerated kidney, acute pleuritis and fibrinous pericarditis, lung oedema with emphysematous foci and disseminated haemorrhages in the lung.

Table 1. Haemogram and serum biochemistry findings in monitoring the patient

Haemogram values range	*Interval 1	**Interval 2	Normal
RBC T.l ($10^{12}.l^{-1}$)	2.93	1.54	5.5—8.5
WBC G.l ($10^{9}.l^{-1}$)	5.47	5.21	6.0—14.0
PCV ($l.l^{-1}$)	0.20	0.18	0.35—0.50
Haemoglobin ($g.l^{-1}$)	70.0	52.0	120—40
ALP(μkat.l^{-1})	1.34	1.92	0.0084—0.410
ALT(μkat.l^{-1})	1.16	2.12	to 0.333
AST(μkat.l^{-1})	1.25 1.63		0.103—0.333
Bilirubin (mmol.l^{-1})	22.15	22.82	0.0—7.7
TP ($g.l^{-1}$)	43.38	38.21	57.0—75.0
LDH (μkat.l^{-1})	0.96	0.52	1.678
The urine analysis			
(urine sample taken by catheterization)			
haematuria	+++	++++	
proteinuria	++	++	
bilirubinuria	++++	++++	
The important laboratory changes are:			
hypoproteinaemia (43.38; 38.21 $g.l^{-1}$)			
hyperbilirubinaemia (22.15; 22.82)			
increased enzyme activities (ALP, ALT and AST)			
severe anaemia (PVC: 0.18 $l.l^{-1}$)			

* — **: 4 hours difference between interval 1 and 2

Diagnosis and treatment

Lyme disease and babesiosis were diagnosed on the basis of anamnesis, clinical and laboratory tests. Of 84 dogs monitored for the presence of anti-*Borrelia* IgG antibodies 30 dogs showed seropositivity by ELISA. One of them developed both Lyme disease and babesiosis (the 6-months-old female Filla Bras.). Of 30 *Borrelia* seropositive dogs the faecal examination determined the presence of *Giardia* spp. in 15 dogs which developed GIT disturbances.

The seropositive dogs which developed Lyme disease were treated with cephalosporins. In the case of 6 the month old Filla Bras. female, the progress of the disease, late visit to the clinic, delay in diagnosis and treatment and the severity of the complications were the reason for its death at few hours after the beginning of the treatment. The coincidence of *Borrelia burgdorferi* and *Babesia canis* were the reason that emergency treatment: fluid therapy, treatment for acute renal failure, Lyme disease with cephalosporins and treatment of babesiosis with diminazen, Berenil-Hoechst, was not completed. Necropsy showed *icterus gravis,* lymphadenopathy, hepatomegaly, degenerated kidney, and lung oedema and disseminated haemorrhages. Treatment (using Metronidazol: Entizol Flagyl 15mg.kg^{-1} body weight twice a day, 10 days) was not 100 % effective in the cases of 15 dogs seropositive to *Giardia* spp. These dogs, due to GIT disorders developed, general weakness, and immunosuppression.

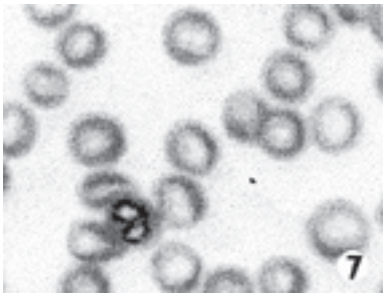


Fig. 7. Babesia canis, only a few parasites were seen after repeated blood sampling, before the death of the animal

CONCLUSIONS

This work gives some data about 84 dogs, on the occurrence of selected tick-borne diseases, Lyme disease and focuses on the few cases with the coincidence of *B. canis* and *Giardia* spp.

Out of 30 dogs showing seropositivity to *Borrelia* IgG antibodies, 15 were seropositive to *Giardia* spp. which resulted in GIT diseases. One of them developed severe damage by the coincidence of the tick-borne pathogens *Borrelia burgdoferi* and *Babesia canis.*

Our result showed the progress in clinical signs and complications when the coincidence of thick-borne pathogens is present. This was the case of Lyme borreliosis and babesiosis in Filla Bras. female the described.

Each diagnosis and differential diagnosis requires to following up with a well-prepared protocol focusing on 1) the close contact of man and animal in the environment, 2) the condition when the *Borrelia*-seropositive dog showed no clinical changes, or the variation of clinical signs which can be present, 3) the presence of *Babesia canis* and situation where it cannot be detected in blood smear in any stages of the disease, 4) the data of animal movement within an endemic area, as well as the anamnestic data of animal transportation are important together with the *Borrelia*-seropositivity and degree of parasitaemia in the coincidence of tick-borne diseases, 5) it is necessary to repeat the treatment for dogs with giardiasis and to prevent the contamination of the environment for weak and immunosuppressed animals.

