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EFFICACY OF INCREASING DOSES OF DIMINAZENE DIACETURATE OR ISOMETAMIDIUM CHLORIDE IN TREATMENT OF MICE INFECTED WITH ISOMETAMIDIUM CHLORIDE-RESISTANT *TRYPANOSOMA CONGOLENSE*

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ABSTRACT

Mice infected with *Trypanosoma congolense* resistant to isometamidium chloride were treated with increasing doses of diminazene diaceturate (DD) or isometamidium chloride (IC). Forty eight mice used for the study were randomly divided into 8 groups of 6 mice each. Groups 1, 2 and 3 were infected with *T. congolense* and treated with 7, 14 and 28 mg.kg^{-1} DD. Groups 4, 5 and 6 were infected with *T. congolense* and treated with 1, 2 and 4 mg.kg^{-1} IC, while group 7 was infected but not treated. Group 8 was the uninfected control. The parasitaemia was zero in all the groups treated with DD and there were no relapses. The parasitaemia was zero in the groups treated with IC but some relapses did occurred. The 4 mg.kg^{-1} IC treated group had the longest mean relapse interval of 16.67 ± 1.91 days when compared with 1 and 2 mg.kg^{-1} IC of 9.00 ± 0.85 and 10.00 ± 1.35 days respectively. The packed cell volume (PCV) of the infected untreated group dropped significantly ($P < 0.05$) when compared with other groups starting from day 12 post infection (PI). However, the PCV of IC treated groups further decreased significantly ($P < 0.05$) from day 32 PI following the relapse. The infection led to a significant ($P < 0.05$) loss in weight on days 8 and 16 PI in all the infected groups. However, the DD treatment reversed the weight decreasing effect of the parasite. The infection led to a significant increase in the rectal temperature on day 8 PI, but was reversed following treatment. The results showed that the IC resistant *T. congolense* was sensitive to DD at all doses tested, but expressed a high level of resistance to IC.

Key words: diminazene diaceturate; isometamidium chloride; resistance; *Trypanosoma congolense*

INTRODUCTION

African trypanosomes are protozoan parasites responsible for both animal and human trypanosomosis. They are transmitted by the bite of an infected tsetse fly. The disease is fatal if left untreated, and chemotherapy which is the major aspect of control measures in Africa is beset with problems of toxicity and increasing incidence of resistance (4, 28, 33). This makes the effectiveness of treatment and efforts to prevent the disease very difficult. Diminazene aceturate (Berenil), isometamidium chloride (Trypamidium), and homidium salt (Ethidium), cymelarsan and suramin are the drugs commonly used for the treatment of African animal trypanosomiasis. Of these drugs, diminazene aceturate is the most commonly used therapeutic agent while isometamidium chloride is most commonly used as a prophylactic agent (16). Therapeutic and prophylatic use of trypanocides has been limited by the numerous problems including toxicity and the development of resistance by the parasites (22).

The emergence of drug resistant trypanosome strains is considered a very serious problem in trypanosomosis control, particularly for the poor farmers in Africa which is the main population group at risk. Delays in the commencement of treatment, underdosing and improper and inefficient monitoring of drug efficacy in the field, have been identified as among causes of relapses and of drug resistance in trypanosomosis (10). Recent surveys in Eastern and Southern Africa (35) and in West Africa (31, 32) have shown that the prevalence of trypanosome drug resistance might be even higher than hitherto reported. There is not much hope of producing a vaccine against the disease in the near future due to antigenic variation (9). The limited availability and affordability of medicines for the treatment of trypanosomes makes the need for research, of

better ways of using available trypanocides to increase their effectiveness, more compelling.

Various strategies have already been applied to enhance the chemotherapeutic activities of trypanocides. These strategies include: change of drugs (19); combination of drugs (7); sanative pairs (46); use of higher doses and repeated treatment (43); and the adjunctive therapy (5). High dose treatments have shown prospects of eliminating infections with trypanosomes which express a high degree of resistance to the drugs (43). This method does not only increase the concentration of the drug to which the parasites are exposed, but it also prolongs the exposure time. However, the major limitation to high dose treatment is the narrow safety margin of most trypanocides with the higher doses becoming toxic (45). Diminazene is quickly excreted (27) and its therapeutic index in most animals is relatively high. Cattle are able to tolerate doses as high as 21 $mg.kg^{-1}$ body weight (bw) without exhibiting signs of systemic toxicity (18). However, diminazene aceturate is notably toxic in dogs (30).

