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VITAL REPRODUCTIVE INDICES IN NON GRAVID FEMALE RABBITS TREATED WITH CRUDE ETHANOL EXTRACT OF *SPONDIAS MOMBIN*

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ABSTRACT

Ten female Chinchilla breed of rabbits with a mean weight of 1.65 ± 0.06 kg, were randomly divided equally into a treatment and a control group. The treatment group received orally 800 mg.kg^{-1} of an ethanol extract of *Spondias mombin* for fourteen days. The control group received only distilled water utilizing the same method of administration. The females were kept separately away from males. Assays of the reproductive hormones before and after treatment with the extract in the treatment group were done. Both groups were sacrificed after fourteen days of oral administration and their reproductive tracts carefully examined. The studies revealed the normal morphology of ovaries, oviduct, uterine horns, and uterine body in both groups. The histological sections of different reproductive segments were normal. Left and right ovaries appeared to show marked oogenesis in the treated group going by the larger number of follicles observed compared to the control. The body weight adjusted mean paired ovarian diameter of the control group (0.51 ± 0.02 cm) was significantly (P ≤ 0.05) different from the treated animals (0.41 ± 0.39 cm) but this did not translate into a significant difference in the paired ovarian weights. The body weight adjusted lengths, widths and weights of other parts of the reproductive tracts did not differ significantly comparing the treated and control groups, except for the length of the right uterine horn and the weight of the right oviduct. The studies showed that all of the hormones did not change significantly, comparing the pre and post treatment in the treated group. It was concluded that 800 mg.kg^{-1} of ethanol extract of *Spondias mombin* appeared to favour folliculogenesis in non-gravid rabbits and resulted in no observed pathological effects on the entire reproductive tract of the rabbit.

Key words: ethanol; rabbit; reproduction; *Spondias mombin*

INTRODUCTION

Interest in the effects of various natural products on reproductive parameters has been on the increase in recent times [1], [2], [12], [17], [18]. This interest in natural products emanate from their advantage over synthetic drugs [16]. Also, productivity in livestock is based upon the ability of animals to reproduce, hence the need to ensure fertility. Fertility remains a key determinant of life time performance [10] and to achieve this, optimization of litter size, good fecundity and treatment and prevention of pathological reproductive conditions are significant [9]. In the rabbit, the normal reproductive tract consists of the ovaries, oviducts, uterine horns joining to continue as a body and then forms the cervix and vagina. Under favourable conditions, does will remain in oestrus for long periods during which time ovarian follicles are continually developing and regressing at more or less the same rate. The active life of a follicle is around 12—16 days [11]. *Spondias mombin*, a tropical fructiferous plant, used for abortifacient purposes, among other uses, by traditional folks, has been termed an antifertility agents by a few authors based on its oxytocic effect [12]. However, its effect on non gravid animals is important to assess for possible positive effects.

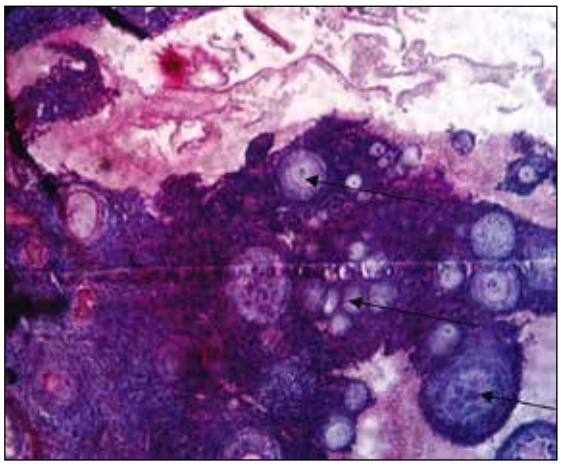

Fig. 1. Histological section of the right ovary of the treatment group with arrows showing follicles at different stages of development.
Periodic acid-Schiff (PAS) stain. Magn. ×100

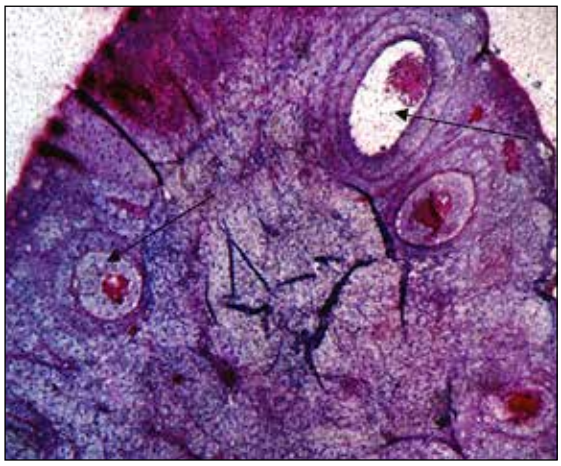

Fig. 2. Histological section of the right ovary of the control group with arrows showing follicles at different stages of development.
Periodic acid-Schiff (PAS) stain. Magn. ×100

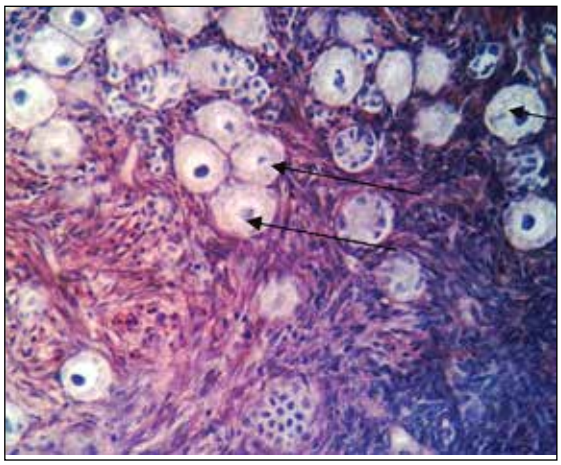

Fig. 3. Histological section of the right ovary of the treatment group. Arrows showing primary follicles.
Periodic acid-Schiff (PAS) stain. Magn. ×400

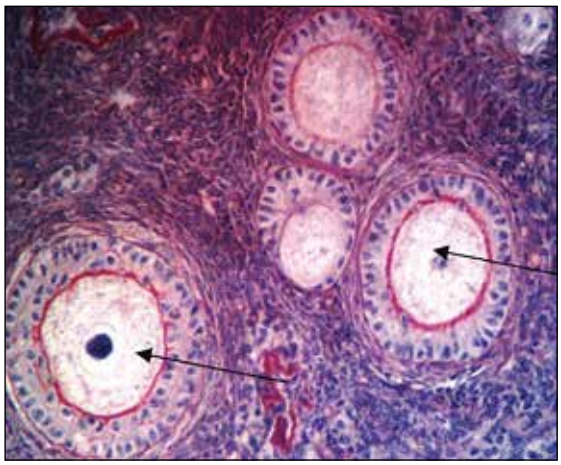

Fig. 4. Histological section of the right ovary of the treatment group. Arrows showing secondary follicles.
Periodic acid-Schiff (PAS) stain. Magn. ×400

This work was aimed at studying the effects of 800 mg.kg^{-1} crude ethanolic extract of *Spondias mombin* on the morphology of the reproductive tract and reproductive hormonal profile of non gravid mature does.

MATERIALS AND METHODS

Spondias mombin leaves were collected, identified and prepared into an extract at the University of Ibadan. Pulverized leaves weighing 3.62 kg were soaked in hexane for three hours in order to remove the fat content. The mixture was decanted and the residue was air dried. The residue was then soaked in ethanol for 3 days, after which, it was filtered. The resultant filtrate was then concentrated *in vacuo* using a rota-evaporator at low temperatures. Ethanol and the residual jelly like dark brownish paste were recovered. This paste was kept in a fume hood for efflorescence of the residual ethanol. A yield of 80 g of dried extract was obtained from which a

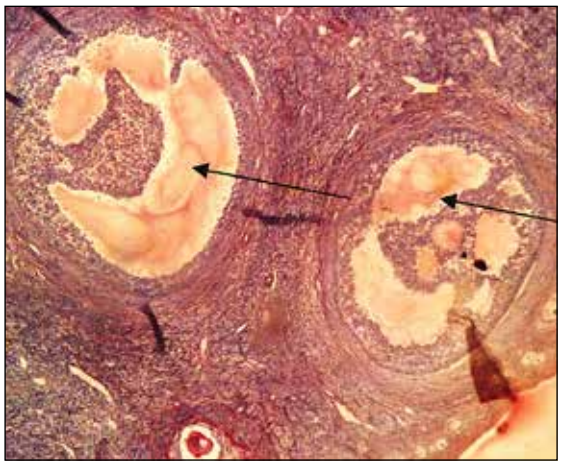

Fig. 5. Histological section of the right ovary of the treatment group. Arrows showing tertiary follicles (antral follicles).
Periodic acid-Schiff (PAS) stain. Magn. ×400

stock solution of 80 g of extract in 100 ml of propylene glycol was constituted. The dose was calculated using the formula:

$$\text{Dose} = \frac{\text{Weight} \times \text{Dosage}}{\text{Concentration}}$$

Experimental animals

Ten Chinchilla breed of rabbits weighing on average 1.65 ± 0.06 kg were utilized. They were pubertal primiparous rabbits. They were kept in standard hutches and fed formulated feed. Water was given *ad libitum*. Fourteen days of acclimatisation was observed during which prophylactic treatment against endo and ecto parasites using avermectin at a dosage rate of 1ml per 50 kg body weight was administered.

Experimental design

The ten rabbits were divided into two groups with five rabbits each. This constituted the extract-treated group and the control group. Ethanol leaf extract was administered orally at a dosage rate of 800 mg.kg^{-1} [15] once daily for fourteen days to the treated group, while the control animals received distilled water during the same period and for the same length of time. Blood samples for hormonal assays were collected carefully from the ear vein of the treated group before the commencement of extract administration and a day after the last administration. ELISA was utilised for assays using hormonal kits. The hormones assayed from the sera were; follicle stimulating hormone (FSH), luteinising hormone (LH), estrogen, progesterone and prolactin. The kits utilised were from Biorex diagnostics (ISO 13485). Batches were; BXEO86OA (Estradiol), BXEO671A (Prolactin), BXEO661A (Progesterone), BXEO631A (FSH), and BXE0651A (LH).

At the cessation of the treatment period, the does from both groups were sacrificed using the guidelines on euthanasia, set by the Committee for the purpose of control and supervision on experiments on animals (CPCSEA, 600 041 Tamil Nadu, India). Afterward, the reproductive tracts were carefully exteriorized. The different segments; the ovaries, the oviduct, the uterine horn, and the uterine body were carefully separated and examined for lesions. Each segment was evaluated for length, width, and weight.

Histopathology and morphometric analysis of the ovaries

All of the extracted ovaries were routinely fixed in 10 % neutral buffered formalin, then dehydrated in graded levels of alcohol, embedded in paraffin wax, sectioned at 5 µm and stained with *Periodic acid-Schiff* (*PAS*). Histological alterations and the number of different follicles were counted at 5 different foci per field of the histological slides of the ovary at ×100 and ×400 magnification with the aid of an Olympus light microscope.

Statistical analysis

The data obtained were collated and analysed into descriptive statistics using GraghPad Prism 5 (Version 5.04). Means, with Standard Error of the Means (SEM) were calculated. Means were compared using one sample *T*-test. A value of $P \leq 0.05$ was considered significant.

RESULTS

Does from both the test group and control group had normal ovaries, oviduct, uterine tubes, and uterine body as determined grossly. The uneven surfaces of the ovaries of all of the does suggested the presence of follicles at different stages of development. There were no evidences of corpus lutea or cystic conditions in any of the ovaries. No defective parts were observed throughout the tracts in any of the animals. Histological sections of the oviduct, uterine tubes, uterine body revealed normal epithelial cells. Left and right ovaries showed marked oogenesis in the treated group, both having a larger number of follicles, compared to the controls (Fig. 1 and 2). Views of different fields of the histological slides of the treated group revealed Primary, Secondary and Tertiary follicles (Fig. 3, 4 and 5). The body weight adjusted mean paired ovarian diameter of the control group was 0.51 ± 0.02 cm (Table 1). This was significantly different from the treated group which had 0.41 ± 0.39 cm ($P \leq 0.05$); however this did not translate to a significant difference in the paired ovarian weights (Table 1). The weight of the right oviduct and the length of the right uterine horn were significantly different ($P \leq 0.05$) in the control group than the treatment group (Table 2 and 3). There was no significant difference between the means of the control and treatment groups in: the length, width and weight of the uterine body; the left

Table 1. Mean (± SEM) diameter and weight of paired ovaries

	Treatment group	Control group	P value
Diameter [cm]	0.41 ± 0.04	0.51 ± 0.02	0.04
Weight [g]	0.07 ± 0.01	0.09 ± 0.008	0.06

$P \leq 0.05$ was considered significant

Table 2. Mean weight in g (x ± SEM) of the oviduct, uterine horn and uterine body in the treated and control groups

	Treatment group	Control group	P value
Right oviduct	0.07 ± 0.03	0.17 ± 0.03	0.05
Left oviduct	0.16 ± 0.05	0.13 ± 0.02	0.52
Right uterine horn	0.18 ± 0.03	0.2 ± 0.06	0.77
Left uterine horn	0.21 ± 0.06	0.22 ± 0.07	0.89
Uterine body	0.28 ± 0.09	0.19 ± 0.04	0.39

$P \leq 0.05$ was considered significant

Table 3. Mean length in cm (x ± SEM) of the oviduct, uterine horn and uterine body in the treated and control groups

	Treatment group	Control group	P value
Right oviduct	4.84 ± 0.58	5.58 ± 0.71	0.45
Left oviduct	4.69 ± 0.27	5.87 ± 0.75	0.18
Right uterine horn	3.47 ± 0.22	4.19 ± 0.12	0.02
Left uterine horn	3.47 ± 0.24	4.08 ± 0.15	0.06
Uterine body	0.3 ± 0.03	0.25 ± 0.06	0.45

P ≤ 0.05 was considered significant

Table 4. Mean width in cm (x ± SEM) of the oviduct, uterine horn and uterine body in the treated and control groups

	Treatment group	Control group	P value
Right oviduct	0.08 ± 0.01	0.11 ± 0.02	0.19
Left oviduct	0.09 ± 0.01	0.11 ± 0.02	0.34
Right uterine horn	0.16 ± 0.01	0.31 ± 0.15	0.37
Left uterine horn	0.17 ± 0.02	0.16 ± 0.02	0.826
Uterine body	0.43 ± 0.01	0.42 ± 0.03	0.53

P ≤ 0.05 was considered significant

Table 5. Mean hormonal concentrations in treatment group (x ± SEM)

Hormones	Pre-treatment	Post-t reatment	P value
Progesterone [ng.ml^{-1}]	0.11 ± 0.09	2.99 ± 2.49	0.28
Estrogen [ng.ml^{-1}]	48.24 ± 28.03	13.26 ± 9.69	0.27
FSH [mlU.ml^{-1}]	1.71 ± 0.31	0.97 ± 0.19	0.08
LH [mlU.ml^{-1}]	55.14 ±18.44	32.76 ± 20.93	0.45
Prolactin [ng.ml^{-1}]	13.96 ± 3.04	23.48 ± 13.27	0.50

P ≤ 0.05 was considered significant

oviduct and uterine horn; and width of the right oviduct and the uterine horn (Tables 2, 3 and 4). All the hormones did not change significantly comparing the pre and post treatment in the treated group (Table 5).

DISCUSSION

The treatment seemed to favour folliculogenesis going by the number of follicles observed in the treated rabbits compared to the control. The mean diameter of the ovaries was significantly higher in the control, but this did not translate into a significant higher weight as expected [8], [4]. The diameter was indeed smaller in treated animals, but they had a greater number of developing follicles. The follicles potentially were available for ovulation, hence more follicles means more possible kids after successful fertilization [11]. The mechanism for favoured folliculogenesis may likely be explained by a follicular dynamic postulation that a higher level of embryo yield occurs in rabbits pretreated with progesterone before being superovulated by gonadotropin [3]. In this study, the progesterone increase (though not significantly) that was recorded after treatment with *Spondias* crude extract might have provided the platform for the observed increase in folliculogenesis, in the presence of FSH. Igwe *et al.* [7] in their study, observed a significant progesterone elevation and higher values than obtained in this study, after intraperitoneal treatment with 750 mg.kg^{-1} body weight of *Spondias* crude extract, lending credence to the progestogenic property of *Spondias*. A postulation of interference with signalling pathways of the Kit Ligand and c-Kit system in the regulation of oogenesis and folliculogenesis could be of consideration also [5].

There was no significant change in the levels of LH, FSH, estrogen and prolactin, comparing the pre-treatment and post treatment results in the treatment group, probably signifying non-interference with the oestrous cycle.″The insignificant effect on estrogen is in agreement with Igwe *et al.* [7], who reported that the 7 and 14-day intraperitoneal treatment of rabbits with 750 mg.kg^{-1} body weight of crude extract of *Spondias* had no significant effect on the serum level of estrogen. This is also in agreement with the report of Oloye *et al.* [15] after rats were treated orally with 800 mg.kg^{-1} body weight and Uchendu *et al.* [17] when rats were treated subcutaneously with 500 mg.kg^{-1} body weight. The estrogen concentration of 1.87 ± 1.60 pg.ml^{-1} after 14 days of treatment, was lower in Igwe *et al.* [7] work, compared to 13.26 ± 9.69 pg.ml^{-1} in this study.

The uterine body weight was not significantly different comparing the treated group and control group of non-gravid rabbits. This also correlates with the non-significant difference in estrogen concentration. Uterine weight increase in rodents has been used as a bioindicator of the presence of estrogens [6].

The weight of the paired ovaries of the treated and control were higher than 0.017 ± 0.003 g recorded by Bitto *et al.* [4] for intact non gravid rabbits. Paired uterine horn of 0.043 ± 0.014 g recorded by Bitto *et al.* [4], was lower than

that recorded for the right and left treated and control in this work and the paired oviduct of 0.008 ± 0.002 g was lower than that recorded for the right and left treated and control in this work. Comparing lengths and widths, Bitto *et al.* [4] record of 8.07 ± 0.409 cm and Ogbuewu *et al.* [13] 8.85 cm for the length of the uterine horn which were higher than the non-derived length measured for the right and left uterine horns in the treatment and control groups in this work. The 6.00 ± 0.794 cm recorded for the length of the oviduct by Bitto *et al.* [4], was however, lower than the non-derived length recorded for the right and left oviducts for the treated and control groups in this work. The 0.933 ± 0.054 cm value recorded by Bitto *et al.* [4] for the width of the uterine horn was higher than the non-derived measurements (measured parts are in proportion with the animal's weight) recorded for any of the right or left uterine horns in the treated and control groups. According to Ogbuewu *et al.* [13] 0.06 g weight for paired uterine horn was quite lower than that recorded for the derived left and right uterine horns (measured reproductive parts were not justified by the overall weight of the animal) of treated and control groups, while the 0.49 g weight of the paired oviducts was higher than that recorded in this work comparing it with the derived right and left oviduct of the treated and control groups.

CONCLUSION

The 800 mg.kg^{-1} ethanol extract of *Spondias mombin* appeared to favour folliculogenesis in non-gravid rabbits and had no pathological effects on the entire reproductive tract of the rabbits.

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EFFECTS OF THREE VARIETIES OF *LABLAB PURPUREUS* SEEDS ON SOME HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS AND HISTOLOGY OF VITAL ORGANS OF RATS

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ABSTRACT

The seeds, immature pods and herbage of *Lablab purpureus* are used for human food and as a feed supplement for livestock. The toxic effects of the oral ingestion of three varieties of *Lablab purpureus* seeds (Rongai white (NAPRI 4), Rongai brown ($P_1$509114) and Highworth black (Grif 12293)) were evaluated in rats. At the end of the experimental period, significant decreases were recorded in the levels of: packed cell volume, red blood cell counts and haemoglobin, in all the rats fed *Lablab purpureus* seeds. Significant increases were observed in the values of: alkaline phosphatase, aspartate amino transferase, alanine amino transferase, gamma glutamyl traspeptidase, urea and creatinine. *Lablab purpureus* seeds also induced: congestion and necrosis of the liver, focal haemorrhages in the kidney and degeneration of the testicular germinal epithelium. The study concluded that the antinutritional/toxic factors present in the *Lablab purpureus* seeds could have deleterious effects on the liver, kidney and testis, as well as, the haemopoietic system, when consumed in the raw state by humans and other animals.

Key words: biochemical; haematology; histology; *Lablab purpureus;* organs

INTRODUCTION

A major problem facing livestock producers in tropical areas is the proper nutrition for their animals, especially during the dry season when pastures, cereal residues and maize stover are limiting in nutritional quality. In most instances, it is during this season that problems such as sickness and weight loss arise due mainly to a poor dietary profiles and the lower occurrence of highly palatable forage crops. One way of improving the nutritional status of livestock is by the proper supplementation with leguminous forages [25]. It has long been established, that ingestion of forage plants in the raw state produces various biochemical and physiological responses, usually accompanied by growth inhibition. This inhibition of growth is believed to be a consequence of inefficient utilization of dietary nutrients caused by the presence of toxic antinutritional factors in plants [14].

