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FOLIA VETERINARIA, 49, 4, 2005

CONTENTS

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 49, 4: 177—181, 2005

THE LEVEL OF SELECTED BLOOD BIOCHEMICAL INDICES IN LACTATING EWES AND COMPOSITION OF MILK

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ABSTRACT

The study investigated the relationship between genotypes and some biochemical parameters in the blood and milk of ewes and lambs. It was conducted from 1999 to 2000 at the State Animal Breeding Station at Uhrusk on various crossbreds (Polish Lowland × Suffolk × Romanowska, Polish Lowland × Suffolk × Booroola). The blood for examination was sampled twice from 33 sheep and 14 lambs. Samples of milk were collected on days 15, 45 and 75 of lactation. The activity of enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and concentration of triglycerides (TG) in the blood of ewes reached an optimum level. However the total protein (TP) content and IgG in sheep blood were significantly higher than the standard values. Examination of milk showed that the level of fat and proteins increased throughout the lactation period. The mean content of proteins in milk at 15, 45 and 75 days of lactation was 4.79 %, 5.02 % and 5.56 %, respectively, and milk fat content in the respective samples reached 5.36 %, 6.45 % and 7.26 %, respectively. With prolonged lactation, lactose content decreased on average from 6.6 % to 4.56 %. The microclimate parameters measured were close to the optimum.

Key words: biochemical indices; lactating ewes; milk composition

INTRODUCTION

Measurable breeding and production outcomes of ewes are affected by genetic selection and various factors of their environment.

Housing conditions and microclimate, feeding, nursing and rearing regimens and animal hygiene are the external factors that determine animal health and productivity. Changes in even one single element of the breeding habitat often exert a negative influence on an animal's organism and can lead to stress that disturbs the organism's homeostasis and affects the processes that, in turn, determine the growth, development, health and productivity of an individual (3, 4, 8, 10, 13, 14). Metabolic disturbances that can be detected by the evaluation of particular biochemical parameters of blood, such as aspartate and alanine aminotransferase (AST and ALT), lactate dehydrogenase (LDH), triglycerides (TG), total protein (TP) and G-class immunoglobulins (IgG) are considered important in this respect (1, 3, 4, 11, 13, 14).

The chemical composition of sheep milk varies, particularly with respect to protein and fat levels (3, 4, 7, 9, 10). The nutritional value of milk is one of the most important factors in proper progeny rearing because milk is the main general food for lambs during the first two weeks of their lives (10).

The aim of our study was to evaluate the selected biochemical parameters in blood of ewes of different genotypes, and the quality of sheep milk up to 75 days of lactation.

MATERIAL AND METHODS

Tests were carried out in the period 1999—2000 in a sheep-house owned by an Experimental Farm in Uhrusk. The material consisted of blood and milk samples from 33 ewes and blood from 14 lambs. The ewes selected were crossbreds of three breeds (PLs × Suffolk × Romanov, Pls × Suffolk × Booroola), between the first and fifth lactation. They were maintained on deep bedding, which was replaced once before taking the sheep out to pasture. Feeding was different in summer and winter. The animals were regularly inspected by an animal husbandry specialist and veterinarian.

The blood for bioassays was sampled twice during the production cycle from the jugular vein to tubes containing a preservative agent and milk was sampled three times during lactation (day 15, 45 and 75).

All parameters investigated were determined in triplicate. The IgG content was determined by a radial immunodiffusion method using a diagnostic test "The binding site: sheep IgG NL RIDKIT". Total proteins, triglycerides and enzymatic activities were determined spectrophotometrically with Liquick Cormay kits. The chemical composition of milk was determined by a Milko-Scan apparatus. The results obtained were processed statistically and compared with basic reference values (15). General microclimate parameters (temperature, humidity, cooling rates, and concentrations of CO_2, NH_3 and H_2S) were determined during the experiment by direct measurements according to the method of Janowski (5).

Results were processed using Statistica software and reported as arithmetic means and extreme values (min.—max.) for each factor within the respective groups. The significance of differences was determined by Duncan's test.

RESULTS AND DISCUSSION

Environmental factors are the stimulator of many biological processes, which occur in an organism and are important for life (1, 3, 4). The process of adaptation to particular conditions is associated with a variety of metabolic and biochemical changes in a body, which are reflected in the level of biochemical indices in body fluids. Their values are determined by the species, breed, sex, age, physiological status, perinatal cycle, season, maintenance system and feeding.

In healthy animals, the determination of serum levels of biochemical indices serves exclusively for checking the interaction of environmental factors with the organism of the animals (4, 8).

Levels of selected biochemical indices in the blood serum of ewes and lamb in the period 1999—2000 are presented in Table 1. It was found that in the first year of study the mean level of AST activity in blood serum of ewes was 83.09 $U.l^{-1}$ and its values varied considerably (45.39 to 144.90 $U.l^{-1}$); in the second year the mean value of this parameter decreased by 11.11 % to 73.86 $U.l^{-1}$. Although both means were within the reference range (40—96 $U.l^{-1}$), some of the values exceeded the optimum limit by more than 30 %.

The mean AST activity in lambs was 85.26 $U.l^{-1}$, therefore in the optimum range. It was observed that enzymatic activity decreased with age.

The results obtained in our study did not confirm those presented by Brzostowski (3) who reported that AST activity clearly exceeded the physiological range during lactation. However, they are consistent with the results of Baranowski *et al.* (1). Single cases showing excessive levels of AST indicate more intensive metabolism and functional load on the liver (2, 12).

The mean ALT activity in lambs reached 19.74 $U.l^{-1}$ and was higher than that in ewes. The differences were small in other samples, and the mean values for the two groups of ewes were 14.67 and 15.62 $U.l^{-1}$ The optimum range of this parameter is 5—17 $U.l^{-1}$. The slightly higher activity of ALT in the lambs could be ascribed to intensive feeding because the quantity and type of fodder can influence ALT activity (1, 2, 4, 7). The level of these parameters can determine the direction and intensity of nitrogen transformation (12). Changes in activities of AST and ALT point indirectly to the disturbances of protein transformation in the liver. It was observed that these activities increased proportionally to protein percentage in the fodder and depended to a slight degree on an animal's dairy efficiency as was confirmed also by our results (1, 2, 3, 11, 14).

The reference range for LDH activity is 504—1049

Table 1. The level of selected biochemical indicators in the blood of ewes and lambs

Category/year	No. of samples		Biochemical parameter					
			AST ($U.l^{-1}$)	ALT ($U.l^{-1}$)	LDH ($U.l^{-1}$)	TG ($mmol.l^{-1}$)	IgG ($g.l^{-1}$)	TP ($g.l^{-1}$)
Lambs/1999	42	Mean	85.26	19.74	371.52	0.70	27.70	ND
		min.—max.	68.09—135	10.47—41.32	256.30—531.50	0.30—1.40	18.20—46.20	ND
Ewes/1999	66	Mean	83.09	14.67	265.58	0.39	22.60	ND
		min.—max.	45.39—144.90	6.98—25.60	181.10—353.40	0.10—0.80	14.60—43.00	ND
Ewes/2000	33	Mean	73.86	15.62	517.06	0.30	ND	74.03
		min.—max.	40.74—131.50	5.24—29.10	310.30—831.00	0.20—0.60	ND	61.30—105.5
Reference values according to (15)		Min.—max.	40—96	5—17	504—1049	0.10—0.30	22-39 lambs	65—79 ewes

ND — not determined

Table 2. The mean level of protein, fat and lactose in sheep's milk in the three lactation stages

Year	Day of lactation								
	15			45			75		
	TP (%)	Fat (%)	Lactose (%)	TP (%)	Fat (%)	Lactose (%)	TP (%)	Fat (%)	Lactose (%)
1999	4.93	5.51	5.59	5.30	6.50	4.72	5.62	8.36	4.44
2000	4.65	5.21	5.11	4.73	6.41	4.68	5.49	6.17	4.67
Mean	4.79	5.36	5.35	5.02	6.45	4.70	5.56	7.26	4.56

U.l^{-1}. Our results approached the lower limit or were even lower, particularly in ewes in the first year of study. The mean value in lambs in the first year of study was 371.52 U.l^{-1}; the mean in ewes in the second year was by 94.69 % higher than in the first one and amounted to 517.06 U.l^{-1}. The increase in enzymatic activity is a very useful marker that reflects liver functional changes (1, 2, 4, 12). Injuries to liver cells result in the increased permeability of cellular membranes and increase in blood levels of enzymes along with an increase in enzymatic activity of damaged liver cells. In addition to that, *mastitis subclinica* may be one of the reasons of increased activity of enzymes. Therefore, the results of LDH activity obtained in our study indicate good condition of ewes and the udder.

Triglyceride (TG) concentration gives information about the transformation of fat in an organism (1, 2, 4, 9, 11, 12). Mean TG level in ewes (0.39 mmol.l^{-1}) was higher in the first year of study (1999) when it exceeded by the reference values. In the second year (2000), it decreased to 0.30 mmol.l^{-1} approaching the upper limit of tolerance. TG level exceeding the upper reference limit was recorded only in single cases in lambs and ewes.

It could be assumed that the rather high TG concentrations resulted from high-energy fodder as suggested also by other authors (12). Immunoglobulins that play the role of antibodies, activate the lysozyme complement, neutralize toxins and inhibit the spreading of some infections. They belong among the most important elements of non-specific immunity (4, 8, 11, 13, 14). Furthermore, IgGs are most sensitive towards the changes in the breeding environment, such as feeding and maintenance conditions.

IgG levels were determined at the first year of study. Its mean value was 22.60 g.l^{-1} in ewes and 27.70 g.l^{-1} in lambs (reference range: 22—39 g.l^{-1}). Considerably wide range was found. The lowest values were below the lower tolerance limit and maximum ones exceeded the upper tolerance limit by more than 100 %. High IgG level indicates good status of the immune system. Grazing of ewes and lambs in the open air and exposure to natural light activates the immune system to produce immune bodies (13, 14). Our assumption is that high IgG levels in our study were associated with the pasture system.

Blood serum total protein (TP) is one of the markers of good animal condition and metabolic function (1, 2, 3, 4, 8, 11). Its mean value was 74.03 g.l^{-1} which is within the reference range (65—79 g.l^{-1}). The lowest value was 61.30 g.l^{-1} which was below the reference range while the maximum one (105.50 g.l^{-1}) exceeded the upper limit by 133.54 %. The results obtained point to a high metabolic rate in the investigated animals.

Basic milk components were determined in three lactation periods. The results are presented in Table 2. The chemical composition of sheep milk is determined by many factors, such as breed, individual traits, feeding and indirectly also the length and sequence of lactations, number of born and nursed lambs, ewe's health (particularly its udder), mammary gland structure, and stress (3, 10). Every component, particularly fat level, varies considerably (10). The cited author observed that fat level increased during milking and lactation. Lactose level decreased along with progressing lactation. However, total protein remained at relatively constant level.

In the first year (1999) of our study, protein concentration increased from 4.93 % on day 15, through 5.30 % on day 45 up to 5.62 % on day 75 of lactation. In the second year (2000), the mean protein level was lower by about 0.40 % compared to the previous one, but also increased with the length of lactation. Protein level changed from 4.65 % through 4.73 % up to 5.49 % in different stages of lactation. Protein concentration increased by 16.07 %, on average, with progressing lactation. An increase in fat content over the lactation period was recorded. Milk fat showed the highest variability compared to other components which was in agreement with other authors (6, 10). In 1999, fat level reached 5.51 %, 6.50 % and 8.36 % on days 15, 45 and 75 of lactation, resp. In the following year, the mean milk fat content was 5.93 % which was by about 0.40 % lower compared to the previous year. A decrease from 5.21 % to 6.17 % was recorded between day 15 and 75 of lactation.

Lactose levels decreased throughout the lactation from 5.59 % through 4.72 % down to 4.44 % in the respective lactation stages in 1999. In 2000, its level also showed a decreasing tendency (5.11 %, 4.68 % and 4.67 %, on days 15, 45 and 75, respectively). The mean lactose level for the two years showed a decrease by about 14.77 %. The results obtained in our study differ from those reported by other authors (9). Mroczkowski (10) has observed a decrease in daily milk yield, fat, protein and lactose levels with progressing lactation. A maximum in these parameters was recorded during the first month of lamb rearing. He has also observed that traits related to milkability showed great individual variations. Lipecka *et al.* (9) have conducted studies on ewes with

Table 3. Estimate of microclimate factors in the sheephouse

Measurement No.	Microclimate parameters: Temperature (°C)	Relative humidity (%)	Cooling (W/m^2)	Gases: CO_2 (%)	Gases: NH_3 (ppm)	Gases: H_2S (ppm)	Illumination coefficient (W:F)
1	10.2	85	258	0.17	7	2	
2	10.4	83	262	0.20	9	5	
3	12.8	77	258	0.22	12	4	
4	12.7	78	252	0.21	13	3	
5	13.5	76	266	0.26	14	4	
6	14.0	74	253	0.27	5	3	1:17
7	10.5	84	264	0.23	7	2	
8	10.7	82	256	0.25	9	4	
9	11.3	80	260	0.24	11	3	
10	11.6	78	258	0.28	13	1	
11	13.6	76	249	0.25	10	2	
12	13.8	75	256	0.24	11	4	

prolific breed ram hybrids and observed that fat, protein and lactose levels in hybrid's milk changed depending on the genotype in particular lactation periods. Milk fat content persisted at a high level till day 14 of lactation (5.9 %—6.4 %) decreased by day 28 in all genetic groups (5.2 %—5.6 %) and then increased gradually ranging between 6.0 % and 8.5 % on day 70.

Protein content in all groups investigated in our study remained in the range 4.6—4.7 % till day 28 and then increased slowly during the study.

The microclimate in confined housings depends not only on macroclimate factors, but also on breeding technology, technical state of a building, performance of animals and affects animal health and productivity (3, 4, 6). According to other authors (6), temperature, relative humidity and cooling values are important environmental factors in animal rearing.

During the study, temperature ranged from 10 °C to 14 °C at relative air humidity from 75 % up to 85 %. The mean cooling value in the sheep-house was 258 $W.m^{-2}$, and slightly exceeded the standard. Concentrations of noxious gases were as follows: 0.23 % CO_2, 10 ppm NH_3, 3 ppm H_2S, all below the maximum acceptable values. Natural illumination coefficient, presented as the ratio of window to floor area was 1:17 and also complied with recommendation (Table 3) (5).

Other authors (4, 12) have reported that technology systems unsuitable to physiological needs of animals caused a reduction in nutrient availability and consequently, increased incidence of various diseases and productivity and economic losses. Therefore, constant monitoring of animal's metabolic indices is a necessary element of prophylaxis and breeding procedures.

CONCLUSIONS

1. The activities of evaluated enzymes, asparaginate and alanine aminotransferase, lactic dehydrogenase and triglyceride, reached optimum values recommended for ewes and their progeny.

2. The content of IgG in blood serum of ewes and lambs in the first year of study was in a wide range: from 18.20 $g.l^{-1}$ to 46.20 $g.l^{-1}$ for lambs and 14.60 $g.l^{-1}$ to 43.00 $g.l^{-1}$ for ewes.

3. The total protein level in sheep blood was high, which indicated good conditions.

4. The mean values in milk were the following: protein content 5.12 %, fat 6.35 %, and lactose 4.87 %. Protein and fat levels increased, and lactose content decreased with progressing lactation.

5. The microclimate indices in the respective sheep-house complied with animal hygiene standards.

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THE DETECTION OF CHROMOSOME ABERRATIONS BY THE FISH METHOD IN BOVINE PERIPHERAL LYMPHOCYTES AFTER *in vitro* GLYPHOSATE-BASED HERBICIDE EXPOSURE

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ABSTRACT

Glyphosate is a broad spectrum, non-selective herbicide, widely applied in agriculture. In our work, bovine whole chromosome 1 painting probe was hybridized with metaphase plates of two healthy bull donors after 24 hour exposure to glyphosate-based herbicide *in vitro*. Aneuploidy of bovine chromosome 1, such as monosomy (2n - 1) and trisomy (2n + 1) were shown in bovine cultures after treatment with a dose of 56 µmol.$^{l-1}$ ($p < 0.001$). Polyploidy (4 n) was a further type of significantly increased numerical aberration ($p < 0.001$), induced after exposure to glyphosate product at this concentration.

Key words: cattle; chromosomal aberrations; fluorescence in situ hybridization; glyphosate; painting probes

INTRODUCTION

Glyphosate herbicide use has grown particularly since 1998 because of the introduction of crops, which are genetically engineered to be tolerant of the herbicide. It is approved throughout Europe for a range of agricultural uses, including pre-emergence and pre-plant applications to various vegetables and arable crops, directed application to weeds in orchards and vineyards, and pre-harvest application in pulses, oilseeds and cereals (9).

It is well documented that surfactants which help glyphosate (active ingredient) to penetrate plant cells, and which are often part of inert ingredients in glyphosate-containing products, are more acutely toxic than glyphosate itself. As far as the health consequences are concerned, some of these patterns of glyphosate can lead to detectable residues (9, 1, 4), which may thus enter the food chain (18). Several studies have demonstrated that glyphosate-based formulations exposure is associated with reproductive problems in laboratory animals (25, 26), such as an overloading of the maternal and foetal antioxidant defense systems in pregnant rats (2).