This study was therefore designed to investigate the effectiveness of increasing (high) doses of diminazene diaceturate and isometamidium chloride in the treatment of mice experimentally infected with isometamidium chloride-resistant *Trypanosoma congolense*.

MATERIALS AND METHODS

Experimental animals

Forty-eight mice which weighed between 22 and 35 *g* were used for this study. The mice were kept in cages in a fly proof Department of Veterinary Medicine Laboratory Animal House and fed with rat chow and water *ad libitum*. They were kept for 12 days for acclimatization.

Trypanosomes

The *Trypanosoma congolense* used was an isolate obtained from National Institute for Trypanosomosis Research, Vom, Nigeria, and was maintained by serial passages in donor mice in that facility. The trypanosome was a primary isolate from a cow in the Gwarzo area of Kaduna State, Nigeria in 2009. The trypanosome has been used extensively in research and previous research has shown that the isolate was resistant to 1 $mg.kg^{-1}$ isometamidium chloride (37).

Experimental drugs

The drugs used were Trypamidium samorin®, isometamidium chloride (IC), (Merial, Lyon-France) and Dimivet® (DD) a diminazene diaceturate manufactured by SKM Pharmaceutical PVT ltd., Bangalore-560001, India.

Experimental design

Forty eight mice were randomly divided into 8 groups of 6 mice each as follows: Groups 1, 2 and 3 were infected and treated with 7 $mg.kg^{-1}$, 14 $mg.kg^{-1}$ and 28 $mg.kg^{-1}$ DD respectively. Groups 4, 5 and 6 were infected and treated with 1 $mg.kg^{-1}$, 2 $mg.kg^{-1}$ and 4 $mg.kg^{-1}$ IC, while Group 7 was infected but untreated, and Group 8 was uninfected and untreated control.

Each mouse in Groups 1–7 was infected with 1×10^6 trypanosomes in 0.2 ml of phosphate buffered solution with diluted *T. congolense* infected mice blood through the intraperitoneal route. The treatment with the DD and IC were done on day 8 post infection (PI). The parameters monitored to assess the therapeutic efficacies of the two drugs and at different doses were; packed cell volume (PCV %), weight, temperature, parasitaemia and parasite clearance time. The parameters, except parasitaemia were assessed every other 4th day, till day 28 PI, when the parameters were assessed every other 8th day. The parasitaemia was determined on day zero and then every other 2 days after treatment.

Detection of parasitaemia

The detection of parasites was done using wet mount microscopy. A blood film was made by placing a drop of blood from nipped tail end of the mouse on a clean glass slide. This was covered with a clean cover slip so that the blood spreads out evenly. The film was systematically searched for trypanosomes under X40 objective.

Determination of packed cell volume

The packed cell volume (PCV) was determined by the microhaematocrit centrifugation technique (14).

Determination of body weight and rectal temperature

The mice were weighed using the electronic weighing balance, while their rectal temperature was determined with a digital clinical thermometer. The temperature was recorded in degree Celsius.

Statistical analysis

The means of all the parameters were compared by one-way analysis of variance (ANOVA) and variant means were separated by Duncan's multiple range test. Probability values less than or equal to 0.05 ($P \leq 0.05$) were considered significant (41).

RESULTS

The *Trypanosoma congolense* infection in mice was established 4 days PI. Treatment with diminazene diaceturate (DD) at 14 $mg.kg^{-1}$ bw was able to clear the parasitaemia within 30 hrs post treatment; DD at 28 $mg.kg^{-1}$ bw cleared parasitaemia within 24 hrs; while DD at 7 mg and isometamedium chloride (IC) at 1, 2 and 4 $mg.kg^{-1}$ cleared the parasites within 48 hrs post treatment. Treatment with DD at all doses led to complete clearing of the parasites while IC treated groups relapsed (Table 1). The 4 $mg.kg^{-1}$ IC treated group had the longest mean relapse interval of 16.67 ± 1.91 days, while 1 $mg.kg^{-1}$ and 2 $mg.kg^{-1}$ gave relapses interval of 9.00 ± 0.85 and 10.00 ± 1.35 days, respectively.