In humans and other animals, the effects of many toxic substances often are manifested in the production of clinical symptoms, as well as gross and histopathologic lesions in mammalian tissues. Although the lesions are rarely characteristic, they often provide definite clues to the nature of the toxicity. One valuable adjunct to the clinical diagnosis of toxicosis is the determination of serum enzymes, which are normally confined to the tissues but have increased concentrations in the serum following tissue injury [16]. For instance, the escape of enzymes as a result of the disruption of the hepatic parenchyma, leads to necrosis and altered membrane permeability, consequently, leading to increased activities of certain enzymes [13].

Lablab purpureus, previously classified as *Dolichos lablab*, is believed to have originated in India and has been widely distributed to many tropical and subtropical countries [12]. It is an annual or short-lived perennial legume sown for grazing and conservation in

tropical environments [9]. The seeds and immature pods are used for human food and the herbage is used as a feed supplement for cattle [15]. However, the seeds of *L. purpureus* contain anti-nutritional factors such as, tannins, phytate, and trypsin inhibitors [4] that reduce nutrient availability and possess toxic potentials. Based on a genetic study with AFLPs (amplified fragment length polymorphism), however, no convincing evidence was found to maintain that this crop had originated anywhere else other than in eastern and/or southern Africa [20]. Despite its wide distribution in the tropics, it is still considered as neglected, in terms of research and development [21].

L. purpureus is associated with several names in different countries. As a result, a proper nutritional assessment of the anti-nutritional factors and other toxic substances in *Lablab purpureus* seeds warrants more research [12]. Further, while the toxicological effects of most other legumes have been very widely studied, those of *Lablab purpureus* have not been well documented. Therefore, this study was carried out to evaluate the toxicological effects of *Lablab purpureus* seeds in rats noting the effects on haematology, serum biochemistry and histopathology as indices for toxicity.

MATERIALS AND METHODS

Source of Plants

The three varieties of *Lablab purpureus* seeds (lablab beans) used for this study were: Rongai white (NAPRI 4), Rongai brown ($P_1$509114) and Highworth black (Grif 12293), and they all were obtained from the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan. The pictures of the *Lablab purpureus* seeds are shown in Fig. 1 below.

Experimental Animals

Twenty weanling male albino rats of the Wistar strain obtained from the rat colony of the Department of Physiology, College of Medicine, University of Ibadan, were used for this study. The rats weighing between 130—180 g, were randomly selected and housed in stainless-steel individual metabolic cages (Associated Crate Ltd, England) at the Animal House of the Department of Animal Science, University of Ibadan. The experimental animals were fed with a normal rat diet and given water *ad libitum*, for two weeks, to allow for the acclimatization of the rats.

At the commencement of the toxicological evaluation, five rats were put on each test diet. The experimental diets contained the three varieties of raw lablab beans; Rongai white, Rongai brown, and Highworth black varieties. Five rats were placed in the control group and were fed rat pellets manufactured by Bendel Feeds and Flour Mills Ltd, Edo State, Nigeria. The feed was placed in small bowls firmly attached to the cages to minimize spillages and spilled feed was collected in the containers placed under the individual cages. All feeds were weighed using Mettler electronic balance (Mettler PM 4000) and the daily feed consumption was computed by collecting the spilled feed and weighing it along with the feed remaining in the bowl. This was subtracted from 10 g to give the actual daily feed consumed. Water was offered in well washed plastic drinking bottles with changes of water made every other day to prevent bacterial and fungal growth. Faecal output and texture were visually examined as excess pellets passed per animal per cage. The experiment lasted seven days during which l0 g of feed were given daily (this is because high mortality rates were observed on the tenth day of feeding during the pilot study initially carried out in our laboratory).

Fig. 1. Lablab purpureus seeds.
Source Nwokocha *et al.* [23]

Haematology and serum biochemical studies

At the end of the seven days of feeding, each rat was bled through the orbital sinus into heparinised bottles for haematological studies and the blood samples collected in clean non-heparinised bottles were allowed to clot to obtain the serum. The serum was separated from the clot and decanted into clean bottles for biochemical analysis. The packed cell volume (PCV) and haemoglobin levels were determined by the microhematocrit and cyanmethaemoglobin methods, respectively [17]. The erythrocyte count was determined by the haematocytometry method [17]. Total white blood cell (WBC) counts were made in a haemocytometer using the WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from Giemsa stained slides viewed from each of the 30 fields of an oil immersion objective of a microscope [11]. Erythrocyte indices including; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined from the values obtained from red blood cells (RBC) count, haemoglobin level and PCV values [13]. From the serum, the total protein was measured using biuret reaction, while albumin was measured by colorimetric estimation using sigma diagnostic reagent (Sigma Diagnostic, UK.), which contained bromcresol green (BCG). The globulin was obtained from the difference of total protein and albumn. Serum urea and creatinine levels were determined using photoelectric colorimeter [11]. The AST and ALT were determined using a photoelectric colorimeter [13]. The alkaline phosphatase activity was measured [30].

Histopathology

All rats were sacrificed and thereafter quickly dissected to remove the liver, kidney and testis. The testes were preserved in Bouin's fluid for 48 h, processed, cut and stained with Haematoxylin and Eosin (H and E). Subsequently, the testes were stored in sample bottles containing 10 % formal-saline (formalin 100 ml, sodium chloride 8.5 g, water 900 ml) for histological examination [24]. The liver and kidneys were also preserved in 10 % buffered formalin dehydrated in ethanol (70 to 100 %), cleared in xylene and embedded in paraffin. All tissue sections were examined under a light microscope after staining with H and E [19], [27].

Statistical analysis

Where applicable, all the values were expressed as the mean ± standard deviation. Statistical analysis was carried out using the PRISM software package (Version 5.0). The statistical significance was assessed by the Student *t*-test and analysis of variance. Values of probability lower than 5 % were considered statistically significant.

RESULTS

The pictures of the three varieties of *L. purpureus* seeds are shown in Fig. 1 above. The effects of feeding raw varieties of *Lablab purpureus* seeds to rats for seven consecutive days, on haematological parameters are presented in Table 1. Significant decreases in the level of; PCV, RBC, and Hb concentrations, were recorded in all of the rats fed with the three varieties of *Lablab purpureus* seeds. The lowest PCV values (30.4 %), RBC ($5.02\times10^{12}.l^{-1}$) and Hb ($9.0\,g.dl^{-1}$) were recorded in the rats fed with the Rongai brown variety. Although, all the rats fed with the three varieties recorded significant increases in the level of MCV, the rats fed with the Rongai brown variety had the highest value (60.8 fl). Significant increases in MCH values were also observed in rats fed with *L. purpureus,* with the rats fed Rongai white and Highworth black varieties producing the highest values of 18.03 pg and 18.60 pg respectively. Further, significant reductions were

Table 1. Haematological parameters of rats fed with three varieties of *L. purpureus* seeds

Parameter	Control	Rongai Brown	Rongai White	Highworth Black
PCV [%]	43.6 ± 1.34	35.4 ± 1.52*	32.4 ± 1.82*	30.40 ± 1.52*
RBC [$\times10^{12}.l^{-1}$]	9.36 ± 0.65	5.02 ± 0.35*	6.46 ± 0.36*	5.76 ± 0.18*
Hb [$g.dl^{-1}$]	13.68 ± 0.53	9.0 ± 0.74*	11.60 ± 0.54*	10.70 ± 0.24*
MCV [fl]	46.75 ± 3.32	60.78 ± 5.07*	54.90 ± 3.11*	56.24 ± 2.40*
MCH [pg]	14.65 ± 0.70	17.96 ± 1.37*	18.03 ± 1.77*	18.60 ± 0.62*
MCHC [$g.dl^{-1}$]	31.50 ± 1.70	29.70 ± 3.35*	32.82 ± 2.14*	33.11 ± 2.10*
WBC [$\times10^{9}.l^{-1}$]	16.80 ± 1.05	8.78 ± 0.62*	12.56 ± 0.44*	10.78 ± 0.30*
Lymph [$\times10^{9}.l^{-1}$]	10.28 ± 0.87	5.68 ± 0.74*	8.34 ± 0.21*	7.68 ± 0.24*
Neut [$\times10^{9}.l^{-1}$]	6.44 ± 0.30	3.16 ± 0.54*	4.30 ± 0.49*	3.43 ± 0.15*
Mono [$\times10^{3}.\mu l^{-1}$]	0.00 ± 0.00	0.06 ± 0.05*	0.00 ± 0.00	0.02 ± 0.04

* — Significant at $P < 0.05$

observed in the values of WBC, circulating lymphocytes and neutrophils, for all the rats fed with the *L.purpureus* seeds. The rats fed with Rongai brown variety had the least values of WBC ($8.78\times10^9.l^{-1}$), lymphocytes ($5.68\times10^9.l^{-1}$) and neutrophils ($3.16\times10^9.l^{-1}$).

The result of the effects of feeding raw varieties of *Lablab purpureus* seeds to rats for seven consecutive days, on serum biochemistry is presented in Table 2. All the rats fed with the seeds recorded significant increases in the values of: alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transpeptidase (GGT), urea and creatinine. The rats fed the Rongai brown variety had the highest values of: ALP (341.8 $U.l^{-1}$), AST (266.5 $U.l^{-1}$), ALT (74.5 $U.l^{-1}$), GGT (29.8 $U.mgprotein^{-1}$), urea (89.0 $mg.dl^{-1}$), and creatinine (1.14 $mg.dl^{-1}$). However, there were significant reductions in the total protein level, albumin and globulin in all of the rats fed with *L. purpureus* seeds. The greatest reductions in the values of total protein (43.8 $mg.dl^{-1}$) and globulin (15.4 $mg.dl^{-1}$) were observed in the group of rats fed the Rongai brown variety of *L. purpureus* seeds, while the rats fed the Rongai white variety had the least value for albumin (25.6 $mg.dl^{-1}$).

The results of the histological changes induced by feeding the three varieties of *L.purpureus* seeds to rats are presented in figures 1 to 12. Severe diffuse necrosis of hepatocytes was observed in the group of rats fed with the Rongai brown variety (Fig. 2), and mild congestion of the hepatic blood vessels was observed in the liver of rats fed with the Rongai white and Highworth black varieties (Fig. 3 and 4). In contrast, no visible lesion was observed in the liver of rats in the control group (Fig. 5).

In the kidney, moderate congestion of blood vessels was observed in the rats fed the Rongai brown varieties (Fig. 6), while moderate vascular congestion and focal haemorrhages were seen in rats fed the Rongai white variety (Fig. 7). Marked tubular degeneration with the presence of protein was observed in rats fed the Highworth black variety (Fig. 8). However, no visible lesion was observed in the kidneys of the control group (Fig. 9).

Lesions observed in the testes include: degeneration and necrosis of germinal epithelium in rats fed the Rongai brown variety (Fig. 10); mild diffuse degeneration and necrosis of germinal epithelium in rats fed the Rongai white variety (Fig. 11); and mild degeneration of seminiferous epithelial cells with the presence of very few cells in the tubules (Fig. 12). No visible lesion was observed in the testes of the control group (Fig. 13).

DISCUSSION

The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans [22]. The reductions in packed cell volume, red blood cell count and haemoglobin level observed in this study suggest that the feeding of the raw varieties of *L.purpureus* has the tendency to cause hematological alteration in rats. This alteration, that is, anaemia, may be due to the effect of antinutritional factors that have been reported to be present in high amounts in *L.purpureus* seeds [12]. Animals that browse this plant may be subjected to depression in erythopoiesis and possibly anaemia over prolonged exposure [1]. The mechanism by which the anaemia is brought about may involve an interference of the antinutritional factors such as tannins, phytate and trypsin inhibitors with the functioning of aminolevulinic acid dehydratase, which is a key enzyme of haeme synthesis [18]. Increase fragility and

Table 2. Serum biochemical parameters of rats fed with *L. purpureus* seeds

Parameter	Control	Rongai Brown	Rongai White	Highworth Black
ALP [$U.l^{-1}$]	77.00 ± 2.65	341.75 ± 35.42*	155.00 ± 2.94*	177.00 ± 6.38*
AST [$U.l^{-1}$]	94.70 ± 11.85	266.50 ± 21.69*	173.50 ± 11.12*	235.50 ±10.08*
ALT [$U.l^{-1}$]	24.25 ± 3.86	74.50 ± 4.73*	40.75 ± 3.5*	53.75 ± 1.89*
GGT [$U.mgprotein^{-1}$]	10.50 ± 1.00	29.8 ± 2.95*	13.8 ± 1.64*	21.5 ± 0.60*
Urea [$mg.dl^{-1}$]	34.75 ± 7.5	89.00 ± 5.00*	45.67 ± 1.53*	65.50 ± 3.87*
Creatinine [$mg.dl^{-1}$]	0.45 ± 0.06	1.14 ± 0.05*	0.53 ± 0.05*	0.83 ± 0.05*
Total Protein [$mg.dl^{-1}$]	80.75 ± 2.99	43.8 ± 2.68*	54.0 ± 3.16*	53.25 ± 2.75*
Albumin [$mg.dl^{-1}$]	47.8 ± 2.06	28.4 ± 1.67*	25.6 ± 2.61*	33.5 ± 1.91*
Globulin [$mg.dl^{-1}$]	33.5 ± 4.04	15.4 ± 2.19*	28.4 ± 2.61*	19.8 ± 1.71*

* — Significant at $P < 0.05$

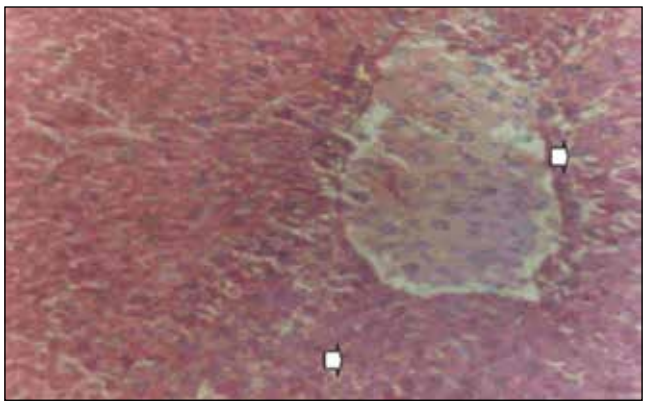

Fig. 2. Liver of rat fed with the Rongai brown variety showing severe diffuse necrosis of hepatocytes (labeled with white arrows). Magn. ×400

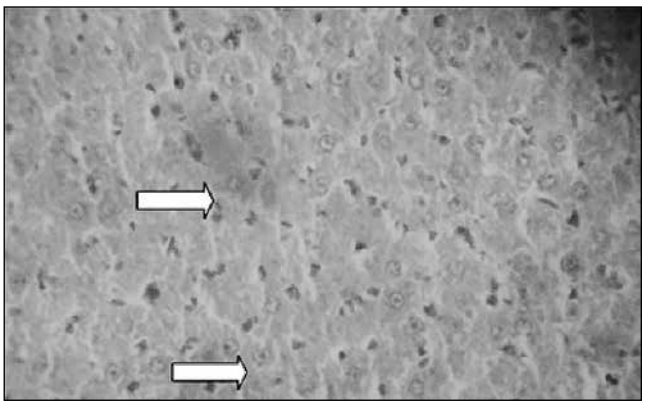

Fig. 3. Liver of rats fed with the Rongai white variety showing mild congestion of hepatic blood vessels (labeled with white arrows). Magn. ×400

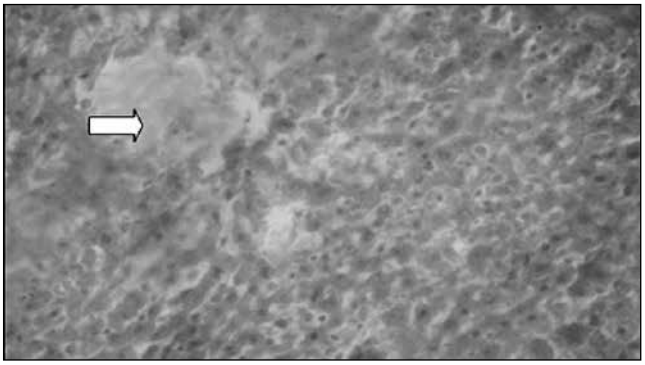

Fig. 4. Liver of rats fed with the Highworth black variety showing mild congestion of hepatic blood vessels (labeled with white arrows). Magn. ×400

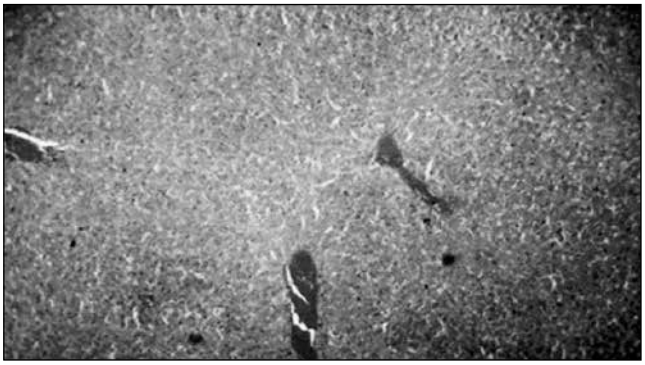

Fig. 5. Liver of rat from the control group with no visible lesion. Magn. ×100

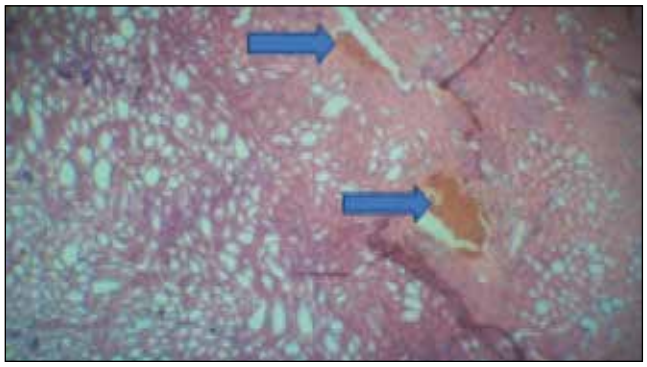

Fig. 6. Kidney of rat fed with the Rongai brown variety showing moderate congestion of blood vessels (labeled with blue arrows). Magn. ×100

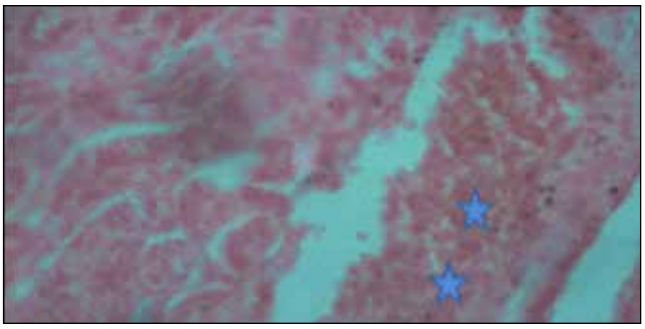

Fig. 7. Kidney of rat fed with the Rongai white variety showing moderate vascular congestion and focal haemorrhages (labeled with blue stars). Magn. ×400

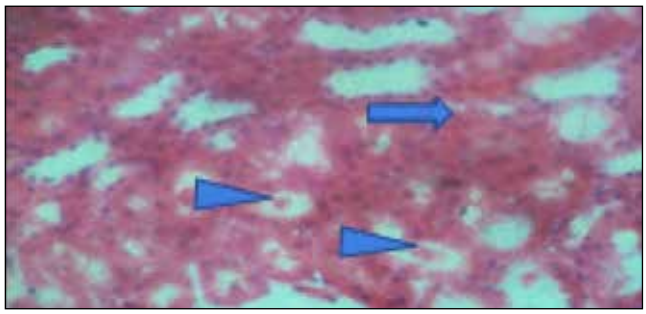

Fig. 8. Kidney of rat fed with the Highworth black variety showing moderate congestion (labeled with blue arrow) and marked tubular degeneration (labeled with blue arrowhead). Magn. ×400

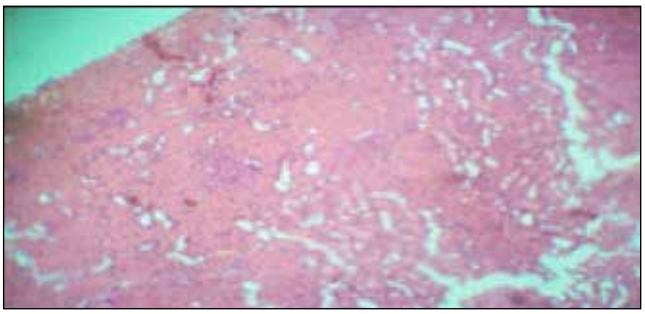

Fig. 9. Kidney of rat from the control group with no visible lesion. Magn. ×100

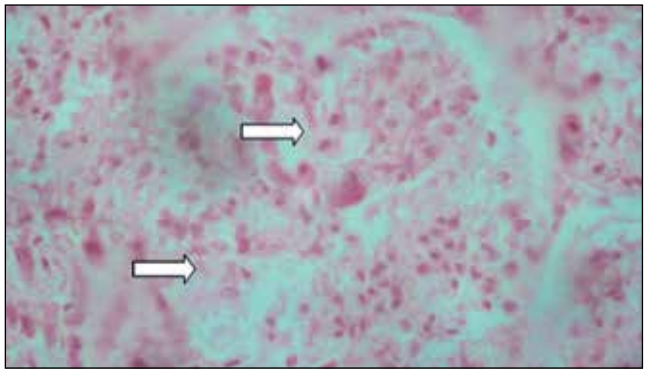

Fig. 10. Testis of rat fed with the Rongai brown variety showing degeneration and necrosis of germinal epithelium (labeled with white arrows). Magn.×400

