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FOREWORD

Contrary to earlier times, an increasing emphasis is laid on the protection of the environment, which became an issue for the human population. One of the reasons is the events of the immediate past when catastrophic floods destroyed extensive parts of Europe and broke 100-year records with regard to both their origin and need for coping with the damage to buildings, infrastructure, and crops.

In each profession there is a space for involvement in issues related to environmental protection. Veterinary medicine can also contribute considerably to solving environmental problems. We already meet for the 5th time to compare and evaluate the results obtained in the past and to use them in coping with future problems. In the past four years some progress has been made in the field of ecology and the related environmental manage-ment and protection, owing to the latest concept of unified actions throughout Europe, based on current legislation.

By organizing our conferences we have tried to follow up and develop further the ideas of the founder of Slovak animal hygiene, Prof. Jan Rosocha, CSc., who already in the nineteen seventies stressed the need for international cooperation and together with Prof. Ferenc Kovács from Budapest, Prof. Johannes Kalich from Munich, and Prof. Tadeus Janowski from Poland founded an International Society for Animal Hygiene. The founders stressed that the interaction between an animal and its environment should be considered as a complex, i.e. the protection of animal health and increase in animal productivity should be related to the management and protection of the environment around the farms, the effective use of wastes from animal production, and the prevention of disseminating infectious diseases.

The negative impact of human anthropogenic activities related to the utilization of chemicals, particularly pesticides and pharmaceutical preparations, is also a sub-ject for our discussion.

The general effort to solve these problems as a com-plex, joining all forces, is reflected in the fact that it was organized in close cooperation with agricultural organizations.

The organizing committee believes that this event will be a successful follow-up to the previous conferences on this theme and that the conclusions of this professional meeting will set targets and trends for further development in environmental protection in the field of veterinary medicine.

Doc. MVDr. Miloslav Ondrašovič, CSc.

FOLIA VETERINARIA, 46, 2, Supplementum: S5—S7, 2002

THE HYGIENE EVALUATION OF THE SOURCE OF DRINKING WATER AND ITS CONTAMINATION

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ABSTRACT

Chemical and microbiological examinations were carried out to assess the quality of drinking water from a well located at the foot of the Slánsky mountains in a region where no danger of contamination with waste-water from human dwellings or agricultural production existed. Despite that, our results indicated a considerable contamination of the water source. Investigation of additional sources of drinking water in the respective region suggested that the deterioration in the quality of drinking water was caused by the contamination of groundwater.

Key words: bacterial contamination; chemical pollution; drinking water; groundwater

INTRODUCTION

Anthropogenic pressure on the environment is reflected negatively in the quality of the sources of drinking water. The assumption that no extensive treatment is required when using groundwater as a source of drinking water can no more be generally applied (3). According to Allen and Geldreich (1), in the period of 1946–1970 more than 50 % of waterborne diseases were spread by infected groundwater. In relation to that Podhrázská and Toman (5) stressed the need for the protection of both ground and surface water sources. They warned that the existing horizontal protection that neglects local conditions is insufficient and should be replaced by differentiated approach. The present study focused on the evaluation of related problems in the selected location using chemical and microbiological examination to obtain more information about the presumed pollution of groundwater.

MATERIAL AND METHODS

The chemical examination included the determination of pH, ammonium (NH_4^+), nitrates (NO_3^-), phosphates (PO_4^{3-}), chlorides (Cl^-), organic matter (COD) and free chlorine (Cl_2) using the methods recommended by the Slovak standard (8). The microbiological examination consisted of determining plate counts of psychrophilic, mesophilic, coliform, and faecal coliform bacteria according to STN (9).

RESULTS

In the period from May 31, 2001, to July 20, 2002, altogether 13 samples of water from the well investigated were examined chemically and microbiologically and compared with the Slovak standard (7). The allowable limits for psychrophilic bacteria in drinking water were exceeded in four samples, for mesophilic in nine samples and for coliforms and faecal coli-forms in nine and eight samples, resp. The chemical examination of the water also indicated pollution of the well as only two samples complied with the requirements on ammonium (NH_4^+). Its levels ranged between 0.25 and 1.06 mg.l^{-1} (mean 0.74 mg.l^{-1}). Chlorides and phosphates exceeded the limit in 2 samples. The level of nitrates complied with the requirements on drinking water (mean 14.9 mg.l^{-1}).

A considerable pollution of the water was indicated by the chemical oxygen demand (COD), which was below the allowed level (max. 3 mg.l^{-1}) only in 3 out of 13 samples and even those were close to the limit (min. 2.9 mg.l^{-1}). On the basis of these results we examined additional water sources in the respective area (Tables 1 and 2).

We found that even the water-mains supplying water to entire villages failed to comply with the requirements on coliforms. Other groups of bacteria were suppressed due to chlorination of water (0.29 mg.l^{-1} free chlorine) (Tab. 2). Organic pollution is indicated by the chemical examination which showed that NH_4^+ level was exceeded in all samples and the COD complied with the standard in only 3 samples.

Table 1. Plate counts of groups of micro-organisms examined in samples of water from the selected region

Sampling site	Source	Psychrophilic (1 ml)	Mesophylic (1 ml)	Coliform (10 ml)	Faecal coliform (10 ml)
Varhaňovce	Well	237*	119	285	45
	Water-main	117*	55	64	0*
Brestov	Well	NP	NP	NP	NP
	Well	295*	184	NP	NP
	Water-main	0*	5*	12	0*
Bunetice	Well	NP	405	NP	380
	Well	NP	279	NP	NP
Ortaše	Well	65*	16*	260	61
Šarišské Bohdanovce	Well	NP	396	NP	44
	Well	138*	196	300	0*
Vtáčkovce	Well	NP	NP	NP	NP
	Well	300*	360	144	0*

* — **allowed levels of micro-organisms according to STN 757111;**
NP — uncountable

DISCUSSION

Our investigations focused on a source of drinking water located at the foot of the Slánsky mountains, about 1820 m from a village. It is a drilled well, 80 cm in diameter. The height of the water column in the well is 67.42 m and the water level is 4.5 m below the ground level. The capacity of the well was not determined. With regard to the considerable distance from the village, conventional pollution of water with wastes from human dwellings or agricultural production is improbable. However, our chemical and microbiological examination indicated considerable contamination of this source. The same applies to other water sources located in the respective region.

The level of NH_4^+, an important indicator of fresh decomposition processes (6), supported our assumptions. The importance of this parameter is not related to its harmful effect on live organisms but to its indication of the immediate pollution of the water source (4). The level of NH_4^+ in groundwater seldom exceeds 0.1 mg.l^{-1}.

Higher concentrations of ammonium ions in groundwaters can be associated with reduction of nitrates of organic origin (10). The existence of such reduction processes in the sources investigated is supported by relatively low levels of NO_3^- and high plate counts of bacteria in our samples. The chemical oxygen demand of the well investigated complied with the standard only in three samples and this parameter was increased also in other sources of drinking water. The chemical oxygen demand corresponds to the level of chemically oxidizable organic substances (2). The results of microbiological examination confirm our assumptions that coliforms were not devitalised even in the water source that contained 0.29 mg.l^{-1} free chlorine (max. allowed value is 0.3 mg.l^{-1}).

The results obtained allow us to conclude that the low quality of drinking water in the respective area resulted most likely from pollution of groundwater.

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Table 2. The chemical examination of sources of drinking water in the selected region (mg.l^{-1})

Sampling site	Source	pH	NH_4^+	NO_3^-	Cl^-	PO_4^{3-}	Cl_2	COD
Varhaňovce	Well	6.4*	0.83	7.0*	111.1	0.22*	0*	3.4
	Water-main	6.5*	0.81	5.3*	11..9*	0.19*	0*	2.9*
Brestov	Well	6.1*	0.81	10.0*	23.8*	1.84	0*	3.1
	Well	6.1*	0.88	9.9*	24.8*	2.06	0*	4.3
	Water-main	6.5*	0.77	3.6*	7.5*	0.24*	0.29*	2.4*
Bunetice	Well	6.2*	0.89	23.0*	14.3*	0.34*	0*	3.2
	Well	6.1*	0.86	3.0*	35.7*	1.50	0*	4.8
Ortaše	Well	6.7*	0.68	5.3*	44.7*	0.03*	0*	3.1
Šarišské Bohdanovce	Well	6.9*	0.75	19.0*	184.6	0.11*	0*	3.9
	Well	6.7*	0.84	2.3*	198.5	0.21*	0*	3.9
Vtáčkovce	Well	6.4*	1.71	4.5*	5.6*	0.21*	0*	2.4*
	Well	6.2*	0.94	6.1*	151.8	0.11*	0*	3.1

* — values complying with the requirements of STN 757 111; * —

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GROUNDWATER POLLUTION BY DAIRY COW FARM

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ABSTRACT

A dairy cow farm housing 300 cows was investigated with regard to the potential risk of pollution of the environment, particularly surface and ground water. By means of piezometers physico-chemical contamination was monitored at winter and summer temperatures over two years at the following sites: opposite a cow-shed (P1), on the plots (P2), at the meadow (P3), at the cultivated area behind the farm (P4) and near the private farm (P5). Increased levels of ammonia nitrogen, nitrates, nitrites, organic nitrogen, chlorides, sulphides, permanganate and bichromate chemical oxygen demands (COD_{Mn}, COD_{Cr}), electrical conductivity and organic carbon were determined in water from the P3 and P5 sites indicating pollution of ground water with organic wastes and sewage.

Key words: dairy cows; ground water; physico-chemical pollution

INTRODUCTION

Animal rearing presents a serious risk to wildlife due to the potential pollution of surface and ground water and the related effects on water fauna and flora. Since 1995 the livestock population in Poland has been increasing. New animal houses were built complying with regulations set by the Ministry for Protection of the Environment, Natural Resources and Forestry, of May 1995 (4). Generally, the breeding farms place higher stress on meeting the economic requirements than on complying with the environmental ones. This may result in pollution of water with faeces, silage juices and other wastes containing biogenic elements.

The objective of the present paper was to determine water pollution caused by a dairy cow farm in the period of 1998—2000.

MATERIAL AND METHODS

The investigation was carried out on a farm with 300 dairy cows with a varying proportion of Holstein-Friesian blood housed on deep litter. The litter was removed every 3rd day, stored in heaps near to cow houses and used for manuring in the growing season.

The geological structure of the soil profile was the following: 0.5 m — soil, 0.5—15 m — losses and mud, 15—200 m siliceous marl and broken marls. The water-bearing stratum was situated on marls at 15 m depth. The arable land was typical chernozem and the soils defined as typical brown while the grassland developed from low bog and deluvial chernozem.

Five piezometers (GEOFIC, Raszyn, Poland) were installed with a drilling mast with the upper edge of piezometer filter below the water surface in the following locations: P1 — opposite the cow-shed; P2 — on the plots; P3 — at the meadow below the living quarters; P4 — at the cultivated area behind the farm; P5 — near a private farm. Water was sampled 10 times with a Giant-type pump after several pumpings to remove any pollutants. Samples were taken at temperatures from –5 to –19 °C and from +10 to +20 °C.

The physico-chemical examination was carried out according to standard laboratory methods (2). The following parameters were determined: ammonia nitrogen, nitrate, nitrite, organic N, phosphorus, iron, manganese, chlorides, sulphides, COD_{Mn} and COD_{Cr}, pH, conductivity, colour, suspended substances and organic carbon.

RESULTS AND DISCUSSION

Pollutants from cattle breeding farms give rise to serious concern due to their gradual, yet steady increase and expansion.

The ground water sampled was polluted with ammonia nitrogen. The highest value at minus temperatures over the 1st experimental year reached 78 $mg.ml^{-1}$ and came from P3. Ammonia nitrogen at all sites indicated

permanent inflow of pollutants from farm to the river supported by the increased presence of chlorides. Nitrates ranged between 0.08 mg.ml^{-1}—240.0 mg.ml^{-1} in the 1st year and between 0.12 mg.ml^{-1}—56 mg.ml^{-1} in the 2nd year. They entered water as runoff from the fertilized fields. Nitrites are an unstable intermediate product of oxidation of ammonia. Levels exceeding 0.01 mg.l^{-1} in ground water indicate pollution. Our examinations at plus temperatures showed that the highest level of nitrites (2.2 mg.ml^{-1}) was recorded in the water collected from P3 in the 1st year and from P5 close to the private farm (4.12 mg.ml^{-1}). This may be ascribed to inappropriate disposal of sewage.

Organic nitrogen may be of plant, animal or sewage origin (6). Its compounds decompose over a long period. The ground water examined showed increased levels of organic N. Samples taken from P3 at the minus temperatures during the 1st year reached 105 mg.ml^{-1} and those from P5 in the 2nd year 130 mg.ml^{-1}.

The levels of phosphorus, iron and manganese in the water collected from the piezometers did not surpass the recommended limits (1, 3, 6).

High concentrations of chlorides were observed in the ground water examined. Their value in samples taken from P3 reached 2800 mg.ml^{-1} (1, 6).

Sulphides were determined only in samples from P3 where they ranged from 1.8 mg.ml^{-1} to 1120 mg.ml^{-1} at plus temperature in the second year of sampling (6).

COD_{Mn} in ground water varied considerably. In the 1st year at minus temperatures it reached 143 mg.ml^{-1} and at plus temperatures 288.0 mg.ml^{-1} in water from the P5, located close to a private farm. In the 2nd year the value interval was still wider ranging from 23 mg.ml^{-1} from P1 to 380 mg.ml^{-1} from P3 (3).

COD_{Cr} values were high in both years of our observation. It reached 3 660 mg.ml^{-1} in the 1st year and 682 mg.ml^{-1} in the 2nd year in water from P3. These results support our suspicion of pollution of ground water with organic matter.

The pH of water from all piezometers was within the limits for drinking water (4). The ground water tested showed substantial electrical conductivity. The highest level of this parameter was determined in water from P3 — 13710 μS.cm^{-1} which indicates a considerable concentration of dissolved mineral salts (4, 5).

Pollution-indicating putrid odour was noted only in the 1st year in water from P3 (1, 6). Water colour ranged between 10 and 30 mg Pt.l^{-1} in the 1st experimental year and between 20 and 30 mg Pt.l^{-1} in the 2nd one (1, 6).

Ground water suspended solids were also high. In the 1st year they reached 82 mg.ml^{-1} and the 2nd year 102 mg.ml^{-1}, both in water from P3. Organic carbon in a water sample from P3 was on the level of 1200 ppm (1, 6).

Farm ground water exceeded the standards for 11 indices. Higher levels were associated particularly with two piezometers, P3 — located at the meadow, and P5 — close to a private farm. The natural slope of the area as well as using the meadow for grazing could influence the results from piezometer 3. In the case of piezometer 5, inappropriate sewage disposal probably contributed to the unfavourable results.

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ODOUR EMISSIONS FROM A FARM WITH FURBEARING CARNIVORES

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ABSTRACT

The study was carried out for over one year on a farm housing 25 thousand common and arctic foxes and 10 thousand minks. The concentration of sulphur compounds, aldehydes and alcohols was measured eight times, considering minus and plus temperatures and air movement. The samples were collected at 7 sites: 15—20 cm over the manure passage surface (I); at 150 cm height between cage rows (II); 100 m (III), 250 m (IV), 450 m (V), 600 m (VI), 800 m from the farm boundary (VII). The main factors deciding about gas emissions and their spreading were identified and attention was drawn to the lack of relevant limits.

Key words: furbearing animals; gas emissions; odour

INTRODUCTION

Animal farms release various pollutants into the ecosystem. Breeding farms are sources of volatile gaseous substances that undergo numerous transformations in the air stimulated by light, dust, or other components. Their emissions depend on farm location, animal species, housing system, herd size, local weather conditions, and others (1, 2, 4, 8, 9). More than 130 gaseous compounds have been identified in animal houses, yet only some of them occur regularly in the outer air. They are produced by complex digestive processes and by the transformation of faeces during their storage and after application on the soil (8, 9). In addition to unpleasant odour they present a serious risk to animal health, attendants and people living nearby. Many substances can be detected by senses at levels below acceptable concentrations while others do not have strong unpleasant smells but exhibit toxic or carcinogenic properties. The mechanism of their influence on organisms has not yet been elucidated sufficiently. It is conditional on the immune status, species susceptibility, age, and the respective substance (1, 7, 9).

The objective of our investigations was to determine air pollution with odourous compounds, i.e. sulphur compounds, alcohols and aldehydes on a farm for furbearing carnivores and in its surroundings.

MATERIAL AND METHODS

Examinations were carried out on a farm housing 25 thousand common and arctic foxes and 10 thousand minks for over one year. Removal of excrements, disinfection, louse control and rat control were performed regularly.

The concentration of sulphur compounds, aldehydes, and alcohols was measured eight times considering minus and plus temperatures and air movement. The samples were collected into tedlar bags at 7 sites: 15—20 cm over the manure passage surface (I); at 150 cm height between cage rows (II); 100 m (III), 250 m (IV), 450 m (V), 600 m (VI), 800 m from the farm boundary (VII).

The material was analysed by a gas chromatograph with flame-photometric detector (FPD). Calibration was performed by the standard method (3).

RESULTS AND DISCUSSION

Our results demonstrated that the sampling site and air temperature were of prime importance to gas emissions. The concentration of sulphur compounds, alcohols and aldehydes was lower at minus temperatures and increased at higher ones. This is related to the higher rate of fermentation processes and the higher volatility of compounds. The distance from an emission source was also an important factor. The highest concentration of sulphur compounds and of most aldehydes and alcohols was recorded over the manure passage surface at both, plus and minus temperatures. Isopropyl mercaptan was the dominating sulphur compound reaching maximum

concentration of 15.5 $\mu g.m^{-3}$ at plus temperatures and 4.2 $\mu g.m^{-3}$ at temperatures below zero. Comparison with the Polish standards showed that acceptable concentrations (AC) of methyl mercaptan (AC30-1 $\mu g.m^{-3}$) and dimethyl disulphide (AC30-5 $\mu g.m^{-3}$) were exceeded. No AC have been set for other compounds identified in our study (5).

Determination of aldehydes showed predominance of decanal (222.0 $\mu g.m^{-3}$) and acrolein (144.2 $\mu g.m^{-3}$) at plus temperatures. The AC30 for acrolein (10 $\mu g.m^{-3}$) was exceeded many times (5). High values were also observed for furfural (18.6 $\mu g.m^{-3}$ at plus temperatures, 9.2 $\mu g.m^{-3}$ at minus temperatures). However, they did not exceed the AC for human exposure (10 $mg.m^{-3}$) (1).

From among alcohols the highest concentrations were recorded for heptanol (60.3 $\mu g.m^{-3}$ at plus and 26.3 $\mu g.m^{-3}$ at minus temperatures). There are no standards for their level which makes it difficult to evaluate their potential harmfulness.

The results obtained approximate those of Nowakowicz–Dębek (2), and Sławoń *et al.* (8) who reported that many gaseous emissions do not carry 500—700 m. As no data is available on the effect of odourous compounds either on animal health and productivity or on farm workers, further investigations appear desirable. Our investigations concentrated on farms for furbearing animals. Detailed knowledge in this respect is necessary due to concordant EU regulations dealing also with furbearing animal welfare in relation to reproductive losses which is the most important factor for breeders (6, 10).

The study indicated another broad area of problems related to the emissions of odourous and noxious gases and their potential effects on animals and humans. This is an area for additional scientific research and new technological solutions.

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A COMPARISON OF SELECTED BIOCHEMICAL INDICES IN DAIRY COWS WITH RESPECT TO HOUSING CONDITIONS

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ABSTRACT

Within a programme of preventive diagnosis and control of health of dairy cows, we evaluated 12 selected biochemical indices in 252 healthy cows in relation to housing conditions (loose, tethered). The animals were divided into three groups: dry, after calving, lactating. Statistical analyses of mean values showed insignificant differences of AST, TP, U, and CR between the groups and types of housing. An opposite trend was recorded for indices of enzymatic profile (ALT, GGT). In all the groups, within the evaluation of energy profile, we observed various degrees of significant differences in mean values. The highest significance was observed for TG and TL (higher mean values in tethered animals). A statistical significance was also observed in dry (Glu), calved, and lactating (TCh), and lactating (BHB) cows (higher mean values in tethered animals). In loose housing, calved and lactating cows showed significantly higher values of NEFA.