ACKNOWLEDGEMENT

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CHRONICLE

Prof. MVDr. Jozef ARENDARČÍK, DrSc. (1922—1993)

Prof. MVDr. Jozef Arendarčík, DrSc., the founder and prominent representative of Slovak veterinary physiology and pioneer in the field of veterinary endocrinology in Slovakia.

He was born on 23rd April, 1922, in Batizovce (district Poprad) to a railwayman's family. He completed his secondary education at the State high school in Spišská Nová Ves in 1941and then studied veterinary medicine at the Veterinary College in Vienna till August 1945. He sat for board exams at the Veterinary College (VŠV) in Brno and obtained there his veterinary Diploma on 7th February, 1946. The graduation ceremony took place in Brno on 11 July, 1946, after he successfully defended his dissertation thesis on the theme: *"The influence of paraldehyde on the suppression of breathing after the administration of barbiturates". He performed the military service at the Military veterinary hospitals in Prague and Bratislava. After its completion, in the period of 1946—1947 he was employed at the Veterinary Department of the Commissary for Agriculture in Bratislava as a junior clerk in the section of infections.*

Then, in the period from 1948—1954, he held the post of a district veterinarian in Stropkov, Vranov, and Turčianske Teplice.

In 1954, on the basis of a contest, he was accepted for the Veterinary Faculty of Agricultural College in Košice as a head of the Department of Comparative Physiology. He stayed with the Faculty as a head of the Department of Comparative Physiology and after its restructuralisation, as a head of the Section of Normal Physiology in the Department of Normal and Pathological Physiology (1980—1987).

He contributed considerably to the building up of the modern Department of Physiology of Farm Animals at the Veterinary College in Košice.

In 1957 he was appointed deputy Docent. On 30 May, 1959, he defended his habilitation thesis on the theme "The study of hepatal activities of some domestic animals" and was appointed Associate Prof. by the Minister of education starting from 1st November, 1959. In 1962 he defended his PhD. thesis on the theme *"The study of chromexcretion and the activity of some haemocoagulation factors in animals"*. He was appointed deputy Prof. starting from 1 July, 1968. In 1970 he defended his doctor`s (D.Sc.) dissertation on the theme *"The titration of gonadotropins of some domestic animals"*. In 1978 the president appointed him university professor as from 1st September of that year.

Prof. Arendarčík made an effort to supply students with suitable books for study. He published the following lecture notes: Arendarčík *et al*.: *The Physiology of farm animals* (In Czech), Prague 1964 and 1966; Arendarčík J.: *The Physiology of farm animals* (In Slovak), Bratislava 1979, 1986.; Arendarčík, J. *et al.: Instructions for practical lessons in comparative physiology* (In Slovak), Bratislava 1979 and 1986.

He was a co-author of a state-wide university textbook – Holub *et al.: The Physiology of farm animals* (Prague 1969). He took part in the publication of additional books, such as Vodrážka *et al*.: *Veterinary medicine for pharmaceutists* (Osveta Martin 1974), Vodrážka, J. *et al*.: *Veterinary medicine and pharmacology* (Osveta Martin 1982 and 1986).

The years 1975—1977 he spent at the veterinary faculty of Makarere university in Kampala (Uganda) as an external examiner in veterinary physiology.

He took an active part in scientific conferences and symposia in Czechoslovakia and abroad.

He participated in the following study- or expert-exchanges abroad: in Vienna, Copenhagen, and Stockholm (1965), in Havana – Cuba (1973), Warsaw and Cracow (1976), and in scientific conferences in Utrecht (1969), Weimar (1969 and 1971), Leipzig and Olsztyn, and Budapest (1971), New Delhi (1974), Moscow (1979), and the World Veterinary Congress in Madrid (1959).

His scientific activities were also very fruitful. First, within the faculty projects, he focused his research on the study of some haematological parameters and their use in practice (1955—1958). From 1959 till 1960 he evaluated methods of investigating of hepatic function *("Research into functional tests of the liver in domestic animals and their diagnostic importance").*

From 1961 until 1990 he was the leader of teams involved in parts of project of the basic state research particularly concerning the hypophyseal and extrahypophyseal gonadotropic hormones.

From 1961 till 1975 he was leader of a research team which became involved in the study of the activity of hypophyseal and extrahypophyseal gonadotropins of domestic animals with respect to their reproduction. From 1969 until 1975 he was involved in the project *"Gonadotropin activity in relation to the cytoarchitecture of adenohypophysis and male sexual organs"*.