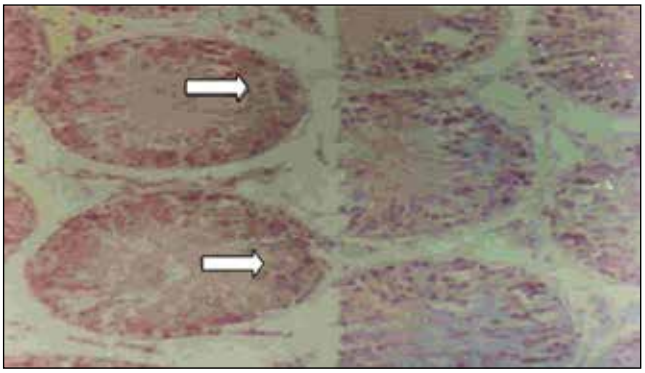

Fig. 11. Testis of rat fed with the Rongai white variety showing mild diffuse degeneration and necrosis of germinal epithelium (labeled with white arrows). Magn. ×400

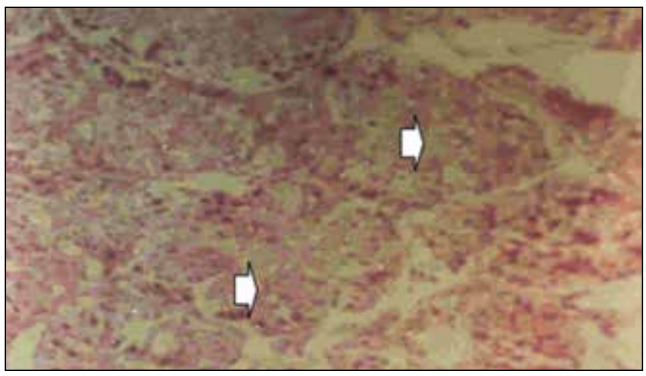

Fig. 12. Testis of rat fed with the highworth black variety showing mild degeneration of seminiferous epithelial cells, and very few cells present in the tubules (labeled with white arrow). Magn. ×400

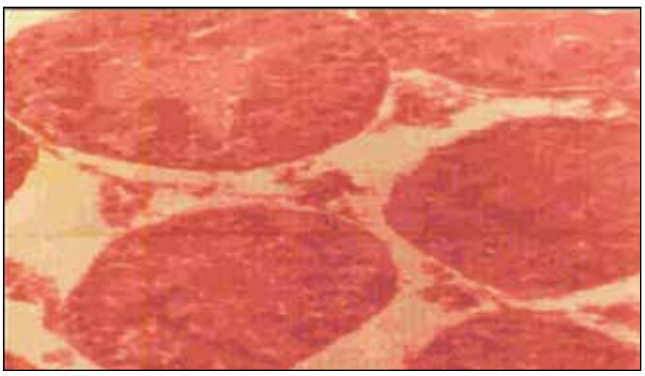

Fig. 13. Testis of rat from the control group with no visible lesion. Magn. ×400

progressive destruction of RBCs due to binding of free radicals has also been reported to cause decreased haematological values [5].

Anaemia connotes a decrease in the oxygen-carrying capacity of the blood and is best characterized by a reduction in haemoglobin level [6], as observed in this study. Anaemia may be relative or absolute. Absolute anemia involves a true decrease in red cell mass, is of hematologic importance, and involves; increased red blood cell (RBC) destruction, increased corpuscular loss, or decreased erythrocyte production [7]. In this study, the anaemia induced by *Lablab purpureus* seeds can best be attributed to the decreased red blood cell production, because jaundice was not observed. Thus, it is not likely that there was increased red blood cell destruction.

The leucogram of rats fed the three varieties of *L. purpureus* showed a significant decrease ($P < 0.05$) in total white blood cell count relative to the controls. Excessive ingestion of a wide variety of plant parts has been found to cause hypoproliferative conditions characterized by a reduced production of all cellular components of the blood. The decrease in white blood cell count observed in this study may have resulted from the suppression of white blood cell production in the bone marrow. Immunosuppressive drugs are usually associated with leucopenia. Therefore, it is possible that raw *L. purpureus* seeds in their raw form may have immunosuppressive properties, especially when consumed for long durations.

In this study, significant increases were observed in the values of ALP, AST and ALT. ALP is often employed to assess the integrity of plasma membrane [3] and is localized predominantly in the plasma membrane of the microvilli of the bile canaliculi [30]. The significant increase in the ALP activity observed may be due to increased functional activity of the liver, probably leading to *de novo* synthesis of the enzyme molecules [32]. AST and ALT are useful marker enzymes of liver cytolysis and damage to the plasma membrane of the liver cells [29]. Their presence in the serum may provide information on organ dysfunction [31]. The general increase in the activity of AST in rats fed the three varieties of *L. purpureus* seeds could be a response by the liver to the assault from the antinutritional factors present in the seeds. In addition, the increases in AST and ALT observed in this study could suggest the ability of the antinutritonal factors to induce cellular leakage and loss of functional integrity of the cell membrane [28]. This is further supported by the severe diffuse necrosis of hepatocytes and mild congestion of hepatic blood vessels, observed in this study. Liver damage is associated with cellular necrosis, increase in liquid tissue peroxidation and elevation of serum levels of many biochemical markers like AST and ALT. Further, the aminotransferases occupy a central position in the amino acid metabolism and help in providing necessary intermediates for gluconeogenesis. Therefore, alterations in their activities, as observed in this study, may have adverse effects on the amino acid metabolism of the tissues and consequently the intermediates needed for gluconeogenesis [32].

The significant reductions observed in the values of; total protein, albumin and globulin, may suggest a deleterious effect of the raw *L. purpureus* seeds on liver functioning. Under normal conditions, most plasma proteins are synthesized in the liver and reduced levels of plasma proteins usually indicate reduced hepatic production. Alteration in total plasma protein is usually due to a decrease in the quantity of albumin [2] but a rise in albumin rarely occurs [8], except in acute dehydration. Albumin is a main contributor to plasma osmotic pressure which is an important determinant of the distribution of the extracellular fluid between the intravascular and extravascular compartments. Decreases in albumin may cause a disturbance of the equilibrium between plasma and interstitial fluid with an attending reduction in the movement of interstitial fluid into the blood. Consequently, excess fluid accumulates in the interstitial space and oedema results.

Generally, a significant decrease in albumin level is usually associated with a decreased synthesis of albumin, as seen in: dysfunctions of the liver; increased volume of the extracellular fluid, as seen in over-hydration or increased capillary permeability; and increased loss of albumin, as observed in severe haemorrhage [10]. However, since significant increases were observed in the levels of the aminotransferases AST and ALT, it is more likely that the observed reductions in albumin, in this study, is due to a dysfunction of the liver, induced by antinutritional factors present in the raw varieties of *L. purpureus* seeds. This is further supported by the histopathologic lesions such as severe diffuse necrosis of hepatocytes observed in the liver of the rats (Fig. 2). Since gamma globulins are produced mostly by plasma cells, the significant decrease in serum globulin, as observed in this study, could suggest a deleterious effect of raw *L.purpureus* seeds on the humoral arm of the immune system.

In conclusion, the results of the haematological, serum biochemical and histopathological examinations suggest that the antinutritional/ toxic factors present in the *Lablab purpureus* seeds could have deleterious effects on the liver, kidney and testis when consumed in the raw state and efforts should therefore be made to adequately process and reduce these antinutritional/toxic factors before consumption of the seeds by humans and other animals.

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SERUM CONCENTRATION OF C-REACTIVE PROTEIN AND HAPTOGLOBIN IN WILD BOARS (*SUS SCROFA* L.)

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ABSTRACT

The objective of this study was to analyse two main positive acute phase proteins in wild boar piglets. Seven piglets, four months of age, were used for this study. To determine C-reactive protein (CRP), we used a method of enzyme immunoassay based onthe "sandwich" ELISA reaction, using specifically marked monoclonal antibodies. The haptoglobin (Hp) was determined by a colorimetric assay. The mean values of CRP and Hp were 263.14 µg.ml^{-1} and 3.08 mg.ml^{-1}, respectively. They were significantly higher than the mean values found in comparative studies. Our results indicate that the analysis of the acute phase proteins can also be used for monitoring the health and welfare of animals in game reserves. In this case, we can speak about the so-called markers of influence of environment and ongoing subclinical diseases.

Key words: acute phase proteins; game reserve; health screening; wild boar

INTRODUCTION

C-reactive protein (CRP) and haptoglobin (Hp) belong to the acute phase proteins (APPs). They are proteins produced by hepatic cells and their production and release into the circulation is regulated by pro-inflammatory cytokines [6]. Changes in the concentration of these proteins in the blood serum indicate external or internal problems, such as inflammation, infection, stress, trauma, surgery, infarction, or progressive tumour disease. CRP is considered a part of the nonspecific congenital immunity, which participates in the protection of homeostasis and restriction of microbial growth. It is a penta-metric reactant synthesised in the liver and its production is primary controlled by interleukin 6. As it reacts with the so-called C protein of the cell wall, it plays a role in opsonin [4]. The plasma concentration of CRP is increased within 4 to 6 hours after the induction of a reaction to an acute phase and reaches its peak after 24 to 36 hours. The values of CRP are usually increased during bacterial infections but not during viral infections [9].

Hp belongs to metal-proteases which are structurally and functionally varying groups of proteins that protect tissues against oxidative stress and act as scavengers of oxide radicals. One of the most important roles of Hp is the binding of free haemoglobin (Hb). This Hp-Hb complex is recognised by CD163, specific surface receptors on the surface of macrophages, and consequently are phagocytised [10]. In this way, it prevents the loss of iron from the organism because the complex does not pass into the urine by glomerular filtration [7]. It has been used as a biomarker in the general assessment of the health status of animals.

The aim of this experimental work was to analyse two main positive acute phase proteins in wild boar piglets for the purpose of monitoring of their health, early diagnostic manifestation of occasional disorders of health and detection of welfare problems.

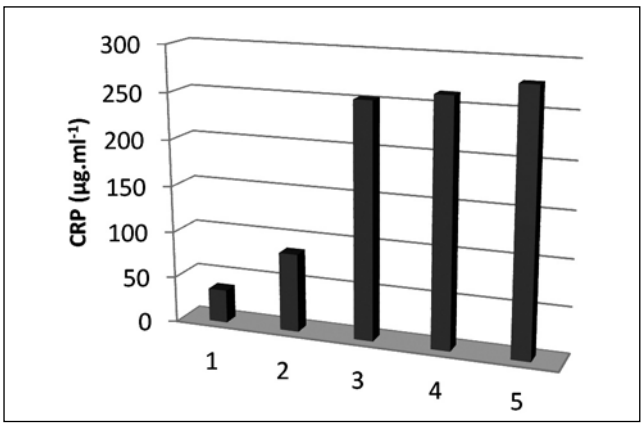

Fig. 1. Comparison of mean values of CRP
1 — clinically healthy piglets [5]; 2 — clinically healthy piglets [1]; 3 — piglets excluded due to growth retardation [1]; 4 — our study group of wild boar piglets; 5 — piglets with confirmed pathological findings [5]

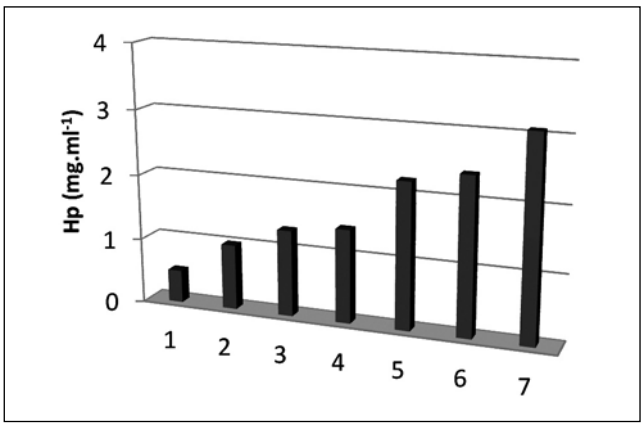

Fig. 2. Comparison of mean values of Hp
1 — clinically healthy piglets [8]; 2 — clinically healthy piglets with lesions found at a slaughterhouse [8]; 3 — clinically healthy piglets [1]; 4 — clinically healthy piglets with lesions found at a slaughterhouse [1]; 5 — piglets with signs of disease [1]; 6 — piglets with signs of disease [8]; 7 — our study group

MATERIAL AND METHODS

Blood samples were taken from 7 boar-piglets at the age of 4 months. The boar-piglets were caught in a breeding game reserve and placed into fenced pens. The piglets were then immobilized by a remote immobilization system Daninject (DAN-INJECT ApS Denmark). Zolazepam-tiletamine (Zoletil®Virbac France) was used as the anaesthetic. Blood samples were withdrawn from venous tangle of eye *sinus ophtalmicus* using standard techniques employed in pigs. The blood samples were then centrifuged and the obtained sera were stored at –20 °C until analysis.

CRP assay

The concentration of CRP was assayed by the sandwich ELISA test, using a commercial test kit (Phase range porcine CRP Assay kit, Tridelta Development Ltd., Ireland). The immobilised antibodies specific against CRP were bound to the bottom of disc holes. After washing, which removed the non-bound material, tetramethylbenzidine (TMB) was added. By this method, the intensity of the produced colouration is proportional to the concentration of CRP in the sample.

Hp assay

The level of haptoglobin in the examined sample was estimated by a colorimetric method, using an available commercial kit (Phase range Haptoglobin Assay kit, Tridelta Development Ltd., Ireland). The haptoglobin present in the specimen combines with haemoglobin at a low pH which reserves the peroxidase activity of the bound haemoglobin. The preservation of the peroxidase activity of haemoglobin is directly proportional to the amount of haptoglobin present in the specimen [2]. The optical densities were read using an automatic microplate reader Opsys MR (Dynex Technologies, USA) at an optical density of 630 nm.

Analysis of results

The results obtained were processed statistically by means of standard models for counting mean value, median value, standard deviation and coefficient of variability using software GraphPad Prism 6.0 and Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The serum concentrations of APPs were high but it was not necessary to dilute and re-analyse any of the samples. CRP in wild boar piglets reached a mean value of 263.14 µg.ml^{-1} (range 135—326 µg.ml^{-1}). For Hp we obtained a mean value of 3.08 mg.ml^{-1} and the respective range was 1.63—4.76 mg.ml^{-1}. The standard deviations, standard errors of mean and coefficients of variation are presented in Table 1.

Table 1. Total values of serum concentration of CRP and HP

APP [1]	n[2]	X min[3]	X max[4]	Mean[5]	Median[6]	SD[7]	SEM[8]	CV[9]
Haptoglobin [mg.ml^{-1}]	7	1.63	4.76	3.08	2.92	1.08	0.41	35.07
C-reactive protein [µg.ml^{-1}]	7	135	326	263.14	266	70.25	26.6	26.7

[1] Acute phase protein; [2] number of animals; [3] minimum value; [4] maximum value; [5] mean value; [6] median value; [7] standard deviation; [8] standard error of the mean; [9] coefficient of variation

Because scientific papers lack the reference values for APP in wild boars, the results obtained in our study were compared with the results of analogical studies that investigated domestic piglets of the same age. Phylogenetically, domestic pig *Sus scrofa domestica* and wild boar *Sus scrofa scrofa* L. are the same animal species. Most often the domestic pig is classified as a sub-species of the wild boar but also as an integral part of the species from which it has evolved. In domestication centres in Europe it originated generally from the wild boars *Sus scrofa scrofa* L. Also, some attributes of game reserve breeding, such as a closed area or higher concentration of animals, simulate conditions of conventional pig breeding. The results of our study showed significantly higher values of CRP in comparison with clinically healthy piglets (Fig. 1) that provided values of 84.88 $\mu g.ml^{-1}$ [1] or even as low as 35.93 $\mu g.ml^{-1}$ [5]. By contrast, the values in wild boar-piglets matched the values in piglets with ongoing pathological processes of various etiologies {252.93 $\mu g.ml^{-1}$ in [1] and 278.54 $\mu g.ml^{-1}$ in [5]}. We attribute this to a non-apparent parasitic diseases (coprologic confirmation of infestation with *Metastrongylus* spp., *Trichuris suis*, *Oesophagostomum* sp. and *Capillaria* sp.) and to environmental factors, particularly to stress induced by frequent disturbances and manipulation with the investigated piglets. Even the lowest measured value of CRP (135 $\mu g.m^{-1}$) was more than double that of the mean value presented in comparative studies in clinically healthy piglets (60.41 $\mu g.ml^{-1}$). The high values of CRP can also indicate inflammatory processes or hidden bacterial infections.

In the case of Hp, the results obtained in our study reached the highest mean level from among all compared groups (Fig. 2) which included clinically healthy piglets, piglets with confirmed pathological findings and clinically sick piglets (respiratory or diarrhoeic syndrome). The values of haptoglobin in the last mentioned group were as follows: 2.23 $mg.ml^{-1}$ [1], 2.4 $mg.ml^{-1}$ [8]. As serum concentration of Hp in wild boar-piglets reached 3.08 $mg.ml^{-1}$, it is highly probable that most of them suffered from a non-apparent disease. Serum concentration of Hp increases significantly with age in conventional breeding of pigs but not in pigs from SPF breeding (Specific Pathogen Free – SPF), i. e. higher values may be related to subclinical diseases in older categories [3] and thus Hp seems to be a potential good marker for detection of subclinical diseases.

CONCLUSION

One of the important aspects of research in present day veterinary medicine, is the ambition to obtain sufficient knowledge and to implement into clinical practice of veterinary care an estimation of certain biomarkers which would enable with adequate accuracy to predict certain diseases, regulate diagnostic procedures, or monitoring processes that initiate the problems. Our study deals with APPs and their potential use in the screening of the health status of entire animal herds. As APPs show low specificity but high sensitivity, their role as potential biomarkers in the field of veterinary care has some foundation. The results obtained showed that the mean values of the determined proteins (CRP 263.14 $\mu g.ml^{-1}$) and Hp (3.08 $mg.ml^{-1}$) were high and adequate attention should be paid to the veterinary care of wild boars in game reserve breeding.

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ALTERNATIVES TO ANTIBIOTICS IN CHICKS (A Review)

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ABSTRACT

In the past few decades, antibiotics have been added to animal diets in order to maintain health and production efficiency. They have also been added to the feed to speed the growth of healthy animals. Unfortunately, the multiyear and expansive use of antibiotics for medical and veterinary purposes may eventually result in the selection for the survival of resistant bacterial species or strains. Since the ban of antibiotic growth promoters in animals in the European Union in January 2006, a number of alternative methods have been investigated. Alternative approaches to sub-therapeutic antibiotics in livestock are: the use of probiotic microorganisms, prebiotic substrates that enrich certain bacteria populations, or combinations of both prebiotics and probiotics. Other alternatives may include strategic use of acidifiers, enzymes, essential oils, microflora enhancers and immuno-modulators. This review focuses on certain aspect of the use of antibiotics in animals, and presents some possible alternatives to antibiotics that are of a non-chemical nature in chicks.

Key words: alternatives to antibiotics; chicken; phytobiotics; prebiotics; probiotics

INTRODUCTION

For the past four decades, antibiotics have been used in animal agriculture to improve growth performance and protect animals from the adverse effects of pathogenic and non-pathogenic enteric micro-organisms. However, recent studies indicate that the use of antibiotic growth promoters in poultry production changes the intestinal microenvironment [31]. Bedford [1] pointed out that the growth-promoting effects of antibiotics in animal diets are clearly related to the gut microflora, because they exert no benefits on the performance of germ-free animals. Furthermore, the continued use of dietary antibiotics was suggested to increase the probability of the development of antibiotic-resistant human pathogens [38]. Hence, antibiotics are being removed from poultry and pig diets around the world, beginning in Sweden in the year 1986 [7]. The European Union (EU) banned the use of subtherapeutic levels of antibiotics in animals to prevent disease, starting with a ban on avoparcin in 1997 [11]. The use of antibiotic growth promoters in the EU were banned in 2006.

Therefore, the search for alternatives to replace in-feed antibiotics has gained increasing interest in animal nutrition formulations in recent years [45]. Various dietary herbs, plant extracts, and especially essential oils (EOs), have been studied for their antimicrobial and growth promotion abilities [5]. Diets supplemented with *Thymus vulgaris* extract increased the amount of acid mucins in the duodenum of chicks and had a positive effect on the local defense against pathogens [4]. The supplementation of oregano EO to the diets of chickens infected with *E. acervulina*, had a modulating effect on some peripheral blood cell indicators and the functional aspects of phagocytic cells [28]. The addition of sage extract to the diets of chickens infected with *Salmonella Enteritidis* PT4 reduced the *Salmonella* count in the liver when compared to infected chick-

ens without sage supplementation [32]. Combinations of probiotic (*E. faecium* 55) and sage extract (*S. officinalis* L.) supplementation significantly reduced the mucin layers in the caecum of chicks challenged with *S. Enteritidis* PT4 [10].