Key words: biochemistry; blood serum; dairy cows; loose and chained housing

INTRODUCTION

A complex programme of preventive diagnosis and checking the health of animals is an inevitable part of farm practice (diagnosis of metabolic disorders, disorders of reproduction and locomotor systems, mastitis) (2). This programme is possible only with the close co-operation of the veterinary surgeon and farmer, because it consists of environmental analysis, analysis of production, reproduction, the animals' health state, and a complex evaluation of any problem, as well as the proposal of corrective measures (3, 8).

The evaluation of biochemical indices requires experience and knowledge of factors influencing these indices (physiological stresses — e. g. advanced pregnancy, parturition, high milk production, composition of diet, age, etc.), as well as pathological states affecting, for example, absorption and utilization of nutrients (4). This work was aimed at evaluating of biochemical indices in relation to type of housing.

MATERIAL AND METHODS

Analyses of 12 selected biochemical indices were done in 252 healthy dairy cows from Slovak farms. The cows were divided into two groups depending on the type of housing: I — loose housing (174 cows), II — tethered housing (78 cows). In both groups, the cows were divided by their production and reproduction stage into the following groups: 1 — dried cows in advanced pregnancy (98 animals), 2 — cows after calving at the beginning of lactation (80 animals), 3 — cows at peak of lactation (80 animals).

Blood samples were collected from the *v. jugularis*. The blood serum was analysed for enzymatic activity of AST (E.C. 2.6.1.1.), ALT (E.C.2.6.1.2.), GGT (E.C.2.3.2.2.), concentrations of total protein — TP, urea — U, creatinine — CR, total cholesterol — TCh, triglycerides — TG, α-hydroxybutyrate — BHB. The blood plasma was analysed for glucose concentration — Glu. The analyses were done by an automatic biochemical analyser ALIZE (Lisabio) using diagnostic kits (Bio Mérieux, Randox). The concentrations of total lipids — TL (Bio Lachema) and nonesterified fatty acids (1) were determined in the blood serum using spectrophotometer SPECOL 211 (Carl Zeiss Jena). Statistical evaluation of the differences between the groups (loose and tethered) was done with the use of Student's *t*-test.

RESULTS

The results obtained for selected indices of enzymatic, protein, and energy profiles are presented in Tables 1—2. When evaluating the indices of enzymatic and protein profiles (Tab. 1) in relation to the type of housing, we found insignificant differences between AST, TP, U, and CR in all three groups of dairy cows (dry, after

Table 1. Indices of enzymatic and protein profiles in the blood serum of cows in relation to the type of housing ($\bar{x}$ ± sd)

GROUP OF COWS	TYPE OF HOUSING		AST μkat.l^{-1}	ALT μkat.l^{-1}	GGT μkat.l^{-1}	TP g.l^{-1}	U mmol.l^{-1}	CR μmol.l^{-1}
DRY	*LOOSE*	**n**	62	54	62	62	62	50
		x	1.53	0.41	0.44^{b}	76.2	3.3	156.2
		sd	0.42	0.10	0.12	6.6	1.2	33.6
	TETHERED	**n**	30	30	30	30	30	24
		x	1.41	0.37	0.37	76.1	3.7	155.0
		sd	0.33	0.08	0.09	9.2	1.9	17.8
AFTER CALVING	*LOOSE*	**n**	56	48	56	56	56	44
		x	2.04	0.34^{a}	0.47	78.7	4.3	113.9
		sd	0.77	0.15	0.18	8.2	1.7	20.7
	TETHERED	**n**	24	24	24	24	24	24
		x	1.84	0.42	0.47	80.2	3.6	122.5
		sd	0.60	0.13	0.12	7.0	1.7	24.5
LACTATING	*LOOSE*	**n**	56	48	56	56	55	44
		x	1.85	0.54	0.59	84.4	4.7	113.9
		sd	0.59	0.13	0.24	9.5	2.2	19.7
	TETHERED	**n**	24	24	24	24	24	24
		x	1.74	0.58	0.58	84.1	4.1	116.9
		sd	0.44	0.14	0.27	7.3	2.3	22.2
	Normal range		**1.3—2.2**	**0.18—0.64**	**0.1—0.5**	**70—90**	**3.6—10.7**	**88—177**

a, b — Student's *t*-test ($a - p < 0.05$; $b - p < 0.01$)

Table 2. Indices of energy profile in the blood serum of cows in relation to the type of housing ($\bar{x}$ ± sd)

GROUP OF COWS	TYPE OF HOUSING		Glu mmol.l^{-1}	TCh mmol.l^{-1}	TG mmol.l^{-1}	BHB mmol.l^{-1}	NEFA mmol.l^{-1}	TL g.l^{-1}
DRY	*LOOSE*	**n**	62	62	62	62	62	62
		x	4.01^{a}	3.40	0.23^{a}	0.57	0.36	3.21 ^{a}
		sd	0.64	1.09	0.11	0.37	0.32	1.03
	TETHERED	**n**	30	30	30	30	30	30
		x	4.29	3.57	0.27	0.61	0.35	3.67
		sd	0.61	0.82	0.09	0.44	0.35	0.88
AFTER CALVING	*LOOSE*	**n**	56	56	56	44	56	56
		x	3.87	2.89^{b}	0.16^{b}	0.73	0.66^{a}	3.15^{c}
		sd	0.66	1.00	0.07	0.51	0.56	1.07
	TETHERED	**n**	24	24	24	24	24	24
		x	3.86	3.88	0.20	0.86	0.45	4.45
		sd	0.71	1.50	0.04	0.65	0.26	1.50
LACTATING	*LOOSE*	**n**	56	56	56	44	56	56
		x	3.99	6.24^{b}	0.13^{c}	0.55^{b}	0.34^{b}	5.49^{b}
		sd	0.75	1.95	0.07	0.39	0.30	1.79
	TETHERED	**n**	24	24	24	24	24	24
		x	3.96	7.60	0.18	0.82	0.20	6.87
		sd	0.41	1.76	0.04	0.36	0.09	1.56
	Normal range		**2.4—4.2**	**2.1—5.2**	**0.17—0.51**	**to 1.0**	**0.1—0.35**	**1.5—5.2**

a, b, c — Student's *t*-test ($a - p < 0.05$; $b - p < 0.01$; $c - p < 0.001$)

calving, lactating). The GGT activity showed significant differences ($p < 0.01$) in the group of dry cows (higher activity in loose housing). A significant difference was observed in ALT activity in cows after calving ($p < 0.05$) with higher values in tethered animals.

More significant differences were recorded when evaluating energy profile (Tab. 2). The highest significance was observed for TG and TL (higher average values in tethered animals). Significant differences in TCh values in cows after calving and lactating cows were recorded ($p < 0.01$) with higher values in tethered cows. The difference in NEFA values were significant in the groups of cows after calving and lactating cows with higher values in loosely housed animals. In all the groups, BHB values were higher in tethered animals with the largest difference found in lactating cows. In tethered dry cows, significantly higher glucose levels were recorded compared with loosely housed cows.

DISCUSSION

In the literature available, there is data missing about the differences in biochemical indices in relation to different types of cows housing. Our results indicate statistically significant differences, particularly in the indices of energy profile. Considering good rearing condi-tions in the farms investigated with diet balanced to milk production and reproduction cycle, we suggest that these differences were not only related to the diet, but also to the type of housing. Energy balance is the most critical factor influencing animals' health, lactation, and reproduction. Traditionally, changes in energy balance are evaluated by live body weight, scoring of body condition, concentrations of ketone bodies. There is wider use of concentrations of NEFA. Clinical experiences in cows during peri-parturient period indicated, that levels of NEFA directly reflect the amount of mobilized fat in negative energy balance. The evaluation of the protein profile is somewhat more complicated. There is a need to analyse a combination of indices including blood urea nitrogen (BUN), CR, TP, albumin, and creatinkinase. In protein deficiency, there is, besides others, alteration of the immune system. Without antibiotic therapy, such dairy cows may die due to any infection (metritis, mastitis, pododermatitis, etc.) (3, 5). The liver function can be checked by determination of enzyme activities (GGT, AST, sorbitoldehydrogenase – SDH) and concentration of total bilirubin in the blood. The concentrations of bilirubin are specific for disturbances in bile outflow and less for damage to liver cells. The activities of enzymes should be evaluated in connection with concentrations of total cholesterol and NEFA. Proteins are required for fat transport from the liver.

In the blood, fat is transported in the form of lipoproteins with very low density (VLDL). Together with fat, the structure of VLDL contains a substantial amount of cholesterol. Therefore, concentrations of cholesterol indirectly indicate presence of VLDL in the blood and, consequently, the ability of liver to produce VLDL. In disturbed production of VLDL, liver infiltration with fat develops (4). Our results should be completed with analyses of indicators of osteopathies and myopathies.

CONCLUSION

The results obtained in the study selected biochemical indices of dairy cows in relation to reproduction cycle and type of housing showed significant differences in ALT and GGT activities, mostly in the dry period and after calving. In particular, indices of energy profile (TG, TL) were significantly higher in all the groups of tethered animals. In tethered lactating cows, concentration of TCh and TL exceeded the normal range. Concentrations of NEFA showed significant differences in loosely housed dairy cows with values above the normal range in cows after calving.

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THE EFFECT OF THEIR ENVIRONMENT ON THE MORBIDITY AND PRODUCTION OF DAIRY COWS

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ABSTRACT

In 2000 and the first half of 2001 we studied the effects of ambient temperature on selected production and health indices in 12 dairy farms with a milk production over 6500 l. In every farm, heat-stress caused a decrease in milk production (14—36 %). We observed decreased food consumption (food intake in late evening only) up to inap-petance, increased water consumption, tachypnoea, tachycar-dia, in some cases increases in body temperature over 40 °C, and reduced responses to external stimuli. Particularly in August, when the heat stress lasted for a longer time, in groups of high-yielding cows, the number of cows remainded with a body condition score 1.5—1.0, worse reproduction indices, and the frequent occurrence of laminitis. The convalescence period was dependent on a number of factors including environment, type of housing, ventilation, system of watering, type of yards, and others. Moreover, this period was strongly influenced by diet composition, the administra-tion of buffers, and management. The peak of lactation was lowered; the lactation curve was flattened or duplicated.

Key words: dairy cows; heat-stress; milk yield; morbidity

INTRODUCTION

Expected changes in the climatic conditions in the environment of high-productive animals are stressed by the adverse effects of heat-stress on their health and production. The main problems in high-yielding dairy farms include diseases of the reproductive system, mastitis, and diseases of the locomotor system, closely related to the nutrition and housing of animals. Imbalance in the diet, without considering reproduction cycle and production level, frequently leads to subclinical acidosis and, consequently, the problems mentioned above (3). In this work, we studied heat-stress, which has an important role in the aetiopathogenesis of these problems in our conditions.

MATERIAL AND METHODS

The heat-stress effects we studied in 2000, mainly in the summer months of June to August, and in the first half of 2001. During this period we evaluated the animals' behaviour, food intake, milk production, and clinical manifestation of heat-stress — respiratory and pulse rates, body temperature, and the occurrence of diseases. An effect of ambient temperature on milk production was evaluated in 12 farms with milk yields over 6500 litres. The daily milk yield was compared in relation to the air temperature measured at $14^{.00}$ hours. To objectivize the study, air temperatures were obtained from Slovak Hydrometeorology Institute in Hurbanovo. The farms were located 80 km from this institute, maximally. The effect of temperature on milk production was evaluated on graphs on the basis of 7-day moving averages.

RESULTS

In every farm, heat-stress caused a decrease in milk production. With increasing air temperature the milk production decreased, and vice-versa. The highest heat-stress was recorded around 14 June with a maximum temperature 36.1 °C (from 8 to 14 June) and from 12 to 21 August with maximum temperature 38.3 °C.

In cows, the heat-stress was manifested by decreased food intake up to inappetance, standing in groups, increased respiratory rate. The cows frequently breathed with open mouth. High-yielding cows during the first 100 days of lactation tolerated the stress less than cows at the end of lactation. Respiration frequencies ranged from 120 to 160 per minute. Frequencies higher than 160 were not countable. Similarly, we observed increased pulse frequencies. Occasionally, the body temperature increased up to 40 °C. We observed reduced responses to external stimuli. The cows ate only in the late evening, when ambient temperature decreased. If the cows were fed three times daily, they did not consume the midday

food. During the summer, particularly in August, when the heat stress lasted for a longer time, the cows' body condition scores decreased by 1 up to 2.

With the heat-stress, water consumption increased rapidly, the cows drank frequently and crowded around the watering places. Because of their unwillingness to movement, they blocked the access of other animals to the water. The cows did not drink in open yards exposed to direct sunshine. In such farms, the consequences of heat-stress were the worst. Convalescence time was longer, the cows loss of body weight was the highest, and reproduction indices worsened markedly. In some farms, restoring body condition lasted up to four months. In groups of high-yielding cows, the number of cows remained with a body condition score 1.5—1.0. There were no deaths among the animals.

Decrease in milk production ranged from 14 to 36 %. The time of restoration of health and production indices was dependent on a number of factors including environment, type of housing, ventilation, system of watering, type of yards, and others. Moreover, this period was strongly influenced by diet composition, the adminis-tration of buffers, and management. The peak of lactation was lowered; the lactation curve was flattened or duplicated.

In several farms delayed improvement of health and production occurred and a quite frequent occurrence of laminitis was recorded.

DISCUSSION

Prognoses of climatic changes in the environment of the animal population suggest a disturbance in the balance and structure of ecosystems, as well as changes in the natural resistance of most macro- and micro-organisms. The environment's autosanition ability will be reduced, the incubation period will be changed in a number of diseases, there will be an absence of year seasons with devitalizing effects on pathogens (e.g. dry winter, hot summer).

From the point of view of these expected changes, heat-stress has an important role by its effect on health and production of high-yielding animals (4).

High-yielding cows are very sensitive to heat-stress due to high food intake, which covers their energy requirements for milk production. During heat-stress, dry matter intake decreases by 8—12 % and milk production by 20—30 %. The total milk yield per lactation is reduced by reduction in the number of litres produced at the peak of lactation, multiplied by 250. Dried cows exposed to heat-stress during the last months of pregnancy deliver calves with lower body weights and they frequently suffer from metabolic disorders after parturition. During the following lactation, milk production of such cows is reduced by 12 %. The reproductive system is adversely affected with reduced conception rates and follicular activity, and increased early embryonic mortality. The cows which underwent heat-stress frequently lost body weight down to body condition score 1 (physiological body condition score is 3.5 ± 0.25 and, when using 5 point scale, the decrease should not exceed 1).

Such cows are exhausted and they suffer from fat cow syndrome, mastitis, laminitis, and subacute rumen acidosis. They consume less food; they are lazy, reluctant to move. If watering places are more than 25 m from their standings, they do not drink. The cows breathe quickly, frequently with open mouth. In subacute rumen acidosis, there are changes in the rumen microflora. In acidic rumen fluid amylolytic microorganisms survive and breake starch down. Because rumen pH decreases below 6, the quantity of cellulolytic microorganisms is reduced. In this way, digestion of cellulose is disturbed leading to a decrease in concentrations of acetic acid (a pre-cursor of milk fat) and the syndrome of low milk fat. Subacute rumen acidosis results in reproduction disorders, claw inflammation, fat cow syndrome, liver abscessation, and others. In cows exposed to heat-stress, the time needed for normalization of vital processes is usually 3—4 months (1, 5).

To reduce the adverse effects of heat stress, the following are recommended: the shading of open yards and feeding troughs, cooling surroundings near standings, feeding places or waiting places in milking rooms with the use of ventilators or water sprayers and fog developers (sprinklers). By this way, it is possible to lower the temperature by 6—7 °C. It is reported, that using this method for three years led to an increase in milk production by 2 litres per cow and day. The amount of sprayed water and time of exposure should be sufficient; however, water should not flow to mammary gland and teats (risk of mastitis). It is important to ensure enough drinking water. One watering place per 20 cows is needed at the distance not more than 10—15 m from resting-places. The places should be cleaned regularly. It is useful to feed quality roughage with ADV (acid-detergent fibre) not below 18—19 %. The ratio should be divided to feed the largest portion in the evening or night when temperature is lower.

Rumen acidosis should be prevented or reduced by the administration of buffers, e.g. bicarbonates or sorbents. Energy requirements can be covered by the addition of fats into the diet. Protein overfeeding should be avoided because of energy demanding the excretion of urea. Mineral nutrition can be adjusted by additions of K (losses in sweat), Na (losses in urine), and Mg. It is useful to enrich the diet with vitamins A and E (2, 5).

CONCLUSION

In the work presented we wanted to detail the adverse effects of heat-stress. In practice, almost in every farm, especially in farms with high milk production and farms in Southern Slovakia, we can find the adverse effects of heat-stress on milk production and the health of dairy cows. The major problems

of heat-stress are decreased food intake and the development of subacute acidosis. Presently, there is an interest in avoiding this stress as we mention in discussion.

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THE INFLUENCE OF WATER EVAPORATIVE COOLING ON HAEMATOGICAL PARAMETERS IN DAIRY COWS DURING HEAT STRESS

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ABSTRACT

Our aim was to find out whether and how much regular water evaporative cooling by sprinkling alleviates heat stress (32 °C) in high-yielding dairy cows. Selected haematological parameters were used for the assessment. The results of haematological parameters showed a significant decrease in haemoglobin, haematocrit and erythrocyte numbers ($P<0.05$) during water evaporative cooling period but leukocyte numbers were not significantly influenced by cooling compared with the hot period without cooling.

Key words: dairy cows; haematological parameters; heat stress; water evaporative cooling

INTRODUCTION

Heat stress depressively influences the milk yield, decreases the food intake, worsens the health condition, and affects the behaviour of dairy cows. Cooling can alleviate heat stress and can improve the thermal balance and both productive and reproductive performances in dairy cows. The most common method is the sprinkler system (3).

Our aim was to find out whether and how much regular water evaporative cooling by sprinkling was needed to alleviate *heat stress in high-yielding dairy cows. Selected haematological parameters were used for the assessment.*

MATERIALS AND METHODS

Four healthy Holstein dairy cows, 2—3 months after calving, with a average daily milk-yield 28 kg (2nd—3rd lactation) were used in the experiment. These dairy cows were housed in the experimental conditions of a climatic chamber, in loose housing with cubicle beds and bedding.

The scheme of the experiment included two periods: 1st hot period without cooling (HP) — 10 days, air temperature 32 °C; 2nd hot period with cooling (HPC) — 10 days, air temperature 32 °C. Cooling was provided every 20 minutes for 50 seconds (4 nozzles). The assessed haematological parameters were: haematocrite value, haemoglobin concen-tration, erythrocyte and leukocyte number. Blood samples were collected three times in each period. The analysis was carried out using Coulter Counter model ZF (Coulter Electronics Ltd, England). The values obtained were statistically evaluated by the F test and the Student *t*-test.

RESULTS

The results are detailed in Table 1. The mean value of haemoglobin was found to be 116.86 $g.l^{-1}$ in HP and 103.38 $g.l^{-1}$ in HPC. There was a significant difference between the parameter in HP and HPC ($P<0.05$). Haemoglobin content correlated positively with haematocrit, the significant difference was between HP and HPC ($P<0.05$). A lower number of erythrocytes were observed in HPC ($P<0.05$) but the leukocyte number was not significantly influenced by cooling (HPC).

Table 1. The means values of haematological parameters

Period	Air temperature (°C)	Haemoglobin ($g.l^{-1}$)	Haematocrit ($l.l^{-1}$)	Number of erythrocytes ($T.l^{-1}$)	Number of leukocytes ($G.l^{-1}$)
HP	32	116.86 (1.024)	0.38 (5.498)	7.56 (0.885)	7.81 (1.042)
HPC	32	103.38* (0.826)	0.32* (2.564)	6.75* (0.504)	8.25 (2.787)

( ) — (standard deviation); * — $P < 0.05$

DISCUSSION

Haematocrit value, haemoglobin concentration, and erythrocyte numbers evidenced a rise in blood concentration in HP. Toharmat *et al.* (5) described an increase in haematocrit values and haemoglobin concentration in summer. Cooling by sprinkling prevented marked changes in the red blood picture. Omar *et al.* (4) reported similar results. Some articles, however, reported that cooling of heat-stressed dairy cows increased blood haemoglobin (2,1). The leukocyte number was insignificantly increased during HPC compared with HP.