From 1976 until 1980 he was involved in investigations of the following: 1). *Endocrine and functional-morphological*

parameters of the hypothalamus-hypophysis-ovaries system in ruminants, and 2). The influence of chronic ionizing radiation on the gonadotrophic function of the hypothalamo-hypophyseal system of sheep. From 1981 until 1985 the topical subjects of his investigations were: 1). Physiological mechanisms affecting the fertility of farm animals investigated by morphological and RIA methods. 2). The effect of protracted irradiation on the reproductive capability of sheep.

From 1986 until 1990: Hormonal spectrum in blood and the hypothalamo-hypophyseal and ovarian systems of ewes after the stimulation of their reproduction abilities. The respective 5-year final reports were always presented and defended on time.

His studies of hypophyseal and extrahypophyseal gonadotrophins (FSH, LH and PMSC) were awarded a prize of the Slovak Endocrinological Society (1971).

From 1975 until 1990 he was a coordinator of the principal project of the state basic research VII-4-1, namely *"Physiological factors affecting the health and reproductive abilities of animals"* (1975—1980) and *"The reproduction of farm animals"* (1981—1990). He was a member of the board determining the key direction of basic research No. VII-4 registered at the Czechoslovak Academy of Sciences (CAS).

He was a tutor to 14 PhD. students of veterinary sciences, a chairman of the committee for the defence of PhD. theses in veterinary physiology, and a chairmen of the committee for the defence of doctor's (D.Sc.) theses in animal physiology.

He was an author or co-author of about 190 scientific and specialized papers published in domestic journals, such as *Czechoslovak physiology, Folia veterinaria, Slovak veterinary journal, Veterinary medicine, Endocrinologia experimentalis (SAS)*, and Endocrinology of Poland (PAS).

From 1964 until 1980 he was an executive editor of the scientific journal *Folia veterinaria.* He was also a member of the Slovak Physiological Society of the SAS and the Slovak Endocrinological Society of the SAS. He became an honorary member of the Czechoslovak Physiological Society at Czechoslovak Academy of Sciences (CAS).

Prof. Arendarčík was awarded the gold medal of Prof. Dr. P. Adámi, the gold medal of the Veterinary College in Košice (1987) as well as silver and later also gold medal of the Slovak Medical Society (1987 and 1992).

For two decades Prof. MVDr. Jozef Arendarčík participated in Union activities not only within Slovakia but throughout the Czechoslovak Republic. His successful unending effort in this area was apprised by several Union awards.

He retired on the 1st September, 1987, but remained a member of staff of the Section of physiology as a research professor-consultant till the end of June 1990. He passed away in Košice after a short and severe disease on 8 December, 1993, at the age of 71 years.

Jantošovič, J., Cabadaj, R., Bugarský, A., Kozák, M.
The Museum of Veterinary Medicine — the University of Veterinary Medicine in Košice

(FOLIA VETERINARIA, 46, 2: 99—100, 2002)

Academician Ivan BRAUNER (1907—1967)

Academician Ivan Brauner — an important personality of Czechoslovak veterinary immunology and epizootiology, double laureate of the State prize, member of the Slovak Academy of Sciences (SAS) and the Czechoslovak Academy of Agricultural Sciences (CAAS), corresponding member of the Czechoslovak Academy of Sciences (CAS) and the German Academy of Agricultural Sciences, Professor of epizootiology at the Veterinary College (VŠV), Košice.

He was born on 22 April, 1907, in Jelenec (district Zlaté Moravce) as the son of a public school teacher. He graduated from the State high school in Komárno in 1925. After graduation, he became engaged in studies of veterinary medicine at the Veterinary College in Brno. He completed his studies on 5 June, 1935 and his graduation took place on 2nd February, 1936 after he successfully defended his dissertation thesis on the theme "*The development of silk-worms irradiated with UV rays*". After completing of his university studies, Dr. Brauner started to work as a practical veterinarian in Kollárovo (Guta), district Komárno. After three years of practice he was appointed a state district veterinarian. Due to everyday contact with practical problems he obtained valuable knowledge about the needs of animals from the veterinary-health point of view. While working in the field, he made an effort to increase his professional knowledge. In 1941 he participated in a specialised course focused on the suppression of sterility of farm animals organized at the Veterinary College in Budapest. The war ended his efforts prematurely. During World War II he was called several times to the service and served as a military veterinarian in several army units.

Veterinary practice did not bring full satisfaction to Dr. Brauner. His yearning for productive, scientific research work brought him, after 10 years of practice, to the State diagnostic veterinary institute in Bratislava. In March 1946 he joined this institution and was assigned to the State diagnostic and serotherapeutic institute in Ivanovice na Hané to become involved in the production of vaccines. There he met with his first success in the form of an inactivated adsorbate vaccine against Newcastle disease which was used on a state-wide scale already in 1949 but did not appear completely satisfactory.