Results from genotoxicity studies of glyphosate have been conflicting (6). Glyphosate did not show any genotoxic activity in a battery of assays (7, 8). However, other studies have observed that glyphosate treatment of human lymphocytes *in vitro* resulted in increased sister chromatid exchanges (3), chromosomal aberrations (12) and indicators of oxidative stress (13).

Cattle are known as a very sensitive animal species to the exposure to various environmental pollutants (22). As demonstrated in several studies (19, 20, 5), cytogenetic analysis of bovine peripheral lymphocytes is a useful tool for the estimation of the exposure of cattle to environmental pollution. In contrast to a conventional cytogenetic method, the development of new DNA fluorescently labelled probes allows the visualisation of a very specific part of a chromosome (telomere, centromere) or the whole specific chromosome, which is involved in different types of chromosomal aberrations. Fluorescence the in situ hybridization (FISH) technique using whole chromosome painting probes (WCPs) is a sensitive method for detecting chromosomal rearrangements, particularly stable chromosomal aberrations (such as translocations and inversions), which do not

result in the loss of chromosome material and which should, therefore, be heritable.

At present, there is no information available about the evaluation of a possible glyphosate-based formulations effect on chromosomal aberrations induction by means of chromosome painting. The aim of the present study was to examine bovine chromosome 1 aberrations in bovine peripheral lymphocytes after a 24 hour glyphosate-based herbicide treatment *in vitro*, using fluorescent-labelled whole chromosome painting probes.

MATERIALS AND METHODS

In the experiment, peripheral blood from two clinically healthy bull donors (Slovak spotted cattle, 6 to 8 months old) was used. The whole blood specimens were cultivated for 72 hours at 38 °C in 5 ml of RPMI 1640 medium supplemented with L-glutamine and 15 mmol.l^{-1} HEPES (Sigma, St. Louis, MO, USA), 15 % foetal calf serum (Sigma, Chemical Co. St. Louis, MO, USA), antibiotics (penicillin 250 U.ml^{-1}, Biotika, Slovenská Ľupča, The Slovak Republic and streptomycin 250 µg.ml^{-1}, Antibiotic Co., Bulgaria) and phytohaemagglutinin (PHA, 180 µg.ml^{-1} Welcome, Dartford, England).

Isopropylamine salt of glyphosate (approximate 62 % by weight, with 38 % inert ingredients-composition not specified) (Monsanto, Antwerp, Belgium) was dissolved in sterile water and added to the lymphocyte cultures for the last 24 hours at concentrations of 28, 56, 140, 280, 560 and 1120 µmol.l^{-1}as reported in a previous study (23). The herbicide dose levels were chosen taking into account the highest doses for testing causing a reduction in the mitotic index (MI) > 50 %.

The slides with metaphase cells were prepared by the standard cytogenetic method. Fluorescence in situ hybridization technique (FISH) was performed for the detection of chromosome aberrations. A spectrum orange-labelled whole chromosome painting probe, specific for the bovine chromosome 1 (prepared in Veterinary Research Institute, Brno, The Czech Republic) was used for hybridization. The painting probe in hybridization mixture (50 % formamide, 2 × SSC, 10 % dextran sulphate, salmon sperm DNA, competitor DNA) was denatured at 72 °C for 10 min and reannealed at 37 °C for 80 minutes. The denaturation of slides was performed in 70 % formamide, 2 × SSC (pH 7.0) at 72 °C for two minutes and following by dehydration procedure (70, 90, 96 % ethanol, -20 °C). After overnight hybridization at 37 °C, the slides were washed in 50 % formamide, 2 × SSC (pH 7.0) at 42 °C, in 0.1 SSC (pH 7.0) at 42 °C and in TNT (Tris-NaCl-Tween 20 buffer, pH 7.0) at 42 °C. The slides were counterstained in DAPI/Antifade (4´, 6´-diamino-2-fenolindol, Q-BIOgene, Middlesex, UK).

A fluorescent microscope Nikon Labophot 2A/2, equipped with dual band pass filter FITC/TRITC was used for probe visualisation. Chromosome aberrations were scored according to Tucker *et al.* (24) and recorded by means of Nikon digital camera (Coolpix 4500, Nikon). The statistical analysis of results was performed using a chi-square test.

RESULTS

The frequencies of chromosome aberrations evaluated in bovine cultured lymphocytes after 24 hours exposure to glyphosate-based herbicide by means of bovine chromosome 1 painting probe are shown in Table 1.

Acentric fragments of chromosome 1 were the most common type of structural chromatid-type of aberrations, but the level of aberrations was not statistically significant increased. Translocations and inversions (stable chromosomal aberrations) were not observed under the conditions of our experiment.

Aneuploidy of bovine chromosome 1, such as monosomy (2n - 1) and trisomy (2n + 1) were shown in bovine cultures after treatment with a dose of 56 µmol.l^{-1} only ($p < 0.001$). A further statistically significant increased type of numerical aberration ($p < 0.001$), induced after exposure to glyphosate product at a concentration of 56 µmol.l^{-1}, was polyploidy (4n). At higher concentrations of product tested (140—1120 µmol.l^{-1}), inhibition in mitotic activity was observed.

DISCUSSION

In our study, a whole chromosome-painting probe for the largest bovine chromosome (BTA 1) was applied to evaluate the involvement of chromosome 1 in the formation of chromosomal aberrations. It is remarkable

Table 1. The frequencies of chromosome aberrations in bovine peripheral lymphocytes after 24 h glyphosate-based herbicide treatment evaluated *in vitro* by means of bovine chromosome 1 painting (% Mean ± SD)

Herbicide concentration (µmol.l-1)	Number of analyzed metaphases	Chromosome 1 aneuploidy (2n ± 1)	Polyploidy (4 n)	Chromatid-type of aberrations
Control	500	0.4 ± 0.06	0.8 ± 0.09	0.4 ± 0.06
28	500	1.4 ± 0.12	1.2 ± 0.11	1.0 ± 0.10
56	500	3.4 ± 0.18***	4.6 ± 0.21***	0.8 ± 0.89
140	350	0.3 ± 0.05	2.3 ± 0.15	0.6 ± 0.07
280	320	0.3 ± 0.06	1.3 ± 0.10	0.9 ± 0.10
560	220	1.4 ± 0.10	0.9 ± 0.09	1.4 ± 0.10
1120	180	0.0	0.6 ± 0.07	0.0

*** — Statistical significance ($P < 0.001$) according to χ^2 test

that on this chromosome, the loci responsible for some serious hereditary diseases have recently been mapped (16, 11).

As was shown in humans, the distribution of radiation as well as chemically induced aberrations among chromosomes did not appear to be random. If chromosome specific sensitivities exist, knowing their order is important and also if such sensitivities are the same for different aneugens (21). In contrast to human chromosomes, almost no information is available both about target breakage chromosomal regions and about possible non-random distribution of chemically induced aberrations among farm animal chromosomes.

The background frequencies of stable structural chromosomal aberrations in cattle had been only recently established (17). The findings of these authors also suggest that cattle have a reduced sensitivity to chromosomal mechanisms, which can cause structural chromosomal aberrations.

Stable aberrations, which are well known as good indicators of exposure to ionic radiation or clastogen, have not been detected under conditions of our experiment. This might be probably explained by the relatively low proportion of the painted genome in cattle examined by means of bovine probe available as well as relatively small numbers of metaphases for each herbicide concen tration. Marshall and Obe (15) have demonstrated, that the stable aberrations are seen at only relatively low frequencies even after treatment with very potent clastogens at dose levels, which give large increases in chromosomal damage.

Our results have shown that induction of chromatide-type aberrations by glyphosate herbicide are similar to those documented Šiviková and Dianovský (23). Using conventional chromosomal analysis, the authors did not observe a significantly increased level of structural chromosome aberrations (breaks) after 24 hours exposure to the same glyphosate-based product.

An interesting finding of this study is bovine chromosome 1 aneuploidy after 24 hours herbicide treatment, which is not easy to detect in conventional stained metaphases. We have observed aneuploidy after treatment with 56 $\mu mol.l^{-1}$ of herbicide, but not at higher concentrations. One of the possible explanations of this could be the decreased mitotic index in exposed cultures with a subsequent impossibility to examine sufficient number of metaphases.

In general, aneuploidy in somatic cells is associated with the development of several cancers. According to Russell *et al.* (21) genotoxicity studies of aneuploidy may potentially produce different results depending on the chromosome selected for analysis if chromosome-specific sensitivities to chemical exposure exist. Our results may indicate that technical glyphosate-based product tested have probably aneugenic properties. It is also important, that one of the possible targets of aneugens are molecules involved in cell cycle control (10), which has been reported in the case of various commercial glyphosate products (14). Glyphosate formulations affected the cell division at the level of CDK1/cyclin B activation that is a universal regulator of the G2/M transition of the cell cycle.

In conclusion, the data presented here show that the FISH-WCP method seems to be a valuable tool for examining farm animal chromosomes after exposure to xenobiotics.

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FATTY ACID ABSORPTION ACROSS THE RUMEN EPITHELIUM IN SHEEP AFTER HORMONAL INFLUENCE UNDER *in vitro* CONDITIONS

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ABSTRACT

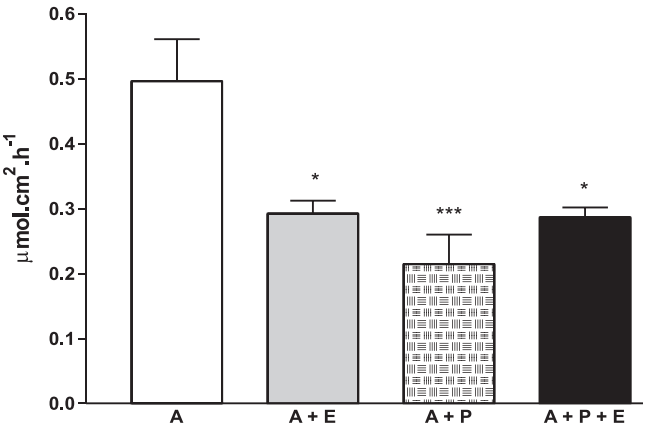
Estradiol (E) is a steroid female sexual hormone derived from acetate and also related to some fatty acids. It was the aim of the experiment to investigate the effect of estradiol on the absorption of acetate (A – 50 mmol.l^{-1} of Thyrode's solution – TS) and propionate (P – 15 mmol.l^{-1} TS) across the rumen epithelium. In the experiment, rumen walls obtained from adult Merino sheep 2 to 4 years old were used. Absorption was observed on an apparatus constructed in our laboratory. Determination of the acids was carried out by gas chromatography (Perkin-Elmer 8500). As compared to its application in combination with propionate (A + P) the acetate absorption rate across the rumen epithelium was significantly higher when applied separately. The addition of estradiol (E – 150 ng.150 ml^{-1} TS) to acetate alone and also to combined A + P decreased acetate absorption. In the case of propionate an amount of 15 mmol and 30 mmol was used. The addition of estradiol at a propionate concentration of 15 mmol did not affect its absorption rate across the rumen epithelium. At a 30 mmol concentration of propionate in the transport solution significant changes occurred in its absorption across the rumen epithelium. The addition of estradiol to a propionate only solution resulted in an almost double increase of its absorption rate. After the addition of estradiol to combined A + P an increase of propionate was also seen, but this increase was not significant. Comparison of the absorption rate with separate propionate showed a significant increase ($P < 0.001$). It can be stated that the absorption rate of both acids was affected by the administration of estradiol from the serous side of the rumen epithelium.

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

INTRODUCTION

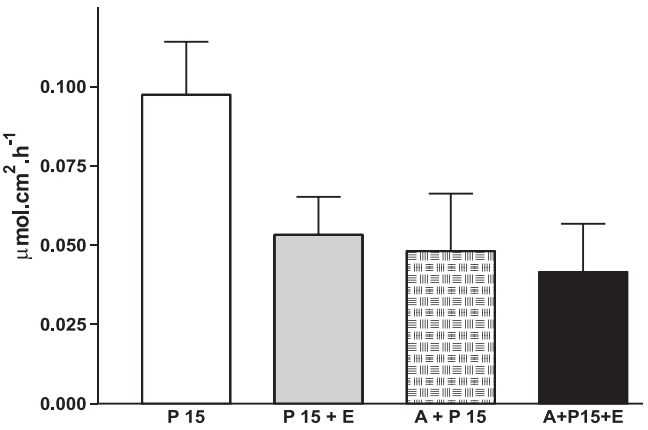
Acetic and propionic acid belong to the short-chain fatty acids (SCFA) which are a usual part of rumen content. They are produced during the fermentation of plant materials in the forestomach of ruminants. In the rumen epithelium they are metabolized or absorbed into the blood stream. Acetic acid is utilized as a source of energy, propionic acid is the only short-chain fatty acid which is glucogenic. It is quantitatively the most important precursor of glucose (1, 2, 3). Practically, total propionic acid is utilized in the liver and does not reach the peripheral blood stream (4).

Estradiol or 17-ß oestradiol is a female sexual hormone belonging to the group of estrogens. This hormone is produced by the gonads, the adrenal cortex, the interstitial cells of the *corpus luteum* and other tissues in both sexes. An especially great number of them are produced by the cells of the theca and also by the granulosa cells in the ovaries and the placenta of pregnant animals. Increased amounts of estrogenous substances are also secreted during oestrus by non-pregnant animals. In the organism they fulfil different functions so that they cannot be compared according to one basic mechanism. From the physiological point of view they are of female phenotype,



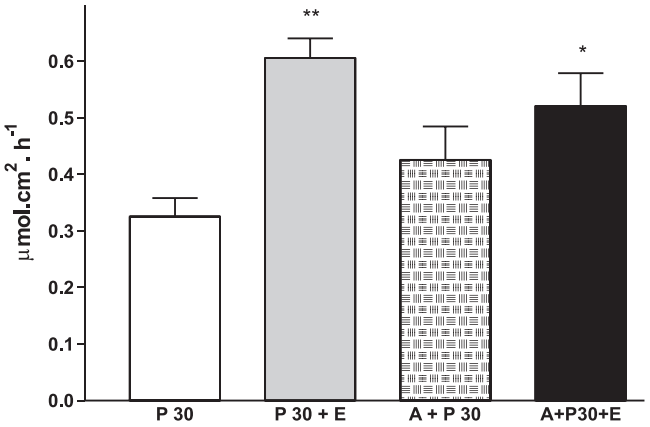
* — P < 0.05; *** — P < 0.001; A — acetate
P — propionate; E — estradiol

Fig. 1. Acetate absorption across the rumen epithelium after administration of estradiol



A — acetate; P — propionate; E — estradiol

Fig. 2. Propionate absorption at 15 mmol level across the rumen epithelium after administration of estradiol



* — P < 0.05; ** — P < 0.01; A — acetate
P — propionate; E — estradiol

Fig. 3. Propionate absorption at 30 mmol level across the rumen epithelium after administration of estradiol

enable full development of the sexual organs and determine the course of the sexual cycle.

Biosynthesis of sexual steroid hormones is derived from acetate and is also related to some fatty acids. It is for that reason that estradiol has been used to investigate the effect of SCFA absorption across the epithelium of the gastrointestinal tract in sheep.

MATERIAL AND METHODS

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

Acetate and propionate absorption was observed on an apparatus constructed in our laboratory (5). However in this experiment 150 ng of synthetic estradiol was added to glass vessels filled with 150 ml of TS with pH 7.4 (serous side). This solution represented the blood stream, i.e. the environment into which the fatty acids are absorbed from the rumen in the mucous-serous direction. The original opening at the other end of Jenette's syringe was used to insert a funnel through which the syringe was filled with 50 ml of TS containing the combined fatty acids (acetate in amount 50 mmol.l^{-1} and propionate in amount 15 mmol.l^{-1} of TS in the first case and 30 mmol.l^{-1} of TS in the second case) with pH 6.9 (mucous side). Each combination was tested six times (n = 6).

Further the experiment continued in accordance with the procedure referred to in (5).

Statistical analysis: The means of the individual parameters were compared using the Tukey-Kramer multiple comparison test (GrapPad Instat Software, Inc., San Diego, USA). The marked differences from the means in graphs represents a standard error (S. E.).

RESULTS

The acetate absorption rate across the rumen epithelium (Fig. 1) was significantly higher when applied separately in comparison to the application of acetate in combination with propionate (A + P). Addition of estradiol to separate acetate decreased its absorption rate but addition of estradiol to A + P in comparison with A + E did not affect acetate absorption.

The addition of estradiol to 15 mmol concentration propionate did not affect the absorption of the latter across the rumen epithelium (Fig. 2). At a 30 mmol propionate concentration in the transport solution significant changes

occurred in its absorption across the rumen epithelium (Fig. 3). The addition of estradiol to the propionate only solution resulted in an almost double increase in the absorption rate. The addition of estradiol to combined A + P also increased the absorption rate of propionate but less. Comparison of the absorption rate with propionate only showed a significant increase.