Heat stress evoked haemoconcentration in dairy cows, haemoconcentration was proved by haematocrit enhance-ment, an increase in red blood cell numbers and an insignificant decrease in leukocyte number. In the second period of the experiment, water evaporative cooling by sprinkling influenced positively the haematological parameters of dairy cows and moderated heat stress.

Acknowledgements

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C-REACTIVE PROTEIN IN THE BLOOD SERUM OF PIGS EXPOSED TO TRANSPORTATION STRESS

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ABSTRACT

The concentration of C-reactive protein (CRP) in 20 pigs (110 kg) transported over a distance of 550 km was studied. Transportation took place in winter time. Covering the distance took 22 h, the same as resting. Blood was col-lected before transportation, after unloading, and at slaugh-ter. During the whole experimental period of 44 h, serum CRP concentration increased 2-fold ($p < 0.0001$). Transportation stress influenced CRP increase by 75.1 % ($p < 0.001$). The resting period did not cause normalization of CRP, which next increased by 16.5 % ($p < 0.05$).

Key words: CRP; pig; stress; transportation

INTRODUCTION

Transporting pigs to slaughter causes many stressful situations (moving, loading, covering the journey distance, unloading). Each of them *per se* initiates stress response. The sequence of events leading to an increasing synthesis of acute phase proteins (APP) is as follows (3): stress exposure → cortisol ↑ (peak of concentration in blood serum ≈ 6 h later) → cytokines (≈ 18 h) ↑ → APP ↑ (≈ 48 h). In pigs, C-reactive protein (CRP) is one of the most reactive APP, apart from haptoglobin, pig-MAP, SAA, AGP, neopterin, α-lipoprotein, albumin, α-1-glycoprotein, ceruloplasmin and transferrin (4).

An increased CRP concentration following infection (6, 11), ACTH administration (2) and weaning stress (8) has been documented. The aim of our study was to determine the effect of long distance road transportation of pigs on the changes in serum CRP concentration.

MATERIALS AND METHODS

The animal material consisted of 20 crossbred pigs (mean bw 11 kg). Road transportation from a farm to the slaughter house took place in December over a distance of 550 km. Bad climatic conditions caused the prolongation of transport time to 22 h as well as the violation of transportation demands (not enough water, feed and straw). After unloading, pigs were located in slaughter lairlage and after 22 h resting time, they were slaughtered. The blood was collected from all 20 pigs in 3 series, before loading (series 1 — control), immediately after unloading (series 2) and at slaughter (series 3). Serum CRP concentration was determined by a commercial kit (Tridelta Phase TM range C-Reactive Protein Assay, Tridelta Development Limited,Greystones, Wicklow, Ireland). The results were elaborated statistically (Statgraph ver. 5.0.)

RESULTS

Throughout the whole experimental period of 44 h (series 3 *vs*. 1), serum CRP concentration increased 2-fold ($p < 0.0001$). Transportation stress (series 2 *vs*. 1) influenced the CRP increase by 75.1 % ($p < 0.001$). Resting period (series 3 *vs*. 2) did not cause normalization of CRP, which next increased by 16.5 % ($p < 0.05$) (Fig. 2).

DISCUSSION

In our experiment pigs were exposed to many stress factors, different in type and intensity. Factors related to transportation conditions were the following: extremely low temperature, lack of feed, water, and straw, shake, vibrations, crowding, slipperiness, etc. Bad climatic con-ditions prolonged the transportation time.

According to Pineiro, M. *et al.* (10), transportation conditions determine serum APP (pig-MAP, haptoglobin) to a higher degree than the length of journey. Comparing two transports, one lasting 48 h and an other one — 24 h, where the first provided excellent conditions (2 m^2/boar, feed, water, sawdust) and the second only average (1.5 m^2/boar, no feed, water, sawdust), it was found that pig-MAP in- creased 1.4 and 3.4 fold and haptoglobin 1.2 and 1.4 fold.

CRP in the control series (70.9 μg.ml^{-1}) exceeded re-ference values. In healthy pigs CRP remains within the range of 2.62—9.92 μg.ml^{-1} (11), or 11—77 μg.ml^{-1} (6), or 8.35—16.8 mg.l^{-1} (2). The increased CRP control value suggests the influence of pre-transportation factors, such as poor welfare at the original farm (7) and/ or unhealthi-ness of pigs (9).

The acute phase response is believed to occur if the concentrations of APP increase by 25% or more (5). According to that definition, transportation stress, causing the increase of CRP by 75.1% (Tab. 1), acted like a disease agent. Assuming CRP as a stress criterion, the intensity of transportation stress was greater if compared to weaning stress reported by Kołacz *et al.* (8).

In the present study the increase of CRP was 75.1% in relation to the control value of series 1, whereas

Table 1. CRP concentration in pigs exposed to transportation stress

Mean	Number of pigs	Series of bleeding	CRP μg.ml^{-1}	95% confidential for means
SEM	20	1	70.9A	56.8—85.1
SEM	20	2	124.29Ba	110.1—138.5
SEM	20	3	144.5Bb	130.3—158.7
Total SEM	60	x	113.2	105.1—121.4
Main effect (p <)	**x**	**x**	**0.0001**	**x**

A—B: p < 0.01; a—b: p < 0.05

weaning resulted in an increased CRP by 16.25%. After weaning, the normalization of CRP was already observed after 10 h, whereas after transportation even 22 h (resting time) was not long enough to achieve the control CRP level (Fig. 1).

It is worth mentioning, that the normalization of APP following infection requires not less than 2—10 days (4). During the resting time, the next CRP increase by 16.5% (series 3 *vs* 2) could result either from the acute phase reaction developed beyond transportation time or the exposure of pigs to the new environment of slaughter

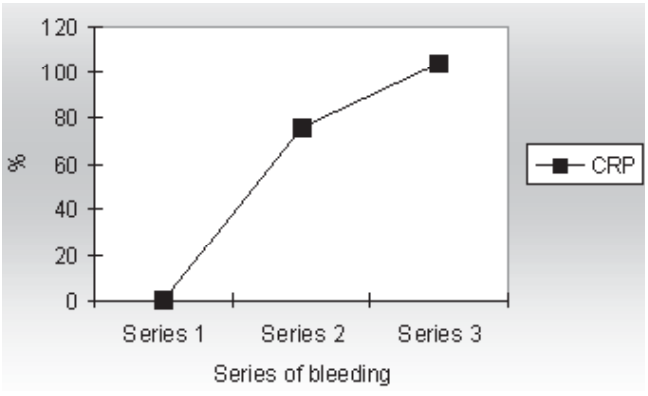

Fig. 1. Percentage changes of CRP concentration in pigs exposed to transportation stress

lairage (demanding adaptive reactions), or both. Brown *et al.* (1) observed very high pigs activity in lairage after unloading (drinking, mounting, exploratory, fighting). In those pigs, liver's glycogen was almost completely depleted.

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THE INFLUENCE OF TRANSPORT STRESS ON THE IMMUNE RESPONSE IN CALVES AT THE AGES OF 14, 21, AND 28 DAYS

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ABSTRACT

We examined the humoral and cellular immune response in calves at the ages of 14, 21, and 28 days (n = 3 × 7) affected by stress evoked by the transport over a short distance: from the dairy farm to the calf barn. The experimental calves under stress conditions (associated with transport and adaptation) have shown higher immune reaction than the control calves. We suggest that such an influence of non-excessive stress may be helpful in the adaptation of the young calves (14 days old) to new environmental conditions, and it indicates that this age can be optimal for transporting the calves to the calf barn.

Key words: calves; immune response; transport stress

INTRODUCTION

The transport stress and its influence on the meat quality of slaughter animals have been extensively investigated in the last decade (2). In Poland the common practice in cattle production is moving calves from dairy barn to calf barn, sometimes over a long distance. The stress associated with the transport and adaptation to the new housing conditions influences the health and immune reactions of animals (1, 4). In the majority of studies carried out the negative results of transport stress in cattle have been described (2, 3, 4).

The aim of the work was to examine the humoral and cellular immune response in calves at the ages of 14, 21, and 28 days. The animals were affected by stress evoked by the transport at a short distance: from the dairy barn to the calf barn.

MATERIAL AND METHODS

The experimental dairy calves (25 % Polish lowland black and white and 75 % Holstein-Friesian) at the ages of 14 (n = 7), 21 (n = 7) or 28 (n = 7) days were transported a distance of 1 km from the dairy barn to the calf barn using the appropriate cage. Calves from the control groups (at the same age as the experimental ones, i.e. 14 days (n = 7), 21 days (n = 6) and 28 days (n = 6)) were left in the dairy barn. The jugular vein blood was collected before and immediately after the transport (30—40 minutes after the first venipuncture) and then in 24th and 72th hour and later on 7th, 14th, and 21st day after the day of the transport. Immediately after the transport and blood collection, experimental and control calves were immunized subcutaneously with ovoalbumin (1 mg.ml^{-1} of saline). The immunization was repeated after one week. Also, at the same time i.e. immediately after the transport and blood sampling, every calf was injected intracutaneously into the left side of the neck with phytohaemagglutinin (100 mg in 0.2 ml of saline). The cutaneous reaction was measured after 24 hours, and ex-pressed as the increase in the lappet thickness.

The level of cortisol in the blood taken before, immediately after and 24 and 72 hours after the transport, was measured using DPC Bierman kit. The specific anti-ovoalbumin IgG_1, IgG_2 and IgM antibodies were detected using ELISA. The polystyrene microplates (Organon-Teknika) were coated with 50 ml of ovoalbumin solution (10 mg.ml^{-1} of carbonate buffer, pH 9.6) and incubated for 3 hours at 37 °C and overnight at 4 °C.

The microplates were blocked with bovine serum albumin (1 mg.ml^{-1} of PBS) for 1 hour, washed using PBS containing 0.05 % of Tween 20 (PBS-T). Calves' sera were diluted 1:100 in PBS-T, and incubated (50 ml per well) for 3 hours at room temperature on the laboratory stirrer. After washing the anti-bovine IgG_1, IgG_2 and IgM antibody conjugates (Serotec) were added for 3 hours. After washing the o-phenylenediamine solution was used as the substrate. The absorbance was read at wavelength 492 nm. The levels of bovine IgG_1, IgG_2 and IgM class serum immunoglobulins were estimated using RID-kit (Binding Site).

RESULTS

In all experimental groups of calves the mean level of cortisol rise immediately after the transport: 3.2 times in 14-days-old calves, 3.95 times in 21-days-old

calves, and 7.1 times in 28-days-old calves. Then after 24 and 72 hours its concentration decreased below the level observed before transport (Table 1). No significant changes in cortisol production were observed in calves of the control groups. It was noticed that the mean cortisol level measured before the transport inversely correlated with the calves' age (Table 1).

Table 1. Mean cortisol levels (mg.l^{-1}) in examined calves

Groups of animals	Mean ($\bar{x}$) and standard deviation (SD)	Before the transport	Immediately after the transport	24 h after	72 h after
Experimental	$\bar{x}$	4.54	**14.57***	1.81	1.43
14 days old	SD	2.79	8.61	2.34	0.81
Control	$\bar{x}$	6.06	**5.03**	4	2.14
14 days old	SD	2.77	4.72	2.48	1.21
Experimental	$\bar{x}$	2.14	**8.45***	2.15	2.16
21 days old	SD	1.85	7.88	2.91	3.03
Control	$\bar{x}$	2.41	**2.83**	1.72	2.15
21 days old	SD	3.34	3.36	1.51	2.29
Experimental	$\bar{x}$	2.3	**16.39***	1.14	0.82
28 days old	SD	1.1	9.1	1.21	1.12
Control	$\bar{x}$	0.71	**0.67**	0.55	0.23
28 days old	SD	0.53	0.9	0.69	0.35

* — $p \leq 0.01$

The skin reaction induced by intradermal injection of phytohemagglutinin measured as the increase in the lappet thickness was significantly more intensive in 14- and 28-day-old experimental than in control calves ($p < 0.05$). No significant differences occurred in groups of 21-day-old calves.

Table 2. The increase of the lappet thickness (mm) at 24 hours after intradermal phytohaemagglutinin injection

Group of calves	14 days old	21 days old	28 days old
Experimental	4.89*	3.18	4.83*
Control	2.18	3.03	3.3

* — $p < 0.05$

On the 21st day after transport, anti-ovoalbumin IgG_1 antibody reaction in the experimental 14-day-old calves significantly exceeded the control group results ($p < 0.05$) (mean absorbance level 0.748 and 0.402, respectively). Twenty one days after transport, in the 21-day-old-calves from the experimental group the antibody reaction (in all tested classes) was significantly higher than in the control calves ($p < 0.05$). No significant differences were found between the antibody reaction in 28-day-old experimental and control animals. The serum IgG_1 level rose from 15.3 to 24 g.l^{-1} in 21-day-old calves both in the control and experimental groups. However, in 28-day-old experimental calves, the mean level of IgG1 class of immunoglobulin raised from 12 to 17 g.l^{-1}, whereas in the control calves of the same age it decreased from 25.5 to 23 g.l^{-1}.

The differences between the experimental and control groups of 14- and 21-day-old calves were not significant. However, the level of this class of immunoglobulins was higher in the experimental than in the control 14-day-old calves. On the other hand, in 21-day-old calves this tendency was the opposite. In the 28-day-old group the serum IgG_1 level was significantly higher in the control than in the experimental before and 7 days after transport ($p < 0.01$ and $p < 0.001$, respectively).

In the all examined groups, the serum IgG_2 levels were higher in the experimental, than in control calves. The IgG_2 level increased rapidly during the study in the experimental calves in every age group (from 1.9 to 3.7 g.l^{-1} before the transport to 3.4—5.7 g.l^{-1} at 21st day of the experiment). The same tendency occurred in control 21-day-old calves (the increase from 1.8 to 2.9 g.l^{-1}), but only slight changes were observed in 14- and 28-day-old control calves (the fluctuation between 1.8—2.6 g.l^{-1}). The differences between experimental and control calves were significant in the 14-day-old group on the 21st day after transport ($p < 0.05$), and in 28-day-old calves in 7th, 14th, and 21st days after transport ($p < 0.05$; $p < 0.01$ and $p < 0.01$, respectively).

The increase of serum IgM concentration was observed in all the groups examined from about 1.9—2.3 g.l^{-1} before the transport to 2—2.7 g.l^{-1} on the 21st day after transport. The IgM levels were slightly higher in experimental than control 14-day-old calves and the difference was significant on the 21st day after transport ($p < 0.05$). No significant differences were found in the serum IgM level in 21- and 28-day-old experimental and control calves.

DISCUSSION

The experimental calves under stress conditions associated with transport and adaptation have shown higher immune reactivity than the control calves. The ovoalbumin immunization, which was introduced directly after transport (within the time of elevated cortisol concentration) induced significantly stronger humoral immune response in stressed 14- and 21-day-old calves than in control animals of the same age. Older calves (28 days of age) showed similar antibody reaction in experimental and in the control group, however, before the transport serum

IgG_1 level was significantly lower in the experimental than in the control group (12 *versus* 25.5 $g.l^{-1}$, respectively). It is worth noticing that stressed 14- and 28-day-old calves showed stronger skin reactions in response to an unspecific mitogen (PHA) in comparison to the control animals. On the other hand, the skin reaction was only slightly higher in the 21-days-old experimental calves *versus* the control group. We concluded that the short-term stress associated with transport and the change of barn increased the cellular and humoral immune reactivity in calves at age of 14, 21 and 28 days. Only the 14-day-old experimental calves showed both elevated cellular and specific humoral reaction in comparison to the control calves. We suggest that such an influence of non-excessive stress may be helpful in the adaptation of 14-day-old calves to new environmental conditions, and it indicated that this age can be optimal for transporting the calves to the calf barn. On the other hand, the role of activation of immune response observed evoked by stress in the processes of adaptation in young animals remains to be elucidated.

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THE INFLUENCE OF METAL POLLUTANTS AND HERBICIDES ON THE SOD-ISOENZYME PATTERNS IN THE LIVERS OF SHEEP, FISH, AND PHEASANTS

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ABSTRACT

The present study investigates the changes in the specific activity of superoxide dismutase (SOD) and in the SOD isoenzyme patterns in the livers of sheep and fish after their exposure to metal pollutants in industrial areas and in the liver of pheasant under the conditions of acute intoxication by herbicides MCPA and ANITEN I. A significant increase in the total SOD activity was observed only in the livers of polluted sheep. In the livers of sheep the new SOD isoforms (pI 5.70; 5.30) were seen in polluted animals as well as in the livers of fish (pI 5.20; 5.10, and 4.45). The isoenzyme pattern of SOD in the pheasants' livers also showed a difference between the isoenzyme patterns in polluted and control groups. In all polluted groups, new isoenzymes were observed.

Key words: animals; isoenzyme pattern; pollutants; su-peroxide dismutase

INTRODUCTION

Animals can be exposed to toxic concentrations of metals and herbicides by the consumption of contaminated feed and water. It is known that pollutants of urban, industrial, mining, and agricultural origin can generate extremely reactive oxygen species (ROS). Living beings are protected from ROS and oxidative damage by several defence mechanisms. The primary antioxidant protection is provided by the enzymes superoxide dismutase converting of superoxide radicals to H_2O_2.SODs are metalloenzymes characterized by the metal ions at their active site. Eukaryotic organisms contain Cu, Zn-SOD and Mn-SOD (4).

The present study investigates the change in the specific activity of SOD and in the isoenzyme pattern of SOD in the livers of sheep and fish after their exposure to metal pollutants in industrial areas and in the liver of pheasants under the conditions of acute intoxication by herbicides MCPA and ANITEN I.

MATERIALS AND METHODS

Chemicals. All reagents, of the highest purity, were from Sigma, Merc and Boehringer. MCPA and ANITEN I are produced by ISTROCHEM (Bratislava, The Slovak Republic). MCPA is 2-methyl-4-chlorophenoxy-acetic acid. ANITEN I is a combined herbicide.

Animals and Diets. Ten Slovak Merino sheep divided into two groups were included in the experiment (3 years old, 44—65 kg b.w.). The control group consisted of five sheep from a nonpolluted area (C group). Another five sheep came from an industrial area polluted by heavy metals (P group). Chub *(Leuciscus cephalus)* about 5 years old were from two rivers. The control group of 12 fish came from the upstream non-polluted area of the river and another group of 12 fish was obtained from a metal-polluted urban area of the river. The pheasants *(Phasianus colchicus),* one year old, were clinically healthy. Twenty-six pheasants were divided into three groups: the control group (C), polluted group A (AI, AII, MCPA), and polluted group B (BI, BII, ANITEN I). They were fed with the standard diet KZ-Bž-n and had free access to water. MCPA and ANITEN I were administered *per os* by gastric tube (Tab. 1). At the end of experiment (24th hour), the pheasants were euthanized by jugular incision. Liver samples from the region of the *vena portae* were taken.

Table 1. Dosage of MCPA (group A) and ANITEN I (group B)

Group	Dose (mg DMA salt of MCPA.kg^{-1} b.w.)	Number
AI	700	5
AII	900	5
BI	200	5
BII	400	5

Legend: Group A (AI, AII, MCPA); group B (BI, BII, ANITEN I)

Table 2. Metal contents in the livers (mg of metal per kg body weight ± SEM)

Sheep				Fish			
Metal	C	P	P/C	Metal	C	P	P/C
Cu	0.366 ± 0.145	188.780 ± 33.701	513.66	Cu	0.010 ± 0.020	8.140 ± 1.465	814.00
Hg	0.016 ± 0.004	0.246 ± 0.106	15.37	Fe	6.400 ± 1.600	74.600 ± 13.428	11.70

Legend: C — non polluted (control) group; P — polluted group; P/C — index of total contamination
Metals were determined in the livers by atomic spectroscopy using a Varian spectrophotometer

Table 3. SOD isoenzyme patterns in tissue extract of the liver

	pI	
Group	C	P
SHEEP	6.20	6.20
	5.45	5.70
	–	5.45
	–	5.30
FISH	4.80	4.80
	4.20	4.20
	4.70	4.45
	5.00	4.70
		5.00
		5.10
		5.20

Legend: Zymograms obtained after separation by IEF in gels with an 8.0—5.0 pH gradient in sheep and in an 6.5—4.0 pH gradient in fish. C — nonpolluted area — control group; P — polluted group

Table 4. SOD isoenzyme patterns in tissue extract of the liver of pheasants

pI				
C	AI	AII	BI	BII
5.50	5.50	5.50	5.50	5.50
6.00	6.00	6.00	6.00	6.00
		6.30		6.30
6.60	6.60	6.60	6.60	6.60
	6.85			6.85
				7.00
7.10	7.10	7.10	7.10	7.10

Legend: Zymograms obtained after separation by IEF in gels with an 8.0—5.0 pH gradient. C — control group; AI, AII, BI, BII — experimental groups.