The infection did not cause a significant ($P > 0.05$) decrease in the packed cell volume (PCV) of the infected groups when compared with the uninfected untreated group by day 8 PI (Table 2). However, the PCV of infected untreated group dropped significantly ($P < 0.05$) when compared with other groups by day 12 through day 16 PI. The PCV of the IC treated groups decreased significantly ($P < 0.05$) from day 28 PI following relapse, whereas the DD treated groups compared favourably with the uninfected untreated group.

The infection led to a significant increase in the rectal temperature on day 8 PI (Table 3) in the infected groups. The temperature however, dropped on day 12 PI following treatment on day 8 PI. Following a relapse, the temperature of

Table 1. The parasitaemia of mice groups infected with isometamidium chloride resistant Trypanosoma congolense and treated with different doses of isometamidiun chloride (IC) or diminazene aceturate (DD)

Dose [mg.kg^{-1}]	0	4	8	10	12	14	Exp 16	days 18	20	22	24	26	28	36	44	52	60	68
DD7	0/6	5/6	6/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
DD14	0/6	6/6	6/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
DD28	0/6	6/6	6/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
IC1	0/6	4/6	6/6	0/6	0/6	1/6	3/6	4/4	4/4	1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
IC 2	0/6	5/6	6/6	0/6	0/6	1/6	5/6	3/3	3/3	2/2	2/2	2/2	2/2	2/2	0/0	0/0	0/0	0/0
IC 4	0/6	6/6	6/6	0/6	0/6	0/6	1/6	2/5	2/5	3/4	4/4	4/4	2/2	2/2	2/2	2/2	2/2	0/0
INF	0/6	6/6	6/6	6/6	6/6	3/3	3/3	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

INF – Infected

Table 2. Mean packed cell volume (PCV %) ± SE of isometamidium chloride resistant *Trypanosoma congolense* infected mice treated with different doses of diminazene diaceturate (DD) or isometamidium chloride (IC)

Exp days	7 mg.kg^{-1} D D	14 mg.kg^{-1} D D	28 mg.kg^{-1} D D	1 mg.kg^{-1} I C	2 mg.kg^{-1} I C	4 mg.kg^{-1} I C	Infected untreated	Uninfected control
0	40.17 ± 1.85	39.83 ± 2.76	40.83 ± 2.77	39.33 ± 2.60	41.33 ± 2.06	41.50 ± 1.76	39.83 ± 1.01	39.67 ± 1.67
4	39.83 ± 2.41	41.00 ± 1.79	40.50 ± 2.91	41.67 ± 2.50	42.17 ± 2.99	39.67 ± 1.84	40.20 ± 1.93	41.50 ± 0.56
8	38.99 ± 0.95	37.42 ± 1.38	36.98 ± 1.48	39.92 ± 1.86	38.56 ± 1.45	39.04 ± 2.90	37.95 ± 1.20	39.99 ± 1.67
12	39.17 ± 1.93^{a}	42.50 ± 1.09^{a}	43.00 ± 2.04^{a}	41.33 ± 1.39^{a}	41.00 ± 1.24^{a}	41.63 ± 2.00^{a}	35.67 ± 1.26^{b}	41.67 ± 1.88^{a}
16	42.67 ± 2.20^{a}	42.00 ± 1.39^{a}	43.67 ± 1.99^{a}	39.00 ± 1.09^{a}	37.17 ± 0.54^{a}	40.60 ± 2.32^{a}	32.00 ± 2.00^{b}	43.50 ± 1.67^{a}
20	40.09 ± 1.46	41.83 ± 1.08	42.00 ± 1.61	39.00 ± 1.45	38.02 ± 0.58	38.60 ± 1.98		41.17 ± 1.40
24	41.33 ± 1.50	42.50 ± 0.76	41.67 ± 1.38	36.11 ± 1.25	37.50 ± 1.50	37.00 ± 2.25		42.08 ± 1.48
28	45.17 ± 1.54^{a}	44.12 ± 1.99^{a}	42.59 ± 1.85^{a}	29.50 ± 2.50^{b}	37.50 ± 1.50^{b}	34.67 ± 2.60^{b}		43.17 ± 1.70^{a}
36	42.33 ± 1.67^{a}	41.83 ± 1.56^{a}	42.67 ± 1.02^{a}		32.50 ± 1.55^{b}	34.08 ± 2.00^{b}		42.03 ± 1.61^{a}
44	40.67 ± 1.89^{a}	42.23 ± 1.28^{a}	41.50 ± 1.76^{a}		31.50 ± 1.08^{b}	33.50 ± 2.50^{b}		41.83 ± 1.48^{a}
52	42.00 ± 1.61^{a}	41.17 ± 1.22^{a}	42.99 ± 0.92^{a}		31.00 ± 1.53^{b}	32.50 ± 1.50^{b}		43.21 ± 1.48^{a}
60	41.59 ± 1.52^{a}	42.00 ± 1.24^{a}	40.28 ± 1.08^{a}		28.03 ± 1.62^{b}	31.50 ± 1.50^{b}		42.12 ± 1.71^{a}
68	40.82 ± 1.26^{a}	44.33 ± 1.33^{a}	42.34 ± 1.19^{a}			29.74 ± 1.50^{b}		40.23 ± 1.60^{a}