Recently the effectiveness of using food supplements, probiotics, and phytonutrients has been demonstrated to fight poultry diseases such as coccidiosis — a parasitic disease that causes annual losses of more than $3.2 billion worldwide [27]. Thus, feeding natural products or probiotics to animals that enhance their defense mechanisms, could effectively reduce or prevent the need for therapy of these enteric infections [19].

Some of the latest scientific breakthroughs and technologies, which provide new options and alternative strategies for enhancing production and improving animal health and well-being, were presented at an international symposium, "Alternatives to Antibiotics: Challenges and Solutions in Animal Production," in September 2012, at the headquarters of the World Organization for Animal Health (OIE) in Paris, France.

The objective of this paper is to briefly review the use of antibiotic growth promoters and the possible alternatives of a non-chemical origin.

Antibiotics

Antibiotics are natural metabolites of fungi that destroy or inhibit the growth of bacteria by altering certain properties of bacterial cellular metabolism. Bacteria are vulnerable to antibiotics that disrupt their normal active cellular metabolism.

Today, antibiotics are essential for the health of humans and other animals. The mode of action of antibiotics as growth promoters is by limitation of the growth of detrimental microbes. They also limit the growth of numerous non-pathogenic species of bacteria in the gut and reduce the production of antagonistic microbial metabolites, such as ammonia [46], which adversely affect the physiology of the host animal. Similarly, there is a change in the thickness of the intestinal epithelium which becomes thinner and hence the nutrient absorption may be enhanced. The minimization of gastrointestinal bacteria may ease the competition for vital nutrients between the bird and the microbes [11].

These effects of antibiotics could lead to improvement of the growth and performance of animals, however, in recent years they have been criticized for their possible role in the occurrence of antibiotic-resistant microbes. Over the years, scientists have developed new technologies that could aid in reducing the use of antibiotic.

Probiotics

A variety of microbial species have been used as probiotics. Species of *Bacillus, Enterococcus*, and *Saccharomyces* yeast have been the most common organisms used in livestock [36]. Probiotics are defined as live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host by improving their microbial balance or the properties of the indigenous microflora [46]. Sanders [34] in her paper discusses the statement that probiotics "improve the balance of microflora" and indicates that an improved balance is often associated with increased faecal levels of lactobacilli or bifidobacteria. This is a measure not of balance but of faecal microbiota alterations. Probiotics may, in fact, ease a return to the normal status after a perturbation of the microbiota. This function more closely supports the concept that probiotics can improve the balance of the microbiota. Further research in this area is needed.

Lactobacillus and *Bacillus* are often the components of probiotic products. There has been an increase in research on feeding *Lactobacillus* to broiler chickens [20]. *Lactobacillus* is a group of normal residents in the chicken intestine, predominant in the small intestine and abundant in the caecum of young birds [14]. *Lactobacillus*-based probiotic may strengthen gut defence functions *via* activation and enhancement of local cell-mediated immunity against certain enteric pathogen [6]. Cultures with even one or two bacteria (*Bacillus, Enterococcus*, *Lactobacillus* spp.) have been shown effective against *Salmonella* [17], [29]. The administration of *E. faecium* 55 in the diet of chickens challenged with *S. Enteritidis* confirmed its antibacterial effect on the bacterial microflora in the small intestine [26].

Lee *et al.* [24] found that in chickens infected by coccidiosis, eating diets supplemented with *Pediococcus*-based probiotics: reduced their oocyte production; upped production of cytokines essential for a strong immune response; and they gained weight.

Edens [9] reported that probiotics act to maintain a beneficial microbial population by two basic mechanisms: "competitive exclusion" and immune modulation. Competitive exclusion involves competition for substrates, production of antimicrobial metabolites that inhibit pathogens, and competition for attachment sites. By directly interacting with the mucosal system of the gut, probiotics can modulate either innate or acquired immunity, or both [8]. The specific immune modulatory effects of probiotics are dependent on the strain or the species of bacteria included in the probiotics.

A fabricated probiotic product has to meet multiple criteria, including high efficacy, low cost, high stability, no safety risk, and ease of application. In practice, probiotic cultures need to have the following characteristics:

1) the ability to colonize the intestine;
2) high growth rate and a low requirement for nutrients;
3) suppress enteric pathogens, by either their cells or metabolites;
4) grow easily on a large scale under commercial conditions;
5) preserve stable activity following the manufacturing process and survive in the feed [17].

To complete all the requirements mentioned above is a goal for scientists and manufacturers, since most products do not have reliable effects in the fight against pathogens. This lack of reliability could be caused by insufficient scientific evaluation, poor manufacturing practice or quality control.

Prebiotics

Prebiotics are non-digestible food or feed ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to the host's health [11]. Gibson *et al.* [13] and Robefroid [33] offered the criteria for a prebiotic as:

1) resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption;
2) fermentation by intestinal microflora;
3) selective stimulation of the growth, activity, or both of those intestinal bacteria that contribute to health and well-being.

Many of the currently used prebiotics are non-digestible oligosaccharides containing one or more molecules of one or a combination of simple sugars [17]. The dominant prebiotics are

fructo-oligosaccharide products (FOS, oligofructose, inulin), gluco-oligosaccharides, mannan-oligosaccharides, stachyose, malto-oligosaccharides, and oligochitosan have also been investigated in broiler chickens [45]. Compared with probiotics, prebiotics are cheaper, possess lower risks, and are easier to handle (no need to maintain them in a viable state) [17], but the use of prebiotics in broiler chicken diets does not have a long history [45]. Some positive changes in digestive enzymes, gut morphology and immune system were noticed in birds given prebiotic-supplemented feed [16], [43].

Intake of prebiotics can significantly modulate the colonic microbiota [12]. Fructo-oligosaccharides (FOS) also may help to control the growth of harmful bacteria such as *Clostridium perfringens*, which is especially important to the poultry industry because this bacterium is a primary cause of necrotic enteritis that has been estimated to cost the worldwide poultry industry $2 billion each year [15]. Further, FOS was shown to support the growth of beneficial bacteria, such as lactobacilli [43], and improve the growth performance of birds; but positive effects were not always observed [43]. Wu *et al.* [42] observed that the optimal FOS level was 2.5 to 5.0 $g.kg^{-1}$ of the basal diet, which had a beneficial effect on body weight gain and feed efficiency, whereas too much FOS (10 $g.kg^{-1}$) caused diarrhoea, thus decreasing the production performance. Xu *et al.* [43] reported that the addition of 4.0 $g.kg^{-1}$ of FOS to the basal diet improved the growth performance of male broilers. Moreover, treatment with 8.0 $g.kg^{-1}$ FOS resulted in poorer performance than 2.0 and 4.0 $g.kg^{-1}$ FOS. Waldroup *et al.* [40], however, reported no effects of FOS on the growth of broilers.

Mannan-oligosaccharides (MOS) are derived from yeast cell walls. They have additional glucan and protein components and may contain mannose which can be partially hydrolysed prior to arrival in the lower digestive tract [17]. Mannose is unique because it is bound by the type 1 fimbriae used by many enteric bacteria to attach to host cells and, therefore, can result in the movement of undesirable bacteria through the intestine without colonization [21]. The benefits of MOS based on their specific properties such as modification of the intestinal flora, reduction in turnover rate of the intestinal mucosa and modulation of the immune system have been claimed. These properties have the potential to enhance: growth rate; feed efficiency and liveability in commercial broiler and turkeys; and egg production in layers [35].

Several studies have been conducted to evaluate the feeding of MOS to poultry and positive effects on growth performance and gut physiology have been observed. Dietary mannan oligosaccharides improved the broiler and turkey performances at a level of 0.0125 to 0.1 % [37]. Yeast cell walls containing MOS, reduced intestinal *Salmonella* concentrations by 26 % in broiler chicks [39] compared with chicks fed an unsupplemented diet. Kumar *et al.* [23] demonstrated that MOS are effective in reducing *Salmonella* infections of birds. MOS were shown to alter mucosal architecture and longer villi were noticed in birds fed MOS-supplemented diets [44].

Spring *et al.* [39] and Kocher *et al.* [22] investigated the effects of MOS on intestinal and faecal microbial populations of broilers but the results were inconsistent.

Yang *et al.* [45] presented that the responses of birds to MOS supplementation were influenced by the type of diet, rearing conditions, as well as the age of the birds.

Phytobiotics

Herbs, spices, as well as plant extracts have been used for ages as food and to treat ailments. Phytobiotics were defined by Windisch and Krosmayr [41] as plant-derived products added to the feed in order to improve the performance of agricultural livestock and four subgroups have been classified:

1) herbs (product from flowering, non-woody and non-persistent plants);
2) botanicals (entire or processed parts of a plant);
3) essential oils (hydro distilled extracts of volatile plant compounds);
4) oleoresins (extracts based on non-aqueous solvents).

EOs enhance: production of digestive secretions, stimulate blood circulation, exert antioxidant properties, reduce the levels of pathogenic bacteria and may enhance the immune status [2]. Antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytobiotics exert their positive effects on the growth performance of animals [45]. These plant-based antimicrobial compounds could be used to replace some antibiotic growth promoters but to be most effective as growth promoters, they must be supplemented to the feed in a more concentrated form than found in their natural source [11]. The positive effects observed *in vitro* justify further research in this area to determine the optimal dietary concentration levels and the mode of action of these plant products to achieve the optimal growth performance and disease resistance in poultry [18].

Blood analysis showed that the addition of sage extract to diets had positive effects on increased levels of bilirubin and ALT activity in chickens [28]. The addition of thyme extract (0.05 %) to the diet of chickens increased levels of total proteins and ALP activity but had no effect on performance [4]. Oregano essential oil caused an increase of calcium ($P < 0.001$) and magnesium ($P < 0.01$) on day 42 of the experiment and the level of serum total protein was higher ($P < 0.05$) in the experimental group on experimental day 29 [30].

The antimicrobial activity of EOs may modulate the gut ecosystem to affect fat digestibility, starch and/or protein digestibility of feeds [18]. Oregano essential oil modifies the gut microflora and reduces microbial loading by supressing bacteria proliferation [45]. There are some claims that oregano essential oil can replace anticoccidial compounds, not because it inactivates coccidia, but because it increases the turnover of the gut lining and prevents coccidial attack by maintaining a more healthy population of gut cells [3].

Lee *et al.* [25] investigated the effects of phytochemicals derived from safflower (*Carthamustinctorius*), a common herbal plant widely used to enhance the health of humans and livestock, on chicken coccidiosis, an infectious disease which affects the growth of chickens by damaging the gut. Their study demonstrated for the first time, that the safflower leaf contains immunostimulatory components and activates the innate immunity of chickens.

The anticoccidial effects of green tea-based diets were evaluated in chickens following oral infection with Eimeria maxima. Jang *et al.* [19] found that the green tea-based diets were effective in the reduction of faecal oocyst output in chickens infected with *E. maxima*, however, the diets were not effective in protecting the loss of body weight caused by the infection when compared to the control group.

Major *et al.* [28] observed a significant decrease in the number of heterophils of *E. acervulina* infected chickens fed diets sup-

plemented with oregano EO compared to other groups on 3 day post infection (dpi). However, on dpi 17, a higher frequency of heterophils was found in *E. acervulina* infected chickens compared to birds treated with the anticoccidial drug.

CONCLUSION

By removing the use of antibiotics in the feed from animal husbandry, there is a greater interest to look for efficient alternatives to maintain or improve the health and performance of animals. To date, scientists have suggested various substitutes such as mentioned in this paper. It can be concluded that the responses of birds to alternatives supplementation are influenced by the age of birds, type of diet, nutritional status, infection, as well as rearing conditions. Furthermore, under certain conditions, the alternatives can even negatively affect the performance. Therefore, many factors need to be considered in order to obtain the maximal growth-promoting effects of alternatives in broiler production. To achieve good health and performance of birds, a combination of strategies, hygiene and feed additives should be used.

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STUDY OF SOME SERUM ENZYME ACTIVITY IN RAMBOUILLET SHEEP DURING SEASONAL MIGRATION TO ALPINE PASTURE OF NORTH WESTERN HIMALAYAN REGION (JAMMU)

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ABSTRACT

This investigation was undertaken to study some physiological parameters in migratory Rambouillet sheep during their seasonal migration from a government sheep breeding farm at Billawar, Kathua, Jammu, to a high altitude pasture at Sarthal, Jammu. The animals were divided into 4 groups; group A (male sheep of 1—2 years), group B (male sheep of 2—4 years), group C (female sheep of 1—2years), and group D (female sheep of 2—4 years). Blood samples were collected during the uphill and downhill migrations and were analyzed for enzymatic activities by using analytical kits. The highest enzymatic activities (aspartate aminotransferase, AST; alkaline phosphatase, ALP; lactate dehydrogenase LDH; and creatine phosphokinase, CPK) were observed at the high altitudes. During the uphill migration, lactate dehydrogenase activities were decreased slightly after 30 minutes of leaving the base station and then significantly decreased ($P < 0.01$) at the mid station. Significantly increased ($P < 0.01$) creatine phosphokinase activities were recorded from the 2nd to the 6th collections; whereas, the activities decreased non-significantly during the downhill migration (from the 7th to 9th collections). All of the enzyme activities were higher in the younger, as compared to the adult Rambouillet sheep. Again, the enzyme activities were higher in the females as compared to the males in both of the age groups.

Key words: age; migration; serum enzyme; sheep; sex

INTRODUCTION

Migratory sheep rearing is very common in the economically weaker sections of the society in the tribal hilly areas like Jammu and Kashmir, India. The sheep migrate from the foothills of the Himalayas to high altitude alpine ranges during the summer months and to the foothills and plains during the winter season [6], [18]. In this process, they undergo several adaptive stresses. These stresses may lead to the release of intracellular enzymes into the blood due to changes in the cell membrane permeability. Thus, the cytoplasmic enzymes will appear in the blood before mitochondrial enzymes and of course, the greater the quantity of tissues that are damaged, the greater is the increase in the blood level of the enzymes [14]. Measuring the activity of enzymes in the blood is best used to understand the level of stress of the animal. Therefore, the present study was undertaken to see the changes of enzyme activity during the migration to high altitude pastures in Rambouillet sheep.

MATERIALS AND METHODS

A total of twenty four Rambouillet sheep were included in this experiment. They were divided into four groups of six sheep each, as follows: **Group** A: male Rambouillet sheep, 1—2 years of age; **Group B:** male Rambouillet sheep, 2—4 years of age; **Group C:** female Rambouillet sheep, 1—2 years of age and; **Group D:** female Rambouillet sheep, 2—4 years of age. All of these animals were

maintained under semi-grazed and semi-stall fed conditions at a farm and on complete grazing during the migration period of five and a half months.

Migratory Route

The sheep flock maintained at the state government sheep breeding farm at Billawar is migratory. Out of 12 months in a year, the animals are maintained at the sheep breeding farm in Billawar only for 6—7 months. During the summer season, they migrate to high altitude pastures in search of nutritious grasses and more favourable environmental conditions. Towards mid-May, the migration starts from the sheep breeding farm at Billawar and these animals continue moving uphill, covering a distance of 4—5 kilometers in a day, up to the mid-station in Mandhar, making night halts at several places during the migratory route. In a period of 6—9 days, the sheep reach the mid-station in Mandhar, where they stay for 8 to 10 days. Finally, after leaving Mandhar, the flock reaches the high altitude pasture at Sarthal within 10—15 days. The flocks then stay at the high altitude pasture until the first week of October. During the downhill journey, the sheep follow the same path as for the uphill journey and finally reached the Billawar farm in the first week of November.

Recording of environmental temperature and relative humidity

A dry and wet bulb thermometer was used to record the environmental temperature and relative humidity at the different stations (Table 1).

Table 1. Mean environmental temperature [°C] and relative humidity [%] at different stations during uphill and downhill migrations

Date of sampling	Station	Temperature [°C]	Relative humidity [%]
10.05.2011	Billawar	35.5	57
18.05.2011	Mandhar	16.3	41
14.06.2011	Sarthal	13	45
15.09.2011	Sarthal	12	22
04.10.2011	Mandhar	22	36
24.10.2011	Billawar	28	37

Blood sampling stations and analysis of enzymes

The blood samples were collected: at the base station (1st collection), 30 minutes after leaving the base station (2nd collection); at the mid station, on the day of reaching (3rd collection), day of leaving (4th collection) and 30 minutes of leaving (5th collection); and immediately after reaching the high altitude pasture at Sarthal (6th collection) during the uphill and at high altitude before starting of the downhill migration (7th collection); at the mid station (8th collection); and on the day of reaching the base station (9th collection) in the downhill migration. About 6 ml of blood was collected from each animal by venipuncture using aseptic measures. The serum was separated and transferred to the storage vials and kept in a liquid nitrogen container at –196°C and transferred to the laboratory and analysed immediately for the respective enzymes, using analytical kits. Aspartate aminotransferase (AST) was analyzed by the UV kinetic (IFCC) method; alkaline phosphatase (ALP) was estimated by the Tris carbonate buffer method [24]; lactate dehydrogenase (LDH) was determined by the DGKC method [10] by using analytical kits manufactured by Erba Mannheim, Solan (H.P), India; and creatine phosphokinase (CPK), by the UV kinetic (IFCC) method using analytical kits manufactured by Siemens Healthcare, Baroda (Gujarat), India. All of the recorded data of the present experiment were analyzed statistically by the method described by Snedecor and Cochran [20].

RESULTS

Aspartate aminotransferase (AST)

During the uphill migration, non-significant increase of AST activities ($IU.l^{-1}$) was recorded from the 1st to the 3rd collection in all of the groups of Rambouillet sheep (Table 2a, b). Decreased activity of the same was found at the 4th collection and then again the activities increased up to the 6th collection in all of the groups. The highest AST activities were found in the high altitude (6th collection). During the downhill migration, a decreasing trend of AST activities was noticed from the 7th collection (starting) to the 9th collection in all of the groups. Significant ($P < 0.05$) decreases in enzyme activities were recorded at the 9th collection (98.67 ± 4.92, 96.56 ± 2.87, 104.20 ± 6.55 and 100.72 ± 4.40 $IU.l^{-1}$, respectively), as compared to the values of the 7th collection (starting of the downhill migration). The AST activities were found higher in male and female sheep of 1—2 years of age, as compared to the same in 2—4 years of age animals. Again, it was found that, the AST activities were non-significantly higher in female animals of both the age groups.

Alkaline phosphatase (ALP)

Perusal of Table 3a, demonstrated that during the uphill migration, ALP activities showed a decreasing trend, from the 1st to the 4th collection (mid-station), then the activities increased up to the 6th collection in all of the four groups of sheep. Similarly, during the downhill migration (Table 3b), the activities of the enzyme decreased at the mid station (8th collection) and then the activities increased non-significantly at the 9th collection in all of the groups of sheep. As compared between the young and adult groups, higher ALP activities were observed in the young (1—2 years of age) compared to the adult (2—4 years of age) Rambouillet sheep. The activities ($IU.l^{-1}$) of the enzyme ranged between 140.51 ± 5.55 to 177.52 ± 5.71 and 145.18 ± 5.17 to 179.43 ± 5.60 in males and females of 1—2 years old sheep (group A and C) and 132.02 ± 5.91 to 172.43 ± 8.23 and 135.60 ± 4.97 to 175.24 ± 4.52 in male and female sheep of 2—4 years of age (group B and D). The ALP activities were found non-sig-

Table 2a. Aspartate aminotransferase (AST) activity [IU.l^{-1}] (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Uphill migration							
	1	2	3	4	5	6	P value [Col.]	CD [Col.]
Group A	102.34± 2.28	104.42 ± 3.48	109.88 ± 4.01	105.53 ± 3.27	106.92 ± 4.54	112.34^{a} ± 5.40	0.53	NS
Group B	101.15 ± 3.14	101.44 ± 2.51	107.23 ± 3.35	104.85 ± 4.58	105.61 ± 3.99	109.67^{a} ± 4.00	0.55	NS
Group C	107.05± 2.52	109.90 ± 3.47	113.32 ± 4.09	109.50 ± 3.14	109.50 ± 3.71	124.71^{b} ± 5.56	0.04	11.21
Group D	104.48 ± 1.79	106.95 ± 3.00	111.01 ± 3.29	107.96 ± 3.85	106.55 ± 3.08	119.02^{b} ± 3.06	0.03	8.92
P Value [Gps.]	0.37	0.30	0.71	0.80	0.90	0.13		
CD [Gps.]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		

Means bearing similar superscripts in rows do not vary significantly.
Gps. — groups; Col. — collections; N. S. – not significant

Table 2b. Aspartate aminotransferase (AST) activity [IU.l^{-1}] (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Downhill migration				
	7	8	9	P value [Col.]	CD [Col.]
Group A	119.09 ± 4.42	112.60 ± 5.13	98.67 ± 4.92	4.66	14.70
Group B	117.50 ± 5.21	110.44 ± 3.85	96.56 ± 2.87	6.78	12.44
Group C	130.74 ± 4.32	121.08 ± 4.06	104.2 ± 6.55	6.94	15.51
Group D	122.53 ±5.01	114.88 ± 3.40	100.7 ± 4.40	6.55	13.15
P Value [Gps.]	0.23	0.33	0.72		
CD [Gps.]	N.S.	N.S.	N.S.		