Preparation of Tissue Extractions. Livers were washed two times with cooled physiological solution, cut into pieces, and homogenized in Ultra-Turrax T-25 homogenizer to make a 25 % (w/v) homogenate in 5 mM Tris-HCl buffer, pH 7.8, containing 0.15 mol KCl, 1 mmol EDTA and 2 mmol GSH. Homogenates were centrifuged 60 minutes at 105,000 × *g using Beckman L8-60 ultracentrifuge, and the supernatants were stored at –50 °C until used for later assays. All procedures were performed at 4 °C.*

Enzyme Assays. Superoxide dismutase (EC 1.15.1.1; SOD) was measured according to Flohé and Ötting (3), at 550 nm (25 °C). Enzyme activity was expressed in U/mg or mU/mg of protein. Protein concentration was determined by the method of Bradford (2).

Statistics. The results are means ± SEM. Statistical analysis was done by Student *t-test with a significance level of* $p < 0.05$.

SOD Isoenzymes Determination. Isoelectrofocusing was carried out with Phast-system equipment (Pharmacia). pI values of the SOD isoenzymes were determined in gels with a pH gradient of 5.0—8.0 and 4.0—6.5, using isoelectric focusing calibration kits. Isoenzymes were localized on the gels by the method of Beauchamp and Fridovich (1) using nitroblue tetrazolium (NBT), riboflavin (RF), and TEMED.

RESULTS

The contents of metals were determined and used as a comprehensive index of total contaminations (Tab. 2). Table 2 shows the accumulation of Cu, Hg and Fe in the livers of polluted animals. The specific activity of SOD and the SOD isoenzymes patterns were determined in the livers of sheep and fish. A significant increase of total SOD activity was observed in the liver of polluted sheep (P: 92.875 ± 26.125 U.mg^{-1} protein; C: 52.840 ± 12.244 U.mg^{-1} protein). Polluted sheep showed a more complex Cu, Zn-SOD isoenzyme pattern than the control animals, displaying two new isoforms (Tab. 3). In polluted fish, the total SOD activity did not significantly change (C: 53.00 ± 18.00 mU.mg^{-1} protein; P: 37.00 ± 19.00 mU.mg^{-1} protein). Three new bands were found in polluted fish (Tab. 3). The specific activity of SOD show no differences between polluted and control groups, in the liver of pheasants after exposure to two types of herbicides in two different doses (C: 157.80 ± 40.990 U.mg^{-1} protein; AI: 153.020 ± 22.760 U.mg^{-1} protein; AII: 199.020 ± 43.480 U.mg^{-1} protein; BI: 106.140 ± 6.420 U.mg^{-1} protein; BII: 139.560 ± 17.310 U.mg^{-1} protein). The new isoforms were detected in all experimental groups (Tab. 4).

DISCUSSION

Although it is known, that oxidative stress induces or enhances the activities of antioxidant enzymes, numerous studies showed wide individual differences in response to various oxidative attacks (6, 8). In our experiments a significant increase in the total SOD activity was observed only in the livers of polluted sheep. Therefore, more sensitive biomarkers of oxidative damages were investigated, namely, the appearance of new isoforms of SOD (5). Eukaryotes have two major kinds of SOD, a dimeric form Cu, Zn-SOD and tetrameric form Mn-SOD (4). Cu, Zn-SOD catalyses the formation of the hydroxyl radical from hydrogen peroxide and can be oxidatively modified, yielding a more acidic protein. The oxidative origin of the new SOD bands was confirmed by the incubation of cell free extracts with different systems generating reactive oxygen species (7).

CONCLUSIONS

The differences in Cu, Zn-SOD isoenzymes between polluted and control groups leads us to propose that the induction of some isoenzymes could be useful biomarkers for monitoring environmental pollution.

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THE INFLUENCE ON THE METABOLIC ACTIVITY OF SHEEP PHAGOCYTES BY THE HERBICIDE CHLORIDAZONE *IN VIVO* AND *IN VITRO*

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ABSTRACT

The effect of the herbicide chloridazone on the metabolic activity of phagocytes (MA) was studied by the iodo-nitro-tetrazolium reductase test (INT) in both, *in vivo* and *in vitro* conditions. In the *in vivo* conditions, sheep were exposed to a single acute dose of 80 mg.kg^{-1} body weight (1/2 LD$_{50}$) and the MA of phagocytes was investigated at 6, 12, 24 h after ingestion. A significant increase in the MA of peripheral blood phagocytes was registered at 12 h ($p < 0.05$). In the *in vitro* experiment, chloridazone was tested at concentrations of 10^{-1}—10^{-6} mol.l^{-1}. At these concentrations of chloridazone, a mild non-significant decrease in the mean values of the index of MA was detected. It suggests that the effects of chloridazone on the MA of phagocytes is not direct, but secondary, resulting from this compound's mechanism of action in the organism.

Key words: chloridazone; herbicide; metabolic activity of phagocytes

INTRODUCTION

Chloridazone is a selective systemic herbicide that enables weed control in fodder beet and sugar beet. In the animal organism, chloridazone uncouples oxidative phosphorylation, and interferes with mitochondrial respiration in the liver (4). The clinical sign of acute intoxication and pathological-anatomical changes have been described by BASF Japan Ltd. (1); Legáth *et al.* (2) and Mlynarčíková *et al.* (4). Chloridazone is not mutagenic, carcinogenic, or teratogenic (5, 6), but there is no information available regarding the immunotoxic effect of this herbicide. In our study we compared the chloridazone effect on the metabolic activity of phagocytes of sheep peripheral blood in both, *in vitro* and *in vitro conditions.*

MATERIAL AND METHODS

Animals. The experiment was carried out on six, clinically healthy 8—9-mo-old sheep (Merino breed) weighing 21–29 kg. The three sheep in the treated group received Chloridazone TP added to vegetable oil orally at the dosage of 80 mg.kg^{-1} b. wt. (1/2 LD$_{50}$). Blood samplings were withdrawn from the jugular vein into 1.5% EDTA 6, 12, 24 h after chloridazone adminis-tration.

Tested pesticide. Chloridazone TP (Istrochem a. s., Bratislava) was freshly prepared before acute intoxication. The features of pesticide are indicated in Table 1 (4, 6).

For the Iodo-Nitro-Tetrazolium Reductase Test, leuko-cytes were isolated from sheep peripheral blood and a quan-titative evaluation of the tetrazolium-reductase activity of phagocytes was carried out according to Lokaj and Obur- ková (3). The results were expressed as the index of metabolic activity of phagocytes (IMA).

Statistical analysis. The Student's *t*-test was used for the statistical analysis of the results.

Table 1. The features of chloridazone

Common name	Molecular formula	Mol. wt.	CAS registry number	Activity	Field doses/ha	Toxicity in sheep LD$_{50}$ per oral (mg.kg^{-1})
Chloridazone	$C_{10}H_8ClN_3O$	221.6	1698-60-8	Herbicide	5–7.5 l 3.2–4.1 kg	160

Table 2. The values of the index of the metabolic activity (IMA) of sheep peripheral blood phagocytes after acute chloridazone intoxication

Parameter	Control	6 h	12 h	24 h
IMA ± SD	3.277 ± 0.707	3.146 ± 0.571	4.824 ± 0.597*	2.875 ± 0.651

Legend: SD — standard deviation; * — $p < 0.05$

Table 3. The values of the index of the metabolic activity of sheep peripheral blood phagocytes after exposure to different chloridazone concentrations in *in vitro* conditions

Concentration of pesticide	Control	DMSO	10^{-1}M	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M
Chloridazone	2.76 ± 1.08	2.58 ± 0.94	2.28 ± 0.95[a]	2.01 ± 0.87[a]	2.68 ± 0.99[a]	2.38 ± 0.99[a]	2.27 ± 0.84[a]	2.20 ± 1.73[a]

Legend: M — $mol.l^{-1}$; [a] — non-significant difference

RESULTS AND DISCUSSION

In the *in vivo* experiment a significant increase in the MA of peripheral blood phagocytes was registered at 12 h ($p < 0.05$) with a following decrease after 24 h post administration (Table 2). The results of the *in vitro* experiment are summarised in Table 3. At these chloridazone concentrations, a mild non-significant decrease in the mean values of the index of MA were detected.

Most pesticides have low mammalian toxicity, but attention should be dedicated to the evaluation of their possible immunotoxic effects. Nowadays there is a tendency to design *in vitro* tests revealing the risk of pesticide immunotoxicity on the different kinds of immune system cells. As issues from the presented results, there is a need to submit the differences between the *in vitro* and *in vivo* influence of chemicals. Short-term change of the IMA 12 h after administration may be connected with the mechanism of the action of chloridazone, that uncouples the oxidative phosphorylation, leading to the condition of hypoxic anoxia especially in the liver cells, in which chloridazone interferes with the mitochondrial respiration (4). It may be supposed that phagocyte mobilization was caused by hypoxic anoxia in the tissues. The effects of chloridazone on the MA of phagocytes was not direct, but secondary, thorough his hepatotoxicity, that was confirmed by the *in vitro* test, in which a significant effect of herbicide on the sheep peripheral blood phagocytes was not recorded.

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THE DETERMINATION OF THE IMMUNOTOXIC AND GENOTOXIC POTENTIAL OF FUNGICIDE DICHLOFLUANID ON SHEEP LEUKOCYTES *IN VITRO*

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ABSTRACT

Dichlofluanid is one of the most widely used fungicides and wood preservatives. Its immunotoxic effect was evaluated *in vitro* on sheep peripheral blood phagocytes by the iodo-nitro-tetrazolium reductase test (INT) at concentrations of 10^{-1}—10^{-8} mol.l^{-1}. The significant inhibitory effect of fungicide on the metabolic activity (MA) of phagocytes was registered at concentrations of 10^{-1} ($p < 0.001$) — 10^{-3} mol. l^{-1} ($p < 0.05$). The genotoxic potential of dichlofluanid was examined by micronucleus assay in sheep lymphocytes at concentrations of 10^{-4}—10^{-6} mol.l^{-1}. The significant increase in the micronucleus frequency was not observed at the concentrations tested.

Key words: dichlofluanid; metabolic activity; micro-nucleus assay; sheep

INTRODUCTION

One of the world most widely used fungicides is dichlofluanid. There is very little information available regarding its genotoxicity and immunotoxicity in livestock which may be directly exposed to pesticides in pastures. In this study an attempt has been made to evaluate the immunotoxic and genotoxic potential of dichlofluanid on the basis of its influence on the phagocyte function and induction of micronuclei in lymphocytes of sheep peripheral blood *in vitro*.

MATERIALS AND METHODS

The tested pesticide. Dichlofluanid (1,1-dichloro-N-[(di-methylamino)sulphonyl]-1-fluoro-N-phenylmethane-sulphenamide), a colourless powder, mol. wt. 333.2, purity 98 % (Supelco, U. S. A.) was dissolved in dimethylsulphoxide (DMSO, LACHEMA, Brno, CzR) and prepared at concentrations of 10^{-1}—10^{-8} mol.l^{-1}. The final concentration of DMSO in the cultures was 0.5 % for the micronucleus assay and 1 % for the Iodo-Nitro-Tetrazolium Reductase Test (INT).

For INT, blood samplings were withdrawn from the jugular vein into 1.5 % EDTA of five clinically healthy one-year-old Merino sheep. Leukocytes were isolated from sheep peripheral blood by the method of Karlson and Kaneko (3) and a quantitative evaluation of tetrazolium-reductase activity of phagocytes was carried out according to Lokaj and Oburková (4). The results were described in the form of an index of metabolic activity (IMA), based on the ratio of actual phagocytic cells (an increased metabolism) to the leukocyte suspension without stress (a basic metabolism).

The micronucleus assay. For the micronucleus assay, heparinized blood (100 IU.ml^{-1}) from four healthy donors (11-month-old female Merino sheep), obtained by jugular vein puncture, was used. Peripheral blood lymphocytes were processed by the modified method of Moorhead *et al. (5).* The induction of micronuclei was evaluated by scoring a total of 1000 binucleated and polynucleated cells per donor and per concentration value.

For the statistical evaluation of the results Student's t-test was used.

RESULTS AND DISCUSSION

A significant decrease in the IMA was registered at concentrations of 10^{-1} ($p < 0.001$) — 10^{-3} mol.l^{-1} ($p < 0.05$; Table 1).

Table 2 shows the levels of micronuclei observed in peripheral sheep lymphocytes cultured in the presence of different concentrations of dichlofluanid.

There is no information regarding the immunosuppressive effect of dichlofluanid. But after the application of fungicide for the treatment of wood furniture the cases of allergic contact skin reaction in men were described (1). The genotoxic effect of dichlofluanid has been demonstrated only on microbial test systems and HeLa S3 cells (2).

Table 1. The values of the IMA of sheep peripheral blood phagocytes in the presence of different dichlofluanid concentrations (* — p < 0.001; * — p < 0.05)**

	Control	DMSO	10^{-1}M	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Concentration of dichlofluanid	2.76 ± 1.08	2.58 ± 0.94	0.91 ± 0.14***	1.43 ± 0.52*	1.29 ± 0.24*	2.15 ± 0.37[a]	2.19 ± 0.17[a]	2.17 ± 0.14[a]	2.55 ± 0.51[a]	2.21 ± 0.02[a]

Legend: M — mol.l^{-1}; [a] — nonsignificant differences

Table 2. Micronucleus frequencies and distributions in the sheep lymphocytes of four donors after exposure to different concentrations of dichlofluanid

Concentration of dichlofluanid (mol.l^{-1})	No. of cells/ donor	Number of MN					Total MN	Micro-nuclei/ cell	Micronucleus distribution				% MN cells
		A	B	C	D	mean			0	1	2	Cells withMN	
Control	1000	10	4	20	13		11.75 ± 6.6547/4000	0.01175	3956	41	3	44	1.10
1.10^{-4}	1000	4	23	34	9		17.50 ± 13.66[a]	70/4000[a]	0.01750	3933	64	3	67 1.68
1.10^{-5}	1000	10	17	18	4		12.25 ± 6.55[a]49/4000[a]	0.01225	3954	43	3	46	1.15
1.10^{-6}	1000	10	17	26	11		16.00 ± 7.35[a]	64/4000[a]	0.01600	3940	56	4	60 1.50

Legend: MN — micronuclei; [a] — nonsignificant differences; A — donor 1; B — donor 2; C — donor 3; D — donor 4

Long-term animal studies determining the toxicological risk of pesticides are time-consuming and expensive to perform; ethical factor also play an important role. Simple, reproduceable, and reliable *in vitro* test systems for the quick screening for the immunotoxic and genotoxic potency of pesticides is therefore necessary. Our results did not reveal any genotoxic effect of dichlofluanid. On the other hand, its inhibitory effect on the metabolic activity of sheep peripheral blood *in vitro* was recorded.

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ENTEROTOXIN PRODUCTION BY *Staphylococcus aureus* IN RELATION TO FOOD HYGIENE AND ECOLOGY

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ABSTRACT

The production of staphylococcal enterotoxins A, B (SEA, SEB) was investigated in the field *S. aureus* isolates obtained from foods and food industry manufactures in several Slovak regions. The ability to synthesize enterotoxins was found in 20 (39.2 %) out of the total number of 51 isolates. Produc-tion of SEA was recorded in 3 (5.9 %), of SEB in 12 (23.5 %), and SEA with SEB in 5 (9.8 %) staphylococcal isolates. Nine (47.4 %) sheep cheese isolates produced enterotoxins, especially SEB (36.8 %). *S. aureus* isolates from pasta were enterotoxigenic in 6 cases (33.3 %).

Key words: enterotoxins; environment; food hygiene; food poisoning; *Staphylococcus aureus*

INTRODUCTION

Of the many metabolites produced by the staphylococci, enterotoxins pose the greatest risk to consumer health. These toxins are elaborated by some strains of staphylococci and if ingested can cause foodborne illness. In veterinary medicine staphylococcal enterotoxins play an important role in cases of bovine mastitis (6) as well as gangrenous mastitis in goats.

Only limited data have been presented (2) about the occurrence of enterotoxigenic *S. aureus* strains in foods in Slovakia. Thus, the aim of our study was to investigate the production of staphylococcal enterotoxins A, B (SEA, SEB) and the presence of respective staphylococcal enterotoxin genes in the field *S. aureus* isolates obtained from foods (sheep cheese, bryndza cheese, pasta, sausages) and food industry manufactures (smears of technological equipment, nasal and throat swabs of food handlers) in several Slovak regions.

MATERIAL AND METHODS

The reference strains (positive controls) used were *S. aureus* FRI 722-SEA (University of Wisconsin, USA) and *S. aureus* CCM 5757-SEB (Collection of Microorganisms, Brno, The Czech Republic). As negative controls, non-enterotoxigenic strains of *S. aureus* CCM 2351 (α-haemolysin) and *S. aureus* CCM 6188 (β-haemolysin) were used. Fifty one field *S. aureus* isolates were obtained from food samples (sheep cheese, bryndza cheese, pasta, sausages) and from food manufactures (smears of technological equipment, throat and nasal swabs from food handlers). Radioimmunoassay (RIA) was performed according to Gondoľ *et al.* (3). Oligonucleotide primers used in the PCR were described by Johnson *et al.* (4). The size of fragments was 120 bp for sea and 476 bp for seb gene.

RESULTS

The production of enterotoxins was found in 20 (39.2 %) of the total number 51 *S. aureus* isolates obtained from food samples and food-processing manufactures. The synthesis of SEA was recorded in 3 (5.9 %), SEB in 12 (23.5 %), and both SEA and SEB in 5 (9.8 %) of the staphylococcal isolates (Table 1). The largest rate

Table 1. The total number of enterotoxigenic *S. aureus* isolates

	Foods	Food processing manufactures	Total
Total number of *S. aureus* isolates	43	8	51
Number of enterotoxigenic *S. aureus*	15 (34.9 %)	5	20 (39.2 %)
Genotypes			
sea^+seb^-	3 (7.0 %)	–	3 (5.9 %)
sea^- seb^+	8 (18.6 %)	4	12 (23.5 %)
sea^+ seb^+	4(9.3 %)	1	5 (9.8 %)
sea^- seb^-	28 (65.1 %)	3	31 (60.8 %)

of enterotoxigenic *S. aureus* was found in sheep cheese (47.4 %), with prevalence of SEB (36.8 %). In the case of 18 isolates of *S. aureus* from pasta, 6 (33.3 %) were found to be enterotoxigenic. Neither synthesis nor the presence of genes coding for SEA or SEB were proved in bryndza, cheese and sausages isolates. One enterotoxi-genic isolate was obtained from the technological equipment smears, 4 isolates from the throat swabs of food handlers. No differencies in results were recorded between RIA and PCR.

DISCUSSION

We have found that the rate of enterotoxigenic *S. aureus* isolates from the total number of 51 isolates was 39.2 %. A similar occurrence of enterotoxigenic strains (36.4 %) was observed by Tsen *et al.* (5) in *S. aureus* from various foods. In comparison with literature which reports that SEA is mostly involved in outbreaks of staphylococcal food poisoning (6), the largest percentage of SEB produ-cing *S. aureus* (23.5 %) isolates was found in our study. In Slovakia, sheep cheese and bryndza cheese are considered to be traditional products, which are mostly made from unpasteurized milk and therefore they can contribute to the sources of staphylococcal enterotoxicosis. In our study the production of enterotoxins was observed in nearly 50 % (47.4 %) of *S. aureus* sheep cheese isolates with a prevalence of SEB (36.8 %). Similar results were presented by Fotta *et al.* (2) who detected enterotoxin production in 54.2 % of *S. aureus* strains from lumpy sheep cheese.

In terms of ecology, staphylococci do not compete successfully in most cases with other food bacteria. In the specific case of cheese manufacture the higher the number of staphylococci in milk used as raw material, the higher the probability that staphylococci will overcome the inhibitory environment of the milk and the competing bacterial flora (1). Salt and other environmental conditions may trigger important regulatory pathways, resulting in the decreased production of toxins. Therefore, a careful control of environmental conditions is needed, especially when evaluating toxin production in *S. aureus.*

As shown by our results, the enterotoxigenic *S. aureus* have occurred in foods and food-processing manufactures in eastern Slovakia. On that account, regular monitoring of SEs genes and estimation of SEs production is necessary to protect the health of consumers.