In 1950 the Minister of agriculture established an Institute for production of vaccines in Slovakia, located temporarily in Bratislava, and Dr. Brauner was appointed the head of this establishment. The establishing of such an institution was stimulated by the serious infectious situation due to the spreading of classical swine fever and the acute need for protective serum against this infection. Under makeshift conditions, a small team consisting mostly of young and eager members, headed by Dr. Brauner, produced in 1951 a sufficient quantity of antiserum and the necessary viral agent for use on a state-wide scale. Then a live vaccine against Newcastle disease was produced from strain H, which was used all over Slovakia in the so-called H campaign which contributed positively towards the control of this feared epizootic.

The new vaccine against Newcastle disease was subjected to additional experiments in the Bratislava Institute. In addition to that, work on the development of a vaccine against foot-and-mouth disease started in Terezín, and was followed by the preparation of an adsorbate vaccine against swine erysipelas. With regard to foot-and-mouth disease, when our territory was endangered in the early fifties, Dr. Brauner was appointed head of a temporary institute for the production of foot-and-mouth vaccine in Terezin and adapted a slaughterhouse in Ústí nad Labem for the production of the respective virus and reconvalescent serum. Under his leadership some hundred thousand litres of successful bivalent vaccine were prepared in 1952 (Přibyl, 1957; Nižnánsky, 1967).

The name of Dr. Brauner is associated with the establishment of a modern enterprise Bioveta in Nitra intended for the production of veterinary biopreparations. He participated in its establishment and was its first director. He initiated successful research leading to the production of biopreparations for specific immunoprophylaxis of serious infectious diseases of farm animals, such as Newcastle disease and fowl plague, cattle brucellosis, rinderpest, infectious paralysis, swine erysipelas, Aujeszky's disease, and others. The production of biopreparations was initiated in 1954. Under Dr. Brauner's leadership new scientists and professionals joined the team and additional laboratories and productive facilities were completed and supplied with the latest equipment. Bioveta in Nitra became one of the largest most modern and successful institutes of its kind in the central Europe. After the completion of the plant in 1957 Dr. Brauner passed the institute to his successor. He acted afterwards as head of the Immunological laboratory of the SAS in Nitra which was transformed later (1959) to the Laboratory of experimental veterinary medicine of the CAAS.

For his successful research activities in the field of development and production of new vaccines Dr. I. Brauner was awarded a State prize (1952). In 1953 he became an academician of the SAS and CAAS. He occupied important positions in both these institutions: the chairman of the Section of agricultural sciences of the SAS, first vice-chairman of the CAAS and later the chairman of the Slovak branch of the CAAS (1959). Besides that he occupied other numerous functions in scientific boards or specialized commissions of

research institutes and universities. In 1957 he was appointed a corresponding member of the German Academy of Agricultural Sciences of GDR. In 1962 he was elected a corresponding member of the CAS; at the same time he became a member of the Scientific collegium for biological basis of agriculture, an important control organ responsible for development of agricultural sciences in the former Czechoslovakia. One year later he was promoted to external Professor of epizootiology at the Veterinary Faculty in Košice (1963). On the occasion of his 60th birthday anniversary the CAS presented him a medal *"Of merit for science and humankind"* (1967). He was also awarded by the Veterinary College in Brno the gold medal of I. J. Pěšina (1966) and by the Veterinary College in Košice the gold medal of P. Adámi *in memoriam* (1969).

After the establishment of the CAAS branch in Bratislava in 1959 he became its chairman. As a head of a small team of research workers in the Laboratory of experimental veterinary medicine he became involved in the problems of immunoprophylaxis of Aujeszky's disease which became a serious threat to intensive pig production. In 1961, together with his co-workers (particularly Assoc. Prof. Dr. A. Žuffa and Dr. R. Škoda), he successfully prepared an attenuated virus and was able to start with the production of a live vaccine. The collective of authors headed by Dr. Brauner was awarded another State prize in 1964. After the abolition of the CAAS (1963) Dr. Brauner headed the Department of veterinary virology of the Virological institute of the CAS in Bratislava.

The extent of his investigative activities is evidenced by experimental and other papers published in journals at home and abroad. He published altogether 41 scientific and specialized papers, 7 of them abroad (3 in China). They focused particularly on epizootiology, virology, immunology and immunoprophylaxis of the most serious infectious diseases of productive animals. In addition to that he published about 100 specialized popularizing contributions.

He published a treatise on *"Animal production and veterinary medicine in the Peoples Republic of China"* (Bratislava, 1957). He was a co-author of two books: *"Infectious diseases of farm animals"* (Bratislava, 1965) and *"Swine diseases"* (Prague, 1966).

In 1967 the SAS awarded him a silver medal *"On merit for science and education"*.