DISCUSSION

Our experiments were concerned with two fatty acids, which from the quantitative viewpoint of production are the most important, i.e. acetic and propionic acid. The observation of acetate absorption across the rumen epithelium revealed an interesting finding. When only separate acetate (50 mmol.l^{-1} of TS) was used its absorption rate across the membrane was much higher than in the case when the same amount of acetate was used in combination with propionate (15 mmol.l^{-1} of TS) but no support has been found for this finding in so far in the known experimental work.

However, we do not suggest that acetate absorption could be so profoundly suppressed by the presence of propionate in the solution used. We rather endorse the opinion that this phenomenon could have been caused by some other factors, which, with the techniques used in the investigation of the SCFA absorption rate, could not be taken into account. It will be necessary to verify this finding by means of some more progressive techniques in future investigation of absorptional relations.

The effect of estradiol on acetate absorption across the rumen epithelium became evident with a slight but significant decrease of its absorption rate when acetate was applied separately. When, however, acetate + propionate were added, the absorption rate was not affected. There are practically no data in literature on similar effects and thus their reasons can only by assumed. As mentioned in the survey of literature the SCFA absorption rate is the larger the longer the carbon chain of the corresponding acid is (6). In this case the absorption rate is greater for acetate than for propionate. However, the amount of acid transported from the digestive tract into the blood stream is in reverse proportion, i.e. it is greater for propionate than for acetate. In addition, propionate is to a considerable extent metabolized in the epithelium (8).

All facts introduced here, supported by the possibility that estradiol might have affected also the morphological properties of the epithelium, could well have been the reason for our finding. The propionate absorption rate through the rumen epithelium was in all cases higher with 30 mmol concentration of propionate in the transport solution than with the 15 mmol concentration. This is probably related to the fact that the absorption rate of the individual acids proportionally increases with their increasing concentration (7).

The effect of estradiol on the propionate absorption rate across the rumen epithelium was not entirely identical. A 15 mmol concentration of propionate in the transport solution did not affect its absorption rate. The absorption rate of propionate across the rumen epithelium proved to be increased ($P < 0.01$) at a concentration of 30 mmol when separate propionate was used. It can thus be stated that estradiol partly increased the propionate absorption rate across the rumen epithelium, but only at the concentration of 30 mmol in the transport solution. We assume that a 15 mmol concentration appeared to be too low for an effect of estradiol to become evident.

Similar experiments with cadmium have been performed: The effect of cadmium on acetate and propionate absorption across the rumen epithelium has been investigated (5). These experiments have mainly theoretical significance. The results acquired are a contribution to broaden the knowledge on short-chain fatty acid absorption across the rumen epithelium.

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THE EFFECTS OF PELLETED LEAVES OF *Phyllanthus amarus* AND *Euphorbia hirta* ON THE HAEMOGRAMS OF RATS

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ABSTRACT

The leaves of the plants, *Phyllanthus amarus* and *Euphorbia hirta* were harvested freshly and allowed to dry under a controlled environment. The dried leaves of these plants were separately pulverized using mortar and pestle until the powdery form of these substances were obtained. These powdery substances were then weighed into specific doses and boiled starch was added to these serving as binders. Using a special device, this product was then moulded into pellet forms. It was these pellets that were fed to the rats. Fifteen rats divided into 3 groups of 5 rats per group were used in this study. While group A rats served as control, the group B rats were fed with the pelleted leaves of *P. amarus* while those of group C were fed with that of *E. hirta.*

Changes in the parameters such as PCV, HB, RBC, WBC, MCHC, MCH, MCV and WBC differential were used to assess the effects of the pelleted leaves on rats. The study showed that there was a significant increase ($P < 0.05$) in the levels of PCV, HB, MCV, MCH, WBC, lymphocytes and neutrophils of animals in groups B and C when compared with the control. This study thus lends credence to the medicinal effects of these 2 plants.

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Key words: *Euphorbia hirta*; haemograms; *Phyllanthus amarus*; rats

INTRODUCTION

Nature provides materials for the treatment of diseases and ailments in the different flora of the world. Every part of the globe explores the rich potency of its local or native flora. The potentialities of Nigerian flora as ready materials for pharmaceutical products and producers of therapeutic ingredients have been variously expressed: Oliver (20), Gbile and Adesina (13).

There is at present revived interest in plants as an excellent source of therapeutic agents. Studies have shown that many materials used as food have some medicinal activities – Odebiyi and Sofowora (19), Gbile and Adesina, (13), Gills (14), Emudianughe and Aderibigbe (11).

Phyllanthus amarus belongs to the family Euphorbiaceae (the spurge family) of which the largest genus is the genus *Euphorbia* The plant has a history of use in Ayurvedic medicine for over two thousand years as well as a wide variety of traditional applications. It is a weed of cultivated land and in waste spaces. It is common to find it growing and spreading freely along road sides, under flower beds and many other places (7). For this reason, grazing animals are prone to consume this plant along with their feed particularly in drier tropical climates where lush, green grass is not often available. It is a plant that can be described as an example of a highly beneficial medicinal plant which is deserving of much more research but one which is fraught with the typical problems

of working with a complicated phytochemically rich plant. This plant is gaining in popularity in many continents as a herbal remedy. Many of the active constituents are attributed to biologically active lignanes, glycosides, flavonoids, alkaloids, ellagitannins and phenylpropanoids found in the leaf, stem and root of the plant. Common lipids, sterols and flavonoids also occur in the plant (18).

Euphorbia hirta Linn. on the other hand is an anthropogenic herb that is commonly seen occupying open waste spaces, roadsides, path sides, and as a weed of cultivation, widespread throughout West Tropical Africa, and dispersed pan-tropically and sub-tropically around the world. It is usually erect, up to 40 cm tall, but it could also be seen lying down. The plant which also belongs to the family Euphorbiaceae contains relatively abundant white latex (7). A number of substances have been detected in the plant: tannins, gallic acid, quercetin, phenols, phyto-sterols, alcohols, alkaloids, etc. (8), (7). Its diuretic and purgative action has been well documented (16). The purgative action of *E. hirta* has led to the postulation and the possibility of its use as an anthelmintic agent (5).

The objective of this study is to assess the safety or otherwise of these plants as medicinal herbs especially that some studies have implicated these plants as being toxic (21, 22, 23). This study is also aimed at determining the effects of processed plant parts on the haematology of rats.

MATERIALS AND METHODS

Animals and Experimental Designs

The animals used in this study were rats of the Sprague Dawley strain weighing between 150 and 200 grams. They were all males and maintained at the Animal House of the Faculty of Veterinary Medicine, University of Ibadan. They were kept in rat cages and fed rat cubes (Ladokun and Sons Livestock Feeds, Nigeria Ltd.) and allowed free access to clean fresh water in bottles *ad libitum*.

Fifteen (15) animals divided into 3 groups of 5 animals per group were used in this study. While group A rats served as control, the group B rats were fed with the pelleted leaves of *P. amarus* while the group C rats were fed with that of *E. hirta*.

Preparation of the Pelleted leaves of the Plants

The leaves of the plants, *Phyllanthus amarus* and *Euphorbia hirta* were harvested freshly for preparation of the pellets. The plants were authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN). These fresh leaves were allowed to dry under controlled conditions in good air draft. The dried leaves of these plants were separately pulverized using mortar and pestle until the powdery form of these substances were obtained. These powdery substances were then weighed into specific doses and boiled starch was prepared and added to the pulverized materials to serve as binders. These were then pelleted using specially design appliance, and the pellets dried at room temperature. It was these pellets that were fed to the rats. To ensure that the animals consume these pellets, they were not given any feed until they had consumed the pellets. The control group did not receive any pellet. This was done daily for 14 days.

Determination of Haematological Parameters

Blood was collected by cardiac puncture from diethyl ether anaesthetized rats into heparinised bottles for haematological studies. Determination of the haemoglobin concentration was as described by Jain (15) using the cyanomethaemoglobin method. Packed cell volume (PCV) was carried out by a conventional method of filling the capillary tubes with blood as described by Schalm *et al.* (24). Erythrocyte count was determined by the haemocytometer method as described by Duncan *et al.* (10). The total leucocytes and leucocytes differential counts were also determined. Erythrocytes indices were determined from values obtained from RBC count, haemoglobin concentration and PCV values.

Statistical analysis

Where necessary, results were subjected to the Student's *t*-test. Results were considered significant at $P<0.05$ – Essex-Sorlie, (12).

RESULTS

The results of this study showed that both *P. amarus* and *E. hirta* caused significant increase ($P<0.05$) in the levels of PCV, Hb concentration, MCV, MCH, WBC, lymphocytes and neutrophils. *P. amarus* however caused a more pronounced effect on the levels of these parameters than *E. hirta* (Table 1).

Table 1. Effects of the pelleted leaves of *P. amarus* and *E. hirta* on the haematological parameters of rats (n = 5)

PARAMETERS	Control	*P. amarus*	*E. hirta*
PCV (%)	36.6 ± 2.2	51.0 ± 0.7[a]	50.0 ± 0.8[a]
Hb ($g.dl^{-1}$)	11.4 ± 0.5	16.7 ± 0.4[b]	16.3 ± 0.8[b]
RBC ($106.\mu l^{-1}$)	6.0 ± 0.3	6.2 ± 0.4	6.1 ± 0.5
MCV (fl)	60.0 ± 3.7	84.0 ± 2.1[d]	84.0 ± 1.1[d]
MCHC (%)	31.0 ± 2.4	33.0 ± 0.4	32.0 ± 0.3
MCH (pg)	19.0 ± 1.6	27.0 ± 3.6[e]	27.0 ± 3.9[e]
WBC ($103.\mu l^{-1}$)	4.7 ± 0.4	10.4 ± 0.5[f]	7.5 ± 0.6[f]
Lymphocytes ($103.\mu l^{-1}$)	2.5 ± 0.8	5.4 ± 1.0[g]	4.1 ± 0.8[g]
Neutrophils ($103.\mu l^{-1}$)	2.0 ± 0.9	4.8 ± 0.6[h]	3.1 ± 0.7[h]
Monocytes (103.µl-1)	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01
Eosinophils ($103.\mu l^{-1}$)	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01

Superscripted items indicate significant values

DISCUSSIONS

The pelleted leaves of the 2 plants, *P. amarus* and *E. hirta* caused significant increase ($P<0.05$) in the levels of PCV, Hb concentration, MCV, MCH, WBC, lymphocytes and neutrophils when compare to the control experiment in the course of this study. It shows that the pelleted

leaves of the 2 plants have the ability to improve the quality of blood of the animals concerned. This ability of the pelleted leaves of the 2 plants to improve the value of the blood of these animals is similar to the effect of haematinics on animals suffering from anaemia. Haematinics are pharmaceutical or chemical compounds functioning to enhance blood formation, and thus, maintain the normal haemoglobin level in the living systems. Elements such as iron, cobalt, zinc, copper, vitamins etc. usually form component of haematinics: Brander *et al.* (6), Adams (1). Phytochemical analysis of the leaves of *P. amarus* and *E. hirta* actually showed that these plants contained iron, copper and zinc in reasonable quantity (3). It may therefore be safe to say that these plants exhibit some haematinic potencies.

The result of this study is however a sharp contrast to the results of other studies of the effects of these plants on the haemograms of rats. For instance, both the aqueous crude extracts as well as the chromatographic fractions of these 2 plants caused dose-dependent reductions in the values of the measured blood parameters (PCV, Hb concentration, RBC, WBC and its differentials) suggesting that these plants could actually caused anaemia in animals that graze them – Adedapo (2), Adedapo *et al.* (4). In fact, many members of the spurge family to which *P. amarus* and *E. hirta* belong are poisonous. For instance, *Mercuralis perennis* (dog's mercury) and *M. annua* (annual mercury) are poisonous. *Mercuralis perennis* gives rise to two distinct syndromes, the first, and the one usually encountered in field case, is a haemolytic anaemia, the second an acute oedematous gastroenteritis in cattle.

In poisoning by *Mercuralis annua*, haematuria is also the most obvious clinical sign. Welchman–D-de *et al.* (26) have reported that 11 lambs in a flock of 400 8-month old Romney lambs died from grazing *M. annua*. Pathological findings including haemolytic anaemia and haematuria were indicative of annual mercury poisoning. Deprez *et al.* (9) have also reported on two cattle farms that animals showed constipation or diarrhoea, dullness, haemolytic anaemia and red urine after the ingestion of *M. annua*.

It has also been reported that the extracts of *Jatropha curcas* (family Euphorbiaceae) caused a progressive reduction in the measured haematological parameters (packed cell volume, red blood cell count and haemoglobin concentration) of rats – Oluwole and Bolarinwa (21).

The findings in this study in which the measured parameters experienced a significant increase may have been due to the method of processing the leaves of these plants. It may be that the boiled starch that was used as binder had some effects on the toxic principles contained in these plants and thus produced the desired effects noted in this study.

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ZINC AND COPPER CONCENTRATION IN THE BLOOD SERUM OF BOARS AFTER THE ADMINISTRATION OF ZINDEP INJ. A.U.V.

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ABSTRACT

The aim of our investigation was to verify the efficiency of the preparation Zindep inj. with its prolongation effect at the insemination centre of boars suffering from hypozincaemia. We analysed the amount of zinc and copper in the complete diet for boars, "KA" and the concentration of these microelements in the blood serum. We collected the blood at 0, 21 and 71 days of the experiment. Preparation Zindep inj. a.u.v. (BIOTIKA, SR) was administered to boars in an experimental group at day zero at a dose recommended by the producer (10 mg of zinc per kg of live weight) by intramuscular application. The dynamics of zinc and copper concentration in the blood serum during the whole period of the experiment was evaluated. The zinc concentration in the complete diet for boars was 119.83 mg.kg^{-1} in dry matter (DM) and copper 24.61 mg.kg^{-1} in DM. The zinc concentration in the blood serum of the boars from the experimental group was at day zero 10.70 ± 0.71 µmol.l^{-1}, at day 21 14.41 ± 0.54 µmol.l^{-1} and at day 71 12.45 ± 0.43 µmol.l^{-1}. The copper concentration in the blood serum of the boars from the experimental group was at day zero 30.24 ± 2.73 µmol.l^{-1}, at day 21 29.48 ± 1.52 µmol.l^{-1} and at day 71 35.19 ± 2.02 µmol.l^{-1}. On the basis of our results we registered an increased amount of zinc and copper in the complete diet for boars "KA". The administration of a zinc-based preparation to 7 boars at a dose of 10 mg.kg^{-1} resulted in a significant increase in the concentration of Zn in the blood serum of the experimental animals on day 21. The difference between the serum levels of Zn in the experimental and control animals remained significant until day 71 of the experiment. According our results, in the blood serum of the experimental and control animals hypercupraemia was found. The difference between the serum levels of copper in the experimental and control animals was not significant until day 71 of the experiment.

Key words: boar; blood serum; copper; nutrition requirements; zinc

INTRODUCTION

Clinical examination is very important for the confirmation of a zinc deficiency in laboratory diagnostics. It is significant in cases, when zinc deficiency is not manifested in clinical forms or is accompanied by other diseases. In these cases the determination of zinc and its metabolites in biological material and in diet is required. Simple collection of samples and their analysis is the most useful method of determining the zinc concentration in the blood serum. Increasing the amount of zinc in a diet should be in correlation with the concentration in biological material at confirmation of a zinc deficiency.

The zinc requirements in a diet vary with the species, breed, age, productive functions and health status of the animals. If we respect these realities the supply of zinc is not always successful. There are states where there are increased needs of zinc (high production, infection disease, stress), if using zinc in a diet by the animals is not sufficient. Primarily disease is provoked by a deficiency of zinc in a diet. Using zinc in secondary deficiencies is influenced by the composition of a

diet, the presence of antagonistic components in a diet, the chemical form of the zinc, digestion (gastrointestinal diseases), and also the zinc concentration in organism (1). Parenteral administration is mainly effective for secondary deficiencies. Despite a sufficient amount of zinc in a diet its efficiency is decreased in other compounds of fodder by the creation of not-utilizing forms (4).

MATERIAL AND METHODS

The investigations of the effectiveness of the preparation Zindep inj. were carried out in 13 boars, 22.41 ± 4.34 months old, of live weight 263.8 ± 17.31 kg. The boars were fed with complete diet for boars "KA" at a dose 3–4 kg.day^{-1} and they had free access to water. The clinical examination of all the animals was performed before the beginning of experiment.

The zinc-based preparation Zindep inj. a.u.v. (Biotika, Slovakia) was administered intramuscularly (at a dose of 10 mg Zn.kg^{-1} body weight) into seven experimental boars. The total dosage for boar of live weight 263.8 ± 17.31 kg was 37—42 ml per animal. This dosage had to be divided into 6—7 single injections with maximum volume 6 ml per injection. The control group consisted of six boars kept under the same conditions as the experimental animals.

Blood samples were taken from the *vena jugularis* and *sinus ophthalmicus* of experimental and control boars. The beginning and duration of the effect of the preparation in accordance with the producer's recommendation were noted at the collection of blood on days 21 and 71.