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THE ECOLOGICAL ASPECTS OF ENDOPARASITOSES OF GAME BIRDS IN CAGE-BREEDING SYSTEMS

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ABSTRACT

Litter samples were obtained from pheasants *(Phasianus colchicus)* and partridges *(Perdix perdix)* bred at the Game Management Center (GMC) in Rozhanovce to determine the endoparasites presented in the birds. The collection of samples was carried out monthly. Within a 7-year period (1995—2001) 1870 faecal samples of pheasants and 750 faecal samples of partridges were examined. Coprological analysis of faeces revealed the presence of *Eimeria* spp. in pheasants and partridges and five species of nematode eggs in phea-sants (*Capillaria* spp., *Syngamus trachea, Heterakis isolonche, Ascaridia* spp., and *Trichostrongylus tenuis*) and three species of nematode eggs in partridges (*Syngamus trachea, Capillaria* spp., and *Heterakis isolonche*).

Key words: cage-breeding pheasants *(Phasianus colchi-cus)* and partridges *(Perdix perdix);* coccidiosis; helmintho-ses; prevalence

INTRODUCTION

The ring neck pheasant *(Phasianus colchicus)* is a common bird of woodland habitats; they are adapted to largely unwooded areas. It is not a species native to Europe, but was introduced from Asia, probably by the Romans. Grey partridges *(Perdix perdix)* are familiar birds of open farmlands in Europe. The native pheasants and grey partridges have suffered a severe decline over the last 30 years. It is related to agricultural intensification, particularly increased pesticide use, which reduces insect abundance, crucial in the diet of young chicks. In many areas of the world, farm-breeding systems of game birds helps to increase the wild pheasant and partridge population.

In the special breeding systems of game birds, the high concentration of birds together with infectious, mycotic and parasitic diseases can cause high losses. In The Czech Republic Kotrlá *et al.* (4) found in pheasant farms 82.5 % prevalence of endoparasites. The most pathogenic species belongs to roundworms (*Syngamus trachea, Capillaria* spp., *Heterakis isolonche, Ascaridia* spp.) and coccidia (*Eimeria* spp.), which are common in wild and reared game birds and may reduce breeding success (3).

Internal parasites can be kept to a minimum by an accurate knowledge of their biological cycles, ways of transmission, and the survival of infective stages of parasites, which can help to prevent the outbreaks of the diseases. Parasites, which have an indirect life cycle, are mainly of importance in range birds and are responsible for severe losses. The purpose of the present study was to determine the most frequent endoparasites with emphasis on the special conditions in intensive cage-breeding systems where different intermediate or paratenic hosts are in high numbers.

MATERIALS AND METHODS

This study began in 1995 in order to detect the prevalence of pheasant and partridge endoparasites at GMC in Rozhanovce. Within a 7-year period (1995—2001) 1870 faecal samples of pheasants and 750 faecal samples of partridges were examined. Faecal samples from pheasants and partridges of different age categories were collected from cages and aviaries of the GMC. The collection of samples was carried out monthly.

All faecal samples were examined by a qualitative flotation method and the McMaster quantitative method according to *Manual of Veterinary Laboratory Methods* (5).

RESULTS

Of 1,870 pheasant faecal samples, 869 were positive (prevalence 46.4 %) and of 750 partridges faecal samples, 258 were positive (34.4 %).

Coprological analysis of faeces revealed the presence of *Eimeria* spp. in pheasants and partridges. In pheasant chicks up to 2-week-old prevalence of coccidiosis was 67 %, in 2—8-week-old chicks it was 71 %. In 2—8-

week-old partridge chicks the prevalence of *Eimeria* spp. was 54 %. Five species of nematode eggs in pheasants were found (*Capillaria* spp. 41.42 %, S*yngamus trachea* 43.28 %, *Heterakis isolonche* 35.76 %, *Ascaridia* spp. 13.45 %, and *Trichostrongylus tenuis* 2.12 %). Three species of nematode eggs were discovered in partridges (*Syngamus trachea* 29.97 %, *Capillaria* spp. 32.24 %, and *Heterakis isolonche* 9.82 %).

DISCUSSION

Coprological examination during our studies (1995—2001) revealed that the most frequent species infesting these cage-breeding birds are *Eimeria* spp., *Capillaria* spp., and *Syngamus trachea.*

The heavy infection of pheasants with *Syngamus trachea* can produce mortality, but significant losses have not been documented in wild situations (2). The rearing of game birds in confinement often results in the contamination of facilities and subsequent heavy tracheal worm infections. Under such conditions mortality can be rather high. In the GMC, gapeworms are a permanent danger to successful breeding.

Capillariid eggs were very frequent findings. The burrowing activity of *Capillaria* in the mucosa caused necrotic lesions and the leakage of plasma protein. If the necrosis was severe enough, diarrhoea, anorexia and cachexia developed. In our findings *Capillaria contorta* from the oesophagus and crop was confirmed. Environmental sanitation is as necessary as treatment, because *Capillaria* (*C. contorta* and *C. caudinflata*) eggs are very durable and have an indirect life cycle. The survival of infective stage is in intermediate hosts — earthworms *(Eisenia foetida, Allolobophora caliginosa)* (6).

The incidence of *Heterakis isolonche, Ascaridia galli,* and *A. compar* was not significant in pheasants; in partridges only *Heterakis isolonche* was found. In The Czech and The Slovak Republics on game-bird farms 83.6 % of pheasants and 11 % of partridges are infected. In the wild 68.5 % of game birds were infected (4).

The incidence of *Trichostrongylus tenuis* in our findings was infrequent as B o c h and S c h n e i d a-w i n d (1) noted. *Trichostrongylus tenuis* infections in The Czech and The Slovak Republics according P á v *et al.* (7) are 2.4 % of the pheasant and 6.5 % of the partridge population.

CONCLUSION

In our study we confirmed the occurrence of endoparasites of pheasants and partridges on the Game Management Center of the University of Veterinary Medicine, which reached maximum intensity of infection in Spring and late Autumn. The incidence of parasites increases in connection with the concentration of pheasants on farms. The most frequent species infecting the cage-breeding game birds were *Eimeria* spp., *Capillaria* spp., *Syngamus trachea, Heterakis isolonche* and *Ascaridia* spp.

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THE CHEMICAL COMPOSITION OF DIETARY YEAST ENRICHED WITH SELENIUM, CHROMIUM, AND ZINC

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ABSTRACT

Dietary yeasts *(Saccharomyces cerevisiae)* enriched with Se, Cr, and Zn were assessed for the content of total protein (TP), ash, raw fat, nitrogen-free extractibles (NFE), and amino acids, as well as selenium, chromium and zinc in the dry mass. The level of nutrients was high (TP 35.9—40.5 %) and the average content of bioelements reached the following: Se — 1534 mg.kg^{-1}, Zn — 8717.3 mg.kg^{-1}, and Cr — 891.1 mg.kg^{-1}.

Key words: chromium; selenium; yeast; zinc

INTRODUCTION

A variety of dietary yeast strains are produced worldwide. These are dry bakery, brewery and whey yeasts, etc. The following species are the most frequently used: *Saccharomyces cerevisiae, Torulopsis utilis,* and *Kluyveromyces marxianus* (8).

Their production, mainly from molasses, is harmful to the environment (7) and the chemical composition very diversified (2). They are an excellent source of proteins (amino acids), B group vitamins and many bioelements.

The aim of the present paper was to assess the chemical content of yeasts, *Saccharomyces cerevisiae* with incorporated bioelements (Se, Zn, and Cr).

MATERIALS AND METHODS

Bakery yeasts *(Saccharomyces cerevisiae)* were incubated in the presence of salts of Se, Zn, and Cr according to the method described by Ryszka *et al.* (4). The optimum conditions for the incorporation of selenium, zinc, and chromium were obtained at 6 or 12 % of incubated yeast content, where 2 % saccharose solution was applied and at an element concentration of 0.25 mmol and incubation time of 30 or 90 minutes. The proposed method of yeast production was characterized by a low production of liquid and solid wastes, and high biomass yields.

The biological material obtained by this method was subjected to chemical examination. Analysis of the basic parameters (dry weight, total protein, raw fibre, ash, nitrogen-free extractibles (NFE) was carried out according to standard methods (1). Amino acid content was assayed by means of a Carlo Erba Amino acid analyser. The content of selenium, zinc, and chromium in the biomass was determined by the ICP method with microwave digestion (3). The results were processed statistically using Statgraphics programme v. 5.0 package.

RESULTS

The chemical composition of 3 kinds of yeasts (D-Se, Y-Zn, Y-Cr) is shown in Table 1.

The Y-Zn yeast had the highest content of ash, total protein and fat, and the lowest content of NFE ($P < 0.05$). Y-Se yeasts had the lowest content of ash, total protein and fat, although they contained the highest amount of NFE ($P < 0.05$). The chemical composition of Y-Cr was similar to Y-Zn.

As a result of the process of yeast enrichment, the mean content of selenium in Y-Se was 1534 mg.kg^{-1} (max. 1728 mg.kg^{-1}), zinc 8717.3 mg.kg^{-1} (max. 15800 mg.kg^{-1}), and chromium 891.1 mg.kg^{-1} (max. 1112.7 mg.kg^{-1}). The analysis of amino acid content (Table 2) showed the highest concentrations for glutamic and aspartic acid and lysine and the lowest for methionine, cystine and tryptophan. The level of amino acids was the highest in Y-Cr (290.27 g.kg^{-1}) and the lowest in Y-Se (271.01 g.kg^{-1}). Significant differences were observed for proline, cystine and phenylalanine ($P < 0.05$).

Table 1. The chemical content of yeasts enriched with selenium, zinc, chromium (n = 5)

Yeast	Y-Se	Y-Zn	Y-Cr
Component (%)			
Dry matter	96.34	95.76	95.19
Crude ash	4.67[b]	6.13[a]	5.04[b]
Crude protein	35.89[b]	40.51[a]	39.71[a]
Crude fibre	trace	trace	trace
Crude fat	0.94[b]	1.21[a]	1.12
NFE	54.84[a]	47.91[b]	49.32[b]
Metabolizable Energy			
Kcal.kg^{-1}	6033.13	5970.22	6010.6
MJ.kg^{-1}	25.24	24.98	25.14
*Bioelement*s (mg.kg^{-1})			
Se	1534.0	0.52	4.23
Zn	263.4	8717.3	265.3
Cr	2.13	3.00	891.1

a—b: P < 0.05

Table 2. Amino acid content of yeasts enriched with selenium, zinc, and chromium (g.kg^{-1}) (n = 5)

Amino Acid	Y-Se	Y- Zn	Y- Cr
Asp	28.02b	30.44	31.89a
Thr	14.38	15.69	15.39
Ser	16.21	16.19	16.67
Glu	42.15	36.87	36.30
Pro	5.35b	9.67a	10.69a
Cys	6.39a	3.44b	3.34b
Gly	11.76	12.40	13.11
Ala	17.20	18.24	18.93
Val	13.33	14.98	14.48
Met	3.67	4.03	3.99
Iso	10.65	12.35	12.07
Leu	21.93b	24.19	24.71a
Tyr	8.32	6.35	6.50
Phe	16.84a	22.47b	27.80b
His	7.89	8.45	8.26
Lys	25.94	22.31	26.94
Arg	15.39a	12.18b	13.02
Try	5.63	6.57	6.29

a—b: P < 0.05

DISCUSSION AND CONCLUSIONS

Our results showed that the nutritive value of yeasts, enriched in bioelements, and their content of protein, fat, ash and energy were comparable to ordinary and brewery yeasts. The amino acid content differed slightly in the yeasts studied, particularly in the case of exogenous amino acids, namely those containing sulphur (6). It is necessary to point out to the high content of zinc, selenium, and chromium, which can substitute supplemen-tation of dietary premixes with these metals (2).

In conclusion, dietary yeasts produced by the waste-free method and enriched with selenium, zinc and chromium, could become a valuable source of proteins and essential bioelements, particularly for monogastric animals.

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THE RELATIONSHIP BETWEEN Cd AND Cu ABSORPTION IN HENS

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ABSTRACT

The aim of the present work was to study the influence of Cd and the interaction of Cd and Cu in tissues and organs of laying hens after Cd administration. Samples were determined by atomic absorption spectrometry. Laying hens were divided into two groups per six animals. In group 1, $CdCl_2$ was administered 0.3 mg.kg^{-1} b.w. Group 2 was the control. In group 1, a significant elevation of Cd ($p \leq 0.05$) levels was found mainly in the liver (9.32 mg.kg^{-1}) and kidneys (26.45 mg.kg^{-1}). The levels of Cu decreased in liver 1.63 times. The results obtained showed a significant correlation between Cd and Cu concentration.

Key words: cadmium; copper; heavy metals; hen; poultry

INTRODUCTION

Risk substances are, at the present time, a constant subject of interest. Endangerment of the environment and food contamination by extraneous substances have a negative effect on farm animal health and yield (12). Observation of changes in animal body is the focus of attention in physiological conditions, as well as after the effect of pathogenic factors. The ability of animals and plants to accumulate different types of substances was shown to be a general physiological phenomenon. The determination of the trace heavy metal content of various environmental samples including blood, natural waters, geological samples, etc. has been continuously performed by various researchers around the world (3, 6). Cadmium has been reported to produce several toxic effects in animals and man (5). Absorption and accumulation of Cd in tissues seems to be determined by a wide range of factors. The nutritional and vitamin status, age, sex (14, 11), but also complex antago-nistic interactions between cadmium and copper have been reported when determining cadmium accumulation and tissue distribution, and the role of copper status on cadmium kinetics (8).

MATERIALS AND METHODS

Twelve laying hens 10 months old, laying hybrid of Tetra SL, from an agricultural farm were included in the experiment. Hens were fed complete feed mixture for laying hens (HYD-10). Feed and water were provided *ad libitum*. The birds were divided into two groups per 6 animals. Group 2 was the control. In the experimental group, $CdCl_2$ was administered daily in water solution as follows: group 1 — 0.3 mg. kg^{-1} b.w. The feed mixture for laying hens as well as biological material obtained later were ashed in a muffle furnace for 8 h at 450 °C and the reproducibility of the method of metal determination was tested by analysing bovine liver reference material (MBH ANAL Ltd., England). Samples were analysed for the presence of Cd and Cu using an atomic absorption spectrophotometer equipped with a graphite furnace with background correction (UNICAM SOLAR 939). The operating parameters used were those recommended by the instrument manufacturer for cadmium and copper. All metal concentrations are expressed on an original matter (wet weight) basis.

The control group and experimental group (1) of laying hens (0.3 mg.kg^{-1}) were bled by jugular section after 5 months.

Statistical analysis: Data are presented as mean, standard deviation (SD), Student *t*-test and correlation analysis (r). Statistical analysis of all data was done using Microsoft Excel 7.0. Statements of statistical significance are based on a probability of $P \leq 0.05$. The results of experimental groups (1) were analysed and compared to control group. The correlation coefficients were calculated to establish correlations between concentrations of Cd and Cu in muscles and organs after 5 months Cd administration.

RESULTS AND DISCUSSION

Accumulation of Cd and Cu in the poultry body is presented in Tables 1 and 2. In our experiment, after 5 month's Cd exposure of poultry, in the experimental group, a marked significant elevation ($p \leq 0.05$) in levels

of this element in the liver and in kidneys was found due to the long-term chronic action of Cd on the poultry organs (9.33 and 26.34 mg.kg^{-1}, respectively). On the other hand in the control group the mean Cd concentra-tions were 0.13 and 0.29 mg.kg^{-1}, respectively. Cd levels in the spleen and ovary increased on average 15.1 and 21.6 times when compared with the control group. On all materials observed there was a statistically significant increase of Cd concentration with level $P \leq 0.05$. These observation correspond with several authors dealing with poultry problems (1). Bokori *et al.* after a 3-week-application of Cd in concentrations of 2.5; 10.5; 30 mg of Cd.kg^{-1} recorded an increase in all observed organs in relation to the increasing Cd concentration.

Table 1. Accumulation of Cd and Cu in poultry in the control group in mg.kg^{-1}

Material	Cd	Sd	Cu	Sd
Breast muscles	0.02	0.01	0.56	0.13
Leg muscles	0.03	0.01	1.13	0.19
Heart	0.04	0.01	3.06	0.43
Liver	0.13	0.03	4.67	0.64
Kidneys	0.29	0.02	2.17	0.36
Spleen	0.06	0.04	0.79	0.15
Ovary	0.05	0.01	0.87	0.26

Studying the interactions of Cu with Cd in the experimental group (0.3 mg of Cd.kg^{-1}) and comparing them the control group we found decreased Cu levels in experimental birds in comparison with the control group. This decrease was most significant in liver (1.63 times). The results obtained correspond with several authors who state that Cd administration influences Cu distribution in the liver and in kidneys (13). Correlation analysis of the indices observed is presented in Table 2.

Table 2. Cd and Cu levels in poultry in the experimental group in mg.kg^{-1} and correlation analysis of the observed elements

Material	Cd	Sd	Cu	Sd	Cd-Cu
Breast muscles	0.11*	0.05	0.56	0.13	0.08
Leg muscles	0.21*	0.13	0.79*	0.26	0.07
Heart	0.32*	0.05	2.82	0.74	0.11
Liver	9.32*	2.01	2.95*	0.43	0.65
Kidneys	26.45*	6.01	2.08	0.18	–0.56
Spleen	0.92*	0.21	0.78	0.23	0.69
Ovary	1.08*	0.38	0.78	0.15	0.09

* — statistically significant at $p \leq 0.05$ level compared with the control

Inter-element correlations of concentrations of Cd and Cu in the experimental group show a strong positive correlations between depositing of Cd and Cu in liver ($r = 0.65$) and in spleen ($r = 0.69$) and a negative value of correlation coefficient in kidneys ($r = -0.56$). Cadmium is a highly toxic metal, which is spread in the environment by human activities. Copper is an essential and potentially toxic element. Deficient intake results in impairment of various biological functions, but both metals are toxic when ingested in excess (15). Recent research documented and explained the interactions between Cd and Cu in the animal body (7, 10). Our results showed that apart from copper levels in the liver, heart and kidney, copper concentrations in muscle were generally low.

The present study also demonstrated, that Cu concentrations in the liver and leg muscle (2.95 and 0.79) were significantly decreased in the experimental group after cadmium application to the poultry diet in comparison to the control group. These results could indicate that the cadmium addition is probably the primary cause of important copper decreases in the chicken body. Essential elements, such as copper play an important biochemical role in the metabolism. Electro-physiological research indicates that some divalent cations, reduce the excitability of the central neurons, probably replacing a calcium ion from the binding places. Although there might be threshold limits of exposure at which toxicity may not be detected, there may be nothing at the molecular level that does not have an adverse effect (4). Thus, even a slight increase of toxic metals within the permissible level, may lead to a disbalance of essential elements. The deficiency of essential elements influences health after exposure to toxic metals and might cause the disbalance of the immune system and disease (9).

CONCLUSION

In conclusion, significant levels of Cd and Cu in liver and leg muscle were found. The study demonstrated the existence of a relation between Cd and Cu. The result obtained with experimental Cd burden characterize an adverse health effect of Cd in the organism as well the effect on dynamic changes of Cu metabolism during higher dietary Cd intake in poultry.

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AN EVALUATION OF THE EFFECTIVENESS OF THE TREATMENT OF FOOD INDUSTRY AND MUNICIPAL WASTEWATERS

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ABSTRACT

The study investigated and evaluated the effectiveness of an aerobic mechanical-biological wastewater treatment plant (WTP) with preliminary denitrification designed for the combined treatment of sewage and wastewaters produced by a milk processing plant. Pollution of raw wastewater (influent) reached 64 800 population equivalents (PE). The removal of organic substances expressed as chemical oxygen demand (CODCr) reached 75—92.0 % and biochemical oxygen demand (BOD5) ranged from 73.5 to 96.5 % which indicated the effectiveness for these two parameters. The effectiveness of the removal of ammonium nitrogen varied from 69.4 to 100 %. The limit for ammonia nitrogen in water discharged to the recipient (10 mg.l^{-1}) was not exceeded in any sample during the 12 months of our investigation. The presented data point to the importance of pre-denitrification in biological treatment of wastewaters.