Gps. — groups; Col. — collections; N. S. — not significant

nificantly higher in female as compared to male Rambouillet sheep of both of the age groups.

Lactate dehydrogenase (LDH)

The present study (Table 4a) revealed that LDH activities were decreased slightly after 30 minutes of leaving the base station (2nd collection) in all of the groups of sheep during the uphill migration. Then the enzyme activities were decreased significantly ($P < 0.01$) during the mid station; whereas, the level increased non-significantly in group A, C and D and increased significantly ($P < 0.05$) in group B at the end of the uphill migration (6th collection), as compared to the values of base station (1st collection). During the downhill migration (Table 4b), LDH activity increased significantly ($P < 0.01$) at the 8th and the 9th collections (mid-station) in all of the groups of sheep. The LDH activities were found significantly ($P < 0.01$) higher in young Rambouillet sheep (1—2 years old) than the adult Rambouillet sheep (2—4 years old) during the 1st (252.62 ± 5.54 IU.l^{-1}), 2nd (251.90 ± 6.24 IU.l^{-1}) and 9th (317.69 ± 9.72 IU.l^{-1}) collections in male animals and in all the collections during migration in female animals. Significantly ($P < 0.01$) higher LDH activities were recorded in the female than the male sheep of both of the age groups.

Table 3a. Alkaline phosphatase (ALP) activity (IU.l^{-1}) (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Uphill migration							
	1	2	3	4	5	6	P value [Col.]	CD [Col.]
Group A	160.52 ± 4.32	156.13 ± 5.34	142.09^{b} ± 5.25	140.51 ± 5.55	141.29 ± 6.75	166.24 ± 4.65	0.00	15.57
Group B	152.78 ± 6.50	147.50 ± 5.01	134.90^{b} ± 5.41	132.87 ± 4.99	132.02 ± 5.91	158.45 ± 7.01	0.01	16.98
Group C	164.24 ± 4.46	160.62 ± 5.18	148.93^{a} ± 5.89	145.18 ± 5.17	146.29 ± 6.48	173.57 ± 5.07	0.00	15.71
Group D	157.24 ± 6.21	155.60 ± 5.64	140.65^{b} ± 5.37	138.64 ± 5.62	135.60 ± 4.97	165.50 ± 4.99	0.00	15.91.
P Value [Gps.]	0.51	0.39	0.37	0.45	0.38	0.31		
CD [Gps.]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		

Means bearing similar superscripts in rows do not vary significantly.
Gps. — groups; Col. — collections; N. S. — not significant

Table 3b. Alkaline phosphatase (ALP) activity (IU.l^{-1}) (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Downhill migration				
	7	8	9	P value [Col.]	CD [Col.]
Group A	168.94 ± 4.84	159.86 ± 7.28	177.52 ± 5.71	0.15	N.S.
Group B	160.09 ± 5.61	150.19 ± 8.50	172.43^{a} ± 8.23	0.15	N.S.
Group C	174.43 ± 5.00	166.10 ± 8.80	179.43^{a} ± 5.60	0.38	N.S.
Group D	164.14 ± 6.55	153.58 ± 7.38	175.24^{a} ± 4.52	0.08	N.S.
P Value [Gps.]	0.32	0.52	0.87		
CD [Gps.]	N.S.	N.S.	N.S.		

Means bearing similar superscripts in rows do not vary significantly.
Gps. — groups; Col. — collections; N.S. — not significant

Creatine phosphokinase (CPK)

In the present investigation (Table 5a), significantly increased ($P < 0.01$) CPK activities were found from the 2nd to the 6th collections during the uphill migration as compared to the values at the base station before migration (1st collection) in all of the groups of sheep. However, during the downhill migration (Table 5b) CPK activities decreased non-significantly up to the 9th collection in all of the groups of sheep. The CPK activities were found higher in young Rambouillet sheep (1—2 years of age) as compared to the values of the adult sheep (2—4 years of age) and between the sexes, female had higher activity.

Table 4a. Lactate dehydrogenase (LDH) activity [IU.l^{-1}] (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Uphill migration						P value [Col.]	CD [Col.]
	1	2	3	4	5	6		
Group A	$_{a}252.62^{a}$ ± 0.54	$_{a}251.90^{a}$ ± 6.24	$_{a}227.93^{b}$ ± 6.48	$_{a}230.12^{b}$ ± 7.50	$_{a}234.30^{a}$ ± 7.38	$_{a}268.18^{a}$ ± 7.74	0.00	19.91
Group B	$_{b}229.29^{a}$ ± 3.96	$_{b}226.85^{a}$ ± 5.00	$_{ad}210.17^{b}$ ± 4.01	$_{ad}212.99^{b}$ ± 5.51	$_{ad}218.81^{a}$ ± 5.68	$_{ad}247.67^{b}$ ± 7.94	0.00	16.01
Group C	$_{c}290.01^{a}$ ± 4.99	$_{c}288.70^{a}$ ± 3.87	$_{b}262.30^{b}$ ± 8.06	$_{b}267.36^{b}$ ± 5.67	$_{b}267.45^{b}$ ± 6.80	$_{b}301.06^{a}$ ± 7.81	0.00	18.51
Group D	$_{d}247.33^{a}$ ± 3.75	$_{d}244.37^{a}$ ± 4.38	$_{cd}223.9^{b}$ ± 5.25	$_{cd}226.43^{b}$ ± 6.63	$_{cd}228.36^{a}$ ± 5.55	$_{cd}255.24^{a}$ ± 11.67	0.01	19.52
P Value [Gps.]	0.00	0.00	0.00	0.00	0.00	0.00		
CD [Gps.]	13.72	14.72	18.23	18.95	19.01	26.57		

Means with similar superscripts in columns do not vary significantly

Table 4b. Lactate dehydrogenase (LDH) activity [IU.l^{-1}] (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Downhill migration			P value [Col.]	CD [Col.]
	7	8	9		
Group A	$_{a}266.61^{a}$ ± 7.48	$_{ad}357.15^{b}$ ± 14.10	$_{a}317.69^{b}$ ± 9.72	0.00	32.82
Group B	$_{ad}246.72^{a}$ ±7.34	$_{ae}327.24^{b}$ ±11.97	$_{be}284.72^{b}$ ± 10.02	0.00	30.30
Group C	$_{b}300.56^{a}$ ±7.40	$_{bd}384.74^{b}$ ±11.75	$_{c}357.29^{b}$ ± 9.15	0.00	29.20
Group D	$_{cd}253.51^{a}$ ± 9.66	$_{ce}335.86^{b}$ ± 8.99	$_{de}307.03^{b}$ ± 13.19	0.00	32.76
P Value (Gps.)	0.00	0.01	0.00		
CD (Gps.)	23.85	35.18	31.60		

Means bearing similar superscripts in columns do not vary significantly

DISCUSSION

The increased AST activities during the uphill migration (Table 2a) might be attributed to increased physical activities associated with the uphill migration [16] which causes the cellular release of the enzyme. Again, the AST activities increased at the high altitude pasture in all of the groups, perhaps indicating that due to the activation of pyridoxine in the liver by the zinc metalloenzyme alkaline phosphatase [16], which was also found increased at the high altitude in the present study (Table 3a, b). Kumar [13] reported that the increase in AST activities at the high altitude pasture was greater in crossbred sheep as compared to exotic breeds. Again, the reason for the higher AST activity in young animals as compared to adults, might be due to the fact that the increased muscular activities during the migration become more stressful to the young one as compared to the adults. In our finding, higher AST activity in young animals corroborate with the earlier findings in Chokla lambs [17] and in Awassi sheep [1]. Similar findings were also reported by Bhat *et al.* [2] in which they found that the AST levels in adult Kashmiri bucks and doe were 19.3 ± 2.5 and 24.3 ± 1.4 IU.l^{-1} and buck-kid and doe-kid were 17.3 ± 0.3 and 19.7 ± 0.2 IU.l^{-1}, respectively. The higher AST activity might

Table 5a. Changes in creatine phosphokinase (CPK) activity [IU.l^{-1}] (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Uphill migration							
	1	2	3	4	5	6	P value [Col.]	CD [Col.]
Group A	243.33 ± 6.87	276.00^{b} ± 6.46	293.00 ± 8.31	283.33 ± 8.46	288.00 ± 9.39	299.50 ± 6.91	0.00	22.65
Group B	226.00 ± 7.84	251.67^{b} ± 8.28	275.67 ± 7.03	265.00 ± 7.51	272.00 ± 9.00	285.67 ± 10.38	0.00	24.41
Group C	250.67 ± 10.13	279.33^{b} ± 5.36	301.50 ± 8.58	289.00 ± 9.56	295.50 ± 11.55	311.00 ± 10.89	0.00	27.74.
Group D	230.83 ± 8.63	255.67^{a} ± 7.63	277.17 ± 8.39	268.83 ± 9.29	275.83 ± 8.57	294.17 ± 8.90	0.00	24.90
P Value [Gps.]	0.18	0.02	0.10	0.19	0.32	0.31		
CD [Gps.]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		

Gps. — groups; Col. — collections; N. S. — not significant

Table 5b. Changes in creatine phosphokinase (CPK) activity [IU.l^{-1}] (Mean ± SD) in different groups of Rambouillet sheep during migration

Collections	Downhill migration				
	7	8	9	P value [Col.]	CD [Col.]
Group A	278.83 ± 7.19	266.67 ± 7.41	258.67 ± 6.90	0.17	N.S.
Group B	270.00 ± 11.18	261.50 ± 8.74	253.50 ± 10.12	0.52	N.S
Group C	292.67 ± 9.23	279.00 ± 6.96	271.83 ± 6.50	0.18	N.S
Group D	283.50 ± 7.52	276.33 ± 6.88	265.67 ± 7.07	0.24	N.S
P Value [Gps.]	0.36	0.34	0.39		
CD [Gps.]	N.S.	N.S.	N.S.		

Gps. — groups; Col. — collections; N. S. — not significant

be due to the increased muscular activity during walking which causes more exertion to female animals as compared to males. In contrast to the present finding, Al-Sadi [1] had reported that there was no significant difference in the AST activities between male and female animals. Similar observations were also reported by Kiran *et al.* [11], who studied the hematological and biochemical profiles of goat and sheep from the district Rahim Yar Khan in Southern Punjab, Pakistan and reported that the age and sex did not affect the activity of AST in goats and sheep. However, Gwaze *et al.* [9] reported higher AST activity in males than in female Nguni goats of South Africa.

Low environmental temperatures at the high altitude pasture (Table 1) and migratory stress might be the reason for high ALP activities (Table 3a, b) as it is involved in maintaining homeostasis and energy generation in the animal body [21], [23]. This finding corroborates with the earlier reports of higher ALP activities at high altitudes [13] in crossbred sheep of Himachal Pradesh during their migration. Similarly, Chahal and Rattan [5] observed a significant increase in the ALP activity at lower environmental temperatures in Corridale rams. Higher ALP activity in young animals was perhaps due to the higher migratory stress on younger, as compared to the adult animals. Similar finding

was also reported by More and Sahni [17], in which they found that the ALP level declined with the advancement of age in Chokla rams. Kumaresan and NdzinguAwa [15] recorded the ALP activity as 3277.7 and 1550.6 IU.l^{-1} in 1–2 years and over 2 years Red Sokoto goat, respectively. Again, in another study, Gwaze *et al.* [9] reported significantly higher ALP activity in the young compared to mature Nguni goats of South Africa. In contrast, other findings revealed higher ALP activity in adults, as compared to young goats [2], [3], [7], [8], [22], which might be due to these experiments being conducted under normal environmental condition without migratory stress. The ALP activity was found higher in females as compared to male goats. Other scientists [9] also reported higher ALP activity in female than in male Nguni goats of South Africa. Higher migratory stress might be attributed to the increased ALP activity in females. In contrast to our observation, different workers [11], [22] reported that age and sex did not significantly affect the level of ALP in different breed of goats and sheep.

Earlier findings [4] reported that after 20 minutes of exercise, the LDH activity decreased from 4.02 to 3.57 μKat.l^{-1} in sheep. Increased physical activity associated with migration might be the reason of a significant increase in LDH activity in the present study (Table 4b). Kumar *et al.* [14] also observed a significantly ($P < 0.05$) increased LDH activities at the mid station in exotic Rambouillet and Polwarth ewes of Himachal Pradesh during the downhill migration. More and Sahini [17] also reported that LDH activities declined with the advancement of age in Chokla lambs, which supported the present findings in Rambouillet sheep. Significantly ($P < 0.01$) higher LDH activities were recorded in female than the male sheep of both of the age groups, which might be due to higher migratory stress on female as compared to male sheep. In contrast to our findings, Kiran *et al.* [11] reported that age and sex did not affect the activity of LDH in goats and sheep of southern Punjab.

Selman *et al.* [19] reported that altered metabolic rates during stress resulted in increased enzyme activities. Knowles *et al.* [12] reported that plasma CPK activity increased maximally after 26 hours of road transportation in cattle. Higher CPK activity during the uphill migration might be due to increased physical activity (muscle contraction). In reverse, in the present study it was found that, during the downhill migration, CPK activities decreased non-significantly up to the 9th collection in all the groups of sheep which might be due to the fact that, during the downhill migration lower CPK activity is associated with less physical activity as compared to the uphill migration. Higher CPK activity in young animals as compared to adult animals perhaps indicated higher basal metabolic rates in young animals as compared to the adults. Again, the CPK activities were found non-significantly higher in female animals of both of the age groups. However, Gwaze *et al.* [9] reported higher CPK activity in males than in female Nguni goats of South Africa.

CONCLUSION

From this study it can be concluded that, all of the four groups of migratory Rambouillet sheep were under stressed condition at the mid station and immediately after the uphill migration at the high altitude pasture which was reflected by a higher level of enzymes in their blood. Among the animals, adult males were more adapted to migratory stress, as compared to females and young ones.

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THE CONNECTION BETWEEN THE INTERNAL VENOUS VERTEBRAL SYSTEM AND THE CRANIAL VENA CAVA IN RABBITS

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ABSTRACT

The aim of this study was to describe the possible variations in the connection between the internal venous vertebral system and the cranial vena cava in rabbits using the corrosion technique. The study was carried out on 40 adult New Zealand white rabbits. The venous system was injected by using Batson's corrosion casting kit No. 17. We found that the connection between the internal venous vertebral system and the cranial vena cava was by means of the vertebral veins and the right azygous vein. The vertebral vein was present as an independent tributary in 36 cases (90 %). In the rest of the cases, it was found as double, triple or forming a common trunk with other veins. The azygous vein was present as an independent tributary of the cranial vena cava in 39 cases (97.5 %). We found also a common trunk formed by the junction of the deep cervical vein, the right vertebral vein and the azygous vein in one case (2.5 %). The azygous vein received 6, 7, 8 or 9 pairs of dorsal intercostal veins. Documenting the anatomical variations in the connection between the internal venous vertebral system and the cranial vena cava in the rabbit will aid in the planning of future experimental studies and determining the clinical relevance of such studies. The internal venous vertebral system has been described as a possible route for metastatic tumours.

Key words: azygous vein; rabbit; tributary; vertebral vein

INTRODUCTION

The knowledge of anatomical variations is important for radiological and surgical procedures in humans and animals due to its practical and theoretical significance for experimental research and surgical practice in experimental and domestic animals [8].

The variations of the internal venous vertebral system and its main veins were best described in humans [11]. The internal venous vertebral system (venous plexus lying within the vertebral canal in the epidural space) was described as a possible route for metastatic tumours in several animals [2]. Rabbits have been used as experimental models for investigating many diseases [4].

The aim of this study was to describe the possible variations in connections between the internal venous vertebral system and the cranial vena cava in rabbit using the corrosion technique.

MATERIAL AND METHODS

The study was carried out on 40 adult rabbits (age 140 days). We used New Zealand white rabbits (breed HY+) of both sexes (female n = 20; male n = 20) with an average weight of 2.5—3 kg in an accredited experimental laboratory at University of Veterinary Medicine and Pharmacy in Kosice. The animals were kept in cages under standard conditions (temperature 15—20 °C, relative humidity 45 %, 12 h light period), and fed with a granular feed mixture (O-10 NORM TYP). Drinking water was available for all animals *ad libitum*. The animals were euthanised by prolonged inhalation

Fig. 1. ***Vena* (*v.) vertebralis dextra* as independent tributary of *v. cava cranialis*.** (1) *v. cava cranialis dextra*, (2) *v. cava cranialis sinistra*, (3) *v. vertebralis dextra*, (4) *v. azygos dextra*. Macroscopic image, dorsolateral view

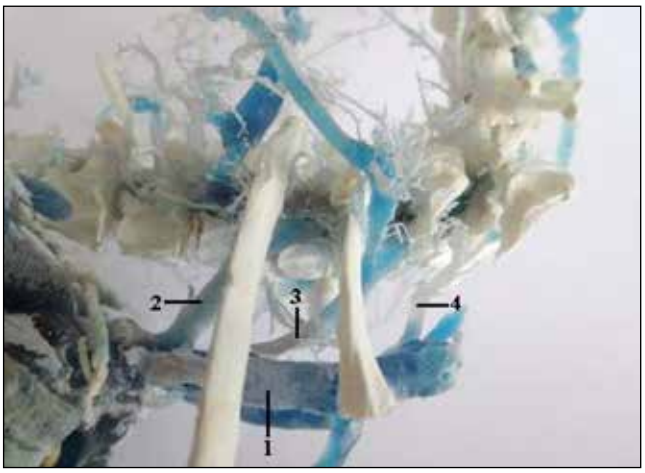

Fig. 2. Triple *v. vertebralis dextra*. (1) *v. cava cranialis dextra*, (2) *v. vertebralis dextra* I, (3) *v. vertebralis dextra* II, (4) *v. vertebralis dextra* III. Macroscopic image, ventrolateral view

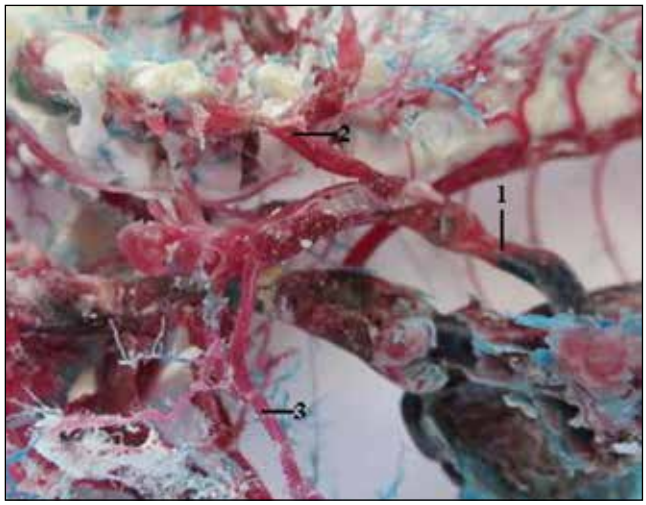

Fig. 3. ***V. vertebralis sinistra* as independent tributary of *v. cava cranialis*.** (1) *v. cava cranialis sinistra*, (2) *v. vertebralis sinistra*, (3) *v. thoracica interna sinistra*. Macroscopic image, dorsolateral view

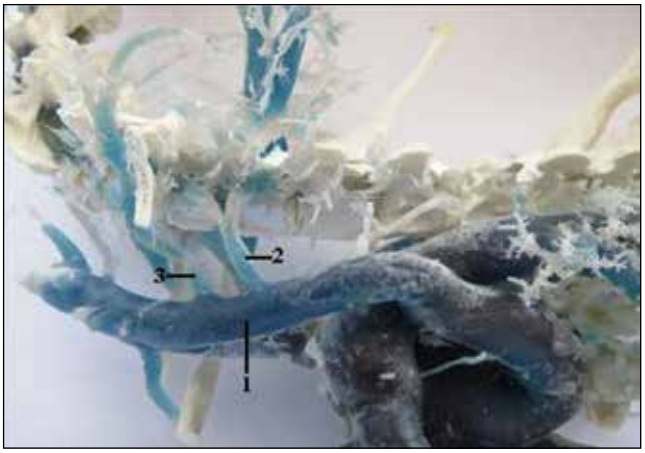

Fig. 4. Double *v. vertebralis sinistra*. (1) *v. cava cranialis sinistra*, (2) *v. vertebralis sinistra* I, (3) *v. vertebralis sinistra* II. Macroscopic image, lateral view

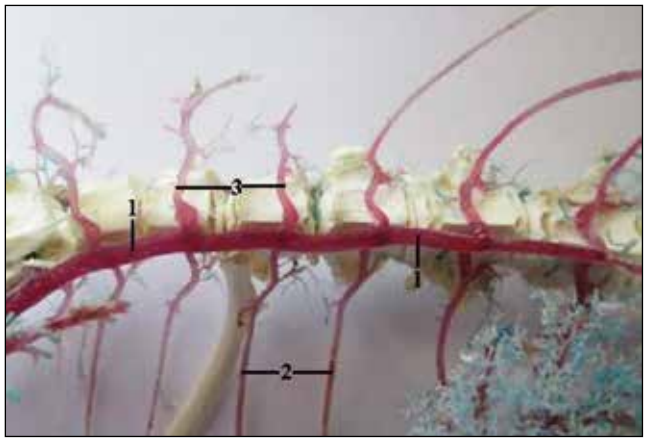

Fig. 5. ***Vv. intercostales dorsales* in number of 6 pairs.** (1) *v. azygos dextra*, (2) *vv. intercostales dorsales dextrae*, (3) *vv. intercostales dorsales sinistrae*. Macroscopic image, ventral view

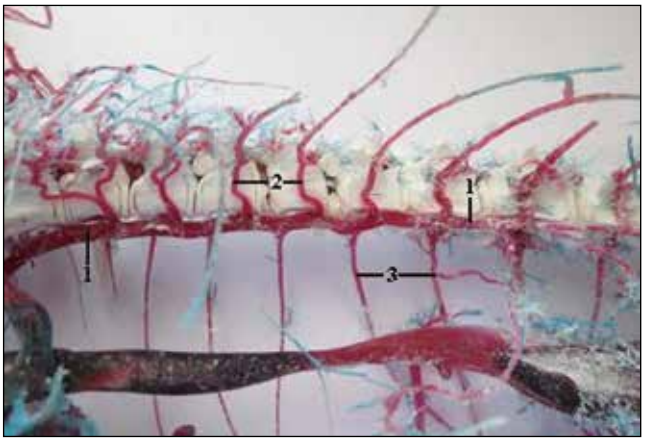

Fig. 6. ***Vv. intercostales dorsales* in number of 8 pairs.** (1) *v. azygos dextra*, (2) *vv. intercostales dorsales sinistrae*, (3) *vv. intercostales dorsales dextrae*. Macroscopic image, ventrolateral view

anaesthesia with ether. Immediately after euthanasia, the vascular network was perfused with a physiological solution. The manual injection was done through the caudal vena cava. Batson's corrosion casting kit No. 17 (Dione, České Budějovice, Czech Republic) in a volume of 35 ml was used as a casting medium. The maceration was carried out in 2—4% KOH solution for a period of 8 days at 60—70 °C. This study was carried under the authority of decision No. 2647/07-221/5.