The organisation and scientific-professional profile of Academician I. Brauner was closely related to his personal characteristics. He was known as a direct, open, kind, and sociable person. He tried to help people with their difficulties and problems. All his life he was a modest person. He did not strive after fame nor did he try to take advantage of the numerous functions he occupied throughout his life. He enjoyed life with all the good things that it may bring to a man (Fried, 1984). His close co-worker Dr. Škoda characterised Dr. Brauner as follows: "*He was a man unusually broad-minded and friendly with many contacts abroad and many good friends among foreign scientists. His constant support of young scientists and friendly approach to younger co-workers and colleagues from practice showed him as a person with pure and good heart"*.

On 6 September, 1967, Academician Ivan Brauner suffered a heart attack and passed away in Bratislava at the age of 60. For all that he represented, it was a great loss to veterinary science.

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(FOLIA VETERINARIA, 46, 2: 101—102, 2002)

NEW BOOKS

Recently, the publishing house Schlütersche GmbH Co. KG, Verlag und Druckerei, Hannover, Germany (E-mail: info@ schluetersche.de), January 2002, introduced a new book in the English language

Clinical Atlas of Ear, Nose and Throat Diseases in Small Animals, 2002, 208 pp., ISBN 3-87706-621-6

Contributors: Merchant, S., Mortellaro, C., White, R. A. S., Taboada, J., Hedlund, Ch. S.

The publication follows the current world-wide trend with many high-quality photographs on art paper, in hard cover.

It is intended particularly for clinical personnel practising in the field of small animals and with regard to animal species it deals with diseases of dogs and cats. The individual chapters of the atlas mentioned below present diseases on the principle of demonstration of disease units as clinical cases respecting the methodical approach of clinical propedeutics and diagnosis. Individual cases are discussed in detail providing information about *Signalment/History, Physical Examination, Differential Diagnosis, Diagnosis* and the *Treatment/Management*. A very important part of the Atlas is the description of cases and clinical status during the treatment (*Outcome*). The therapy is demonstrated using an interdisciplinary approach so that in many cases the classical treatment or surgical interventions are described and illustrated in great detail. Each case is supplemented with a *Discussion.*

The *Table of Contents includes the following chapters:*

1. *The Ear, with the presentation of 16 clinical cases.*
2. *The Nasal Cavity and Paranasal Sinuses, discussing 12 cases.*
3. *The Larynx, with 5 cases.*
4. *The Trachea, which includes 5 cases.*
5. *The Oral Cavity, with the presentation of 9 cases.*
6. *The Pharynx, with 9 cases.*

Each of the 56 cases is supplemented with numerous and realistic high-quality colour photographs that record the clinical state, diagnosis and relevant microphotographs in the cases indicated. The photographs frequently show clinical findings and the results of radiographic examination, endoscopy, and other special examination methods. The majority of pictures can be considered unique.

The *"Clinical Atlas of Ear, Nose and Throat Diseases in Small Animals"* dealing with dog and cat disease units, should be included in the group of publications that enrich veterinary medicine as a whole and particularly the "*small animal*" practice. The Atlas can be used as a very suitable aid in the education of veterinary students because it demonstrates very clearly and perceptively the clinical approach to a number of disease states that may not be encountered by students during their university studies.

Assoc. Prof. DVM. Marián Kozák, PhD.
Head of the First Internal Clinical Department of UVL in Košice

Dik, K. J., Gunsser, I.: ***Atlas of Diagnostic Radiology of the Horse. Diseases of the Front and Hind Limbs.*** **Second edition (in English). Schlütersche GmbH Co. KG, Verlag und Druckerei, Hannover, Germany**
(E mail: info@ schluetersche.de), 2002, 300 pp.
ISBN 3-87706-651-8

Radiographical examination of horse limbs is an important part of diagnosis of lameness. In addition to the high-quality of radiographs, great importance is ascribed to their correct interpretation. These are the reasons why this Atlas is an important aid for veterinarians concerned with the orthopaedics of horses as a part of their clinical practice.

The Atlas focuses on radiographical pictures of various pathological states of limb joints and bones. On the basis of the topography of the limbs, the monography is divided to 10 basic chapters and contains 702 radiographs supplemented with 82 vivid drawings. The descriptions of radiographs discuss the clinical significance of findings and provide additional detailed information. Each chapter contains radiographs of fractures, inflammatory and degenerative changes, and tumour diseases of bones. Some diseases are illustrated at different projections and their significance is stressed in order to prevent a wrong diagnosis resulting from a superficial reading of the radiographs. Diseases of joints and of some injuries are supplemented with both monochrome pictures and pictures with contrast substance that specify further the pathological findings.

A considerable part of the chapter dealing with the *Hoof* is devoted to various findings associated with laminitis, ossification of hoof cartilage, and breakage of hoof bone. It also illustrates and describes artefacts resulting from insufficient treatment of hoof before examination. The diagnose of diseases are supported by many photographs, e.g. the presence of foreign objects, the sinography of fistulae and wounds and their possible consequences — the lysis of the distal sesamoid bone, the rupture of the *flexor digitorum profundus* tendon, the lysis and sequestration of hoof bone.