Key words: aerobic treatment; dairy wastewaters; pre-denitrification, sewage

INTRODUCTION

Treatment of wastewaters is an important part of environmental protection; particularly the treatment of wastewaters from agriculture and the food industry and also sewage because these wastewaters increase the risk of both chemical pollution and still more important of microbial and parasitic conta-mination.

The Act No. 138/1973 of the Code stresses the importance of wastewater treatment. According to this act all producers of wastewaters must take measures to prevent excessive pollution of recipent water courses.

Currently, the biological treatment with activated sludge is the most frequently used method of aerobic treatment of wastewaters (1, 2). According to Horváthová (5) biological treatment is most effective for wastewaters containing mostly organic biodegradable substances.

The aim of our study was to evaluate the effectiveness of a wastewater treatment plant (WTP), treating wastewaters produced by a milk processing plant together with sewage, in relation to the potential pollution of the recipient and the preliminary denitrification stage.

MATERIAL AND METHODS

Samples of wastewater from the combined treatment of sewage and wastewaters from milk processing were collected as pooled 24-h samples over 12 months covering all important points of the treatment system and all seasons. The examination focused on the effectiveness of the treatment with regard to basic chemical and microbiological parameters.

The chemical investigation included the determination of pH, chemical oxygen demand (CODCr), biochemical oxygen demand (BOD_5), total nitrogen (Nt), ammonia nitrogen ($N\text{-}NH_4$), nitrate nitrogen ($N\text{-}NO_3$) and chlorides. The determinations were carried out according to STN 83 0540 (8) and by the methods of Horáková *et al.* (4)

The microbiological investigation consisted of determining the plate counts of psychrophilic, mesophilic, and coliform bacteria according to S T N 83 0531 (9). Before the inoculation of samples into solid and liquid media decimal dilutions of samples in sterile saline solution were prepared.

RESULTS AND DISCUSSION

The results of the chemical and microbiological examinations of samples obtained in our study were evaluated with respect to the retention time in the treatment system. According to Chudoba *et al.* (2) the retention time is an important factor in the treatment. The real retention time in the activation tank was 11 hours. The treated water then passed to the sedimentation tank where it remained for an additional 4.7 hours and was discharged through a measuring device into the recipient.

The pollution of wastewaters varied considerably throughout the year and depended on the production of wastewaters by the milk processing plant. An pollution of WTP influent was approx. 64 800 population equivalents (PE). The mean daily production of wastewaters from milk processing was 263 m^3 (BOD^5 1500 mg.l^{-1}, max. 300 mg.l^{-1} or 394.2 kg.day^{-1}).

The highest values of BOD_5 were determined in waste-water from milk processing. They varied considerably, similar to CODCr values, in relation to the quantity of processed milk. After dilution with sewage, the concen-tration of organic substances decreased considerably. BOD_5 in the influent ranged from 70 to 350 mg.l^{-1} and in the effluent between 0.3 and 53.8 mg. l^{-1}. The highest acceptable value in the effluent (29 mg. l^{-1}) was surpassed in spring and autumn.

The combining of milk-processing wastewaters with sewage was reflected in a considerable decrease in CODCr. The values of CODCr in the treated wastewater ranged between 27.9 and 210 mg.l^{-1}. The maximum accep-table value in the effluent (53 mg.l^{-1}) was surpassed in spring and summer when the effectiveness reached only 75.0 and 77.5 %, resp. On the other hand, the highest levels of $N\text{-}NH_4$ were determined in combined wastewater (19.6—21.3 mg.l^{-1}). The effluent limit for this parameter (10 mg.l^{-1}) was not exceeded during our observations. This indicates the importance of preliminary denitrification which appears a suitable solution for treatment of these types of wastewaters. According to Loehr (6), a reten-tion time of 8—11 hours is needed to achieve a conversion of ammonia in the activated sludge system, however, for wastewaters produced by rendering plants 30 h may be required.

The course of nitrification within the mechanical-biological treatment is reflected in the values of nitrate nitrogen in the treated wastewater. The limit set for ef-fluent (50 mg.l^{-1}) was exceeded in spring. The effecti-veness of its removal ranged from 30 to 85 %. This indicates considerable variations of nitrogen substances in the effluent related to the quantity of processed milk.

Results of the microbiological examination showed that the plate counts of investigated groups of bacteria decreased by 2—3 orders of magnitude.

The treated wastewater discharged to the recipient complied with the requirements of Government Decree of SR No. 242/93.

The results obtained allowed us to draw the following conclusions:

— retention time is an important factor with regard to effectiveness of the treatment and evaluation of potential interventions aimed at its improvement;

— wastewaters from milk processing considerably affect the quality of influent intended for treatment and their effect depends on the quantity of processed milk;

— preliminary denitrification supports the removal of nitrogenous substances and appears more effective in comparison with the conventional arrangement of treatment stages;

— it is important to ensure that the wastewaters discharged into the recipient do not reduce the quality of water in the recipient particularly due to the high content of suspended substances, changes in pH, substances with high oxygen demand, toxic and mutagenic substances, and the presence of pathogenic micro-organisms.

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DECOMPOSITION PROCESSES IN STORED PIG SLURRY SOLIDS TREATED WITH DIFFERENT DOSES OF NATURAL ZEOLITE

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ABSTRACT

The study was conducted on pig slurry solids separated in the first stage of pig slurry treatment with activated sludge. Two kg portions of slurry solids were treated with 1, 2, 3, and 4 % by weight of natural powder zeolite clinoptilolite and stored for 10 weeks in thermally insulated plastic bags without the access of air except for mixing and sampling after 1, 2, 3, 4, 5, 6, 7, 8, and 10 weeks of storage. The untreated control was handled in the same way. Throughout the experiment, the temperature in the core of substrates remained below 33 °C, and no positive effect of zeolite was observed. Zeolite treatment affected significantly ($P < 0.01$) the dry matter content and residue on ignition. The pH values of water extracts were the highest (6.43—6.82) in the control and lowest (5.77—5.80) in the substrates with 4 % and 3 % of zeolite for most of the experiment. The level of extractable ammonia was the lowest in the substrates with zeolite and the highest in the control. The latter indicates considerable potential of zeolite to decrease environmental pollution after the application of pig slurry solids to cropland.

Key words: ammonia; decomposition; pig slurry solids; zeolite

INTRODUCTION

The solid fraction (SF) of pig slurry is a substrate with a relatively high content of nutrients and an abundant microbial population. Because of its high content of water, it should be mixed with a sufficient amount of bulking material and composted before it is applied to cropland or else it can become a source of spreading of infections and parasites and of environmental pollution (5).

Zeolites are natural materials with a unique three-dimensional structure which allows them to adsorb water and various ions and release them gradually into the environment. They have been tested as treatments for excrements and soil, in wastewater treatment, and in other applications related to their ion-exchange properties (3, 6).

The aim of our study was to investigate the influence of different doses (1—4 % by weight) of natural powder zeolite clinoptilolite on pig slurry solids stored under the conditions that may occur in practice and present some risk to the environment.

MATERIAL AND METHODS

Experiments were carried out on 2 kg portions of SF treated with 1 %, 2 %, 3 %, and 4 % by weight of natural powder zeolite clinoptilolite (SF-1, SF-2, SF-3, SF-4) mined in Nižný Hrabovec, Slovakia (main fractions: 0.125—0.250 mm 76.9 %; 0.25—0.5 mm 10.8 %; CEC 0.77 mol.l^{-1}). The substrates were stored for 10 weeks in thermally insulated plastic bags without access to air except during mixing before sampling after 1, 2, 3, 4, 5, 6, 7, 8, and 10 weeks of storage. The untreated control (SF-C) was handled in the same way.

The temperature in the core of substrates was continuously recorded (Commeter System, Rožnov pod Radhoštěm, CzR). Chemical examination included determination of dry matter (DM, drying at 105 °C to constant weight), residue on ignition (or ash, 550 °C, 4 h), pH (10 g SF + 90 ml dist. H_2O, 3 min. mixing, filtering) and extractable ammonia ($N\text{-}NH_4^+$, 100 g SF + 500 ml H_2O, 3 min mixing, filtering, distillation).

All determinations were carried out at least in duplicate and the results presented are average values. Statistical analysis was carried out by paired Student's *t-test.*

RESULTS AND DISCUSSION

Temperature in the stored substrate is one of the most important indicators of the decomposition processes and the level of sanitation (7). The most important factors affecting its level are aeration, water content, pH, and satisfying the nutritive demands of micro-organisms. In our experiment no significant positive effect of zeolite

on temperature profile was observed. The highest temperature (32.3 °C) was reached in SF-C after 53 days of storage while in substrates with zeolite (SF-1 to SF-4) the maximum temperatures ranged from 30.3 to 32.1 °C. The temperatures recorded were not sufficient to ensure sanitation of the respective substrates.

The content of dry matter (DM) increased during the first week due to the release of some retained liquid and then decreased gradually towards the end of the experiment. This parameter was affected by zeolite which adsorbs water and is able to release all of it only at temperatures higher than those used for determination of DM (105 °C). The effect was dose-dependent. This was not advantageous in our case due to high initial water content (SF-C: 13.69 %). The final DM content reached 14.0 % in SF-C and 15.7—19.5 % in zeolite-treated substrates, which is too low for optimum decompo-sition of organic matter.

The residuum on ignition or ash (550 °C) is the inorganic portion consisting of various inorganic minerals and trace metals in the form of carbonates, bicarbonates, sulphates, phosphates, nitrites and others that should not be affected by composting (1). During composting, netto losses of organic matter occur and the proportion of ash increases. Our results indicated that the ash content was affected by zeolite ($P < 0.01$) and was dose-depen-dent. In our experiment the residue on ignition increased in the control from 8.74 % to 15.13 % in SF-1 from 9.74 % to 21.95 % and in SF-4 from 12.74 % to 41.49 % (results for SF-2 and SF-3 were between those obtained for SF-C and SF-4). Evidently, the processes leading to formation of volatile compounds were supported by zeolite and these compounds were released during mixing. This effect has been observed consistently in all our experi-ments with this material (8).

The pH values determined in water extracts were in the range suitable for composting (2). For most of the experimental period (weeks 1—8) pH level was the highest in the control (6.43—6.82) and the lowest in SF-3 and SF-4 (5.77—5.80). These values indicated anaerobic processes and higher accumulation of organic acids in zeolite-treated substrates. They may be released during mixing or serve as substrates for aerobic microorganisms. After their utilization the pH increases again (4). The conclusions drawn from the level of pH were in agreement with the results obtained for residues on ignition. The level of ammonia in water extracts (Fig. 1) of individual substrates indicated an important role of zeolite during the decomposition of organic matter. The differences compared to the control group were significant for SF-3 ($P < 0.05$) and SF-4 ($P < 0.01$). Ammonia is adsorbed to large internal surface of zeolite particles and slowly released to the environment. This may prevent environ-mental pollution of soil, water, and air due to ammonia and eventually also nitrates related to improper storage, handling, and land application of excrements.

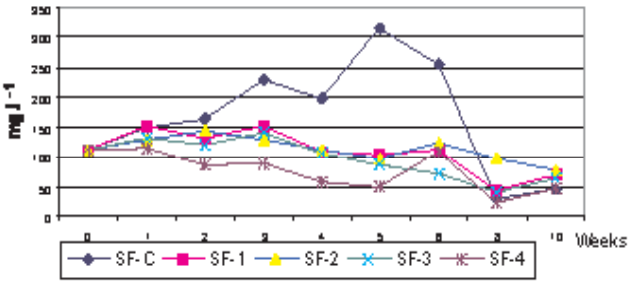

Fig. 1. The level of water-extractable ammonia in the control and in experimental substrates

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FOLIA VETERINARIA, 46, 2, Supplementum: S45—S47, 2002

THE PRODUCTION OF WATER VAPOURS AND HEAT BY LAYERS IN CONVENTIONAL CAGES

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ABSTRACT

Production of water vapours and heat was investigated in a conventional house for layers with four deck cages and a capacity for 17 000 birds. The respective values were calculated on the basis of temperature and relative humidity outside and inside the house over a 10-day period. The external temperature ranged from 6.0 to 25.0 °C and relative humidity from 46 to 90 %. The internal values of temperature and relative humidity varied between 11.0 and 25 °C and 54 and 80 %, resp. The calculated production of water vapours during 1 hour intervals ranged from 2.24 to 6.23 g.h^{-1}.kg^{-1}, the daily means ranged from 3.48 to 4.35 g.h^{-1}.kg^{-1} and the total mean was 3.91 g.h^{-1}.kg^{-1}. Pro-duction of heat in the same intervals varied within 2.66—8.74 W.h^{-1}.kg^{-1} with daily means ranging between 5.32 and 8.74 W.h^{-1}.kg^{-1} and the total mean reaching 5.78 W.h^{-1}.kg^{-1}.

Key words: caged layers; humidity; production of heat; ventilation

INTRODUCTION

Production of noxious components, buildings quality, bioclimate, and ventilation in layer halls are closely interconnected to both the productivity and welfare of layers and the economy of rearing (4). Considering this, the calculation of values for determining optimum ventilation cannot be based solely on observations in climatic chambers or hypothetical considerations but should also take into account the real conditions. Although the results of observations made under practical conditions are affected negatively by a number of difficult-to-predict factors this disadvantage is compensated for by the fact that we obtain results from a large number of birds, the ethological manifestations of which correspond to the respective technological system.

MATERIAL AND METHODS

Our investigations focused on a layer hall with the capacity for 17 000 layers housed in 4-deck cages. Over the period of 10 days, temperature and relative humidity was recorded in 2 locations inside the house in the stream of outgoing air and also outside by means of thermohygrographs. Values recommended for calculating the production of water vapours and heat at the respective ventilation allowed us to obtain information about the temperature and relative humidity of air at 1 hour intervals:

$Mwj = V_o.\Delta x$ (g.h^{-1}.kg^{-1}); $Qprj = V_o.\Delta i + Q_K$ (W.h^{-1}.kg^{-1})

where Mwj, Qprj — production of water vapours and heat per unit of body weight

V_o — ventilation in m^3.h^{-1}.kg^{-1}

Q_K — heat losses by construction

Δx, Δi – the difference in the content of water vapours (g.m^{-3}) and heat (w.m^{-3}) in the incoming and outgoing air.

RESULTS AND DISCUSSION

The range of calculated temperatures and the relative humidity of air inside and outside the house during our observations is illustrated in Fig. 1, and the respective differences in the content of water vapours and heat (Δx, Δi), calculated at 1 hour intervals, together with the level of ventilation (m^3.h^{-1}.kg^{-1}) is shown in Fig. 2.

Figures 3 and 4 present the mean values for the production of water vapours and heat at internal air temperatures ranging between 15 and 25 °C. They also show variations over the period of investigation and comparison with data of other authors.

The relatively wide range of calculated values of water vapour production of 3.99 g.h^{-1}.kg^{-1} and heat production of 4.08 W.h^{-1}.kg^{-1} can be ascribed not only to different production by layers but also to the delay in the reflection of considerable changes in the outer environment and in the bioclimate inside layer hall. However, the mean values should be considered reliable.

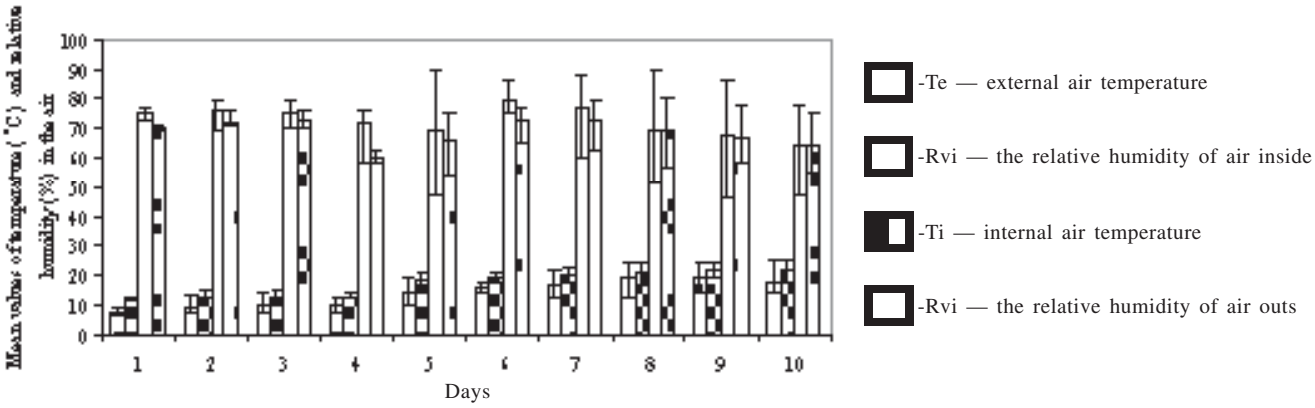

Fig. 1. Temperature-humidity levels in a hall for layers

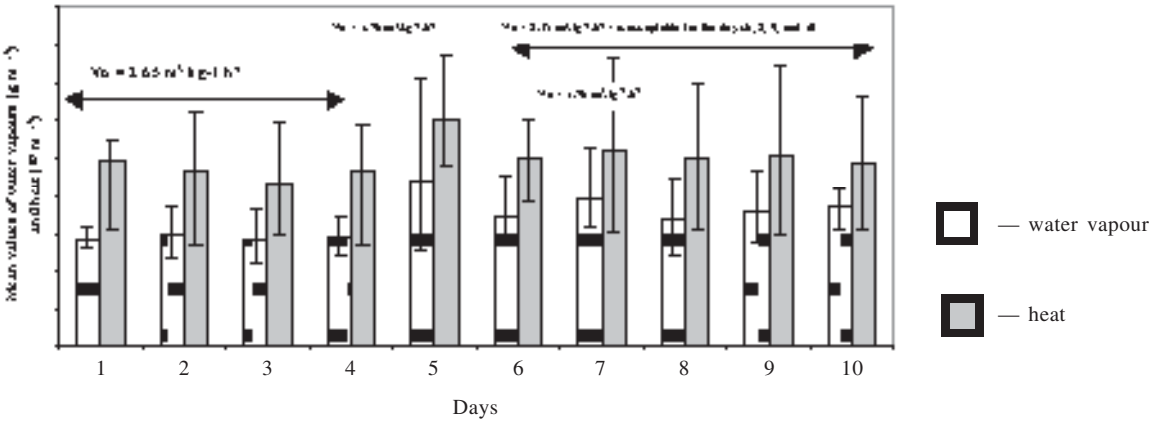

Fig. 2. Differences in temperature and humidity between the incoming and outgoing air in a hall for layers

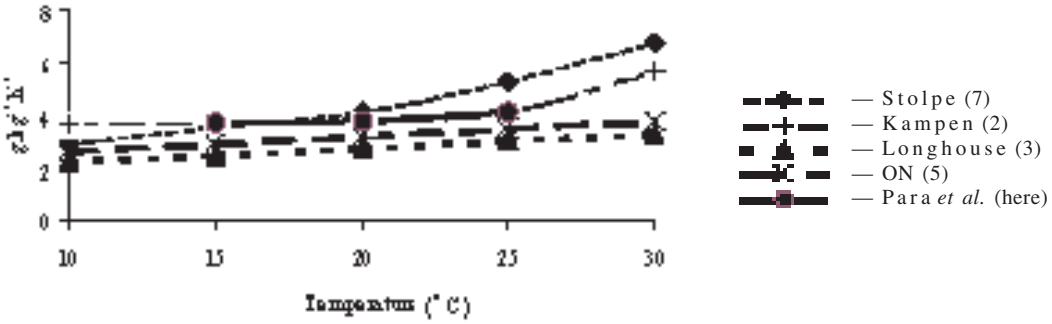

Fig. 3. The production of water vapours in a hall for layers

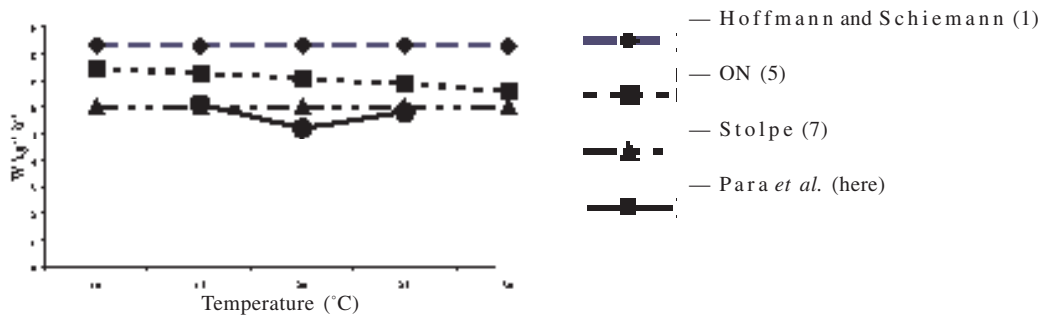

Fig. 4. The production of heat in a hall for layers

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FOLIA VETERINARIA, 46, 2, Supplementum: S48—S49, 2002

TEAT STRESS BY CALF-SUCKLING AND MACHINE-MILKING

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ABSTRACT

Our aim was to find out, by means of a thermographic method, the effect of machine-milking and calf-suckling on the teats. Generally, the teat temperature showed a significant increase ($P<0.05$) after milking and suckling. The effect of calf-suckling on the temperature of teats is dependent on age — calves in the colostrum period stressed teats significantly less than older calves ($P<0.05$), older calves stressed teats more than machine-milking ($P < 0.05$).