The determination of Zn and Cu in the blood serum and in the complete diet was carried out by the AAS (atomic absorbance spectrophotometry) method using an apparatus Perkin Elmer (5000). The values of Zn in blood serum are expressed in μmol.l^{-1} and those in a diet in mg.kg^{-1} in DM.

The samples for determining the content of both were processed in accordance with the method used by Institute of Animal Physiology of the Slovak Academy of Science in its laboratory of mineral metabolism (11).

The samples of a complete diet for boars "KA" in amount 2 grams were mineralized in platinum dishes, in an oven at 450 °C with overnight incubation and then burned after addition NH_4 NO_3 at 550 °C for four hours. The ash of samples was consecutively solubilized in sand bath by addition and evaporation 17 % HCl, concentrated HF and solubilisate was transmitted and adjusted into final volume 25 ml by mixture HCl : HNO_3 : deionized H_2O (1 : 2 : 7). Consecutively developed mineralisate was measured by the AAS method.

The results of the analysis of zinc in the blood serum are presented as mean values and standard deviations (± SD). GraphPad Prism 3.0 (1999) using Student's *t*-test was applied to compare the results (14).

RESULTS AND DISCUSSION

According to Vrzgula (18), the daily demand of zinc for young boars is 90 mg of Zn per kg in DM and for adult boars 70 mg of Zn per kg in DM. Šimeček *et al.* (16) and Brestenský (6) has presented the daily requirements of zinc for boars as 80 mg of Zn per kg in DM. Animals in heavy conditions have the highest requirements for zinc with maximum production rate. The zinc requirements for pigs are significantly higher at a level up to 100 mg of Zn per kg in DM (10). The complete diet for boars manufactured by the producer presents 60 mg of Zn per kg in DM. On the basis of our results the amount of zinc in a diet for boars is 119.83 mg of Zn per kg in DM, so an increased amount in the diet.

The clinical signs of zinc deficiency arose when the serum zinc concentration was 7—10 μmol.l^{-1} (5). The reference values of zinc in the blood serum pigs are 16—45 μmol.l^{-1} (4), 15.3—35.2 μmol.l^{-1} (18), 12.3—18.5 μmol.l^{-1} (17), 30—45 μmol.l^{-1} (19).

Before the administration of the preparation, the marked hypozincaemia was registered in the blood serum of the experimental and control animals (Fig. 1). The concentration of zinc in the blood serum was 9.30 ± 0.36 μmol.l^{-1} in control group (n = 6) at day zero and in 10.70 ± 0.71 μmol.l^{-1} in experimental group (n = 7). The differences between mean values of the control and experimental group are not statistically significant (P = 0.12; P > 0.05).

A statistically significant increase in Zn content in the blood serum of the experimental animals in comparison with the control was observed on day 21 (P < 0.0001). The concentration of zinc in the blood serum of boars on day 21 of the experiment was 14.41 ± 0.54 μmol. l^{-1} in the experimental group and in the control group, 10.70 ± 0.28 μmol.l^{-1}.

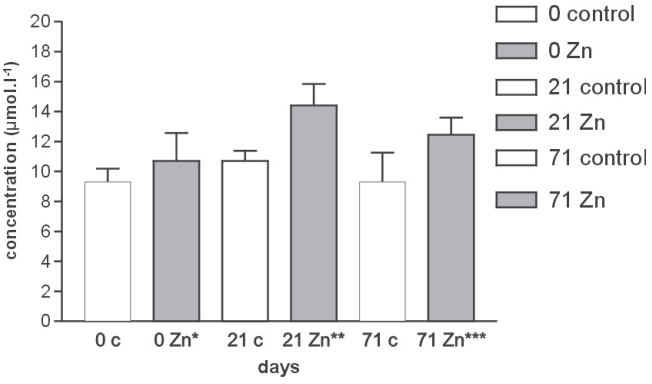

Fig. 1. Concentration of zinc in blood serum of boars (μmol.l^{-1}) on days: 0, 21 and 71; (mean ± SD); * — P > 0.05; ** — P < 0.0001; * — P < 0.01**

The difference between the serum levels of Zn in the experimental and control animals remained significant until day 71 of the experiment (P = 0.0041; P < 0.01) (Fig. 1). The concentration of zinc in the blood serum of boars in the experimental group was 12.45 ± 0.43 μmol.l^{-1} and in the control group 9.30 ± 0.80 μmol.l^{-1}.

Cigánková *et al.* (7) have discovered statistically significant hypozincaemia in boars after 100 days of feeding with barley. The concentration of zinc in the blood serum of boars was 8.97 ± 1.65 μmol.l^{-1}. This hypozincaemia was caused experimentally by a deficiency of zinc in the fodder plant (barley). Primarily, the concentration of zinc in barley was 30.14 mg.kg^{-1} in DM and a statistically significant increase of Zn content in the blood serum was achieved on day 20 (22.13 ± 1.45 μmol.l^{-1}). The zinc content in the blood serum decreased on day 60 to 18.46 ± 1.056 μmol.l^{-1}. The difference between the serum levels of Zn in experimental and control animals remained significant until day 60. The reference values of zinc in the blood serum of boars were achieved after the administration of the preparation.

Similar results have been recorded by Vrzgula (18) in an experiment with sows that suffered from hypozincaemia. A statistically significant increase in the zinc content in the blood serum of experimental animals in comparison with the control was observed as early as on day 8. The concentration of zinc in blood serum of sows was 8.46 ± 1.02 μmol.l^{-1} on day zero; 16.56 ± 3.12 μmol.l^{-1} on day 30; 13.45 ± 4.19 μmol.l^{-1} on day 60; 11.45 ± 1.46 μmol.l^{-1} on day 90. A statistically significant zincaemia was registered in comparison with the control until 90 day of the experiment.

However, in our experiment a significant increase in the concentration of Zn in the blood serum of the experimental animals in comparison with the control had occurred by Day 21 and the serum levels of Zn remained significant until day 71 of the experiment, we did not reach the serum zinc reference values cited by Vrzgula (18) and Bíreš (4).

Zinc is absorbed according to the need of an organism (17). Zinc in cereals has a low and variable absorbality for pigs and poultry of around 60 % when compared with inorganic sources (1, 2).

Šimeček *et al.* (16) have reported the daily requirements of copper for boars at 10 mg.kg^{-1} in DM. Underwood and Suttle (17) have reported at 5—6 mg Cu.kg^{-1} in DM.

The reference values of copper in the blood serum of the boars have been reported by Gaal *et al.* (9) 18—34 μmol.l^{-1}, Baumgartner (3) 25—40 μmol.l^{-1}, Vrzgula and Sokol (19) 24—42 μmol.l^{-1}.

On the basis of our analysis the values of copper concentration in the blood serum of boars in the control and experimental groups are in the range of reference values cited by the authors we have mentioned above.

A decreasing concentration of copper in the blood serum of boars in the experimental group was registered on day 21 (after administration Zindep inj.). The concentration of copper in the blood serum of boars in the experimental group was 29.48 ± 1.52 μmol.l-^{1} and in the control group 26.10 ± 1.63 μmol.l^{-1}. The decrease of copper concentration in comparison with the control group is not statistically significant (P = 0.16, P > 0.05).

The concentration of copper in the blood serum of the boars in the experimental group on day 71 was 35.19 ± 2.02 μmol.l^{-1} and in the control group, 31.87 ± 2.09 μmol.l^{-1}. The difference between mean values of the control and experimental group is not statistically significant (P = 0.28; P > 0.05).

Underwood and Suttlle (17) have reported that the normal values of zinc in the blood serum of pigs ranged from 12.3 to 18.5 μmol.l^{-1}, but individual variability can be high and many factors other than dietary zinc affect concentrations. In accordance with these results, the reference values of zinc in the blood serum of boars were achieved after administration preparation Zindep inj. until the end of our experiment.

Underwood and Suttle (17) and Suttle *et al.* (15) have presented reference values of copper in blood serum of pigs 16.5—20 μmol.l^{-1}, considerably lower than others. In comparison with their results, our blood serum concentration of copper in the experimental and control animals can be considered as hypercupraemia.

According to Underwood and Suttle (17) 50 mg of Zn per kg in DM is adequate for the nutrition requirements of pigs, apart from cases, when their diet is supplemented by an excessive amount of copper. The need of 150 mg of Zn per kg in DM is suitable at a higher amount of copper in the diet for pigs.

The values of zinc in blood serum can be misrepresented, if the animals are under stress conditions during blood collection. Serum or plasma zinc values must obviously be used with caution in the diagnosis of zinc deficiency in farm animals. Since serum iron declines and copper rises under the influence of most stressors (17).

On the basis of our analysis, the amount of copper in the diet for boars is 24.61 mg Cu.kg^{-1} in DM, which means increasing the amount in the diet. The concentration of copper in blood serum of boars (Fig. 2) in the control group on day 0 (before administration Zindep inj.) was 30.3 ± 2.31 μmol.l^{-1} and in the experimental group, 30.24 ± 2.73 μmol.l^{-1}. The difference between the mean values of the control and experimental group is not statistically significant (P = 0.98; P > 0.05).

Significant hypozincaemia in all boars, values 9.30 ± 0.36 — 10.70 ± 0.71 μmol.l^{-1}, was found before the start of our experiment. The concentration of zinc in the blood serum of boars in the experimental group was 14.41 ± 0.54 μmol.l^{-1} on day 21, enhanced about 3.54—3.87 μmol.l^{-1}.

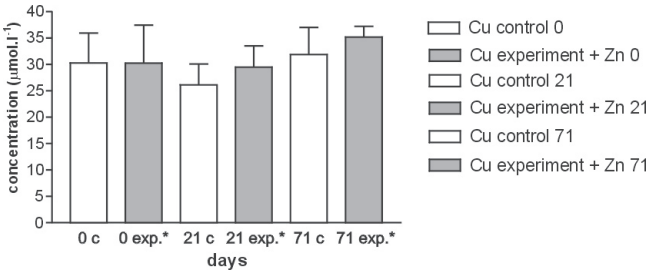

Fig. 2. Concentration of copper in blood serum of boars (μmol.l^{-1}) on days: 0, 21 and 71; (mean ± SD); * — P > 0.05

The amount of zinc utilisation from the digestive tract decreases with the presence of phytates in a diet based on cereals. The substance of a decreased use is the production protein-zinc-phytate complex that is resistant to hydrolysis *in vivo* (8). An increase of activity phytates in the diet increases the uptake of zinc in the organism and the primary antagonist of zinc-phytate is eliminated (17).

Feeding trace elements bioplexes (Fe, Cu, Zn, and Se) have had a positive effect on the statistical increase of the concentrations of Fe, Cu, Mn and Zn in the blood serum of the experimental group of pigs (13). Supplementation of diet with organic forms of minerals bound to amino acids or short-chain peptides is one of the methods that can be used to increase the availability of trace elements in pigs. The organic-bound trace elements are more resistant to reactions with other chemical compounds during digestion, more soluble, and therefore they are more easily absorbed and integrated into biological reactions and body structures (12, 13).

The administration of the preparation Zindep inj. in a dose recommended by the producer, 10 mg of zinc per kg of live weight by intramuscular application, induced no local changes at the site of injection.

The producer of the preparation reports a long-term duration effect and from this point of view it is not suitable to carry out repeated administration longer than 3—4 months (Biotika, the Slovak Republic).

On the basis of the results in our experiment the concentration of zinc in blood serum of boars significantly increased on day 21, but decreased earlier than day 71 after administration of the preparation Zindep inj. to boars suffering from hypozincaemia before the start of experiment. From this point of view repeated administration of Zindep inj. is suitable after 2 months with confirmation of a decrease in zinc concentration in blood serum under its reference values.

CONCLUSION

Hypozincaemia in boars, in our experiment, was the consequence of a secondary zinc deficiency. This type of deficiency is influenced by the composition of a diet, by the presence of antagonistic components in the diet (copper), by chemical forms of zinc (organic or inorganic), by digestion (the diseases of digestive system), but also the initial concentration of zinc in the blood serum of an organism. The state of hypozincaemia can be connected with the redistribution of zinc in the organism for its current demands. There are e.g.: the integrity of dermal units, participation in the defence against infection diseases, in healing wounds and regeneration of tissues, in the production of semen. The level of zinc in the blood serum decreases under the influence of the majority of stressful factors.

It is possible to solve the insufficient saturation of the organism by zinc by revaluing its concentration in the diet and by assessment of its concentration in biological materials. Its total requirements has to take note of the all zinc functions in organism. At the same time we should take note of the possible relationships with other micro-elements, especially with the interaction of zinc and copper.

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THE EFFECT OF VOLATILE AIR POLLUTANTS ON THE IMMUNOGLOBULIN LEVEL IN THE POLAR FOX

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ABSTRACT

Investigations were conducted to identify the influence of gaseous air pollutants emitted from a farm on immunoglubulin levels in polar foxes *Alopex lagopus*. Foxes reared on a farm in cages in a pavilion system constituted the control group, while the experimental animals were kept in a chamber with restrained air movement. According to chromatographic analysis of the air, the foxes kept in the chamber were exposed to higher levels of the relevant gaseous pollutants than those kept in the pavilion system. The experimental females exhibited a higher IgM level (82.1 mg.dl^{-1}) compared to the control (66.5 mg.dl^{-1}). The differences were significant ($P < 0.01$). The IgG levels in the control group differed according to sex. The differences in IgG levels between the groups were insignificant. Statistical differences ($P < 0.01$) were detected between the samples in both groups. The IgA values in respective groups did not vary significantly.

Key words: air pollution; immunoglobulins; polar fox

INTRODUCTION

The continuous advance of breeding methods, feeding conditions and other aspects related to modernization in animal husbandry affect both the conditions of keeping animals and their performance. However, the exposure of animals to various indoor factors has not been reduced and is frequently reflected in the impairment of their functions. Many disturbances result not only from the presence of infectious or parasitic agents but also from chemical pollutants. Various volatile gaseous substances released into the environment or produced by mutual chemical reactions of primary pollutants penetrate the animals as xenobiotics and have various primary, secondary and metatoxic effects.

The respiratory tract protects itself and the rest of the body against aerial challenges by three mechanisms: the mucociliary apparatus, alveolar phagocytes, and secretory antibodies (15). Although fox farms frequently face problems of environmental pollution, few reports on the impact of gaseous volatile substances on the health of farmed foxes are available in the literature.

The system responsible for defence against adverse factors is the immune system. It activates mechanisms which eliminate antigens through immunologically active forms. Within the immune (humoral) response, class M antibodies are produced initially, and are followed by classes G, A or E. Still, the level of immune response depends on a complex of immunopathological factors (1, 7, 9, 16, 17).

The present study focused on the effect of increased concentrations of air pollutants on immunoglobulin level in polar foxes (*Alopex lagopus*).