In addition to routine projections of the *coronal joint*, the stress photographs also provide better illustration of joint instability, subluxation, and complete luxation.

The chapter the *Fetlock* pays considerable attention to various types of fractures starting with breakages in the area of the metacarpus growth plate (Salter-Harris I and II types), through incomplete longitudinal intraarticular fractures of the metacarpus and fetlock bone up to avulsion fractures of the proximal sesamoid bones. Additional details are provided for diagnosis of proximal sesamoitidis.

The common chapter presents findings on *metacarpal* and *metatarsal bones* of the thoracic and pelvic limbs.

The *Carpal joint* chapter illustrates, besides other basic diseases, also the developmental growth diseases of foals — epiphysitis and angular deformities. The chapter ends with radiographs of tumour diseases — *osteochondroma, osteoblastoma and synovial sarcoma.*

The Ulnar joint is accompanied with illustrations of fractures, osteomyelitis and osteochondrosis.

The most extensive chapter deals with the *Heel joint*. This is one of the most frequently radiographed joints in horses in association either with a diagnosis of lameness or with an examination of horses before purchase. The diseases that most frequently affect this joint include osteochondrosis and degenerative diseases. The Atlas shows development of osteochondrosis in various individuals during their growth.

Knee joint diseases are another frequent cause of lameness of pelvic limbs. This chapter also shows, besides pathological findings, the development of individual bones in foals. The interpretation of foal radiographs is demanding and it is difficult to distinguish physiological findings from osteochondral changes.

The book ends with the chapter dealing with fractures of the *pelvis* and problems associated with *coxal joints*.

The Atlas is a valuable contribution to hippiatrics practice. Along with atlases presenting radiographical pictures of normal structures, it allows one to obtain good knowledge of pathological findings and helps to determine their significance.

The monography presents almost all osseous pathological states encountered in horses. The radiographs are on a high qualitative level and allow one to make very good diagnosis of disease. They are based on the long-term theoretical knowledge and clinical and radiographical expertise of the authors. We recommend this monograph not only to veterinarians but also to veterinary students.

The monograph with its form and content raises the body of materials regarding radiographical diagnosis of the osseous diseases of horses to a higher qualitative level.

Assoc. Prof. DVM. Valent Ledecký, PhD.
Head of the Clinical Department of Surgery, Orthopaedics and Roentgenology
Assist. Prof. DVM. Martin Mihály

(FOLIA VETERINARIA, 46, 2: 103—104, 2002)

Guidance for Contributors

All artricles, which conform to the Uniform Requirements for Manuscripts submitted to Biomedical Journals will be reviewed.

The house style, which differs in respect of References, is set out below.

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Where papers cited are "in press", copies should accompany the manuscript submitted. The editor reserves the right to make literary corrections: texts will be returned to the author(s) for major rectification, in line with the recommendations of the referees.

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Acknowledgements. Those who have given technical assistance, or moral or financial support, or supplied equipment or materials, or engaged in translation or general supervision, etc. should be recognized in the Acknowledgements (cf also McNab, S.M. Coping with Clutter in a Scientific Paper. *European Science Editing,* 1992; 45: 8)

Conflict of Interest. If a study evaluates a pharmaceutical product, a medical or scientific device, or any other commercial manufacture, the authors must disclose, in a confidential covering letter to the editor, any and every financial interest (e.g. employment, consultancy, share-holding, board membership, etc.) they may have in the company that manufactures the product discussed or in a rival firm and/or commodity.

References. Only the work used should be mentioned. At the end, the references should be listed in alphabetical order by the first author's surname. List the first six authors followed by *et al.* References should be set out thus.

Journals: Surname(s) and initial(s) of the author(s), year of publication, full title of the paper, title of the journal (in italics), volume, and relevant pages. (See examples below.) The issue number should be quoted in parentheses only if the pagination of the journal is by issue rather than by volume.

Books: Surname(s) and initial(s) of the author(s) and/or editor(s), year of publication, full title of the book and edition (if not the first), publisher and place of publication, and pages.

These references should then be numbered. In the text, these numbers are used instead of names and dates for citations, e.g. "All space-flight embryos ….. showed normal embryogenesis (3, 6) and post-hatch development (5)." Only if the writer's name is a necessary part of the sentence should it be used, e.g. "Jones (7) discovered that …….". If the date is essential, it too should form part of the text, e.g. "Then in 1997 Jones (7) made a breakthrough." This alphabetical – numerical style for references is to make the text flow: to separate the science from the customary clusters of nominal citations.

Examples:

1. Ahlborg, B., Ekelund, L. C., Nilsson, C. G., 1968: Effect of potassium-magnesium aspartate on the capacity of prolonged exercise in man. *Acta Physiol. Scand.,* 74, 238—245.