Key words: calf-suckling; machine-milking; teats; ther-mography

INTRODUCTION

The most stressed part of the udder are the teats. Repeated teat compressions may cause mechanical and circulatory changes in teat tissue and hyperaemia in the teat wall (3,1,6).

There are a number of factors in machine-milking that may influence the teat condition, but calf-suckling is regarded as much more "friendly" to the teats (5).

Our aim was to find out, by means of a thermographic method, the effect of machine-milking and calf-suckling on the teats.

MATERIALS AND METHODS

Three groups of animals were used in the experiment: the group 1 (machine-milking, 42.6 kPa) — 10 dairy cows; the group 2 (calves in the colostrum period, age 5 days) — 6 dairy cows +6 calves; the group 3 (calves in milk period, age 20 days) –4 dairy cows +12 calves. Thermograms of teats (the tempera-ture of teats) were obtained at the following intervals: before suckling (milking), after suckling (milking), 1—5 minutes after suckling (milking) for 2 days in every group. The values obtained were statistically evaluated by the F test and the Student *t*-test.

RESULTS AND DISCUSSION

The results are showed in Fig. 1. Generally, the teat temperature showed a significant increase ($P<0.05$) after milking and suckling. But the statistically significant differences were found between the groups. Calves in the colostrum period stressed teats significantly less than older calves ($P<0.05$), older calves stressed teats more than machine-milking ($P<0.05$).

The effect of suckling on the teats has not been explored as the effect of machine-milking, and thermographic measurements of suckling have not been published. Our results show that the suckling of calves in the milk period induces a significantly higher temperature in teats compa-red with calf-suckling in the colostrum period and machine-milking. The effect of calf-suckling on tempera-ture of teats is dependent on age. This supports the findings of Krohn (4). This author recommended only short-term suckling. Flower and Wear (2) reported similar results.

Acknowledgements

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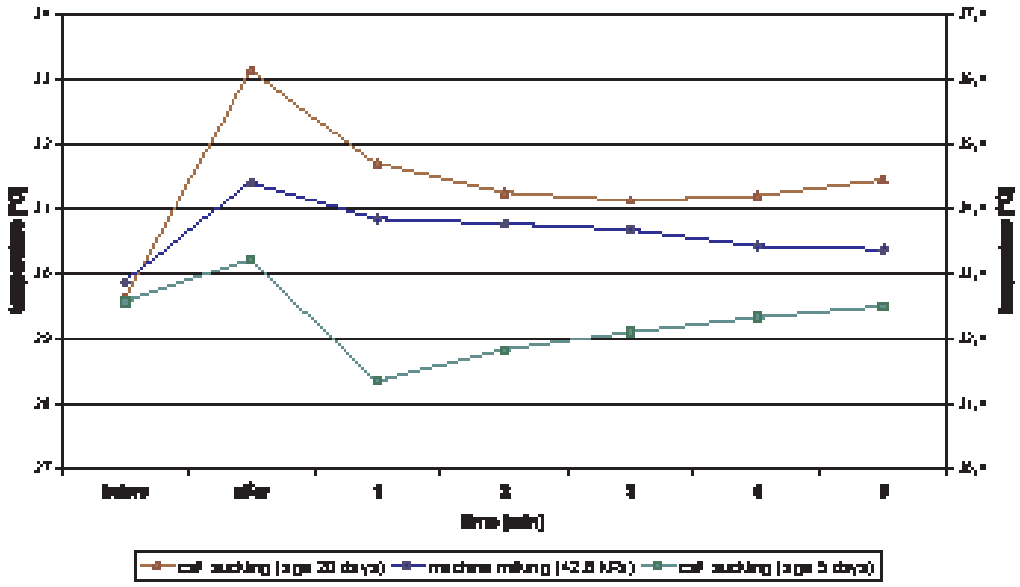

Fig. 1. The course of temperature changes in teats depending on calf-suckling and machine-milking

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FOLIA VETERINARIA, 46, 2, Supplementum: S50—S52, 2002

THE EFFECT OF A SINGLE DOSE OF WHOLE-BODY GAMMA RADIATION ON CHANGES IN SELECTED SERUM PARAMETERS OF LABORATORY RATS

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ABSTRACT

Male rats of Wistar SPF breeding were observed for the changes in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the concen-trations of glucose and cholesterol in their serum after exposing them to a single dose of whole-body gamma radia-tion of 6.0 Gy. Analyses were carried out after 6 h, 24 h, 48 h and on day 7, 14, and 21 after irradiation. The choles-terol level and the activity of ALT increased in the terminal period. In this period a significant increase in serum glucose was also observed. The activity of AST had decreased signi-ficantly after 48 h and on day 21 after irradiation.

Key words: ALT; AST; cholesterol; gamma radiation; glucose; rats

INTRODUCTION

Human and animals are constantly exposed to a range of negative environmental factors, such as chemical compounds, heavy metals, exogenous substances, radiation, and others (3, 6), that affect their health and life more or less.

All live organisms are exposed to radiation from natural sources. The use of ionizing radiation in medicine and technical applications increases their total radiation exposure due to sources that have beccame a part of the production basis and our everyday life. This points to the undeclining importance of the study of the harmful effects of ionizing radiation even in peaceful periods not only on mammals (7) but also on other animal species (2).

The present study investigated the changes in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and concentration of glucose and cholesterol in the serum of laboratory rats exposed to a single dose of whole-body gamma radiation of 6.0 Gy.

MATERIAL AND METHODS

The experiment was carried out on laboratory male rats of Wistar SPF breeding, of mean initial weight of 180 g (VELAZ Prague breeding station, CzR). The animals were put to previously disinfected (4) plexit cages with wooden shavings and kept there for one week to get used to the experimental conditions (temperature 22—24 °C, relative humidity 60—70 %, and natural lighting) (5).They had free access to water and feed. Their rations consisted of LD pellets (8).

The animals were exposed to a single dose of whole-body gamma radiation of 6.0 Gy (Chisostat, ^{60}Co-source; Chirana, The Czech Republic) directly in the adjusted plexit cages. Control rats were exposed to sham irradiation, i.e. they were handled in the same way as the experimental ones except for the irradiation.

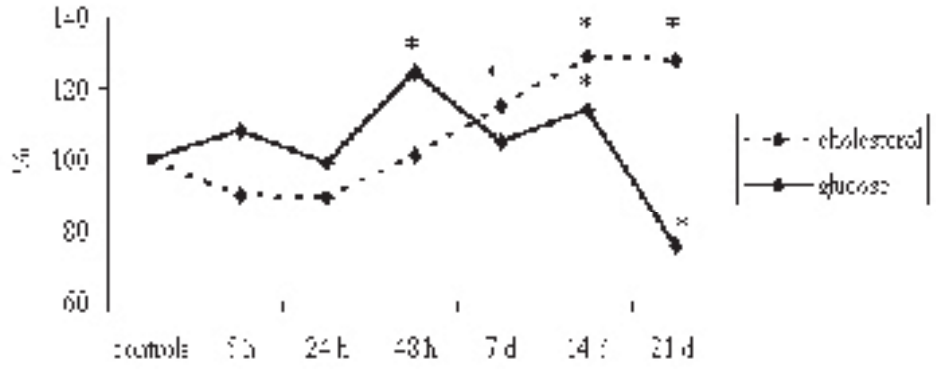
Investigations were carried out on animals killed by de-capi-tation (1) at the following intervals: 6 h, 24 h, and 48 h and on days 7, 14, and 21 after irradiation. Mixed blood was col-lected into Petri dishes. The serum obtained was used to determine the concentrations of glucose and cholesterol and the activity of ALT and AST (using Bio-la-tests LACHEMA Brno, CzR).

The statistical significance of differences between the expe-rimental and control (non-irradiated) rats was evaluated by means of the non-paired *t*-test. The experiment was carried out in winter.

RESULTS AND DISCUSSION

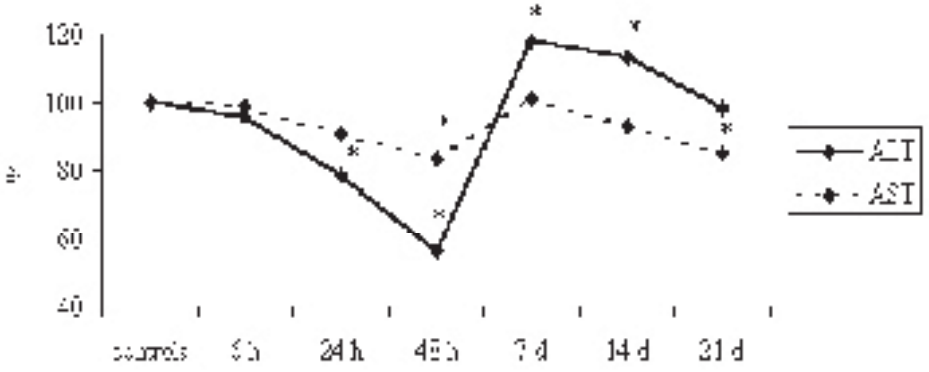
The concentration of cholesterol (Fig. 1) decreased insignificantly 6 h, 24 h, and 48 h after irradiation and increased significantly on the 7th, 14th, and 21st days after irradiation.

The concentration of glucose (Fig. 1) after irradiation showed an increase in comparison with the control



*** Differences between irradiated and control rats were significant (P < 0.05)**

Fig. 1. Changes in glucose and cholesterol concentration in rat serum after irradiation with a dose of 6.0, expressed in per cent



*** Differences between irradiated and control rats were significant (P < 0.05)**

Fig. 2. Changes in the activity of ALT and AST in rat serum after irradiation with a dose of 6.0 Gy, expressed in per cent

after 6 h and 48 h and on the 7th and 14th days. The differences were statistically significant after 48 h and 14 days. A decrease was observed after 24 h, however, the decrease was significant only on day 21 after irradiation.

The activity of ALT (Fig. 2) was decreased after 6 h, 24 h and 48 h and on day 21 after irradiation. The diffe-rences were significant only after 24 and 48 hours. A statistical increase in the activity of ALT was detected on day 7 and 14 after irradiation.

The activity of AST (Fig. 2) had increased insignifi-cantly on the 7th day after irradiation. After the remaining periods of observation the activity of AST was decreased but the differences were significant only after 48 h and on day 21 after irradiation.

The increase in glucose concentration suggested total post-irradiation hyperglycaemia associated with increased gluco-neogenesis of amino acids released from tissue proteins after irradiation. Similarly, the changes in the activity of AST and ALT probably resulted from the change in gluconeogenesis in the irradiated body as both the enzymes play an irreplaceable role in that process. This is more pronounced with ALT than with AST.

Changes in concentration of saccharides and enzymatic activity in irradiated animals are accompanied by total hyperlipaemia which raises serum cholesterol, as was also observed in our study.

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STRESS AND AMINO-ACID VALUES OF POULTRY MEAT

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ABSTRACT

The amino acids content was determined in the breast muscle of layers which were kept under experimentally simulated stress conditions and consumed feed mixture (HYD-06-KZ granulated) — original and fortified with vitamins C (Ascorbic acid 2.5 g per head and day), A (Retinol 10 000 IU per head and day), D_3 (Cholecalciferol 4 800 IU per head and day) and E (Tocopherol 40 mg per head and day) using the conventional column chromatography method with ion exchangers (AAA T 339). The results indicated that the poultry supplied with vitamins at the appropriate level were able to cope more successfully with the stress (electric current, Narcotan). The values of amino acids in poultry fed with fortified feed increased in comparison with the control. A significant increase ($p < 0.001$) was recorded for proline, arginine and glycine. Poultry fed with common feed mixture showed a decrease in the values of amino acids ($p < 0.05$) threonine, glycine, alanine, isoleucine, leucine, lysine and arginine after the exposure to stress factors.

Key words: amino-acids; poultry; stress; vitamins

INTRODUCTION

The participation of hormones in coping with a stress situation results in many metabolitic changes. Immediately after the change in the natural environment the stage of shock arises. Concentrations of circulating catecholamines, histamine, and serotonin are changed with the following increase in oxidation in the CNS and fatty acids and glucose in myocard. The production and secretion of ACTH (adenocorticotrophic hormone) rises and comes to the synthesis of the steroid hormones of the adrenal cortex (2, 4, 5). In such conditions mostly catabolic processes arise (glycogenolysis — lipolysis) and then enzyme hydrolysis of the cell proteins in organs and tissues follows.

The question of quick rearing of poultry crops up with the requirement for the increased production of poultry meat. This way of poultry meat production is closely connected with problem of limited movement and an increased load on body (1, 3). The question arises if under these stress conditions the general health of the birds is not adversely affected and if muscle matter produced in this way is really of full-value.

MATERIAL AND METHODS

The study was aimed at the analysis of the content of amino acids in the breast muscle of both poultry fed with common feed mixture and poultry supplied with mixture fortified with vitamins C, A, D_3, and E, and exposed to experimentally induced stress conditions. In the first experiment ($n = 18$), layers were fed with conventional feed mixture (HYD-06-KZ granulated). In the second experiment ($n = 20$), layers were fed with the same mixture supplemented with vitamin C (Ascor-bic acid 2.5 g per head and day), vitamin A (Retinol 10 000 IU per head and day), vitamin D_3 (Cholecalciferol 4 800 IU per head and day) and vitamin E (Tocopherol 40 mg per head and day). Layers were divided to three groups. The first was the control group ($n = 6$), the second was stressed by electric current of voltage 1250 V and intensity 16 mA. The layers of the third group were stressed by 3.5 % Narkotan. Amino acids were analysed by means of conventional column chromato-graphy using ion exchangers (AAA T 339).

RESULTS

There was no statistically important decrease in aminoacid content, in poultry fed by usual feeding mixture, when we compared control group and group stressed by an electric current even if a slight fall was recorded. A statistically significant $p < 0.05$ decrease in the values in threonine, glycine, alanine, isoleucine, leucine, lysine and arginine was recorded in group stressed by 3.5 % Narcotan compared with the control group.

In the second experiment where food of layers was fortified with vitamins C, A, $D_{3,}$ and E, all the values

of aminoacids were increased in the group of birds stressed by an electric current but also by Narcotan with the exception of essential isoleucine. A very significant increase ($p < 0.001$) in the levels of aminoacids was recorded in proline, arginine and glycine. We observed a significant increase at the level of importance ($p < 0.01$) in alanine, valine and leucine. Poultry that was fed by food supplemented with vitamins helping the body to cope with unexpected physiological overload, managed the simulated stress surprisingly well.

DISCUSSION

The stress reaction encompasses increased secretion of STH (somatotropic hormone) induced by dopaminergic stimulation (2). The biological significance of the elimination of STH during stress consists in proteo-anabolism compensating for the action of glucocorticoids (4, 5). STH increases proteosynthesis by means of which the positive nitrogen balance in an body could be documented. A decrease in the amino acid serum level occurs in parallel. Further, STH ensures the transport of amino acids into muscles and supports the production of proteins in the muscles through stimulation of RNA and DNA synthesis (5) which can explain an increase in the level of some aminoacids in the stressed poultry which consumed feed supplemented with vitamins.

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THE INFLUENCE OF RADIATION ON THE REPRODUCTION OF SHEEP

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ABSTRACT

We focused on changes in the hypothalamic neurosecretion, morphology of brain ventricle ependyma and in the sexual apparatus of sheep after irradiation, and hormonal stimulation. We observed sheep in anoestrus. Synchronization was ensured with Agelin for 10 days. On day 5 after the instillation of sponges, we started with irradiation lasting for 5 days (2.5 Gy dose) and on day 10 we stimulated the sheep with SG and FSH. The samples from hypothalamus and the sexual apparatus intended for REM, were processed according to Murakami *et al.* (4) and additional 20 sheep were kept under observation for ovulation and potential gravidity. Hormonal treatment in combination with irradiation produced qualitative changes, more marked in the ependyma than in the hypothalamus. Despite the higher number of ovulations in irradiated and hormonally stimulated sheep, the examination of viability of irradiated oocysts showed the absence of gravidity in all 20 sheep after mating. This indicated that the dose of 2.5 Gy was not harmless to the reproductive system of sheep.

Key words: Agelin; ependyma; FSH; light and REM; radiation; SG; synchronization

INTRODUCTION

The available literature contains studies that deal with the influence of radiation on the hypothalamo-hypophyseal system as the controlling mechanism of sexual functions of animals. All of them reported that histological changes occur in the irradiated organisms depending on the type of radiation, its intensity, length of application, and the total irradiation dose (2, 3, 1). The aim of our study was to observe the effects of low irradiation doses that imitate the situation in our environment in which the animals are exposed to radiation from different sources.

MATERIAL AND METHODS

The experiment was carried out on 40 Slovak Merino sheep in the anoestrus period. The sexual cycle of the sheep was synchronized with Agelin sponges (Agelin Spofa, Prague) introduced for 10 days. Sheep were irradiated with X-rays (2.5 Gy) for 5 days starting on day 5 after the introduction of sponges. On the 10th day after the insertion of Agelin sponges the sheep were stimulated hormonally with Serum Gonadotropin (Bioveta Ivanovice na Hané, CzR) and FSH (Folicotropin Spofa, Prague). Samples of the hypothalamus and sexual apparatus from 20 sheep were processed and additional 20 sheep were observed for ovulation and potential gravidity. Samples of brain and sexual apparatus intended for REM were processed according to Murakami *et al.* (4) and observed under REM Stereoscan Cambridge 2A and Jeol.

RESULTS

Our study showed that the administration of synthetic hormonal preparations in combination with irradiation caused only slight stimulation of hypothalamic structures toward higher neurosecretion but induced more marked qualitative changes, particularly at the level of the habenula. The upper third of the suprachiasmatic region of control sheep contained long cilia completely covering the apical ends of ependymal cells. Experimental sheep had only a few cilia in various locations in this region and their distribution was not uniform.

The rostral and medial parts of the lateral ventricle of experimental sheep exhibited focus-like, in some places more extensive, proliferations of ependymal cells to 2—4 layers. In some sections of the third brain ventricle, particularly in the *recessus infundibularis,* the apical surface of the cells was damaged which resulted in the disintegration of cytoplasm and the release of cellular nuclei. The radiation of sheep in combination

with their hormonal stimulation caused a slight increase in the number of ovulations but out of 20 sheep none was gravid after mating.

Detailed analysis of the damage to ovaries, caused by irradiation and subsequent hormonal stimulation, showed empty demarcated areas and a reticular structure in the cortical layer that indicated the disappearance of primary follicles. It is interesting that unchanged primary follicles were found in some places. Marked changes were observed in the nuclear membrane of oocytes in the Graaf follicle which was shrivelled and invaginated into the nucleus. Although haematomas were observed round the vessels in the yellow corpuscle tissue, both cellular types of secretory nuclear cells were preserved.

DISCUSSION

No marked morphological changes in ependyma were observed in the infundibulum region in which the processes related to sexual activity take place. The presence of a considerable number of vacuoles in the cytoplasm of still preserved ependymal cells pointed to their increased secretion activity. Observations of the influence of radiation and hormonal stimulation on ovaries indicated differences in the sensitivity of the organ itself. Haemorrhagias in the CL tissue occur due to high sensitivity of vessel endothelium. Although the irradiated and hormonally stimulated sheep exhibited higher number of ovulations, investigation of viability of the irradiated oocytes after mating showed that none of 20 sheep remained gravid which indicated that even the 2.5 Gy dose was not harmless to the reproductive system of sheep.