MATERIALS AND METHODS

The study was conducted on a fox farm situated in the south-eastern part of Poland. During the experiment about

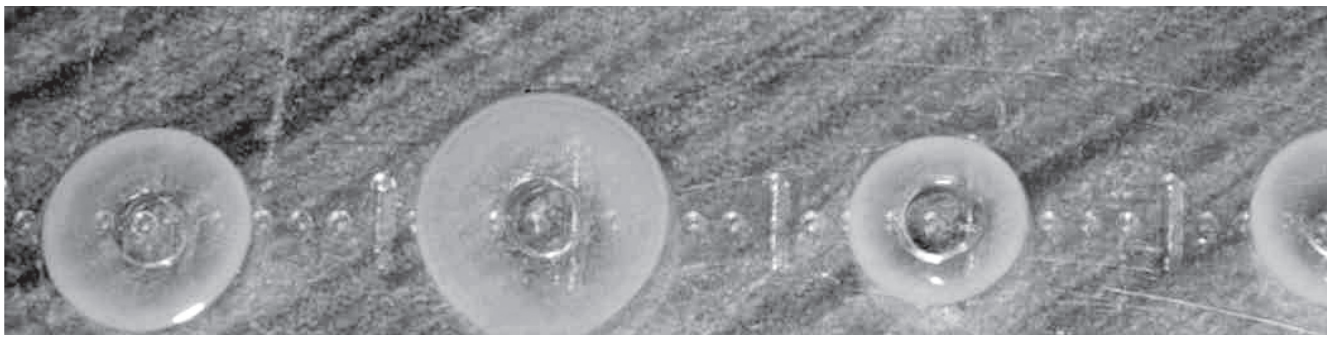

Fig. 1. Diameters of precipitation arcs

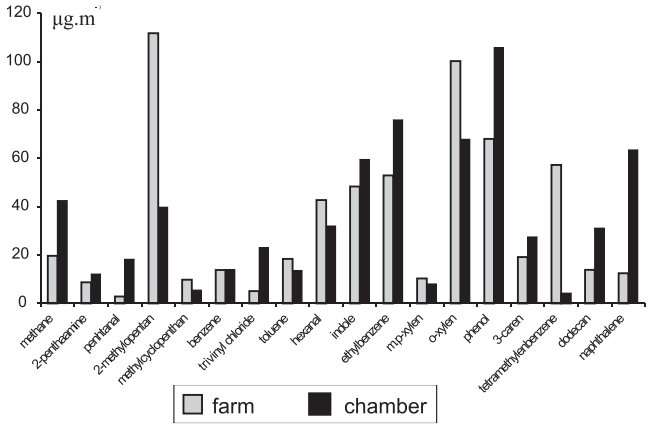

Fig. 2. Mean values of other gaseous compounds identified on farm and in confinement (chamber) in µm³ [acc. to Nowakowicz-Dębek *et al.*, 2004]

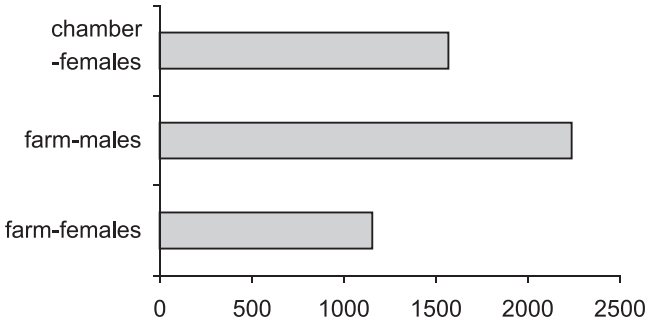

Fig. 4. IgG level in the polar fox (*Alopex lagopus*) (mg.dl^{-1})

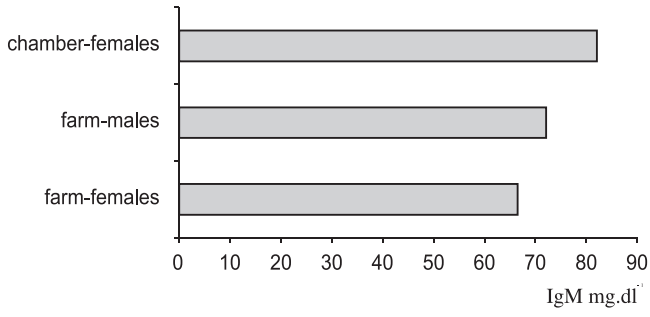

Fig. 3. IgM level in the polar fox (*Alopex lagopus*) (mg.dl^{-1})

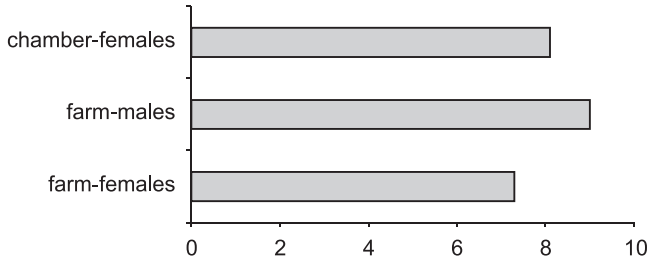

Fig. 5. IgA level in the polar fox (*Alopex lagopus*) (mg.dl^{-1})

fifty polar foxes (*Alopex lagopus*) were kept on this farm. The animals (n = 12) caged in a farm pavilion system made up the control group (six males and six females). The experimental group consisted of six female foxes selected at random, placed in an air-conditioned chamber with controlled air flow. Permanent monitoring of volatile air pollutants was insured for both groups based on calorimetric techniques and gas chromatography (3, 11, 12).

Over the investigation period, the animals were fed the same feed according to the feeding standards for fur animals and their age (2). Supervision of veterinarian and animal husbandry technician was ensured and appropriate sanitation measures were taken. Blood for examination was withdrawn twice from the foot vein (*vena saphena parva*). Serum level of classes M, G, and A immunoglobulins was determined by the method of SRID VMRD, Inc. (6) (Fig. 1).

Statistical analysis was performed on the grounds of a linear model of variance analysis for the double classification with interaction. The results are presented in graphic form.

RESULTS AND DISCUSSION

The immune system, through its interaction with other structures and systems of an organism, maintains a functional balance of the body. Homeostasis is particularly essential when environmental pollution comes into the picture. With regard to biological monitoring, it is recommended to look for new indicators with high reliability capable of detecting the influence of toxic contaminants on the animals reared.

The monitoring of air quality achieved in this and our previous studies confirmed that higher levels of

volatile pollutant emissions were found in a confined space (chamber) compared to the farm pavilion system (Fig. 2) (11, 12). In the air in the chamber ammonia levels were also increased (13).

Thus, the existing gaseous air pollutants constituted a factor potentially disturbing animal functional balance and the immune system was expected to react accordingly. The first contact of foxes with environmental antigens was manifested in the primary immune response as results from Figure 3 demonstrating the level of class IgM antibodies in the respective groups. In the females kept in cages (control group) the mean IgM level was 66.5 mg.dl^{-1} (0.67 g.l^{-1}) while in those from the experimental group mean level of IgM reached 82.1 mg.dl^{-1} (0.82 g.l^{-1}). The differences in the IgM values between the groups were significant ($P<0.01$) and were in the range 0.1—2.7 g.l^{-1} reported by Deptuła and Buczek (4) in dog serum.

Within a few days of antigenic action, the animals produced IgG antibodies or antibodies of other classes as indicated by IgM decrease on the second sampling in the experimental foxes. The activation of the successive immunoglobulins synthesis is controlled, among others by T-helper lymphocytes, the appropriate ligands between lymphocytes or cytokines (7, 9).

The level of IgG immunoglobulins, contributing to the secondary immune response in foxes, is presented in Figure 4. Levels of these antibodies in dogs, as reported by Deptuła and Buczek (4), are found within the interval 10.0—20.0 g.l^{-1}. The IgG levels produced in foxes from the control group differed with sex (females 1155.8 mg.dl^{-1} or 11.56 g.l^{-1}); males 1137.5 mg.dl^{-1} or 22.38 g.l^{-1}). The two-factor variance analysis showed significant differences ($P<0.01$) in the IgG level between the females and males kept in the pavilion system. Interactions between samples were detected in the control group of foxes. On the second collection from females of the experimental group a marked increase in IgG level (mean value 21.08 g.l^{-1}) was recorded and it proved to be higher compared to the control. The mean IgG value in the females from the experimental group was 1568.3 mg.dl^{-1} (15.68 g.l^{-1}). No significant differences between the groups were observed. However, the results of successive samples from females of both groups differed significantly.

Transfer of antigens across mucous membranes induces the synthesis of antibodies IgA or IgE, which are present in the secretions. Neutralizing attributes were ascribed to the dog's IgA and the respective levels in dog serum were in the range 0.01—1.5 g.l^{-1} (4).

The IgA level in the serum of the examined animals is presented in Figure 5. Our results showed that there were sex-related differences in mean IgA levels in the control group of foxes (females 7.3 mg.dl^{-1}, males 9.0 mg.dl^{-1}). The mean IgA level in experimental females was 8.1 mg.dl^{-1}. The differences between groups were insignificant.

According to Deptula and Buczek (4), immunoglobulin classes found in dogs resemble those in humans. Still, the function of each subclass in the organism has not yet been clearly defined.

The causes for human and animal health disturbances as well as their mechanismus can be multiple. They are, however, closely related to the environment of the organisms. This assumption has been confirmed by the investigations of Erdel *et al.* (5), who have determined immunobiomarkers in children exposed to volatile pollutants in a confined space. Susceptible children exhibited changes in these markers associated with high concentrations of volatile contaminants. They observed increasing levels of G class antibodies and leukocytes and decline in IgM. Similar relationship was observed in the present study on foxes exposed to gaseous pollutants in an experimental chamber.

The immune system is affected also by other systems, e.g. endocrinal or nervous. These systems, through the secretion of appropriate hormones, can affect immune functions. In many cases, animals are stressed due to unsuitable rearing conditions, i.e. their animal welfare is compromised.

Lechowski *et al.* (8) have reported dependence of some biochemical parameters in pigs on housing arrangement, transfer between the cages or transportation before slaughter. The authors have indicated that the stress related to the changing of cages and animal separation decreased the IgG fraction significantly and increased APP (protein acute phase) and lysozyme activity. Such changes were not observed after transporting animals for slaughter. Gammaglobulins were determined as protein "negative" towards APP because their level declined under the influence of environmental factors.

Similar dependence also appeared in humans subjected to psychological stress. The changes reported in studies dealing with IgA, IgM, and IgG levels indicated the influence of stress factors (10).

The effect of volatile air pollutants on lysozyme activity and selected liver profile parameters in the polar fox has been investigated in our previous studies (13, 14). Lysozyme activity as one of the non-specific immunity elements was significantly increased in animals exposed to increased air pollution and reflected activation of a defense response in the organism of foxes. On the other hand, differences in levels of ALP, AST, ALT, GGT, LDH and bilirubin between control and experimental animals were insignificant and remained within the reference range. The levels reached in three successive samples indicated that some balance and adaptation to different maintenance conditions may have developed in the organism of foxes.

The results of our investigations imply that the immune system response is closely connected to the organism's habitat. Even temporary deterioration of the fox maintenance conditions adversely affected their immune system. Yet, the values of subsequent antibody generations, as environmental immuno-biomarkers, need to be correlated, among others, with respect to the sex or species susceptibility.

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IMMUNOMODULATORY THERAPY IN CANINE SKIN DISEASES

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ABSTRACT

In the study effect of the immunostimulation by beta-1, 3,-D-glucan in dogs suffering from demodicosis (*Demodex canis*) and pyoderma (*Staphylococcus intermedius*) on functional activity of phagocytes and lymphocytes was evaluated. These skin infections were associated with different degree of the immunosuppression. Glucan application resulted into improvement of the immunological parameters and in some cases also with clinical recovery of the dogs affected.

Key words: demodicosis; dog; glucan; immune system; pyoderma

INTRODUCTION

Immunosuppression plays the important role in pathogenesis of some infectious diseases. Many recurrent and persistent infections can develop as the result of inherited or acquired abnormality of the immune system reactivity (15). Demodicosis, deep pyoderma of German shepherds, cutaneous mycosis, purulent dermatitis, atopy are the most common canine skin diseases associated with the alteration of the immune reactivity (11). In such cases besides causative therapy there is necessary to assess an immunomodulation in case when is indicated (4). Selenium, zinc, vitamin E and C, glucan, levamisole, bacterial products belong to the immunomodulators recommended.

The aim of our study was to observe the effect of immunostimulation therapy (glucan) together with the etiological treatment on the level of non-specific immunity in dogs with selected skin diseases associated with decreased immunocompetence.

MATERIAL AND METHODS

Animals: I. Twelve dogs with an average age of 2.7 years of different breeds (5 German shepherds, 2 mops, 3 dobermans, 2 boxers) and sexes (7 males, 5 females) with uncomplicated generalized demodicosis (*Demodex canis*) confirmed by clinical and parasitological examination (skin scraping). Dogs were divided into 2 groups (group D; group D + G).

Treatment: Group D, n = 6: amitraz in concentration of 12.5 % per 51 of water (Ectodex lig. a.u.v., Hoechst Roussel Vet, Germany) in two-week interval locally.

Group D + G, n = 6: amitraz + glucan (beta-(1,3),D-glucan; Imunoglukán (Pleuran s.r.o., Slovakia)) p. o. at total dose of 100 mg per day during 4 week period.

Animals: II. Ten dogs with an average age of 4.7 years of different breeds (7 German shepherds, 3 dobermans) and sexes (6 males, 4 females) suffering from pyoderma (*Staphylococcus intermedius*) confirmed by clinical and bacteriological examination verified by the BBL Crystal identification system (Becton Dickinson, USA). Dogs were divided into 2 groups (group P; group P + G).

Treatment: Group P, n = 5: antibiotic treatment (amoxicillin + clavulanate) during ten days, 12.5 mg. kg^{-1} b.w. twice daily.

Group P + G, n = 5: topical antibiotic treatment during ten days + glucan (beta-(1,3),D-glucan; Imunoglukán (Pleuran, s.r.o., Slovakia)) p.o. at total dose of 100 mg per day during 4 week period.

Scheme of sampling: day 0 (sampling 1), week 4 (sampling 2), week 7 (sampling 3).

Results of examination in animals of groups I and II were compared with group C (10 healthy dogs without skin lesions, average age 3.8 years, 5 males, 7 females, German shepherds).

The evaluation of parameters of cellular immunity

Blastogenic response of blood lymphocytes to mitogens. Lymphocytes were separated from venous blood on the Ficoll density gradient (Pharmacia Biotech AB, Sweden). The viability of the isolated cells was determined by trypan blue exclusion and exceeded 97 %. Most (> 95 %) isolated cells were mononuclear cells. The cultivation (culture medium contained 10 % of autologous serum), mitogen stimulation and the measurement of blastogenic response of lymphocytes by the fluorescence method were performed according to Nakanishi *et al.* (7). Concanavalin A (Con A, Sigma Chemical Co., USA) was used for stimulation in the concentration 25 $mg.ml^{-1}$ (10).

The level of the blastogenic response of the lymphocytes was expressed as the stimulation index (SI). The SI was calculated according to the formula SI = (A – C)/(B – C); A = mean FI (fluorescence intensity) with mitogen, B = mean FI without mitogen, C = background FI. The FI was measured by a spectrofluorometer (Jasco FP-550, Japan).

The phagocytic activity of blood neutrophils was examined as described by Větvička *et al.* (18). 0.1 ml of fresh heparinized blood (5 U of heparin.1 ml^{-1} of blood) was mixed with 0.05 ml of 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 mm, ARTIM Prague) and incubated for 1 hour at 37 °C with occasional shaking. The phagocytic activity (PA) of neutrophils (Ne) was expressed as the percentage of the neutrophils phagocytizing 3 and more MSHP, and as the index of phagocytic activity (IPA) representing the ingestion ability of neutrophils (the ratio of the number of phagocytized MSHP and the number of potentially phagocytizing Ne).

Statistical analysis. Results were expressed as the mean and standard deviation. Significance of differences was evaluated by the Student's *t*-test.

RESULTS

In all groups of dogs examined various degree of initial alteration of the immune parameters was found in relation to the character of the disease process. In dogs with generalized demodicosis significant decrease of blastogenic response of lymphocytes after mitogen stimulation was observed (Fig. 3). Phagocytic activity and phagocytic index were lower in comparing to control, but not significantly (Figs. 1, 2). In dogs suffering from pyoderma (P) significant decrease of phagocytic activity of neutrophils (Fig. 4) and blastogenic activity of lymphocytes (Fig. 6) were found. Ingestion capacity of neutrophils expressed as the phagocytic index (Fig. 5) was in dogs affected higher when compared with healthy dogs.

The application of beta-(1,3)-D-glucan together with causative therapy influenced positively altered immune parameters as follows: In dogs with generalized demodicosis (Fig. 7) a slight elevation of phagocytic activity and phagocytic index appeared after 4 weeks of glucan application comparing to dog treated with amitraz only. Seven weeks after initial sampling these activities have been improved and were comparable with values found in healthy animals. Blastogenic response of lymphocytes after mitogen stimulation increased after glucan application but not significantly and even in the end of observation did not reach values of healthy animals (Figs. 1, 2, 3).

Dogs affected with pyoderma (Fig. 8) in most cases showed an improvement of decreased parameters of functional activities of both cell populations after 4 weeks of glucan application; ingestion capacity of phagocytes remained unchanged. When compared to animals treated with antibiotics only, the immunity improvement was in correlation with the clinical recovery of patients that appeared earlier in dogs treated also with glucan (Figs. 4, 5, 6).

DISCUSSION

Alteration of the immune reactivity of organisms is accompanying phenomenon of many infectious and parasitic diseases of skin. Marking suppression of the T-lymphocyte response to mitogens was documented in generalized demodicosis (1, 8, 9, and 10). Moreover in such affected dogs depressed functional activity of blood phagocytes was described (6, 12).

A decrease of some parameters of cellular immunity was reported in dogs in relation to the form and duration of the infection (16). Dysfunction of neutrophils is common finding in dogs with pyoderma (2, 5, and 17).

Our results can confirm different degree of immunosuppression that reflects character of the infectious process on the skin, when reduction of the immune reactivity of an organism can occur or, on the other side, some activities can be enhanced. Alteration of the immune system reactivity results into worsening of the primary process and secondary infection that complicates and prolongs therapy.

In such cases, when immunosuppression is confirmed, besides causative therapy the immunomodulation is indicated. Practical use of immunomodulatory substances in veterinary medicine requires an assessment of various aspects. First of all there is absolutely necessary to monitor healthy and immunological condition of animal suspected. The second is a decision about suitable immunomodulator, its doses and scheme of application.