RESULTS

We found that the connection between the internal venous vertebral system and the cranial vena cava was by means of the right and left vertebral vein and the right azygous vein.

The vertebral vein conveys the blood from the cervical and cranial thoracic regions. As an independent tributary, the right vertebral vein opened into the cranial vena cava in 36 cases (90 %; Fig. 1). It was double in two cases (5 %). In one case (2.5 %), we found a common trunk formed by the right vertebral vein, the right deep cervical vein and the right azygous vein. In the same corrosion cast, we found the second right vertebral vein as an independent tributary which opened into the cranial *vena cava*. This vein received a communicating branch coming from the first right vertebral vein and branch going out of the transverse canal of the cervical vertebrae. The right vertebral vein was triple in one case (2.5 %; Fig. 2).

The left vertebral vein, as an independent tributary of the cranial vena cava was found in 36 cases (90 %; Fig. 3). In 3 cases (7.5 %), a double vein was present (Fig. 4) and in one case (2.5 %), as a triple vein. In one case (2.5 %), we found the left vertebral vein receiving two tributaries at the level of the head of the first rib.

The right azygous vein starts its formation at the level of the first lumbar vertebrae by the junction of the first, second and third pairs of lumbar veins. The vein entered the thoracic cavity ventrally to the vertebral column. Its thoracic segment received the dorsal intercostal veins in the number of 6 pairs in 2 cases (5 %; Fig. 5), in the number of 7 pairs in 10 cases (25 %), in the number of 8 pairs in 24 cases (60 %; Fig. 6) and in the number of 9 pairs in 4 cases (10 %). It emptied in the cranial vena cava in 39 cases (97.5 %). The common trunk formed by the junction of the right deep cervical vein, the right vertebral vein and the right azygous vein was found as a tributary of the cranial vena cava in one case (2.5 %).

DISCUSSION

Up until now the vertebral vein was described as a single independent tributary of the cranial vena cava [9], [10]. We found the vertebral veins as independent tributaries in 36 cases (90 %). In the rest of the cases, it was found as double, triple or forming a common trunk with other veins. Only one author described that the right vertebral vein was a tributary of the right costocervical vein [1]. Many works doubt the presence of the vertebral vein [5]. Krause [7] described the replacing of the vertebral vein by small vertebral branches coming out from the vertebral canal between the first, second and third thoracic vertebra and emptying independently into the cranial vena cava.

The internal venous vertebral system is in direct connection with the vertebral veins. Metastatic abscesses and metastatic tumours can appear in locations that do not seem to be in line of a direct spread from their primary focus. This type of spread is known as paradoxic metastasis. The internal venous vertebral system is today denoted as the way of paradoxic metastasis in patients with bone lesions with diagnosed carcinoma of the penis [2]. The clinical significance of the internal venous vertebral system is also obvious in patients with vein thrombosis of the thoracic limb [12].

The azygous vein was described as an independent tributary of the cranial vena cava [1], [5], [6], [7], [9], [10]. We found the same situation in 39 cases (97.5 %). We found also a common trunk formed by the junction of the deep cervical vein, the right vertebral vein and the azygous vein in one case (2.5 %). In all cases, the azygous vein started by the junction of bilateral first, second and third pair of lumbar veins. Craigie (5) described the point of arising at the level of the junction of the first pair of lumbar veins. The azygos vein received 6, 7, 8 or 9 pairs of dorsal intercostal veins. In only one study was the number of intercostal veins described [7]. In the present study, they were found in the number of 7 on the right side and in the number of 6 on the left side.

The azygous vein is generally known as the by-pass between the cranial and caudal vena cava. In different species of animals, the efferent venous system of the adrenal glands was in direct connection with the internal venous vertebral system and by this way with the azygous vein [3]. The documentation of variations in the azygos vein is of great importance in CT and MRI investigation of the mediastinum [11].

CONCLUSIONS

It is important to report and document different anatomical variations of the azygous vein and the vertebral veins that may occur, because some anomalies of these veins can easily be confused with pathological conditions such as aneurysm, tumours and enlarged lymph nodes. Documenting the anatomical variations in the connection between the internal venous vertebral system and the cranial vena cava in the rabbit will aid in the planning of future experimental studies and determining the clinical relevance of such studies.

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CANNABINOID RECEPTOR 2 AGONIST (JWH-133) INHIBITS PROLIFERATION OF CEM AND JURKAT-T LEUKEMIC CELLS: A SHORT PRELIMINARY *IN VITRO* INVESTIGATION

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ABSTRACT

Previously, it has been shown that cannabinoids (CB) exert significant anti-cancer properties. It is well known that the unwanted psychotropic effects of CBs are mediated *via* the CB_1 receptors, thus selective targeting of the CB_2 receptor would avoid side effects in leukemia treatment. Therefore, the aim of our study was to evaluate the effect of selective CB_2 receptor agonist, JWH-133, on CEM and Jurkat-T leukemic cells using the MTT-test.

Moreover, the expression of the CB_2 receptor was confirmed by RT-PCR. The present study showed that JWH-133 demonstrated significant cytotoxic effects at micromolar concentration in both cell lines. Nevertheless, further *in vivo* research should be performed to evaluate the underlying mechanism of action and find the optimal therapeutic concentration for the clinical use.

Key words: blood cell; cancer; MTT

INTRODUCTION

At the present time medications based on cannabinoids (CB) are registered as useful adjuvants to conventional anti-tumor chemotherapy [1], [13]. Since the CB receptors (R) were cloned, the influence of CBR modulators on tumor cell proliferation has been started to be investigated. In numerous studies it has been described that CBR ligands have both anti- and pro- cancer effects [3]. However, still more studies point to the anti-proliferative effects of CBs in tumor cells [5], [14].

In previously published studies it was shown that THC-induced apoptosis is CBR mediated [6], [8]. In particular, it was shown that THC, a non-specific CBR agonist, induces apoptosis in Jurkat cells *via* the CB_2R [6]. It is well known that the unwanted psychotropic effects of CBs are mediated *via* the CB_1R. From this point of view, the selective targeting of CB_2R would avoid the side effects in cancer treatments.

Since leukemia is an unaffordable disease with poor prognosis, the basic research should be focused on new molecules which might have the potential to reverse this situation [7]. This study was, therefore, aimed at assessing the effect of selective CB_2R agonist, JWH-133 {3-(1,1-Dimethylbutyl)-1-deoxy-Δ8-tetrahydrocannabinol}, on the proliferation of the following leukemic cell lines: CEM and Jurkat-T.

MATERIALS AND METHODS

Reagents

MTT, 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was purchased from Sigma-Aldrich Chemie (Steinheim, Germany), Collagenase II was from Invitrogen (USA), JWH-133 {3-(1,1-Dimethylbutyl)-1-deoxy-Δ8-tetrahydrocannabinol} (99% purity determined by GLC) was kindly given by Prof. J. W. Huffman (Clemson University, Clemson, SC, USA). Trizol was purchased from Invitrogen. One-step RT-PCR kit (#210212) was purchased from Qiagen (Valencia, CA, USA).

*both authors contributed equally to this work

RNA isolation, cDNA preparation by RT and CB_2R amplification by PCR

The total RNA was extracted from both of the indicated cell lines, using Trizol according to the manufacturer's instruction. The RT-PCR was done according to the protocol provided by the Qiagen one-step RT-PCR kit (#210212). Briefly, for each sample, 150 ng of total RNA was mixed with RT-PCR buffer, dNTP mix (10 mM of each dNTP), primers (10 mM each) and enzyme mix. For CB_2R detection forward primer 5'-TTTCCCACTGATCCCCAATG-3' and reverse primer 5'-AGTTGATGAGGCACAGCATG-3' and for Beta-actin detection forward primer 5'-ACCAACTGGGACGACATGGAGAAAATC-3' and reverse primer 5'-GTAGCCGCGCTCGGTGAGGATCTTCAT-3' were used. Thermal cycling parameters were; 30 min at 50 °C for reverse transcription, and 15 min at 95 °C for initial PCR activation. PCR cycles were used as follows: 30 sec at 95 °C, 30 sec at 55 °C and 30 sec at 72 °C. The amplified DNA was fractioned by electrophoresis in 2 % agarose gel and stained with ethidium bromide.

Cytotoxicity assay — MTT

The cytotoxic effect of JWH-133 was studied by using a colorimetric microculture assay with the MTT end-point (10). Briefly, 8x104 cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in the culture medium containing the tested chemicals at final dilutions of 1/5 to 1/500. After 72 hours of incubation, 10 µl of MTT (5 $mg.ml^{-1}$) (Sigma, Germany) were added to each well. After an additional 4 h, during which insoluble formazan was produced, 100 µl of 10 % sodium dodecylsulphate were added in each well and another 12 h were allowed for the formazan to be dissolved. The absorbance was measured at 540 nm using the automated MRX microplate reader (Dynatech Laboratories, UK). The absorbance of the control wells was taken as 100 % and the results were expressed as a percentage of the control. The experiment was repeated three times.

Statistical analysis

For all experiments, the mean values and standard deviations were calculated. To evaluate the statistical significance, ANOVA followed by Tuckey-Kramer post-hoc test were employed. The statistical significance was considered to be present if $P < 0.05$.

RESULTS

RT-PCR analysis

The CB_2R was expressed in both cell lines. Its expression is shown in Fig. 1.

Cytotoxicity assay

Survival of tumor cells exposed to various concentrations of JWH-133 is shown in Table 1. JWH-133 significantly decreased CEM survival at the concentration of 10 and 100 $\mu mol.l^{-1}$ and Jurkat survival at 100 $\mu mol.l^{-1}$.

DISCUSSION

Cannabinoids have been shown to inhibit the proliferation of several cancer cell lines, included leukemic [4], [2], [11], [12], [14]. Reviewing the potential mechanism of action, it was shown that THC (delta-9-tetrahydrocannabinol) induces apoptosis in leukemic cells [8]. Nevertheless, the psychotropic effect of THC points to its limited use in clinical practice. However, in our present study we showed that the CB_2R is expressed in both tested leukemic (CEM and Jurkat-T) cell lines (Fig. 1) and that the selective CB_2R agonist also has antiproliferative effects in these cells (Table 1).

In addition to studies with leukemic cells, the inhibition of solid tumor growth by selective activation of the CB_2R was

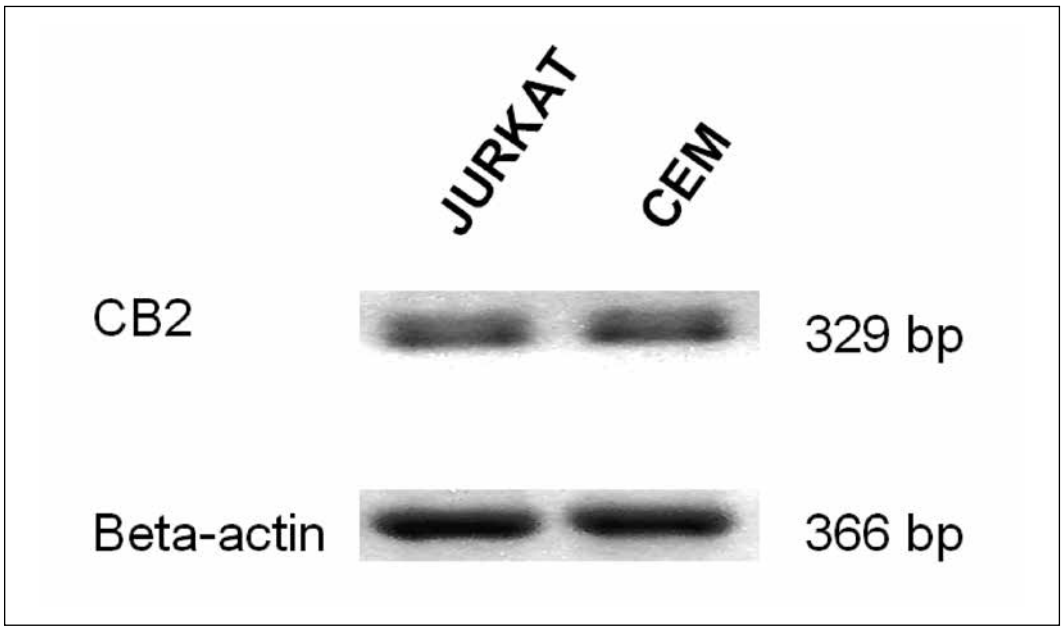

Fig. 1. CB_2R is expressed in both tested cell lines

Table 1. Results of cytotoxicity assay (*P < 0.05; **P < 0.01)

	100 μmol.l^{-1}	10 μmol.l^{-1}	1 μmol.l^{-1}	100 nmol.l^{-1}	10 nmol.l^{-1}	1 nmol.l^{-1}
CEM	31.66 ± 29.83**	87.66 ± 10.21*	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Jurkat	47.66 ± 27.42**	96.66 ± 5.77	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

observed as well. *In vitro* experiments carried out with C6 glioma cells showed that following JWH-133 treatment, selective activation of the CB_2R receptor signaled apoptosis *via* enhanced ceramide synthesis de novo was present [9]. Similarly, in prostate cancer cells involvement of CB_2R-mediated induction of cell death and an anti-proliferative effect was observed [13]. Interestingly, we also demonstrated reduced proliferation and colony formation following JWH-133 treatment in A549 lung cancer cells [14].

Furthermore, an inverse relationship between CB concentration and cell proliferation was also shown [3]. While micro-molar concentrations of CBs inhibited the proliferation of both lung tumor cells and glioblastoma, multiforme cells nano-molar concentrations improved their growth. However, we did observe such effect in this current investigation in a concentration range from 1 μmol.l^{-1} to 100 μmol.l^{-1} (Table 1).

On the other hand, it was also shown that a combination approach of THC and chemotherapy could be a valuable tool in cancer treatments [5]. From this point of view, it would be meaningful to test whether JWH-133 could act synergistically with conventional chemotherapy, since such treatment would avoid the psychotropic side effects of CB_1R stimulation.

In conclusion, our study showed that JWH-133, selective CB_2R agonist, has anti-tumor activity at micromolar concentrations in leukemic cell lines. Since JWH-133 is a non-psychoactive agent further research should be performed to facilitate its clinical use.

ACKNOWLEDGEMENT

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VASCULAR VARIATIONS OF THE RABBIT KIDNEYS AND THEIR CLINICAL SIGNIFICANCE

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ABSTRACT

The aim of this study was to describe the origin, localisation and variations of kidney arteries and veins in the rabbit as the subject of a scientific investigation. The study was carried out on 40 adult New Zealand white rabbits. We prepared corrosion casts of the rabbit arterial and venous systems. Spofacryl was used as the casting medium. In 75 % of the cases, the origin of the *arteriae renales* was located at the level of the third lumbar vertebra and in the remaining 25 % of the cases, the *arteria renalis dextra* branched off at the level of the second lumbar vertebra. In 10 % of the cases, we observed that the number of the *arteria renalis sinistra* was doubled. We recorded also in one case, the presence of the *arteria renalis accessoria* for the *ren dexter.* In 10 % of the cases, we observed that the number of the *vena renalis sinistra* was doubled. In 5 % of cases, two *venae renales sinistrae* arose from the kidney and subsequently, about 1 cm from the opening to the *vena cava caudalis,* they united to form a single vein. In 5 % of the cases, two *venae renales sinistrae* arose from the kidney and subsequently, approximately 1 cm away from *hilus renalis,* they united. The variations of the number of renal arteries were partially homologous to the human, but variations of the renal veins were localized on the other side as in humans.

Key words: origin; rabbit; renal artery; renal vein; variation

INTRODUCTION

Knowledge of the variations of renal vascular anatomy has importance in exploration and treatment of; renal trauma, renal transplantation, renovascular hypertension, renal artery embolisation; angioplasty or vascular reconstruction for congenital and acquired lesions; surgery for abdominal aortic aneurysm; and conservative or radical renal surgery. Numerous reports have appeared in the literature describing variations in the renal vascular anatomy [13].

Variations in the renal vasculature have etiologic, diagnostic, and therapeutic implications. Etiologically, multiple renal arteries represent persisting mesonephric arteries. Diagnostically, failure of opacification of a portion of the kidney upon arteriography raises the possibility of; an infarct, avascular tumour, or intrarenal haemorrhage in the presence of a normal variant [2].

Therapeutically, as each multiple renal artery is a terminal vessel, its lesion may produce segmental ischaemia with subsequent hypertension. In conservative surgical procedures, besides haemorrhage and the loss of renal parenchyma, a serious consequence of renal arterial lesions is the development of hypertension. The presence of multiple renal arteries increases the complexity of renal transplantation. It has been reported that kidneys presenting anatomic vascular variations are associated with a significantly higher failure rate than kidneys with a single artery [15]. In the past, a number of studies focused on the investigation of vessels in rabbit kidneys which have been extrapolated to humans [8].

The aim of this study was to describe the origin, localisation and variations of kidney arteries and veins in the rabbit, as this

laboratory animal is frequently the subject of scientific studies and the results have been extrapolated to humans.

MATERIALS AND METHODS

The study was carried out on 40 adult rabbits (age 140 days). We used New Zealand white rabbits (breed HY+) of both sexes (female n = 20; male n = 20) with an average weight of 2.5—3 kg. They were housed in an accredited experimental laboratory at the University of Veterinary Medicine and Pharmacy in Kosice. The animals were kept in cages under standard conditions (temperature 15—20 °C, relative humidity 45 %, 12 h light period), and fed with a granular feed mixture (O-10 NORM TYP). Drinking water was available for all animals *ad libitum*. Twenty rabbits of both sexes were used to prepare corrosion casts of the kidney arterial system and another group of the same size and composition served to investigate the venous system. The animals were euthanised by prolonged inhalation anaesthesia with ether. Immediately after euthanasia, the vascular network was perfused with a physiological solution. During manual injection of the arterial system through the ascending aorta, the right atrium of the heart was opened in order to lower pressure in the vessels to ensure a well perfused injection. Before injecting the venous system, we fixed the venous valves by means of 10 % formalin injected through the caudal vena cava, to prevent their reverse closure. Fixation of the venous system was maintained for three hours. Subsequent injection was carried out through the caudal vena cava. Spofacryl (polymethylmethacrylate, SpofaDental, Czech Republic) in a volume of 35 ml was used as a casting medium. The maceration was carried out in 2—4 % KOH solution for a period of 5 days at 60—70 °C. This study was carried out under the authority of decision No. 2647/07-221/5.