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galactomannaus of Trichophyton mentagrophytes and Trichophyton rubrum. *Biochem. J., 323, 297—305.*

Language Style

1. Be prepared to use the first person ("I" or "We"), but do not overuse it. (e.g. "We studied 24 Slovak Merino ewes.")
2. The excessive use of the passive voice is a principal cause of dullness in scientific writing. Use it sparingly, and prefer the active voice ("We conclude that…") to the passive ("It can be concluded that…") whenever justifiable.
3. Use the past tense for reporting observations, completed actions, and specific results ("We observed no significant changes.")
4. Use the present tense or the present perfect for generalizations and generalized discussion. ("This suggests that…")
5. Employ the specialist vocabulary of your discipline(s). "The dynamic development of biological sciences has…had a positive influence on the current knowledge of the activated mechanisms…in the case of human and animal organisms" can be rendered succinctly as "the rapid growth of biological science has enabled us to understand the functions of human and animal bodies better." Convoluted and roundabout expression does not impress and may well irritate the reader.
6. Remember that many readers will not be native-speakers of English. If you are an ESL (English as Second Language) author, apply the principles of English style and syntax when writing, and be mindful that the correct word order is important in English sentences. Make sure that your sentences are sentences: do not lose control of their structure.
7. Be simple and concise; where possible use verbs instead of abstract nouns. Break up long noun clusters and "stacked modifiers" (strings of adjectives before nouns without clues about which modifies which). Logically ordered and lucidly expressed ideas will make your meaning clear: obfuscation will not assist the reader.

Units of Measurement

1. Measurements of length, height, weight, and volume should be reported in metric units.
2. Temperatures should be given in degrees Celsius; blood pressures in millimetres of mercury.
3. All haematological and clinical chemistry measurements should be recorded in the metric system or in the terms of the International System of Units (SI).

Abbreviations and Symbols. Use only standard abbreviations. Avoid abbreviations in the title and abstract. Abbreviations and acronyms should be used only if they are repeated frequently. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement, e.g. positron emission tomography (PET).

Numerals and Dates. Whole numbers from one to ten should be written as words in the text, not as numerals, e.g. "Experiments were carried out on four male Rhine geese…" Numerals should be used for numbers above ten, except in the titles of papers and at the beginning of sentences, where they must appear as words. Dates in the text should be written as follows: 29 September 2000.

Nomenclature and Terminology. Medicines must be shown by there generic name followed by the proprietary name and manufacturer in parentheses when they are first mentioned, e.g. Apramycin (Apralan 200; Elanco, Austria).

Authors should respect international rules of nomenclature. For animal species and organisms, the recommendations of the International Code of Zoological Nomenclature, London 1999, should be observed. Linnaean names should be used for plant species. Anatomical terminology should agree with the nomenclature published in the *Nomina Anatomica Veterinaria* 4th edn. (1994) ed. **Habel, R. E., Frewein, J.,** and **Sack, W. O.,** World Association of Veterinary Anatomists, Zurich and Ithaca, New York.

Latin terms and other non-English words should be italicized in the manuscript. Use the British Standard 2979:1958 for the transliterations of Cyrillic characters in the references as well as the text.

Photographs and Illustrations. These should be on separate sheets, each with a label pasted on its back, bearing the author's name, the figure number, and an arrow indicating the top of the figure. Black-and-white photographs should be clear and sharp, suitable for reproduction. Photomicrographs must state the magnification and stain technique. Illustrations should be drawn in black ink on white paper in a form suitable for photographic reproduction. The main objects, changes, and findings should be shown by an arrow or some other symbol explained in the legend.

Graphs and Tables should contain essential data not given in the text. In the journal, graphs will have an overall width of no more than 8.5 cm and be drawn on pages 17.5 cm. wide. The size of the letters in legends should suit these dimensions. Computer-drawn graphs are preferred—laser-printed on diamond paper. Statistics (with tables of parameters) must be enclosed. Captions for figures, including graphs and photographs, and all legends should be subscribed. Tables should be typed with double-spacing on separate sheets of paper and numbered consecutively in the order of their citation in the text. Within each table, lines should separate only the headings from the body of the table, and the body of the table from any totals, averages, etc. Titles for tables should be superscribed, and explanatory matter placed in footnotes, using the symbols and sequence recommended. Number explanatory texts to graphs, figures, and tables with Roman letters or Arabic numerals. Each such text should be enclosed on a separate sheet of paper and placed in order. The author should indicate with pencil in the left-hand margin of the manuscript where the supporting material should be inserted.

Ethical Considerations. When reporting experiments on animals, indicate whether the Institution's or Research Council's Guide for, or any national law on, the care and use of laboratory animals was followed. Manuscripts should describe the measures taken to minimize or eliminate pain and distress in animals during experiments and procedures. If the editors deem that animals have been subjected to suffering unjustified

by the scientific value of the information sought, they will reject the paper on ethical grounds.