Levamizol was successfully used in dogs with demodicosis associated with decreased immunocompetence (6). Other known immunostimulators are glucans. Glucans are polysacharides isolated from yeasts and fungi (*Saccharomyces cerevisiae, Pleurotus ostreatus*). They stimulate humoral and cellular immunity, mechanisms

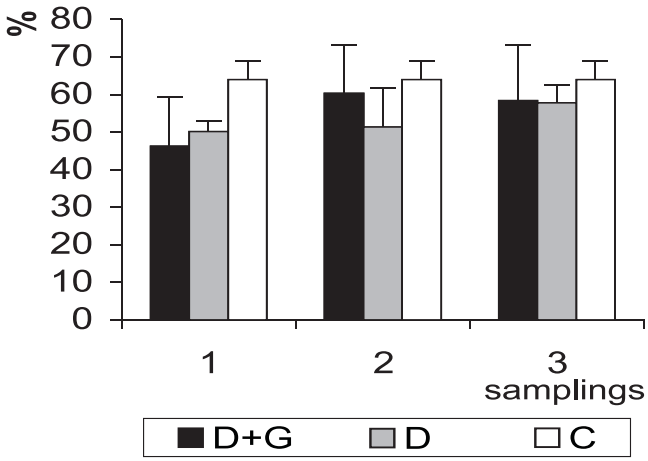

Fig. 1. Phagocytic activity of neutrophils in dogs with demodicosis before and after treatment

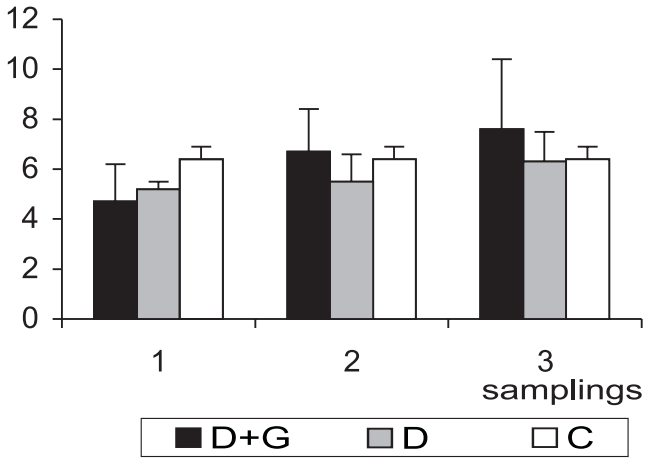

Fig. 2. Index of phagocytic activity in dogs with demodicosis before and after treatment

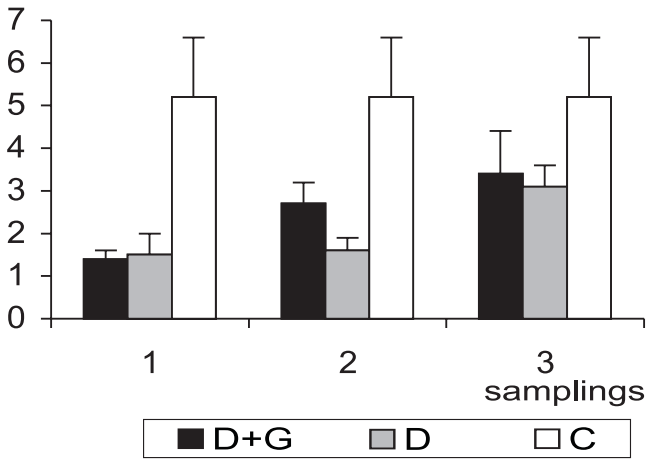

Fig. 3. Stimulation index of lymphocytes in dogs with demodicosis before and after treatment

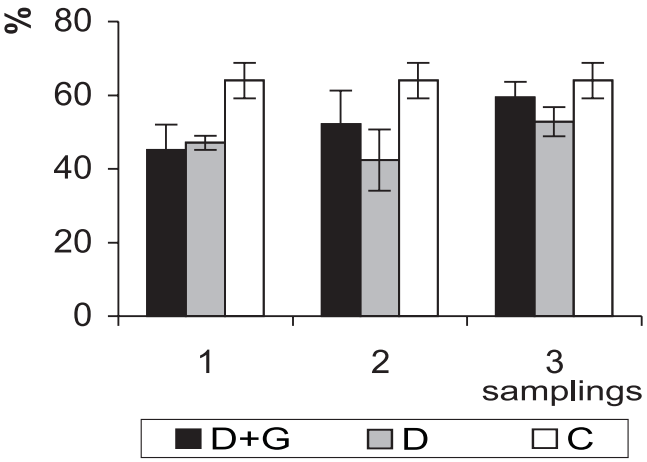

Fig. 4. Phagocytic activity of neutrophils in dogs with pyoderma before and after treatment

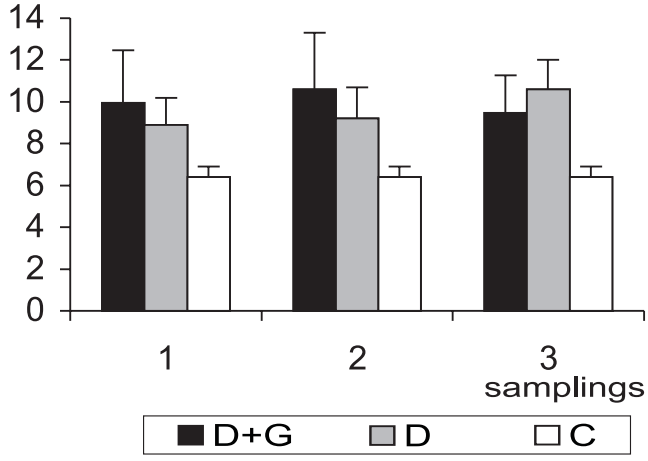

Fig. 5. Index of phagocytic activity in dogs with pyoderma before and after treatment

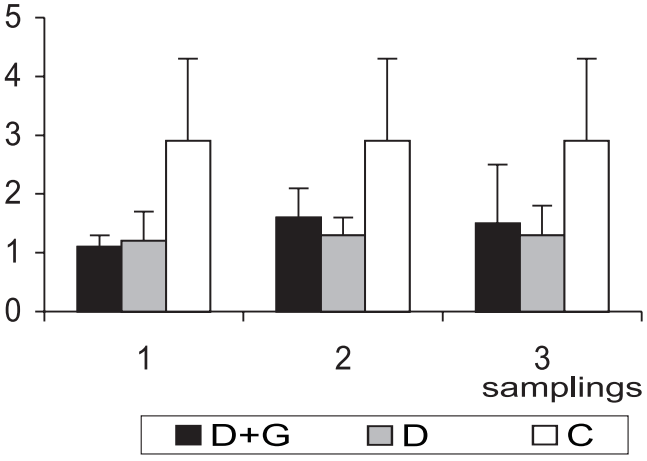

Fig. 6. Stimulation index of lymphocytes in dogs with pyoderma before and after treatment

of non-specific immunity and haematopoiesis (3). Glucan influences IL-1 secretion by macrophage that is a decisive cytokine for T-lymphocyte activation in antigen-presentation process and for IL-2 production (13). Glucans enhance protection against infectious (19) and parasitic (14) diseases.

Beta-(1,3),D-glucan used in our study showed positive effect on altered immune parameters that was in correlation with the clinical recovery of skin lesions in pyoderma affected dogs.

We can conclude that canine skin diseases (demodicosis and pyoderma) are associated with changes of the

Fig. 7. Demodicosis–skin lesions in 5 year old German Shepherd

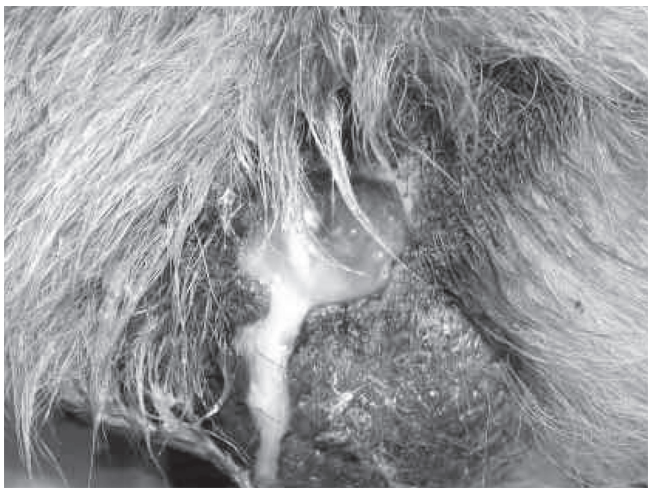

Fig. 8. Pyoderma – skin lesions in 4.7 year old boxer

immune reactivity. We recommend immunomodulatory therapy that makes causative therapy more effective and can reduce secondary complications of the disease. Beta-(1,3),D-glucan was proved as a suitable immunostimulators for clinical practice of small animals.

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IMMUNOLOGICAL CHANGES DURING *Eimeria procera* INFECTION IN A NON-SPECIFIC HOST

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ABSTRACT

A study of the most pathogenic species of *Eimeria* found in the caeca of partridges (*Eimeria procera*) was carried out on ten-day old chickens. In the histological sections of four intestinal regions (duodenum, jejunum, ileum and caeca), first generation schizonts were found in the epithelial cells at 12, 36, and 60 hours post-infection (hpi) with the highest concentration in the duodenum and caecum. To estimate the immunological response after administration non-specific coccidia to the chicken, subpopulations CD3+, CD4+, CD8+, and BU1b+ in the peripheral blood and spleen were ascertained by flow cytometry. The total number of peripheral blood leukocytes was not significantly increased during the whole experiment in the infected group, whereas the actual number of lymphocytes showed a slight increase at 12 and 48 hours pi. The cell subpopulation values of infected chickens exceeded the number of the control animals in the peripheral blood except of BU1b+. In the spleen only CD3 positive cells were stimulated in infected chickens. The results demonstrated a moderate immune reaction and immunogenecity of a non-specific host – chickens – to the partrigde *E. procera* infection.

Key words: chickens; *Eimeria procera*; immunity; lymphocyte subpopulations; non-specific host

INTRODUCTION

The host and site specificity of species of *Eimeria* are characteristically marked. It is rare for one of these parasites to occur naturally in more than one host. *Eimeria* infection induces an immune response in the host, including both humoral and cell-mediated immunity that lead to protection. Although it is generally accepted that this acquired immunity is species specific, Augustine and Danforth (2) have confirmed that chickens repeatedly inoculated with *E. adenoeides* develop a measure of immunity that partially protects them from a subsequent moderate challenge with *E. tenella* oocysts. Reciprocal studies, in which turkeys were immunised with *E. tenella* or *E. acervulina* have shown a failure of protection against a challenge with *E. adenoides* (4). The mechanisms preventing the intracellular development of *Eimeria* in non-specific hosts are not totally defined. One of these mechanisms is the host immune system with a dominant role of cell-mediated immunity in the host-protective response to *Eimeria* infection (17, 15, 12).

The infection of *E. procera* in chickens and the nature of immune responses are not known. Therefore, this study was designed to determine whether *E. procera* can develop in a chicken host and to follow the changes in lymphocyte subsets in blood and spleen during this infection.

MATERIALS AND METHODS

Chickens

A total of thirty-six ten-day-old White Leghorn chickens were randomly divided into experimental and control groups, containing eighteen birds each. The birds were kept in isolation in floor pens of 1 m^2 per group on wood shavings that were changed every day of the experiment. The pen was lit continu-

ously. The temperature was maintained at that required for the age of the birds (32 °C at first week and was reduced weekly by about 2 °C). The chickens had free access to water and a commercially available nutritive mixture including anticoccidial medicaments (BR1; Kŕmne zmesi Tomáš Latta, Košice). All chickens were coccidia-free before the experiment.

Reagents

Mouse anti-chicken monoclonal antibodies (Moab) CD3, CD4, CD8 T-lymphocytes (Serotec, Austria), and BU1b (Southern Biotechnology Associates, Inc., Birmingham, USA) expressed on B-cells were used for flow cytometry.

Parasites

The experimental infection was carried out with a pure culture of partridge sporulated oocysts *Eimeria procera*. Pure cultures were obtained by means of single oocysts isolation on agar (7).

Experimental design

The chickens in the experimental group were orally infected by 3 × 105 sporulated oocyst of partridge *E. procera*. Coccidia free chickens were used as control animals. Six birds from each group were cardiac punctured and then killed by cervical dislocation on 12, 36, and 60 hpi. The peripheral blood, and spleen were used for flow cytometry analysis. All parts of the intestine (duodenum, jejunum, ileum, and caecum) were taken for histological examination.

Histological examination

Intestinal samples were fixed in 10 % buffered formalin and subjected to routine processing, 5 µm thick section were cut and stained with haematoxylin-eosin. Evaluation was done by light microscopy.

For preparation of semi-thin sections the intestine was immediately immersed in a fixative solution consisting of a mixture of 2.5 % paraformaldehyde in 0.1 mol cacodylate buffer (pH 7.2). Samples were postfixed in 0.1 % OsO_4, dehydrated by an increasing ethanol series, and embedded in Durcupan. Semi-thin sections 1 to 2 µm thick were made on a Pyramitom LKB, stained with 0.5 % toluidine blue, and then evaluated under a light microscope.

White blood cell count (WBC)

Leukocytes were counted by a routine laboratory method using Fried – Lukáčová solution (6), (475 µl plus 25 µl blood). Differential cell counts were made on blood smears after Hemacolor (Merck, Germany) staining by counting 100 cells per slide.

Flow cytometry in the peripheral blood and spleen

Splenic cells were removed by teasing through a 70 mm mesh screen (9) and then isolated on a density gradient-Telebrix (1.077 $g.ml^{-1}$; SEVAC, Prague, the Czech Republic). Telebrix was also used for the separation of lymphocytes from the peripheral blood. After separation, the lymphocytes were twice washed with phosphate buffer saline (PBS). Fifty µl of cellular suspension (1 × 106 lymphocytes in PBS) and 50 µl of specific Moab (Table 1) were mixed and incubated at 4 °C for 30 minutes. After incubation the cells were washed twice in the PBS and pellets were mixed with 25 µl of the secondary antibody (FITC-conjugated goat antimouse immunoglobulin; Dako, Denmark) in dilution of 1:50 and incubated in the dark as described above. The cells were washed twice in the PBS and resuspended in 0.5 ml of 1 % paraformaldehyde in PBS.

Table 1. Primary monoclonal antibodies used in the experiment

Specificity	MoAbs	Isotype	Dilution
CD3	RTMCA1378	mouse IgG1	1:50
CD4	SRTMCA1473	mouse IgG1	1:25
CD8	SRTMCA1377	mouse IgG1	1:25
BU-1b	8370-01	mouse IgG1	1:25

Samples were analysed by the FACScan flow cytometer (Becton Dickinson, Germany). Data on 1 × 104 viable cells were collected using the Cell Quest programme. For the peripheral blood the actual lymphocyte counts were computed as follows: WBC count × % of the relative lymphocytes × % lymphocyte subpopulation.

Statistical analysis

All data were tested by a paired Student's *t*-test. Differences between the mean values for the groups of chicken were considered significant, when probabilities of less than 0.05 were obtained.

RESULTS AND DISCUSSION

By histological examination in *E. procera* infection, first generation schizonts were found in the epithelial cells of intestinal villi. Partridge *E. procera* infected the non-specific host – chickens in the cranial part of the intestine and caeca, at the areas, which are typical

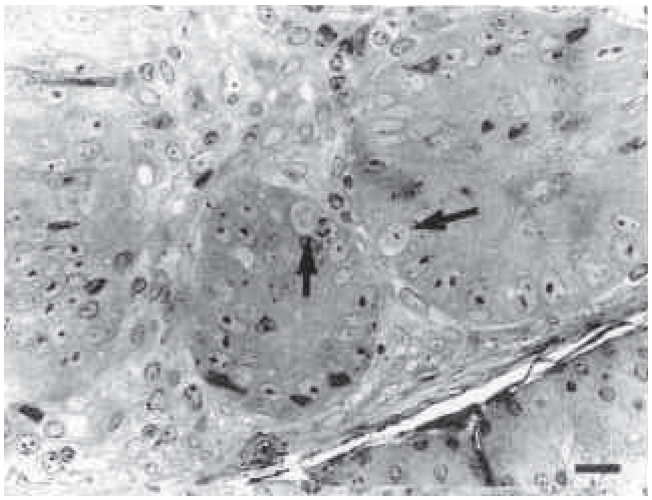

Fig. 1. First generation schizonts of *Eimeria procera* (arrow) in epithelial cells of chicken caecum twelve hours post-infection. Semi-thin section stained with toluidine blue (bar = 1 mm)

Table 2. Numbers of lymphocytes and their subsets in the peripheral blood of *Eimeria procera*-infected chickens in comparison to the control group ($G.l^{-1} - 1.109.l^{-1}$ ± standard deviation; * — P < 0.05)

Hours pi	Lc	Ly	CD3	CD4	CD8	BU1b
			E. procera			
12	9.90 ± 1.72	5.29 ± 0.44	1.14 ± 0.39	1.00 ± 0.44	0.74 ± 0.30	0.11 ± 0.09
36	16.72 ± 0.83	8.61 ± 1.11	1.02 ± 0.33	1.58 ± 0.50	1.25 ± 0.68	0.21 ± 0.06
60	15.50 ± 1.23	8.14 ± 0.48	1.65 ± 0.88	2.13 ± 0.35*	1.25 ± 0.34	0.14 ± 0.09
			Control			
12	8.33 ± 1.72	5.12 ± 1.11	0.89 ± 0.41	0.79 ± 0.43	0.45 ± 0.32	0.11 ± 0.09
36	14.77 ± 0.98	8.29 ± 0.09	1.87 ± 0.41	1.08 ± 0.51	1.20 ± 0.49	0.57 ± 0.15
60	14.03 ± 2.38	9.58 ± 1.88	1.23 ± 0.53	0.94 ± 0.49	0.84 ± 0.45	0.29 ± 0.11

for the natural hosts (Fig. 1). Staining procedures using parasite-specific monoclonal antibodies showed that sporozoites of turkey coccidia survived within the intestinal cells of the chicken for up to three days, but failed to develop any further (3).