RESULTS

The arteriae renales, as paired organ arteries supplying the kidneys arose from the lateral walls of the *aorta abdominalis*. The a*rteria renalis sinistra* was longer than the *arteria renalis dextra* which was related to a more lateral localisation of the left kidney. We observed that in all cases, the *arteria renalis dextra* originated more cranially than the *arteria renalis sinistra* (Fig. 1). In 75 % of the cases, the origin of the *arteriae renales* was located at the level of the third lumbar vertebra and in the remaining 25 % of cases, the *arteria renalis dextra* branched off at the level of the second lumbar vertebra. In 95 % of the cases, the origin of the *arteriae renales* was located caudally from that of the *arteria mesenterica cranialis.* In two cases, the *arteria renalis dextra* originated at the same level as the *arteria mesenterica cranialis*. In 10 % of the cases, we observed that the number of the *arteria renalis sinistra* was doubled (Fig. 2), both originating at the level of the third lumbar vertebra. We recorded also one case of the presence of the *arteria renalis accessoria* for the *ren dexter* (Fig. 3). This originated from the ventral wall of the *aorta abdominalis* between the fourth and fifth lumbar vertebra and entered the kidney at its caudo-medial margin. After entering the *hilus renalis, the arteria renalis* gave off individual *arteriae interlobares.*

The a*rteria abdominalis cranialis* was a paired artery originating from the *arteria renalis*. In 65 % of the cases, the *arteria abdominalis cranialis dextra* originated from the *arteria renalis dextra* ahead of the *arteria abdominalis cranialis sinistra* originating from *arteria renalis sinistra*. In 30 % of the cases, they both originated from the *arteriae renales* at the same level. In 5 % of the cases, they originated directly from the lateral wall of the *aorta abdominalis,* cranially to the origin of the *arteriae renales*.

The localisation and frequency of the opening of the *venae renales* into the *vena cava caudalis* were coincident with the origin of the *arteriae renales* from the *aorta abdominalis. The venae renales* were situated more caudally than the *arteriae renales*. The v*ena renalis sinistra* was longer than the *vena renalis dextra* (Fig. 4). The v*ena abdominalis cranialis sinistra* opened into the *vena renalis sinistra* in all of the cases, while the *vena abdominalis cranialis dextra* opened into the *vena renalis dextra* in 65 % of the cases. In 35 % of the cases, *the vena abdominalis cranialis dextra* opened into the *vena cava caudalis*. In 10 % of the cases, we observed that the number of *vena renalis sinistra* was doubled. In 5 % of the cases, two *venae renales sinistrae* arose from the kidney and subsequently, about 1 cm from the opening to the *vena cava caudalis,* they united to form a single vein. The v*ena abdominalis cranialis sinistra* also joined this common vein. The v*ena ureterica cranialis sinistra* opened into one of the *vena renalis sinistra* (Fig. 5). In 5 % of the cases, two *venae renales sinistrae* arose from the kidney and subsequently, approximately 1 cm away from the *hilus renalis,* they united into one vein. The v*ena abdominalis cranialis sinistra* and the *vena ureterica cranialis sinistra* also opened into this common vein (Fig. 6). The v*ena ureterica cranialis sinistra* opening into the *vena renalis sinistra* was observed in 20 % of the cases.

DISCUSSION

Senecail *et al.* [17] described that the left anomalies of the renal vein may represent real traps in the interpretation of abdominal imaging, particularly in CT scanning, or in magnetic resonance where they are not always recognized. The abnormal imaging may be the source of technical difficulties in diagnostic or therapeutic angiography [6] and may modify the values obtained by catheter sampling for suprarenal hormonal levels [16], [17].

An angiographic evaluation of the renal arterial supply must be carried out routinely before kidney donor nephrectomy, in order to identify any arterial variants or covert parenchymal disease [5], [14], [15].

The anatomical knowledge of the renal arteries, veins and their variations are of extreme importance for the surgeon that approaches the retroperitoneal region, mainly in the face of the current frequency of the renal transplant surgeries, also these anatomical variations should be kept in mind by clinicians and academics that may manipulate this anatomical area [4].

The basic form of the vascular arrangement of kidneys has two vessels on each side: one artery and one vein for each

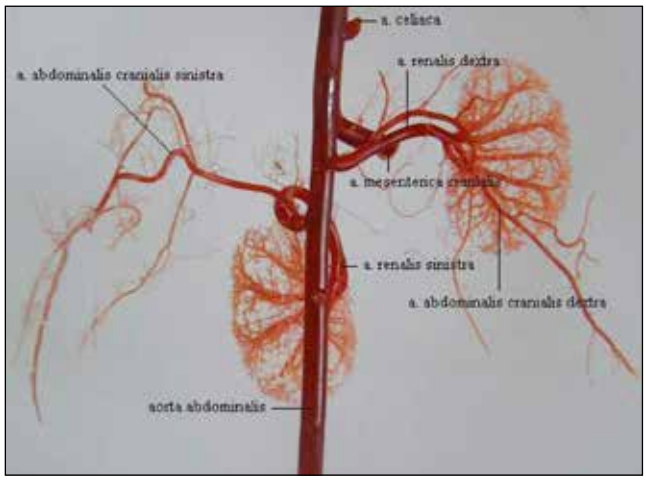

Fig. 1. Renal arteries as branches of the aorta abdominalis. The a*rteria renalis dextra* originates more cranially than the *arteria renalis sinistra*. The a*rteriae abdominales craniales* are branches from the *arteriae renales*. Macroscopic image, dorsal view (a. — arteria)

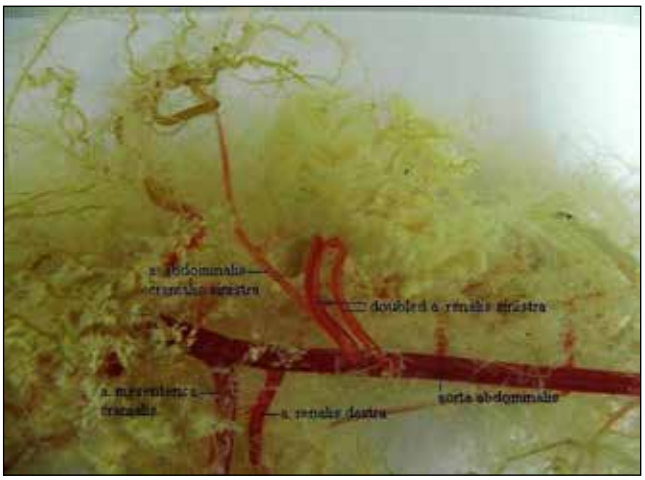

Fig. 2. The doubled *arteria renalis sinistra*. Macroscopic image, ventrolateral view — arteria)

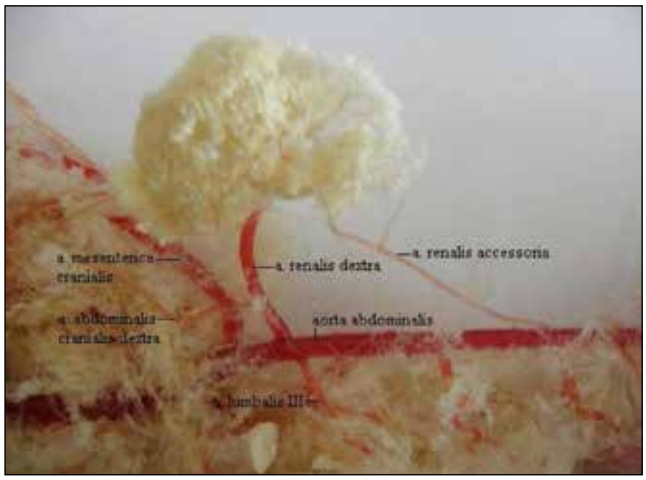

Fig. 3. The a*rteria renalis accessoria* of the right kidney. Macroscopic image, dorsolateral view (a. — arteria)

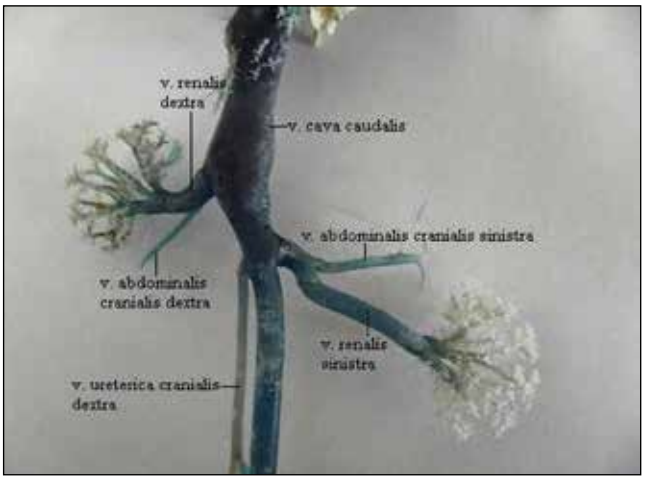

Fig. 4. The v*enae renales* opens into the *vena cava caudalis* and the *venae abdominales craniales* opens into the *venae renales*. The v*ena ureterica cranialis* as a single tributary of the *vena cava caudalis*. Macroscopic image, ventral view (v. — vena)

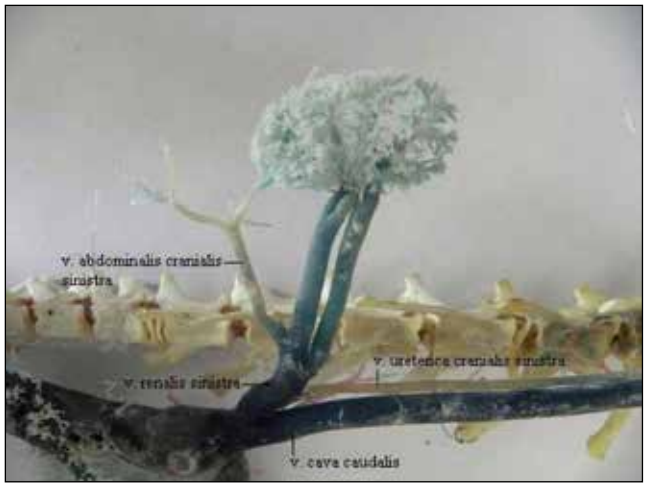

Fig. 5. Two *venae renales sinistrae* arise from the kidney and subsequently, about 1 cm from the opening to the *vena cava caudalis,* they unit to form a single vein. Macroscopic image, ventrolateral view (v. — vena)

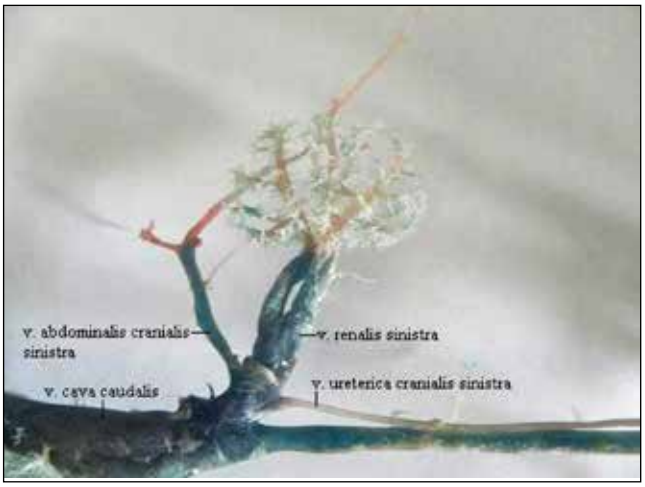

Fig. 6. Two *venae renales sinistrae* arise from the kidney and subsequently, approximately 1 cm away from the *hilus renalis,* they unit into one vein. Macroscopic image, ventral view (v. — vena)

kidney [10], [12]. Although rabbits are laboratory animals relatively frequently used for studies, there is a considerable gap in the angiologic literature regarding the issue of vasculatory variations of rabbit kidneys. Papers dealing with this issue have been published only sporadically [9]. Studies involved in similar observations in humans are more frequent [2], [3], [4]. Ozkan *et al.* [11] described of 855 consecutive patients with anomalies of the human renal artery in a large angiographic evaluation: in 76 % of the cases, one single renal artery was present bilaterally; in 19 % of the cases, multiple renal arteries were present unilaterally; and in 5 % of the cases, multiple renal arteries were present bilaterally. Multiple renal arteries were present more often on the right. In our study, we found that in rabbits, the single renal artery was bilaterally present in 85 % of the cases and multiple renal arteries were present unilaterally in 15 % of the cases. We didn't find bilaterally multiple renal arteries. Multiple renal arteries were present on both sides in equal proportions.

Baptista-Silva *et al.* [1] described an anatomical study of the renal veins that was on the right side; the renal vein was double or triple in 38.79 % and the left renal vein was always unique. In rabbits, we found in 10 % of the cases that the number of left renal vein was doubled.

CONCLUSIONS

Our observations which pointed to double numbers of both *arteriae renales* and *venae renales,* were obtained using a relatively significant statistical set of data. The total accuracy of preoperative evaluation with using digital subtraction angiography is 91—95 % and with using CT angiography was 95—97 % [7]. But the total accuracy by intraoperative findings in our study by preparing corrosive casts was 100 %.

Variations of the number of renal arteries were partially homologous to humans, but variations of the renal veins were localized on the other side as in human. We find left-sided multiple renal vein, but in humans most were often right-sided multiple renal vein [1]. The presence of multiple renal veins on the other side is less responsible for the use of rabbit as a simple model for example of ischemic renal injury.

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SARCOCYSTOSIS IN MALLARDS IN THE SLOVAK REPUBLIC

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ABSTRACT

The mallard (*Anas platyrhynchos*) is a common specie of water fowl. It is the specie of water birds most sensitive to sarcocystosis. Sarcocystosis in mallards is a parasitic disease caused by a protozoan parasite (*Sarcocystis rileyi)* that forms typical macrocysts in the pectoral muscles of parasitized birds. We collected and examined 63 samples of pectoral muscles from mallards, acquired by hunting from October 2010 to January 2012 in the following localities: Bratislava, Šaľa, Michalovce, Bystré and Čierne nad Topľou, and the Hunting district of Tvrdošín. We examined all of the samples macroscopically during the parasitological dissection and microscopically after artificial digestion. We confirmed the presence of *S. rileyi* in 14.3 % (9/63) of the tested animals. Seven positive samples originated from the hunting district of Tvrdošín. We detected macrocysts in their pectoral muscles. Two positive samples from Šaľa were confirmed by the finding of microcysts. Our results confirm the current presence of sarcocystosis in mallards in the Slovak Republic; therefore it is necessary to inform the public, mainly hunters and consumers about this parasitosis.

Key words: *Anas platyrhynchos*; macrocysts; mallard; microcysts; sarcocystosis; *Sarcocystis rileyi*

INTRODUCTION

Sarcocystosis is caused by the intracellular protozoan parasites of the genus *Sarcocystis* (Apicomplexa). The parasite has an indirect two-host life cycle that involves a definitive and an intermediate host. The definitive hosts are carnivores (predators) in which intestinal infection occurs. Herbivores and omnivores (prey) play the role of intermediate hosts. In theirs muscle tissues, sarcocysts contain hundreds to thousands of developing bradyzoites. Sarcocysts are oval, whitish cysts that vary in size from microscopic to visible. The bradyzoite stage is infectious for the definite host which becomes infected following ingestion of infected muscle tissues. The bradyzoites are released in their intestine, enter the *lamina propria* and undergo gametogony to form oocysts that mature in the host's cells and are shed in the faeces. The oocyst contains two sporocysts with four sporozoites in each and may disintegrate during excretion. The intermediate hosts become infected when they ingest oocysts or sporocysts. The sporozoites released in the intestine penetrate into the bloodstream and multiply in the walls of small blood vessels. Then they invade the skeletal or cardiac muscles, form the sarcocyst wall, multiply as merozoites for several generations and develop into bradyzoites.

More than a hundred *Sarcocystis* species are known to infect domestic and wild animals. Water fowl, in particular the mallard (*Anas platyrhynchos*), are frequently infected with *Sarcocystis rileyi* [2] that forms typical macrocysts in the pectoral muscles [5], [6]. Birds can be infected by feeding on infective faeces of the definitive host, stripped skunk, (*Mephitis mephitis*) or during building their nests [4].

The mallard is a partially migratory bird species. Slovak breeding individuals occur throughout the territory to the altitude of about 1000 m, mainly in lowlands, backwaters, water reservoirs, but also in running waters. Slovak mallard populations move south for the winter and individuals from northern latitudes arrive to our territory. Mallards migrate after the drop of temperature (September, October) due to the freezing of water and minimization of food sources in their habitats. The main migratory routes from European countries lead towards the Mediterranean and Africa. In the spring (March, April) the reverse situation occurs when birds return back to the north and east for the breeding season. Due to abnormal climatic conditions in winter, migration exceptions can be recorded [13].

Sarcocystosis of birds (*Anseriformes*) originated in the North America, where it was detected in the 1940's. The parasitosis has spread to Africa, Asia and Europe because of the bird's migrations [2], [11]. *Sarcocystis rileyi* was confirmed in the pectoral muscles of mallards and graylag geese which were hunted in Lithuania, Poland, Austria, Bulgaria and Germany [3]. The definitive host has not been confirmed in Europe, however the fox (*Vulpes* spp.) or raccoon dog (*Nyctereutes procyonoides*) could be a possible definitive host [12]. Sarcocystosis was found in Slovakia for the first time in 2004. It was an accidental finding of macrocysts in pectoral muscles of a mallard from Malacky (personal information). The first record of the parasite by Oberhauserová *et al.* [12] described the presence of macrocysts in mallards hunted in the Hunting district of Tvrdošín in 2009 [12].

The aim of our study was to study the prevalence of sarcocystosis in mallards living in territory of the Slovak Republic.

MATERIALS AND METHODS

We monitored the occurrence of sarcocystosis in mallards in selected localities from October 2010 to January 2012. The main studied locality was the Hunting district of Tvrdošín. We also examined samples from control localities in western (Bratislava, Šaľa) and eastern areas of the Slovak Republic (Čierne nad Topľou, Bystré nad Topľou, Michalovce) (Fig. 1). The investigated birds were of different ages, ranging from 3—4 months to about 1 year of age. In total, we examined 63 samples of pectoral muscles from mallards. We used the method of helminthological dissection to detect the presence of macrocysts in the pectoral muscles and the standard artificial digestion method for the detection of microcysts in tissues. Briefly, we homogenized *m. pectoralis* (15 g) with digestive juice (1 litre buffer + 50 mg trypsin). After 30 minutes of the digestion, the samples were filtered and centrifuged (5 min at 1000 r. p. m.). After this step, the samples were washed twice with distilled water and centrifuged again. The sediment was examined for microcysts using a light microscope with a magnification of 100—400 × [10].

RESULTS AND DISCUSSION

We confirmed the presence of *S. rileyi* in 9 samples of 63 examined pectoral muscles of mallards. The total prevalence was 14.3 % (Table 1). We recorded the presence of typical macrocysts in 7 samples, that were obtained from Orava, the Hunting district of Tvrdošín (Fig. 1). The visible, rice grain-like, 5—7 × 2 mm cysts were localized parallel with the muscle fibers. Microscopically, we observed fusiform microcysts in 2 samples from Šaľa (Fig. 1). They had a cellular structure and measured 400 µm. We recorded detritus of cysts with banana shaped bradyzoits. As both positive specimens from Šaľa were 3—4 months old, the microcysts were most likely the cysts of type I—IV or juvenile stages of *Sarcocystis rileyi.* The young birds cannot have macrocysts in their muscles because the cyst development lasts 3 and more months [8], [9]. We did not evaluate the prevalence dependency on the gender because the sampled group was not balanced (2 males, 7 females). Some authors reported that females are more frequently infected than males [4]. This statement may relate to the fact that female ducks build their nests and have a close contact with the infective material. On the other hand, other authors found that drakes were more often infected with *S. rileyi* [1].

The prevalence of sarcocystosis in mallards from the Hunting district of Tvrdošín was 25.93 %. We suppose that the positive mallards found in Orava could have migrated from Poland. Mallards hunted in Orava were members of

Table 1. The prevalence of sarcocystosis in mallards in Slovakia

Location	Number of examined mallards	Number of positive samples	Presence of microcysts	Presence of macrocysts	Prevalence %
Bratislava	5	0	0	0	0
Bystré n./Topľou	10	0	0	0	0
Čierne n./Topľou	4	0	0	0	0
Michalovce	5	0	0	0	0
Šaľa	12	2	2	0	16.67
Tvrdošín	27	7	0	7	25.93
Total	**63**	**9**	**2**	**7**	**14.29**

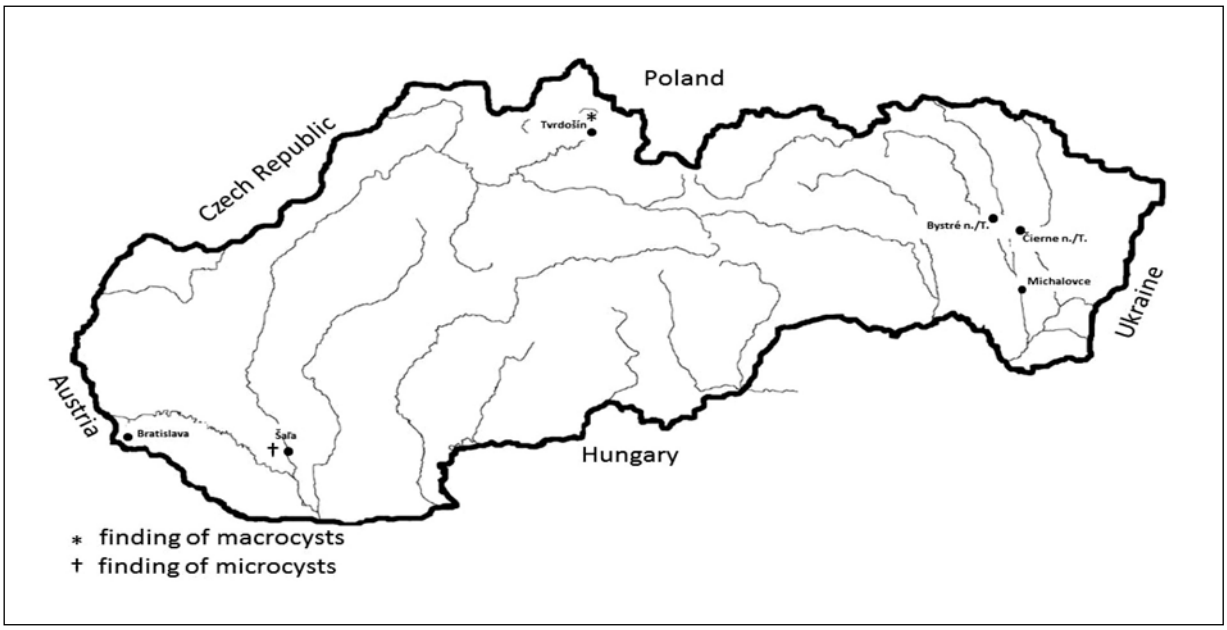

Fig. 1. Localities of collection of mallards examined for sarcocystosis in Slovakia and locations of positive findings

autochthonous nesting population because, according to the local birds and game manager, the mallards from the northern region had not yet flown to the mentioned area at the time of this study. Findings of the parasite in the pectoral muscles of mallards reflect their way of life. During winter the mallards potentially infected with *S. rileyi* migrated from the northern localities to the southern regions [11]. Tvrdošín is close to the Polish border. In Poland, sarcocystosis was confirmed for the first time in 2003 with the prevalence of 0.7 % [14].