The journal encourages integrity in science. Questionable and fraudulent claims will not be entertained.

Experimental Hazards. Authors should draw attention to any dangers involved in carrying out their experiments, and should detail the precautions taken to guard against such hazards.

Statistics. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the results reported. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty. Discuss the eligibility of experimental subjects. Give details about randomization. (Cf. the statistical guidelines for authors in *The Australian Veterinary Journal* Vol. 76, No. 12, December 1998, p. 828.)

Types of Papers. Please state clearly which category of paper is being submitted. (If an author believes that his/her article or short communication is of outstanding topicality and importance, he/she should indicate this in a covering letter. It may merit fast-track publications.)

A. Standard Full Length Papers. Full papers should be concise. They should not exceed 12 pages (A4) including tables, graphs, illustrations, photographs, and references.

The Title Page. The papers should be headed with the full title, which should accurately and concisely describe the topic in no more than two lines. This should be followed by the surname(s) and initials of the author(s) and the name and place(s) of their employment. (If the work was carried out in an institution other than the place of employment, this should be noted in the body of the text.) Acknowledgements (see above) should be typed in a separate section, separated by a reasonable space. A short title (the running head) of no more than forty characters (counting letters and spaces) should be included at the foot of the page. Each manuscript should be thematically complete: serialization is discouraged.

The Abstract. The second page should carry an abstract, which should be self-contained and not exceed 250 words. It should briefly incorporate the purpose and relevance to veterinary science of the work, basic procedures, the main findings, and principal conclusions. It should emphasize new and important aspects of the study or observations.

Key words. Key words should be listed below the abstract, from which they are separated by a one-line space. They should consist of three to ten words in alphabetical order, written in lower case and separated by semi-colons.

The Introduction. State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

Material and Methods. Describe your selection of observational or experimental subjects (including controls) clearly. Identify the age, sex, state of health, and other important characteristics of the subjects.

Identify the methods, apparatus (with the manufacturer's name and address in parentheses), and procedures in sufficient detail for other workers to reproduce the experiment. Quote established methods, including statistical methods; provide references and brief descriptions for methods that have been published but are not well known; describe new or substantially modified methods in full; give reasons for using them, and evaluate their limitations. Precisely identify all drugs and chemicals used, including generic name, dose, and route of administration.

Results. These should be as succinct as possible and presented in a logical sequence in the text, with graphs and tables. Emphasize or summarize only the important observations in the text. Do not duplicate in the text all the data in the graphs and tables.

Discussion. Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results sections. Include in the Discussion section the implications of the findings and the limitations, together with their significance for future research. Relate the observations to other relevant studies.

Link the conclusions with the aims of the study, but avoid unqualified statements and conclusions not completely supported by the data. Avoid claiming priority and alluding to work that has not been completed. Recommendations, when appropriate, may be included.

B. Notes and Short Communications

Such manuscripts should have the same form as full papers, but are much shorter. Separate headings are needed only for the Acknowledgements, Key Words, Abstract, Main Text, and References. These scripts fall under the following main headings and should be marked accordingly.

1. Technical Notes. Such notes should record a new method, technique, or procedure of interest to veterinary scientists. They should include the reason(s) for the new procedure, a comparison of results obtained by the new method with those from other methods, together with a discussion of the advantages and disadvantages of the new technique. A technical note should not exceed six printed pages, including figures and tables.

2. **Research Communications.** These are short articles, no more than four printed pages, which should introduce novel and significant findings to the commonwealth of veterinarians.

3. **Observations.** Research of this kind contributes to knowledge, but not to the advancement of ideas or the development of concepts. In some cases, these papers underpin what may seem obvious, with statistical data. Such communications should not exceed four type-set pages.

4. **Current Issues.** Papers that deal with issues of topical interest to veterinary scientists will be considered. Issues may include items on environmental concerns, legislative proposals, etc.

C. Review Articles. These should provide a substantial survey, with an appropriate historical perspective, of the literature on some aspect of veterinary medicine. Alternatively, such articles may review a topic of veterinary interest which may not come within the normal purview of many veterinarians (e.g. Asefa Asmare, A., 2000: The Camel, ……, *Folia Veterinaria, 44, 4, 215—221). Authors submitting review manuscripts should include a section describing the methods used for locating, selecting, extracting, and synthesizing data. These methods should be summarized in the abstract.*

D. Book Reviews may be submitted. They should bring a new text to the readership and evaluate it.

E. Letters to the Editor. These are items of scientific correspondence, designed to offer readers the chance to discuss or comment on published material and for authors to advance new ideas. Should a letter be polemical, a reply or replies for simultaneous publication may be sought from interested parties.

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