Sporozoites of the chicken coccidia also invade a foreign host, the turkey, in the same intestinal sites as in the natural host (1). These facts suggest that sporozoites of the chicken coccidia, like those of the turkey coccidia, would be able to elicit cross-species protection. In our previous work we were able to observe first generation schizonts after *Eimeria colchici* chickens' infection on 60 hpi (13).

The total number of peripheral blood leukocytes (Table 2) was not significantly increased in infected chickens during the whole experiment. This is in accordance with the findings of higher values in *E. colchici* infected chickens. The increase of lymphocytes in *E. colchici* (14, 8) and the decrease in *E. procera* infected birds were found 60 hpi (Table 2).

Kogut and Eirmann (11) have demonstrated that the suppression of T-lymphocyte activity in a non-specific host early during an infection with a heterologous species of *Eimeria* permits the complete intracellular development of the parasite. In our experiment parasite induced immune response with reflection in the changes of T-lymphocytes, especially CD4+ T-cell subset in the peripheral blood (Table 2). CD4+ T-cells were important in the control of primary infection with *E. tenella* in natural host-chicken (16). The proportion of CD3+ cells had slightly increased in the peripheral blood (Table 2) and spleen (Table 3) in *E. procera*-infected group.

It is known that the CD3 receptor plays a role in the transfer of signal into lymphocytes, and in the presentation of antigen together with CD4 or CD8 T-lymphocytes. Changes of CD8+ cells were not significantly higher in the blood of infected chickens than in the spleen, where these cells had similar values to the control group. A role for CD8 cells was hypothesized to become functional after a challenge infection (5). *E. procera*-infected chicken showed a higher ratio of CD4/CD8 T-lymphocytes.

These changes, together with higher values of CD4 and CD8 cells indicate the prevalence of cell mediated immune mechanisms in experimentally infected non-specific hosts. On the other hand, the level of BU1b subpopulation, expressed on B-cells, as well as on a subset of chicken macrophages (10) did not exceed the values of the control group.

Table 3. Relative percentage of lymphocytes subsets in the spleen of *Eimeria procera*-infected chickens in comparison to the control group (mean ± standard deviation)

Hours pi	CD3	CD4	CD8	BU1b
		E. procera		
12	36 ± 5	24 ± 1	38 ± 3	10 ± 7
36	47 ± 10	30 ± 3	46 ± 8	11 ± 4
60	57 ± 8	30 ± 4	45 ± 15	4 ± 3
		Control		
12	33 ± 4	28 ± 3	39 ± 5	9 ± 6
36	43 ± 8	28 ± 4	48 ± 2	22 ± 5
60	52 ± 9	31 ± 2	50 ± 4	10 ± 5

On the basis of these results it may be summarized that infection of chickens with *E. procera* had immunomodulatory effect. In comparison with our previous results (14) we can claim, that *E. colchici* have elicited more intensive immunoreactivity in chickens than *E. procera*. Further investigations could show a cross-specific protection against a challenge with host-specific coccidian.

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THE DIAGNOSTIC EFFECTIVENESS OF CONTRAST MEDIA IN THE EXCRETORY UROGRAPHY IN DOGS

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ABSTRACT

A clinical study was conducted to investigate the use of four different positive contrast media, Omnipaque (Nycomed Imaging As.), Optiray (Maliincrodt Medical GmbH), Ultravist (Schering AG), and Urografin (Schering AG) in normograde excretory urography. Investigations were carried out on thirteen dogs. Each group of dogs was administered a different contrast medium. Radiographs were obtained immediately after the administration of contrast media and after a lapse of 5, 10 and 15 minutes. The effectiveness of the application was evaluated by assessing the radiographs of abdominal cavities in ventro-dorsal and latero-lateral positions. None of the dogs showed pronounced side effect after the application of contrast media. Ectopic ureter was diagnosed in three and a prostate cyst in one of the patients. Our results showed that Omnipaque and Ultravist were the contrast agents most suitable for the examination of the urinary apparatus by intravenous contrast excretory urography. Their advantage is that visualisation of the kidneys and ureters achieved by these media is sufficiently long and contrastive.

Key words: contrast medium; dog; excretory urography; X-ray films

INTRODUCTION

Contrast media have been used widely in veterinary medicine. They are frequently used in diagnostics of diseases of the digestive and urinary systems, damage and diseases of the spinal column, vessels, bronchi, lymphatic system and the bile tract. The use of contrast media in roentgenology allows one to obtain positive or negative contrast against other visualised tissues. Such imaging is a valuable diagnostic tool. Nephrology and urology uses positive and negative contrast to an equal extent. Contrast media can be applied in a normograde and retrograde way (7).

Examination with the use of a contrast medium consists of intravenous administration of the medium, roentgenological observation of its accumulation and later excretion by urinary tract organs (1).

Examination is indicated when there is a suspicion of neoplastic or cystic changes in kidneys, morphological damage to the urinary system manifested as haematuria, at suspect nephrolitiasis, infiltrative or inflammatory diseases of the renal parenchyma, suspect trauma of ureter, suspect ectopic ureter and *incontinentia urinae* (3).

The contrast methods use either negative (radiolucent) or positive (radioopaque) agents. The negative contrast media include: air, oxygen, nitrous oxide and carbon dioxide. They are used in pneumocystography, pneumoperitoneography, pneumopericardiography and pneumoventriculography. Positive contrast media include insoluble salts of heavy metals (barium sulphate) and organic iodine compounds (7).

On the basis of chemical structure, contrast media are divided to four groups (monomeric ionic, monomeric non-ionic, dimeric ionic and dimeric non-ionic).

The groups differ in their chemical structure and physical-chemical properties. These properties determine their osmotoxicity, chemotoxicity and ionic toxicity. Toxic effects

are associated mostly with the central nervous system, cardiovascular system and kidneys (6).

All water-soluble contrast media used in urography and angiography are substances with extracellular action excreted by the kidneys in a non-metabolised form by the mechanism of glomerular filtration. Approximately 85–90 % of the administered dose could be found in urine during the first six hours after administration. By 24 hours, 95–100 % of the administered medium is excreted in the urine. Less than 2 % can be found in the faeces (2).

Contrast media allow one to examine kidneys and other organs of the urinary tract by the method of excretory urography, cystography or uretrography.

The aim of our clinical study was to compare the effectiveness of contrast media used in the excretory urography in dogs with regard to accentuating the tissue structures in radiographs taken to diagnose some diseases.

MATERIALS AND METHODS

Four contrast media were evaluated with regard to their diagnostic effectiveness in excretory urography (Table 1).

Table 1. List of administered contrast media

Name of the contrast medium	Active ingredient	Content of iodine
Omnipaque (Nycomed Imaging As)	Iohexol	240 mg I.ml^{-1}
Optiray (Maliinckrodt Medical GmbH)	Ioversol	300 mg I.ml^{-1}
Ultravist (Scgering AG)	Iopromide	300 mg I.ml^{-1}
Urografin 76 % (Schering AG)	Sodium and meglumine salt of amidotrizooic acid	370 mg I.ml^{-1}

After intravenous administration of all four media we focused on the following:

a) rate of excretion by kidneys: the most rapid, optimum and the slowest,

b) clearance time — from kidneys through ureter into the urinary bladder,

c) evaluation of the visibility of contrast,

d) presence or absence of complications during administration or after excretion of the contrast medium from the body.

On the basis of obtained radiographs, considering the time lapse between intravenous administration of the contrast medium and our evaluation, the presence or absence of the contrast medium in the urinary apparatus was evaluated using the following scale:

0 — absence, + — slight visibility, ++ — good visibility, +++ — especially good visibility of the contrast medium in the urinary apparatus.

The respective media were administered by needles and cannulas adjusted to the size of dogs, sterile tampons and Septonex spray a.u.v. as a disinfectant. Radiographic examination was carried out using an X-ray machine Chiralux — 2 Chirana with cartridges X-omatic, Green Kodak 400 films, type Green Kodak and Fuji of, dimensions 30 × 40 cm. Radiographs were developed by a developing automat AR 510. Lysholm aperture was used. For each patient, specific values (kV, s, mAs) were set on the X-ray machine according to age, weight, nutrition state and dog breed.

The clinical study was conducted on dogs of various categories. Five of them were the patients at the Clinic of Surgery, Orthopaedics and Roentgenology and the Ist Internal Clinic of the University of Veterinary Medicine in Košice. The remaining dogs were clinically healthy and were included in the clinical study. Before examination, the dogs fasted for one day and during the last four hours they had no access to water. Temperature, pulse, and respiration rate was determined before administration of the contrast media and the results were within the physiological range.

Niemand *et al.* (5) have recommended applying 1.5 ml.kg^{-1} body weight of a contrast medium according to the content of iodine (450—800 mg I.kg^{-1} b.w.). The maximum dose is 35 grams iodine per dog. The dose of contrast medium used in our study was determined according to recommendations mentioned above and according to our own experience.

A native radiograph in ventro-dorsal projection was obtained before the administration of the contrast medium.

The contrast medium was administered to *vena cephalica antebrachii* of a shaved limb. The full dose of the contrast medium was administered in the form of a rapid intravenous bolus. Immediately after administration, the first radiograph was obtained in a ventro-dorsal projection. Additional radiographs were taken after 5, 10 and 15 min, also in the ventro-dorsal projection without external abdominal compression by a sand bag.

When evaluating the radiographs with a negatoscope, we considered the rate of elimination of the contrast medium and visibility of contrast. We used the above mentioned four-point scale. Our evaluations are summarised in four tables according to dog groups and the contrast media. We recorded for each patient the visibility of contrast in relation to time after administration of the contrast medium and localisation of the eliminated contrast medium in the urinary system of dogs.

RESULTS

On the basis of the data summarised in Table 2 the contrast medium Omnipaque appeared as very suitable for the examination of the urinary apparatus by contrast excretory urography as it became visible in the urinary system shortly after administrations and persisted in it for sufficient time. Administration of this medium was very simple and dogs did not show any negative side effects. On the basis of this examination we diagnosed ectopic ureter in two patients and a prostate cyst in one patient.

Results in Table 3 show that the contrast medium Optiray was less suitable for examination of the urinary apparatus by contrast excretory urography. This contrast medium became visible in various time intervals in individual patients and the visibility of contrast differed in

Table 2. Evaluation of the diagnostic effectiveness of the contrast medium Omnipaque

Dog	Dose	Time after admin.	Immediately after administration	Visibility in a radiograph 5 min after	10 min after	15 min after
1	500 mg I.kg^{-1}	**Evaluation**	++	+++	+++	+++
		Location	kidneys	kidneys, ureter partially urinary bladder	kidneys, ureter partially urinary bladder	kidneys, ureter partially urinary bladder
2	500 mg I.kg^{-1}	**Evaluation**	++	+++	+++	+++
		Location	kidneys, ureter partially urinary bladder	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder
3	500 mg I.kg^{-1}	**Evaluation**	0	+++	+++	+++
		Location		kidneys, ureter	kidneys, ureter urinary bladder	ureter urinary bladder
4	500 mg I.kg^{-1}	**Evaluation**	++	+++	+++	+++
		Location	kidneys	kidneys, ureter	kidneys, ureter urinary bladder	urinary bladder

Table 3. Evaluation of the diagnostic effectiveness of the contrast medium Optiray

Dog	Dose	Time after admin.	Immediately after administration	Visibility in a radiograph 5 min after	10 min after	15 min after
5	600 mg I.kg^{-1}	**Evaluation**	0	0	0	+
		Location				kidneys
6	800 mg I.kg^{-1}	**Evaluation**	+++	+++	++	++
		Location	kidneys, ureter	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder
7	600 mg I.kg^{-1}	**Evaluation**	+	+	+	++
		Location	kidneys, ureter	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder

individual dogs and increased with time after administration. Negative reaction to this contrast medium occurred in one dog in the form of oedemas in the facial region and on top of the head.

With regard to our results, the contrast medium Ultravist was evaluated as suitable for examination by excretory urography as it passed to the urinary apparatus immediately after administration and persisted there for sufficient time needed to carry out radiographical examination of the urinary tract.

The information obtained indicated that the contrast medium Urografin appeared less suitable for examination of the urinary tract by contrast excretory urography. Elimination of this contrast medium occurred at a very high rate which was not suitable for this type of examination. Its administration was associated with considerable pain which was not observed with the other media. Moreover, one of the dogs vomited shortly after administration and showed a local inflammatory reaction in the place of administration on the following day.

Our results showed that excretion of the contrast media by kidneys was the quickest with Optiray and Urografin, optimum with Ultravist and the slowest with Omnipaque. Elimination from kidneys through ureter to the urinary bladder took 5 minutes with contrast media Optiray, Ultravist and Urografin and 10 min with Omnipaque. The visibility of contrast was best with Omnipaque and good with the remaining contrast media. Marked side-effects were recorded after administration of the Optiray and Urografin contrast media.

DISCUSSION

Radiographic examination belongs among special and specific examination methods in veterinary medicine as it confirms or rejects the clinical diagnosis. It is specific because it provides information about the physiology and pathology of tissues, particularly osseous and pulmonary ones. However, by using contrast media in radiographic examination of soft tissues one can obtain a large body of information also about their pathology.

No specific contrast media have been developed for use in veterinary medicine. It is possible to use those which have been subjected to our tests of effectiveness. By comparing the individual contrast media we have

Tab. 4. Evaluation of the diagnostic effectiveness of the contrast medium Ultravist

Dog	Dose	Time after admin.	Immediately after administration	Visibility in a radiograph 5 min after	10 min after	15 min after
8	600 mg I.kg^{-1}	**Evaluation**	++	+++	+++	+++
		Location	kidneys ureter	renal pelvis, ureter urinary bladder	renal pelvis, ureter urinary bladder	renal pelvis, ureter urinary bladder
9	600 mg I.kg^{-1}	**Evaluation**	++	++	+++	++
		Location	kidneys ureter	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder	urinary bladder
10	600 mg I.kg^{-1}	**Evaluation**	+	+	+	++
		Location	kidneys, ureter partially urinary bladder	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder	kidneys, ureter urinary bladder

Tab. 5. Evaluation of the diagnostic effectiveness of the contrast medium Urografin

Dog	Dose	Time after admin.	Immediately after administration	Visibility in a radiograph 5 min after	10 min after	15 min after
11	740 mg I.kg^{-1}	**Evaluation**	++	+++	+++	++
		Location	kidneysureter urinary bladder	urinary bladder	urinary bladder	urinary bladder
12	740 mg I.kg^{-1}	**Evaluation**	++	++	++	+++
		Location	kidneys	kidneys, ureter partially urinary bladder	urinary bladder	urinary bladder
13	740 mg I.kg^{-1}	**Evaluation**	+	++	++	++
		Location	kidneys, ureter partially urinary bladder	kidneys, ureter urinary bladder	urinary bladder	urinary bladder

concluded that Omnipaque and Ultravist appeared most suitable for examination of the urinary tract by contrast excretory urography as they complied with all criteria set for such media. One of their important advantages is their good tolerability and low occurrence of undesirable side effects and complications.

Manhire *et al.* (4) has used contrast media containing sodium and meglumin salts of ioxaglate (Hexabrix) and iohexol (Omnipaque) and observed that Hexabrix induced nausea and vomiting in 24.5 % of patients while no such reaction was observed with Omnipaque. Our experiments showed that vomiting occurred only in dogs, which were administered Urografin, the contrast medium with sodium and meglumine salt of amidotrizooic acid as an active ingredient. Urografin, as an ionic contrast medium, exhibits high osmolality and viscosity compared to other media investigated in our study, which explains the pain associated with its administration and the undesirable post-administration side effects, such as vomiting and local inflammatory changes on limbs at the site of administration.

Of other negative side effects we should mention one allergic reaction manifested as oedemisation in the face region and on top of the head after administration of the non-ionic contrast medium Optiray with effective ingredient ioversol. According to the package leaflet of the contrast medium Omnipaque (active ingredient iohexol), other negative side-effects have been observed in human medicine, such as warm sensation and transient "metal taste". Such information cannot be obtained in veterinary medicine. Only one of our patients vomited after administration of contrast media.

Examination with the use of contrast media allowed us to make a reliable diagnosis in clinical patients, which confirms high effectiveness of these media in radiography. We diagnosed congenital ectopic ureters which ran intramurally and opened to the vagina. Similarly in dogs with a prostate cyst we could differentiate prostate, prostatic cyst, urinary bladder and kidneys. For the present, we are unable to evaluate the clinical effectiveness of the use of contrast excretory urography in kidney diseases as we did not encounter such a case during our 2-year observations. According to Kučera (3) contrast agents are excreted at different periods due to the disturbed filtration of the blood.