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METABOLIC CHANGES IN BROILER CHICKENS AFTER IRRADIATION

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ABSTRACT

Investigation of the effect of radiation on animal organisms helps us to develop methods of protection against its unfavourable influences. The presented study focused on changes in the concentration of total proteins, corticosterone, and the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum of broilers irradiated with gamma rays. The serum activity of (AST) had significantly decreased on day 5 and had significantly increased on day 30 after irradiation. The serum corticosterone had increased at all intervals, significantly on day one after irradiation. The increase in serum corticosterone can be ascribed to the stress action of ionizing radiation on the broilers.

Key words: ALT; AST; broilers; corticosterone; gamma radiation; total proteins

INTRODUCTION

The peaceful use of nuclear energy, testing of nuclear weapons, the application of ionizing radiation and radioactive substances in diagnosis and therapy, and the development of mobile communication technologies are important sources of ionizing radiation affecting not only the human population but also the environment. The study of the effect of ionizing radiation is an important task in these days. More detailed knowledge of the biological influence of radiation and the determination of its effects on individual metabolic events will enable us to develop protective measures against the adverse action of ionizing radiation and suggest suitable protective and therapeutic procedures.

MATERIAL AND METHODS

The experiment was carried out on 35 day old broiler chickens. From hatching up to day 35 they were reared in previously disinfected (5) experimental facilities (7) and supplied with feed and water *ad libitum.* The rations consisted of BR I and BR II commercial granulated feed. The broilers were handled at the Faculty of Natural Sciences of UPJŠ in Košice, directly in adjusted plexit cages, using an apparatus CHISOSTAT ^{60}Co-CHIRANA (CzR).

The 35 day old experimental broilers were exposed to a single dose whole-body gamma radiation of 4.5 Gy (output 0.295 Gy/min). Analyses were carried out on days 1, 5, 14, and 30 after irradiation. Control broilers were exposed to sham radiation, i.e. they were handled in the same way except for irradiation with gamma rays. They were killed by decapita-tion (3, 8). The mixed blood was collected in Petri dishes placed on ice and blood serum was obtained to determine the total proteins, corticosterone and the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Six birds from each group were analysed on average. The significance of differences between experimental broilers and the controls were evaluated by the non-paired *t*-test. The experiment was conducted in summer.

RESULTS AND DISCUSSION

Total serum proteins (Table 1) had decreased insignificantly on days 1, 14, and 30 and significantly on day 5 after irradiation. The serum activity of ALT decreased insignificantly on days 1, 5, and 30 and significantly on day 14 after radiation compared to the controls. The serum activity of AST had significantly decreased on day 5 and had increased on day 30 after irradiation. Serum corticosterone level showed an increase at all observation intervals. The increase was significant on day 1 after irradiation.

Buckland *et al.* (2) investigated corticosteroids in the peripheral plasma of immature White Leghorn chickens by competitive protein-binding assay. They determined corticosterone under various stress conditions. The basic corticosterone concentration in 39-day-old chickens was

Table 1. Serum concentration of total proteins, ALT, AST, and corticosterone in radiated and control chickens, ± SD

		Day 1	Day 5	Day 14	Day 30
Total proteins	radiated	27.04±1.909	33.748 ± 2.571	27.042 ± 2.327	26.86 ± 2.177
[g.l^{-1}]	controls	28.73±2.772	28.73 ± 2.76	28.79 ± 2.173	28.78 ± 2.16
ALT	radiated	0.086±0.029	0.102 ± 0.011	0.136 ± 0.017	0.091 ± 0.015
[μkat.l^{-1}]	controls	0.109±0.013	0.109±0.013	0.109±0.0121	0.108±0.012
AST	radiated	0.537±0.046	0.486±0.033*	0.591±0.087	0.774±0.020**
[μkat.l^{-1}]	controls	0.616±0.035	0.614 ± 0.035	0.634±0.026	0.630±0.026
Corticosterone	radiated	86.34 ± 38.43*	24.99 ± 13.24	37.61 ± 18.18	35.65 ± 12.86
[pmol.l^{-1}]	controls	15.73 ± 3.188	15.55 ± 2.99	16.01 ± 3.21	15.68 ± 2.85

* — $P < 0.05$, ** — $P < 0.01$

6.77 ng.ml^{-1}. The application of dexame-tazone (0.5 mg.kg^{-1} body weight) significantly decreased plasma corticosterone down to 3.95 ng.ml^{-1}. All stressors significantly increased the levels of plasma corticosteroids. The prevention of access to water caused a 2-fold increase in corticosterone to 15.87 ng.ml^{-1}, cold stress induced an increase to 16.56 and 24.34 ng.ml^{-1} (3 h/1.2 °C and 6 h/1.2 °C, resp.) and heat stress increase to 15.18 ng.ml^{-1} (6 h/37.9 °C). Sex of birds did not influence levels of plasma corticoids.

Plasma corticosterone starts to increase rapidly several minutes after irradiation. The maximum increase was observed 150 min after irradiation with lethal doses. This so-called "first increase" depends on the dose used, type of radiation, and conditions during irradiation and also on the time of the day. In comparison with the control animals, the irradiated ones show specifically higher first increase. The second increase is recorded in the period of intestinal damage after the development of radiation sickness. After irradiation with a medium dose, several additional corticosterone peaks may be observed in plasma and a lower number of them in adrenals (4).

Radiation with low and lethal doses increases transaminase activity in the liver, particularly that of alanine aminotransferase (E.C. 2.6.1.2) and aspartate aminotransferase (E.C. 2.6.1.1) (6). An increase in the activity of these enzymes indicates a prevalence of catabolic processes in irradiated bodies, associated not only with changes in cellular permeability (1), the development of serious histological changes in hepatocytes during the first three days after irradiation, but also with the stimulation of synthesis of *de novo* transamination enzymes induced by the increased secretion of the adrenal cortex after irradiation.

The investigation of enzymatic synthesis in irradiated animals provides information about physiologically important influence of ionizing radiation on proteins.

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THE OCCURRENCE OF *Listeria monocytogenes* IN RAW MILK AND ITS SURVIVAL UNDER VARIOUS STORAGE CONDITIONS

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ABSTRACT

The study is aimed at the isolation of *Listeria monocytogenes* from raw cow's and sheep's milk, cottage cheese from unpasteurized sheep's milk, and bryndza-cheese, and at the survival of the reference strain of *Listeria monocytogenes* CCM 5576 under various storage conditions. As the results show, the strain was able to survive in milk at a temperature of 70 °C for 13 minutes. The growth of *Listeria monocytogenes* was noticed within the range of pH from 4.0 to 11.0. However, at pH-values below 4.6 the growth was considerably reduced. The addition of sodium chloride in an amount of 1—7 % did not inhibit the growth of *Listeria monocytogenes*.

Key words: cheese; *Listeria monocytogenes*; milk; pH; resistance; sodium chloride; temperature

INTRODUCTION

Listeria monocytogenes is responsible for about 0.5—1.0 % of all food-borne bacterial diseases in well-developed countries. However, a high average mortality (30 %) ranks listeriosis in the first position (6) and makes this infection especially dangerous for the consumer's health (2).

Raw cow's, sheep's and goat's milk, as well as cottage cheese from sheep's milk are often contaminated with Listeria monocytogenes. These non-pasteurized products are determined for further processing, especially for the production of bryndza-cheese. The presence of *Listeria monocytogenes* has been permanently reported in this group of dairy products within the past few years, thus serving as a possible source of food-borne illness (5).

MATERIAL AND METHODS

Twenty one samples of raw cow's milk, four samples of sheep's milk, ten samples of cottage cheese from sheep's milk, and eight samples of bryndza-cheese were examined for the presence of *Listeria monocytogenes* according to the official government standard (1).

Furthermore, the survival of the reference strain of Listeria monocytogenes CCM 5576 at both temperatures of 5 °C and 70 °C, various pH-values (from 4.0 to 11.0), as well as various concentrations of sodium chloride (from 1 % to 7 %) were observed. The reference strain was also tested for its resistance to selected antibiotics and sulphonamides, as well as for its sensitivity to two disinfectants commonly used in the dairy industry.

RESULTS

The presence of *Listeria monocytogenes* was determined in four samples of raw cow's milk (19 %), two samples of sheep's milk (50 %), and one sample of cottage cheese from unpasteurized sheep's milk (10 %).

It has been found that the reference strain is able to survive in milk at a temperature of 70 °C for 13 minutes. On the other hand, the storage of milk inoculated with *Listeria monocytogenes* CCM 5576 in an amount of $10^2.ml^{-1}$ at a temperature of +5 °C for 5 days caused an average increase in *Listeria monocytogenes* counts by one radix. The growth of Listeria monocytogenes was noticed within the range of pH from 4.0 to 11.0. However, at pH-values below 4.6 the growth was considerably reduced. The addition of sodium chloride in an amount of 1—7 % did not inhibit the growth of *Listeria monocytogenes*.

As to resistance, the reference strain of *Listeria monocytogenes* was extremely sensitive to chloramphenicol and oxacillin; less sensitive to penicillin, tetra-

cycline, streptomycin, erythromycin, lincomycin, and bacitracin; and fully resistant to neomycin, polymyxin and sulphamethoxidin. Among the two disinfectants tested, MIKAL GU showed a bacteriocidic effect against *Listeria monocytogenes* in concentrations higher than 8 %. On the other hand, no such effect of MIKASEPT KP was noticed in concentrations from 0.1 % to 1.5 %.

DISCUSSION

The quality of dairy products is considerably influenced by the quality of raw milk (3). Therefore, bryndza-cheese, as the typical Slovak product made from non-pasteurized sheep's milk, can become easily contaminated with *Listeria monocytogenes* from raw sheep's milk. Both handling and storage of milk at chilling temperatures are the basic requirements in order to maintain milk quality and safety. On the other hand, chilling is accompanied by the development of psychrotrophic bacteria (4) including *Listeria monocytogenes*. Thus, its original count usually increases during the storage of milk and milk products.

Neither the decrease in pH-value due to cheese-ripening, nor the addition of sodium chloride used for the preservation of bryndza-cheese (2—3 %) and unpasteurized cottage cheese during the winter season (5—6 %) inhibit the development of *Listeria monocytogenes*. Because of these facts, the suitability and efficiency of disinfectants used in the dairy industry are of great importance.

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THERE IS A POSSIBILITY TO PROTECT THE ENVIRONMENT OF KOŠICE AGAINST THE MENACES OF MISTLETOE, VERMIN BIRDS, AND EMISSIONS

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ABSTRACT

In our report we deal with the possibility of protecting the environment of the city of Košice against white mistletoe, vermin birds, and emissions. The mutual interaction of all three factors damages mainly the trees of that environment, which are located predominantly around the highways, in the city parks and on the outskirts of the city Košice. Trees damaged by white mistletoe parasitism are not able to realize their maximum vegetation, process of growth, and the satisfactory fulfilment of the sanitary function of the environment, in particular to produce oxygen, environmental moisture, and to form shadows. Among other trees that are very sensitive are poplars, birches, willows, appletrees, and some others. The transfer of the white mistletoe from one tree to the other is, realized predominantly by such vermin birds as crows, rooks, magpies, jays, etc.

Key words: birds; emissions; environment; mistletoe; moisture; oxygen; shadow

INTRODUCTION

White mistletoe (*Viscum album,* further VA only) is a phyto-therapeutic with an effect on the heart, gastrointestinal tract (GIT), Central nervous system (CNS) and on the uro-genital system (UGS) (6,5). It contains viscatoxin, acetylcholine, choline, tyramine, some valuable amino acids, polypeptides, histones and some other agents. A healthy environment is important not only for the life of animals, and plants, but also to secure the required and optimal production and reproduction of individual species, although in both types of phyla, but mainly in the animal ones (1,3,). It is very well known e.g. that various animals can survive without food for several days (or weeks). Furthermore, some animals can survive without water for several hours (and longer). A most critical situation occurs with oxygen. It is known that higher animals are not able tolerate the absence of oxygen for longer than 2.5 min. and so they very frequently perish, and this is the reason why O_2 is so important.

The purpose of our studies was to point out the problem of endangering the animal environment and especially oxygen production by Košice's trees, thanks to the mutual interaction of VA, vermin birds, and emissions in our city.

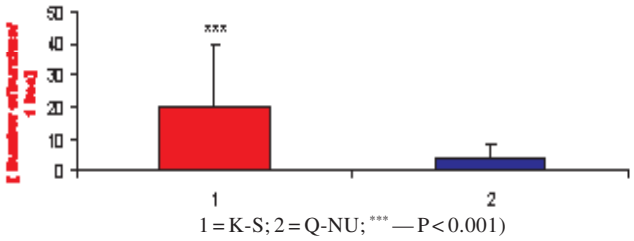
MATERIAL AND METHODS

The information about the extent and intensity of the occurrence of VA are necessary for focused pharmacological use. That is why we have investigated the frequency of its presence in the region of Košice (in two localities, A and B). We undertook our studies of the occurrence of phytotherapeutic VA we did throughout one year. However, we took their photos predominantly at the end of February and in some other cases also in June or in other seasones. Our study has been oriented predominantly for investigating its extent of occurrence as well as to obtain a picture of the influence on the environment of Košice city, and on its intensity of occurrence on the other hand. For our studies we have used a monocular telescope, an Exa-II camera, (Ihagee Dresden, Germany), as well as a Pentax camera of Japanese provenance and a 35 mm Colour Print film (ISO-100) also of Japanese provenance for documentating the state of the city.

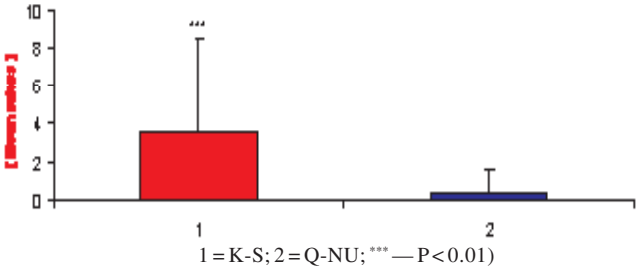
RESULTS AND DISCUSSION

The extent of VA is very great in Košice and it has reached a very serious state (Graphs 1 and 2). It is disseminated through the whole area of the city, but predominantly along main roads, than in parks, in the cementeries, as well as on the outskirts of the city. Besides its great extent, it is relatively high also in its intensity of parasitism, and it means risking the health of trees, which produce oxygen.

In the Graph 1 we introduce the occurrence of VA bunches on the trees attacked, and in the Graph 2, we introduce the means of the occurrence of VA in the total quantities of trees in Košice's territory. Veterinarians are interested in some facts connected with the VA, if



Graph 1. An overview of the occurrence of white mistletoe's bunches in the attacked deciduous trees in the localities A and B



Graph 2. Mean occurence of white mistletoe of the total number of the deciduous trees in the localities A and B

Fig. 1. *Viscum album* in the locality A

Fig. 2. *Viscum album* in the locality B

it has an influence on the production of life supporting O_2 for animal species. Under the intensive attack of VA, trees may lose their ability to produce oxygen, useful humidity of air, and the beneficial shade for animals (7, 3). We think that the environment in Košice is heavily influenced by VA and by vermin birds which may transfer VA. The emissions also have a jeopardizing effect (9).

There are several possible ways of improving the endangered environment. The best way how to improve the jeopardized environment should be connected with the regulation of vermin birds by surgery castration, together with the appropriate pruning of sick trees. It may also be useful to plant trees that are more resistant to VA and emissions. Otherwise, more trees will die and endanger the environment. We must add, that similar problems as we have, are also present in the western hemisphere of the globe. However, the difference is that in the Pacific coast apart from *Viscum album,* there are some very dangerous other species of mistletoe, e.g. *Phoradendron rubrum*, as well as *Arceutobium laricis,* and others (1, 4, 2, 8) which attack from 35 to 50 % of their forest trees.

CONCLUSION

Although we can protect the jeopardized environment by influencing various factors, we think that possibly the surgery castration of birds, cutting of sick tree branches and the reduction of emissions may be the best way to protect the jeopardized environment of Košice.

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THE ORGANIZATION OF CATTLE REARING FROM THE VIEWPOINT OF THE ECOLOGICAL FUNCTION OF AGRICULTURE

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ABSTRACT

Economic aims in food production under continually changing conditions of external and internal milieu are preferred while less care is given to the ecological function of agriculture. In this paper we demonstrate the influence of cattle rearing on the environment, the technologies utilized, and the possibilities adjusting technologies in cattle rearing to the natural behaviour of animals, preserving the natural fertility of the soil, and the contribution of cattle rearing to the production of ecological food.

Key words: cattle rearing and ecology; housing capacity; housing of animals; economical aspects in cattle rearing without market production of milk

INTRODUCTION

Cattle rearing is vitally important in providing nutrition for people and as business cattle products represent one third of total incomes. It fulfils an important function and its future development in The Slovak Republic will depend on the conditions agreed in the terms of the negotiation before acceptance by the EU.

MATERIALS AND METHOD

To prepare this paper we have used statistical data available for the years1989—2000 concerning the number of cattle, housing capacity, production and reproduction indices, and reached level of costs. On the basis of analysis, synthesis, comparison and our calculations we present our conclusions on the effect of cattle rearing on the environment, the possible adjustment of rearing technologies to the natural behaviour of animals, preserving the natural fertility of the soil and contribution of cattle rearing to the production of ecological food.

RESULTS AND DISCUSSION

From the 70s in the last century industrial ways of the rearing of farm animals with high concentration and specialization, using complex mechanization and automatization were introduced in The Slovak Republic. Traditional material like bricks, wood that together with straw bedding created the required microclimate in sheds were changed by monolithic concrete buildings and the housing of animals without bedding started to be practised.

The above-mentioned changes had an adverse influence on animal welfare, which was reflected in the impairment of the health state and life span of the animals. These changes have a negative influence on the environment as well. From the total number of houses — 11 853 with the capacity 1 956 066 heads in cattle rearing, 5 057 houses were suitable (42.7 %), 5 038 were suitable after reconstruction and modernization (42.5 %) and 1 758 houses were not suitable (14.8 %) in 1989. At present there is no survey about the technologies utilized in cattle rearing in The SR and housing spaces are used at the level of 75—80 % with big differences between regions. For example in rearing of milking cows in Košice-city region housing spaces are used in 56.0 %, in Žilina region in 101.0 %, in the rearing of young cattle in Bratislava region in 73.3 %, in Žilina region in 111.6 %, in fattening of cattle in Nitra region in 48.3 %, in Žilina region 102.0 % (1).

Majority of cows in rearing of milking cows in The SR are kept using stanchion housing and mostly with

traditional technologies of rearing characterized by high labour intensivenees. In cowsheds with stationary feeding, the time needed for one milking cow is 22—26 minutes and one worker can serve 14—16 cows. This norm is increased to 18—20 cows in cowsheds with mobile feeding. More significant increases in work productivity can be reached in free housing and milking provided in milking parlours, where the time needed for one milking cow is 10—12 minutes, one tender has 30—36 cows and with production 3 600 l, it makes 119 000 l of milk for one tender (3).

Admission to EU (without regard to the yet unsolved subsidies policy) and the opening of markets without protective measures means, for The SR achieving of the level of states in western Europe, where the time needed for milking one cow is 6 minutes and the production of milk for one tender is 360 000 l of milk a year. In terms of the reserves, which enable us to improve the ecological aspect of cattle rearing is the solving of housing, microclimate, the technological equipment of cowsheds and its adjustment to the needs of animals.

Individual categories of cattle are sensitive for change of rearing conditions. Despite the reduced production in 10—20 % free housing with bedding in rearing of milking cows and creating such technology which leads to the utilization of selfserving activity of animals with a limited influence of the environment and man on the lives of animals appears to be more suitable compared to stanchion housing (2).

From the viewpoint of ecological food production, the rearing of cows without market production of milk appears to be an interesting husbandry form. Though in The Slovak Republic it is a new production specialization, in former German Democratic Republic (DDR) it was successfully realized in the 80s of the last century. After analysing proper expenses in CzR for the year 2000, we came to the conclusion that not all natural conditions are suitable for cow rearing without the marketable production of milk. The reason lies in the different costs. This production specialization can be advantageous only if the products obtained from its implementation will be sold at the prices of ecological products.

CONCLUSION

Future development of cattle raising will depend on agreed production quotas for The SR in negotiations with EU. Present backwardness of The SR behind the level of developed countries points out at priority of economic function.

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