The other area with 2 positive cases detected by our study was the region of the Danube lowland, near the Austrian border. In Austria, sarcocystosis was reported for the first time in 2002. In Europe, sarcocystosis is a typical parasitic infection of birds in Lithuania, being reported repeatedly since 2003. The prevalence of sarcocystosis in Lithuania was 17 % in 2006 [6], [7]. This fact corresponds with the more positive geographical aspect there. Lithuania offers better conditions for the life of water fowl than Slovakia.

The results of our study confirmed sarcocystosis in mallards as a current parasitosis in the Slovak Republic. Therefore, it is necessary to inform the public, particularly hunters and consumers about the risk of infections. An effective therapy of the intermediate hosts in nature is impossible. The most important prophylactic measure is to avoid feeding the dogs with raw water fowl meat.

CONCLUSION

Sixty-three mallards (*Anas platyrhynchos*) from Slovak localities (Tvrdošín, Bratislava, Šaľa, Michalovce, Bystré nad Topľou and Čierne nad Topľou) were collected and examined for the presence of sarcocystosis between 2010 and 2012. Nine birds were infected which indicate a 14.3 % prevalence of sarcocystosis. Seven positive findings originated from the Hunting district of Tvrdošín. In these mallards, *Sarcocystis rileyi* macrocysts were macroscopically observed in the breast muscles during their complete anatomical dissections. Two findings of *Sarcocystis* spp. cysts were microscopically recorded in mallard ducks hunted in the locality of Šaľa.

The presence of the parasite in mallard ducks hunted in the above mentioned localities is related to their winter migration from northern regions, where they can become infected, to the southern countries. Slovakia is a transit region for these birds. Tvrdošín is located close to the border with Poland, where sarcocystosis in birds has previously been reported. The Danubian Lowland, where the Šaľa location is situated, is the nesting ground for waterfowl situated close to Austria, where the occurrence of sarcocystosis has also been recorded.

The results of our study clearly confirmed that sarcocystosis of mallard ducks is present in Slovakia; therefore it is necessary to inform the public, mainly hunters and consumers about this parasitosis.

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ABDOMINAL SURGERY AND LIPID PEROXIDATION IN DAIRY COWS WITH ABOMASAL DISPLACEMENT

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ABSTRACT

Our study was aimed at examining the effect of abdominal surgery stress on lipid peroxidation (TBARS — Thio Barbituric Acid Reactive Substances) in dairy cows suffering from the left abomasal displacement (n = 10) and abomasal volvulus (n = 10). Blood samples were drawn from the jugular vein prior to surgery, immediately and then 15, 30, 60, 90 minutes, and 2, 5, 10, and 24 hours after reposition of the abomasum. The surgical stress resulted in significant elevations of plasma cortisol ($P < 0.05$), with the highest values found 15 minutes after the abomasal reposition in both groups. In addition, the surgery transiently enhanced the plasma levels of TBARS ($P < 0.001$) and the highest TBARS plasma levels ($0.91 \pm 0.18\ \mu mol.l^{-1}$) were recognized 60 minutes after reposition of the abomasum in dairy cows with the left displacement of the abomasum. There were no significant changes in the ferric reducing ability of plasma (FRAP) in dairy cows. These data indicate that the stress reaction caused by the surgical correction of the left displacement of the abomasum can be associated with higher production of TBARS. The data of cows with the abomasal volvulus indicate that the stress effects of omentopexy are not strong enough to overwhelm the stress response to pre-surgical events (transport, sickness, new environment). Nevertheless, we can conclude that stressful events associated with the surgical correction of displaced abomasums can be responsible for the enhancement of the peroxidation rate.

Key words: cortisol; dairy cows; omentopexy; TBARS

INTRODUCTION

Stress response may include changes in feeding behaviour, hypertension, reproductive dysfunctions, inefficient feed conversion, gastric and intestinal ulcers, electrolyte imbalance and immune deficiency [1]. The major stress alterations involve enhanced secretion of glucocorticosteroids and increased sympathetic nervous system activity.

A number of studies also demonstrated that the stress reaction has stimulating effects on free radical production, thus contributing to an increased lipid peroxidation in animals [2]. In cows with abomasal volvulus, the post-surgical reperfusion of the ischemic abomasum could be an additional risk of enhanced lipid peroxidation [3]. These reactions can be monitored by the analysis of the lipid peroxidation products, and antioxidant status of the organism. The production of free radicals is supported by several stress factors and can finally result in the onset of health disorders in cattle. Oxidative stress resulting from the increased production of free radicals and reactive oxygen species, and a decrease in antioxidant defence, leads to the damage of biological macromolecules and disruption of the normal metabolism and physiology.

Moreover, there are several data describing the periparturient period which claim that this period is related to a oxidative⁄antioxidative imbalance [4]. Displacement of the abomasum belongs to the diseases with the highest incidence during the postparturient period.

The present investigation was undertaken to study the effects of surgical stress on thiobarbituric acid reactive substances and the

antioxidative capacity in dairy cows suffering from abomasal displacement.

MATERIALS AND MEHODS

Ten Holstein-Friesian dairy cows, mean age 5.26 years, admitted for treatment of left abomasal displacement (LAD) and ten Holstein-Friesian dairy cows, mean age 4.74 years, admitted for the treatment of abomasal volvulus (AV), were used in this study. Abdominal surgery (omentopexy) was performed in a standing position [5] in cows with LAD 16–24 hours after admission and in cows with AV 1—2 hours after admission. Procasel (2 % procain-hydrochloride) was used as local anaesthesia. The mean duration of preparation for surgery lasted approximately 30—40 minutes, and the surgery involved approximately 30 minutes. Blood samples were taken from the jugular vein prior to surgery, immediately and then 15, 30, 60, 90 minutes, and 2, 5, 10, and 24 hours after the reposition of the abomasum.

The plasma cortisol levels were determined by a radioimmunoassay kit (Coat-A-Count [125I]; Diagnostic Products Cooperation, L.A., USA). The intensity of lipid peroxidation was measured fluorometrically by the thiobarbituric acid reacting substances test (TBARS) in EDTA plasma [6]. The total plasma antioxidative capacity was measured in samples (in triplet) by the modified FRAP (ferric reducing activity of plasma) assay [7]. Briefly, 0.09 ml H_2O and 0.03 ml plasma (EDTA) were added to 0.9 FRAP solution. The absorbance was measured at 593 nm after incubation for eight minutes (37 °C). 1 M $FeSO_4 . 7\ H_2O$ was the standard solution.

Statistical analysis was carried out by the one-way analysis of variance (ANOVA).

RESULTS

The surgical stress in LAD cows resulted in significant changes in plasma cortisol concentrations ($P < 0.05$). The highest cortisol levels were reached within the first hour after reposition of the abomasum and were approximately five times higher than those found prior to the stress. A return to the levels below the initial ones was recognised 10 hours after the repositioning of the abomasum (Table 1). The ANOVA revealed ($P < 0.05$) the enhanced effects of the surgical stress on the plasma concentrations of thiobarbituric acid reactive substances. The maximum mean levels of TBARS were measured between one and two hours after reposition of the abomasum. Twenty four hours after reposition, the TBARS values returned to the pre-surgical levels. In contrast, there were no significant changes in the ferric reducing ability of the plasma (FRAP) in dairy cows. However, the FRAP values tended to decrease within the first 40—60 minutes after abomasal reposition and to increase to pre-surgical values afterwards.

Significant changes in plasma cortisol concentrations in AV dairy cows ($P < 0.05$) were seen during the study. The highest cortisol levels were reached 15 minutes after the repositioning of the abomasums. However, these values did not differ from pre-surgical ones. The typical basal cortisol values could be recognised only at the end of the study, 24 hours after abomasal repositioning (Table 2). The ANOVA did not reveal any effects of the surgical stress or the abomasal reposition on both plasma concentrations of the thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of the plasma (FRAP) in dairy cows (Table 2).

DISCUSSION

The mean plasma cortisol levels of 1—10 $mg.l^{-1}$ were found in dairy cows during the first month after calving [8]. In another study, it was concluded that 69.4 % of 307 baseline cortisol samples had concentrations below 3 $mg.l^{-1}$, whereas 13.7 % of the samples contained 6 $mg.l^{-1}$ cortisol or more [9]. Similar cortisol concentrations have also been defined by others as baseline values [10], [11]. The mean cortisol values prior to surgery in LAD cows, and from 5 hours after the abomasal replacement can, therefore, safely be considered to be in the normal range of baseline concentrations. The significant effect of surgery on the increase in plasma cortisol levels was similar to that previously reported in cattle after laparotomy [12]. The pattern of cortisol concentrations seen after surgery in this study was similar to the pattern seen in 5—6 month-old cattle after amputation dehorning [13]. In contrast, the cows with abomasal volvulus demonstrated higher mean cortisol values prior to surgery in comparison to the LAD cows and it might be a result of both sickness and transport stress of the animals prior to surgery. The effect of surgery on the plasma cortisol was minimal, however, cortisol levels fell to the physiological baseline range by the end of the trial.

The measurement of thiobarbituric acid reacting substances (TBARS) is one of many techniques and tests which have been used to measure lipid peroxidation in biological specimens. In the TBARS test, lipid peroxidation is purposefully propagated in the specimen *in vitro* and the total malondialdehyde-thiobarbituric acid (MDA-TBA) adduct formed at the end of the procedure is taken to be due to the sum of the pre-existing bond of free MDA plus that formed from degradation of pre-existing lipid hydroperoxide in the sample [14]. One of the disadvantages of the TBARS test is that MDA is only formed by fatty acids which contain three or more double bonds. In our experiment with LAD cows, the surgery resulted in a transient increase of lipid peroxidation in dairy cows. TBARS levels culminated within two hours after the abomasal replacement, i.e. within a very short time period after the stress. Similar changes of lipid peroxides (malondialdehyde) could be seen immediately after the exercise stress in male subjects [15]. In our experiment with AV cows, the surgery did not result in any significant changes of the plasma TBARS. However, the pre-surgical levels of TBARS were lower than the baseline values found in the cows with LAD which also proves the theory the lipid peroxidation in our model is primarily result of a stress response of the animal and not of metabolic reactions related to tissue reperfusion. Recently, it was reported that dairy cows suffering from abomasal volvulus did not show a

Table 1. Plasma concentrations of cortisol, thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of plasma (FRAP) in dairy cows (n = 10) stressed by omentopexy of the left displacement of abomasum (mean ± SD)

	Cortisol [mg.l^{-1}]	TBARS [mmol.l^{-1}]	FRAP [mmol.l^{-1}]
Before surgery	6.21 ± 6.08	0.71 ± 0.11	262 ± 57
Immediately AR	14.0 ± 7.21	0.84 ± 0.11	247 ± 54
15 minutes AR	32.4 ± 18.1	0.82 ± 0.14	243 ± 56
30 minutes AR	30.8 ± 19.8	0.90 ± 0.19	244 ± 51
60 minutes AR	25.1 ± 15.4	0.91 ± 0.18	253 ± 45
90 minutes AR	17.3 ± 13.1	0.85 ± 0.12	256 ± 42
2 hours AR	16.8 ± 13.9	0.91 ± 0.18	258 ± 52
5 hours AR	8.79 ± 5.21	0.89 ± 0.20	278 ± 51
10 hours AR	4.18 ± 2.89	0.80 ± 0.19	268 ± 66
24 hours AR	3.92 ± 3.52	0.69 ± 0.15	264 ± 58
ANOVA	$P < 0.05$	$P < 0.05$	$P > 0.05$

AR — after replacement of abomasum

Table 2. Plasma concentrations of cortisol, thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of plasma (FRAP) in dairy cows (n = 10) after surgery of the abomasal volvulus (mean ± SD)

	Cortisol [mg.l^{-1}]	TBARS [mmol.l^{-1}]	FRAP [mmol.l^{-1}]
Before surgery	30.0 ± 26.6	1.04 ± 0.24	308 ± 89
Immediately AR	29.0 ± 21.0	1.01 ± 0.26	292 ± 82
15 minutes AR	44.5 ± 23.8	0.95 ± 0.24	275 ± 83
30 minutes AR	39.5 ± 22.9	1.04 ± 0.29	267 ± 76
60 minutes AR	22.9 ± 15.2	1.00 ± 0.25	275 ± 84
90 minutes AR	15.9 ± 13.3	0.97 ± 0.26	271 ± 79
2 hours AR	19.9 ± 13.3	1.02 ± 0.26	277 ± 75
5 hours AR	19.3 ± 15.4	0.95 ± 0.27	267 ± 77
10 hours AR	15.0 ± 14.4	0.81 ± 0.16	264 ± 75
24 hours AR	8.30 ± 10.1	0.78 ± 0.11	277 ± 56
ANOVA	$P < 0.05$	$P > 0.05$	$P > 0.05$

AR — after replacement of abomasum

severe abomasal ischaemia [16]. The level of lipid peroxidation does not depend only on increased free radical production but it is also strongly affected by the level of antioxidant protection. It is well known that higher levels of nutritive antioxidants, including tocopherol, b-carotene, and ascorbic acid, can efficiently decrease the level of lipid peroxidation [17]. Post-parturient decrease in erythrocyte malondialdehyde was reported in dairy cows which was associated with the increase in plasma vitamin E [18].

The FRAP assay is quick and simple to perform and it can provide basic information about the antioxidative status of animals. Individual plasma antioxidants contribute to the total FRAP value as follows: ascorbic acid 15 %, a-tocopherol 5 %, uric acid 60 %, bilirubin 5 %, albumin 5 %, protein 10 %, and others 5 % [7]. Plasma concentrations of these antioxidants depend on both dietary intake and the synthetic capacity of specific organs, in particular the liver. In this study, we could find only the tendency to the lower FRAP values within the first two hours after replacement of the abomasum in LAD cows. Thus, the total antioxidative activity of plasma which represents the non-enzymatic antioxidative systems could indicate an increased requirement. Similarly to the TBARS reaction we could not recognise any significant dynamic in plasma FRAP levels in cows with abomasal volvulus.

CONCLUSIONS

In conclusion, these data extend our existing knowledge by indicating that the stress reaction caused by surgical correction (laparotomic omentopexy) of the left displacement of the abomasum is associated with a transient increase in lipid peroxidation. In addition, our data indicated that stress reactions caused by surgical correction (omentopexy) of abomasal volvulus is not strong enough to overwhelm the stress response to pre-surgical events (transport, sickness, new environment). The question whether there is a need for preventive measures against lipid peroxidation in surgically stressed animals, i.e. antioxidant administration, remains open for further studies.

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

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

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Manuscripts. Papers should be written in English and the spelling should follow the preferred form in *The New Shorter Oxford English Dictionary* (1993). Three copies of the text (preference: CD ROM, secondrate: disc-form) and one copy of the photographs and illustrations, tables or graphs (in digital form) should be sent to the Executive Editor, *Folia Veterinaria*, The University of Veterinary Medicine, Komenského 73, 041 81 Košice, The Slovak Republic.

The text should be double-spaced and typewritten on one side of A4 paper, 30 lines, 60 strokes, with margins of at least 25 mm.

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

Authorship. In accordance with the criteria for authorship recommended by the International Committee of Medical Journal Editors (*JAMA* 1997; 277, 927—934; *Can. Med. Assoc. J.* 1997; 156: 270—7 and 1997; 156: 571—574) each author must have (a) participated substantially in the conception and execution of the work, (b) contributed significantly to the drafting and/or revision of the manuscript, and (c) agreed with the final version, in order to accept public responsibility for the article. In cases of multiple authorship, authors should provide a description of what each contributed. This information may be published. The order of authorship on the byline should be a joint decision of the co-authors. Authors should be prepared to explain this order.

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

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

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

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

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

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

EXAMPLES

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

Black, H., Duganzich, D., 1995: A field evaluation of two vaccines against ovine pneumonic pasteurellosis. *New Zeal. Vet. J.*, 43, 60—63.

Brown, L. W., Johnson, E. M., 1989: Enzymatic evidence of alkaline phophatase. In **Caster, A. R.:** *Enzymology*. Plenum Press, New York, 99—101.

Ikuta, K., Shibata, N., Blake, J. S., Dahl, M. V., Nelson, R. D., Hisamichi, K. *et al.*, 1997: NMR study of the galactomannaus of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. *Biochem. J.*, 323, 297—305.

Language Style. Be prepared to use the first person ("I" or "We"), but do not overuse it. (e. g. "We studied 24 Slovak Merino ewes.")

The excessive use of the passive voice is a principal cause of dullness in scientific writing. Use it sparingly, and prefer the active voice ("We conclude that…") to the passive ("It can be concluded that…") whenever justifiable.

Use the past tense for reporting observations, completed actions, and specific results ("We observed no significant changes.")

Use the present tense or the present perfect for generalizations and generalized discussion. ("This suggests that…")

Employ the specialist vocabulary of your discipline(s), but do not allow this technical jargon to turn into gobbledegook. "The dynamic development of biological sciences has… had a positive influence on the current knowledge of the activated mechanisms… in the case of human and animal organisms" can be rendered succinctly as "The rapid growth of biological science has enabled us to understand the functions of human and animal bodies better." Convoluted and roundabout expression does not impress and may well irritate the reader.

Remember that many readers will not be native-speakers of English. If you are an ESL (English as Second Language) author, apply the principles of English style and syntax when writing, and be mindful that the correct word order is important in English sentences. Make sure that your sentences are sentences: do not lose control of their structure.

Be simple and concise; where possible use verbs instead of abstract nouns. Break up long noun clusters and "stacked modifiers" (strings of adjectives before nouns without clues about which modifies which). Logically ordered and lucidly expressed ideas will make your meaning clear: obfuscation will not assist the reader.

Avoid "dictionary" and "computer English" — transverbation based upon an incorrect choice of words in a dictionary or word bank. (One computer produced this: "Natural immunity is not bound on antecedent individual skill by your leave pathogen and him close non-pathogenic microorganism").

Units of Measurement. Measurements of length, height, weight, and volume should be reported in metric units.

Temperatures should be given in degrees Celsius; blood pressures in millimetres of mercury.

All haematological and clinical chemistry measurements should be recorded in the metric system or in the terms of the International System of Units (SI).

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

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

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

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

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

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

Graphs and Tables should contain essential data not given in the text. In the journal, graphs will have an overall width of no more than 8.5 cm and be drawn on pages 17.5 cm wide. The size of the letters in legends should suit these dimensions. Computer-

drawn graphs are preferred-laser-printed on diamond paper. Statistics (with tables of parameters) must be enclosed. Captions for figures, including graphs and photographs, and all legends should be subscribed. Tables should be typed with double-spacing on separate sheets of paper and numbered consecutively in the order of their citation in the text. Within each table, lines should separate only the headings from the body of the table, and the body of the table from any totals, averages, etc. Titles for tables should be superscribed, and explanatory matter placed in footnotes, using the symbols and sequence recommended. Number explanatory texts to graphs, figures, and tables with Roman letters or Arabic numerals. Each such text should be enclosed on a separate sheet of paper and placed in order. The author should indicate with pencil in the left-hand margin of the manuscript where the supporting material should be inserted.

Ethical Considerations. When reporting experiments on animals indicate whether the Institution's or Research Council's Guide for, or any national law on, the care and use of laboratory animals were followed. Manuscripts should describe the measures taken to minimize or eliminate pain and distress in animals during experiments and procedures. If the editors deem that animals have been subjected to suffering unjustified by the scientific value of the information sought, they will reject the paper on ethical grounds.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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