Results obtained after the administration of four types of contrast media in excretory intravenous urography in dogs indicated that their use in veterinary medicine has potential. The contrast media Omnipaque and Ultravist appeared to be more effective although good results were also obtained with Optiray and Urografin. Our experiences support the use of this method for the diagnostics of urologic diseases in dogs. However, evaluation of contrast media requires some experience in this field.

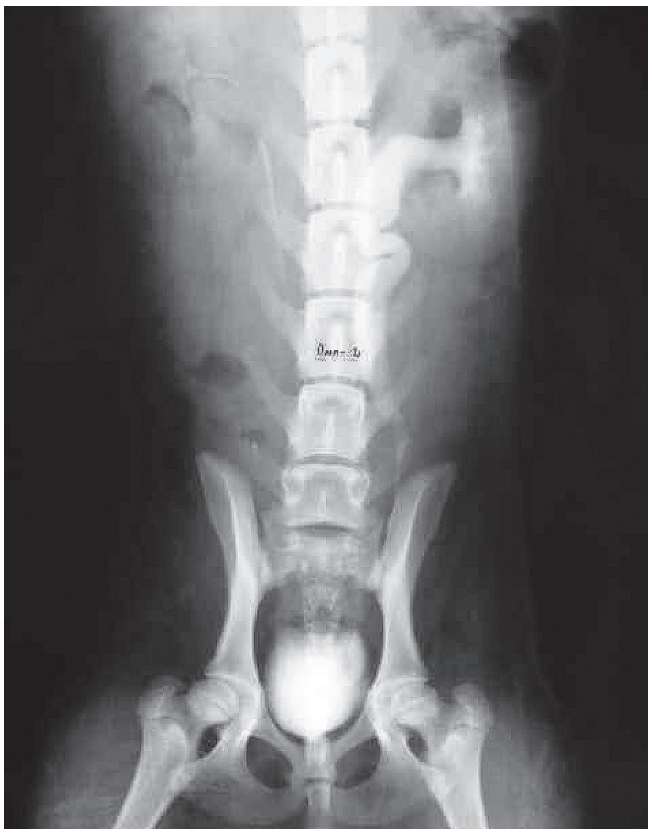

Fig. 1. Omnipaque 10 minutes after application. There is visible concentration of the contrast media in the urinary bladder

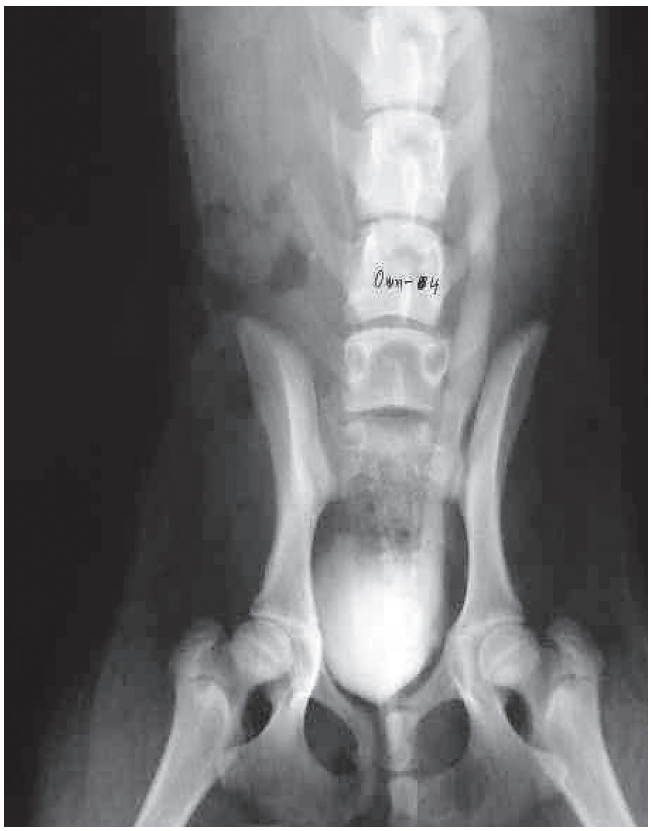

Fig. 2. Omnipaque 15 minutes after application. Notable is great dilatation of the left ureter

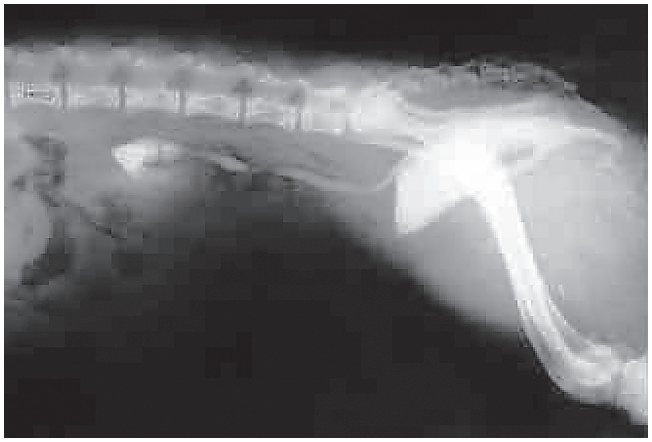

Fig. 3. Omnipaque is 15 minutes after application still in the kidneys. Distended left ureter is entering into the vestibulum vaginae – an ectopic ureter

CONCLUSIONS

Our study focused on the clinical use of four types of contrast media in excretory urography in dog s and the evaluation of their diagnostic effectiveness. The respective contrast media were Omnipaque, Optiray, Ultravist and Urografin.

We evaluated the rate of elimination of the contrast medium from the urinary apparatus of the dog, clearance time of the medium from kidneys through ureter to the urinary bladder, visibility of the contrast and occurrence or absence of complications during and after administration and after elimination of the contrast medium from the body.

On the basis of our investigations the contrast media Omnipaque and Ultravist (compared to Optiray and Urografin) exhibited good effectiveness in the examination of the urinary apparatus of dogs by intravenous excretory urography in practical veterinary medicine.

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MANIPULATION OF RUMEN NITROGEN METABOLISM (A REVIEW)

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ABSTRACT

This review deals with some factors influencing enzymes involved in nitrogen metabolism in the rumen. Urease inhibitors reduce the rate of ammonia nitrogen release from dietary urea to increase its utilization in proteosynthesis in ruminants. Ionophores inhibit Gram-positive bacteria that produce ammonia or lactic acid; antibiotics inhibit pathogenic bacteria in the rumen and bacteriocins affect amino acid degradation. Essential oils, saponins, tannins or yeast can alter ruminal fermantation as well. Essential oils decrease the rates of NH3 production from amino acids in ruminal fluid by inhibiting hyper-ammonia producing bacteria. Saponin-containing plants appear to be useful as a means of suppressing the bacteriolytic activity of rumen ciliate protozoa and thereby enhancing the total microbial protein flow from the rumen. Dietary tannins adversely affect fermentation by bacteriostatic and bactericidal activities and by inactivating ruminal enzymes. Yeasts have some positive effect on urease, aspartate and alanine aminotransferasese and proteases activities and the total protozoal count in the rumen. Experiments with divalent ions showed that they could interfere with enzymatic activities. Feed additives can decrease fermentation losses (e.g., ammonia or methane) in order to increase animal productivity.

Key words: feed additive; protein metabolism; rumen enzyme; ruminant

INTRODUCTION

High levels of animal productivity cannot be sustained by forage alone. Ruminant nutritionists have sought methods for decreasing fermentation losses (e.g., ammonia) or increasing the production rate and molar proportion of volatile fatty acids. Ruminants are fed with a variety of additives* (e.g., ionophores, bacteriocins, inhibitors, bioactive substances) to alter fermentation. These supplements are widely used. This review deals with the description of factors including several feed additives influencing enzymes involved in nitrogen rumen metabolism *in vitro* and *in vivo*.

UTILIZATION OF AMMONIA BY RUMEN BACTERIA

The nutritional requirements of ruminants are different from those of monogastric animals. Rumen microbes can synthesize enough amino acids and peptides from the inorganic nitrogen of ammonia or other nitrogen source and carbon skeletons and sulphur precursors. The assimilation of ammonia by rumen microbes depends on lots of factors such as rumen pH (38),

* Feed additives are products used in animal nutrition for purpose of improving the quality of feed and the quality of food from animal origin, or to improve the animal performance and health, e.g. providing enhanced digestibility of the feed materials.
Council Regulation (EC) 1831/2003 sets out new rules for the authorization, supervision and labelling of feed additives.

rumen ammonia concentration (20) and rumen ammonia-assimilating enzyme acitivity (4, 39). De Veth *et al.* (38) have reported that the rumen digestibility of pasture dry matter was optimized at pH 6.35, and the synthesis of microbial protein was optimized at pH 6.13.

Ammonia is the main precursor for microbial protein synthesis in the rumen and, although the required concentration remains controversial, NH3 must be presented in excess of microbial requirements for optimal fermentation to occur. Results of Eschenlauer *et al.* (5) have demonstrated that different methodologies and different substrate concentrations have provided an explanation for different apparent rates of ruminal NH3 production reported in different studies and identified a diverse range of hyper-ammonia-producing bacteria in the rumen of sheep.

RUMEN UREASE HYDROLYSES UREA TO AMMONIA

Many papers have dealt with the study of the effectiveness of various inhibitors on the activity of rumen urease. Reducing the rate of ammonia nitrogen released from dietary urea would increase its utilization in ruminants both *in vivo* (27, 22, 28, 43) and *in vitro* (16). Faixová and Faix (7) have observed that both cadmium and copper appeared to be inhibitory factors for urease activity *in vitro* studies. But the rumen microflora may often be capable of adapting to chronic administration of urease inhibitors, thereby limiting its practical use in improving the utilization of dietary urea *in vivo* (17). Whitelaw *et al.* (42) have reported that even substantial changes in urea recycling had only a minor effect on the overall N economy on the animal *in vivo*.

Ammonia utilization by rumen microbes depends on ruminal ammonia-assimilating activity, too.

Several ammonia-assimilation reactions by rumen bacteria are known. In *Ruminococcus flavefaciens* and *Prevotella ruminicola* glutamate dehydrogenase (GDH) appears to be the predominant route of ammonia assimilation irrespective of ammonia concentration, and peptides modulate GDH activity in *P. ruminicola* (13).

Glutamate dehydrogenase plays an important role in safeguarding the balance between ammoniac nitrogen and amino nitrogen and acts as a kind of adaptation system in relation to the metabolic situation in the ruminant organism.

FEED ADDITIVES IN RUMINANT NUTRITION

Various methods for decreasing fermentation losses (e.g., methane or ammonia) or increasing production rate and the molar proportion of volatile fatty acids have been introduced in nutrition in order to increase animal productivity.

Heat-treated proteins decrease ruminal deamination and provide an additional source of amino acids (6, 5).

Ionophores (e.g., monensin) inhibit Gram-positive bacteria that produce hydrogen, ammonia, or lactic acid (1, 10, 31).

Antibiotics such as salinomycin (19), sulfastimidine (33) or oxytetracycline (34) had influence on rumen function. Sadhu *et al.* (33) have reported that oral administration of sulfadimidine at 200 $mg.kg^{-1}$ bodyweight for six successive days caused a significant increase in rumen fluid pH and methylene blue reduction time and a significant decrease in total protozoal count and glucose fermentation rate in buffalo calves. Later, rumen fluid analysis and biochemical estimation in calves administered oxytetracycline at 20 $mg.kg^{-1}$ bodyweight for five consecutive days revealed a significant reduction in protozoal motility, total protozoal count and glucose fermentation rate. No significant changes were observed in rumen fluid pH and methylene blue reduction time (34).

Bacteriocins influence cellulose digestion, amino acid degradation and even starch fermentation (15).

Panda *et al.* (26) have observed that dietary supplementation of yeast (*Saccharomyces cerevisiae*) had some positive effect on urease, aspartate and alanine aminotransferases and protease activities and total protozoal count in the rumen in crossbred cattle calves. Similar results have been reported by Kamra *et al.* (12) and Nursoy and Baytok (23).

DEFAUNATION

Defaunation alters protease, urease, amylase and other enzyme activities of rumen fluid as well ammonia nitrogen level (25, 14, 2, 32, 35).

NATURAL MANIPULATORS

Various natural plant compounds are capable of affecting ruminal fermentation.

Makkar *et al.* (18) have observed that tannin-containing exctract of oak leaves decreased rumen urease, cellulase, protease, amylase, glutamate dehydrogenase, alanine aminotransferase, aspartate aminotransferase activities, but increased glutamate synthetase. The inhibition was higher when substrate rather than enzyme was added for start of the reaction *in vitro*. Later it was found that although condensed tannins in *Lotus corniculatus* reduced the population of some proteolytic bacteria, total microbial protein and microbial protein outflow to the abomasum were unchanged in sheep (21). Dietary tannins adversely affect rumen metabolism by bacteriostatic and bactericidal activities and by inactivating enzymes, e.g., carboxymethyl cellulase, proteases, and glutamate dehydrogenase (29).

Wallace *et al.* (41) have reported that dietary essential oils cause a decrease of rates of NH_3 production from amino acids in ruminal fluid taken from sheep and cattle receiving the oil, yet proteinase and peptidase activities are unchanged. Hyper-ammonia producing bacteria were the most sensitive of ruminal bacteria to essential oils in pure culture. Saponin-containing plants appear to be useful as means of suppressing the bacteriolytic activity of rumen ciliate protozoa and thereby enhancing total microbial protein flow from the rumen.

These studies illustrate that natural products such as essential oils and saponins are two types of plant secondary

compounds having great potential as 'natural manipulators' of rumen fermentation. Hristov *et al.* (11) have investigated the effect of bioactive agents (oils, saponins, tannins, bentonite and ionophores) on ruminal fermentation and protozoal activity *in vitro* as potential feed additives to improve feed efficiency. Results indicate that they could alter total free amino acid level, enzyme activities (carboxymethyl cellulase, xylanase and amylase) and protozoal numbers.

An exctract of Yucca shidigera (De-Odorase, Alltech Inc.) given to sheep reduced the concentrations of ammonia and urea in ruminal fluid. The extract appeared to bind to ammonia and did not affect urease activity or change volatile fatty acid profiles or pH (30).

INORGANIC IONS

Ruminal enzyme activities were found to be influenced by a number of inorganic ions.

Ions, like copper, cadmium, and zinc are well known in binding to the -SH groups in the proteins or enzymes, thereby interfering with enzymatic activity.

Engle and Spears (3) have reported that copper did not affect ruminal fermentation in *in vivo* studies, whereas Forsberg (9) have observed that Cu concentrations of 21 $\mu g.ml^{-1}$ could decrease fermentative activity and growth of certain populations of bacteria in *in vitro* studies. Furthermore, Odenkirchen *et al.* (24) have reported that supplementing 2 g $CuSO_4$ per animal per day is recommended as the maximum dose in cattle to overcome copper deficiency. Faixová and Faix (7) using strained ruminal fluid have reported that Cu concentrations of 5 $mmol.l^{-1}$ in rumen fluid are found to inhibit both urease and glutamate dehydrogenase activities in sheep. Recently Faixová *et al.* (8) observed that Cu concentrations of 0.383 $mg.l^{-1}$ of rumen fluid stimulated alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase and γ-glutamyltransferase activities of rumen fluid in *in vivo* studies.

Copper is an essential element required for a number of biochemical functions but ingestion of quantities of Cu slightly higher than required may cause accumulation in the tissues and haemolysis. Goat are more sensitive to high copper supplementation than other farm animals (36).

Wallace and McKain (40) have reported that copper, chromium and mercury inhibited *Prevotella ruminicola* dipeptidase activity to 15, 15 and 5 % of control activity.

This is in agreement with the results in ewes reported by Spears and Hatfield (37), who have observed that copper, zinc and cadmium ions are found to inhibit urease in the rumen fluid *in vitro* experiment whereas barium, nickel and manganese appear to be slightly stimulatory at both the high and low concentrations. Strontium, calcium and cobalt are inhibitory at high concentrations.

Wallace and McKain (40) have reported that cobalt, manganese and zinc stimulated *P. ruminicola* dipeptidase activity by 189, 30 and 26 %, respectively. Results of Faixová *et al.* (8) show that Zn concentrations of 5.9 $mg.l^{-1}$ of rumen fluid stimulated ruminal enzyme activities in *in vivo* studies.

CONCLUSION

Ruminant animals and ruminal micro-organisms have evolved together for millions of years, and the rumen is inhabited by diverse and interdependent populations of bacteria, protozoa, and fungi. As ruminal micro-organisms are highly competitive, the ruminal community is normally quite stable.

However, in the past fifty years, humans have drastically alterted the diet that ruminants consume. The use of non-protein nitrogen such as urea in ruminant diet has been a cost effective method of providing nitrogen and has improved the economics of animal agriculture; however, the ammonia formed when excess urea breaks down in the rumen could lead to acute toxicity and in turn, have increased the need for feed additives to counter this problem.

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