^{abc} – Different letters in superscript in a row indicate significant difference between the means at the level of $P < 0.05$

Table 3. Mean temperature (°C) ± SE of of isometamidium chloride resis ant *Trypanosoma congolense* infected mice treated with different doses of diminazene diaceturate (DD) or isometamidium chloride (IC)

Exp days	7 mg.kg^{-1} D D	14 mg.kg^{-1} D D	28 mg.kg^{-1} D D	1 mg.kg^{-1} I C	2 mg.kg^{-1} I C	4 mg.kg^{-1} I C	Infected untreated	Uninfected control
0	36.37 ± 0.20	37.38 ± 0.34	36.73 ± 0.43	37.22 ± 0.50	37.65 ± 0.27	37.03 ± 0.34	37.53±0.38	36.85±0.39
4	37.38 ± 0.39	37.45 ± 0.31	38.48 ± 0.14	37.78 ± 0.24	37.65 ± 0.33	37.83 ± 0.23	37.32 ± 0.26	36.67 ± 0.19
8	37.20 ± 0.23^{a}	36.87 ± 0.53^{a}	38.42 ± 0.29^{b}	38.90 ± 0.55^{b}	37.48 ± 0.31^{b}	37.88 ± 0.34^{b}	38.42 ± 0.67^{b}	36.01 ± 0.40^{a}
12	36.65 ± 0.34	37.08 ± 0.38	36.45 ± 0.26	37.08 ± 0.14	37.05 ± 0.29	37.36 ± 0.32	37.64 ± 1.20	36.07 ± 0.38
16	36.97 ± 0.23	37.03 ± 0.19	36.85 ± 0.24	37.32 ± 0.31	37.53 ± 0.24	37.58 ± 0.21	38.54 ± 0.20	36.87 ± 0.22
20	37.02 ± 0.32	36.77 ± 0.36	36.52 ± 0.26	36.98 ± 0.18	37.90 ± 0.30	37.09 ± 0.14		36.93 ± 0.29
24	37.00 ± 0.09	37.12 ± 0.14	37.00 ± 0.16	37.90 ± 0.13	37.50 ± 0.17	37.60 ± 0.20		36.78 ± 0.08
28	36.48 ± 0.18	37.00 ± 0.14	37.10 ± 0.16		37.90 ± 0.20	38.10 ± 0.22		36.85 ± 0.18
36	37.05 ± 0.12	37.10 ± 0.14	37.00 ± 0.18		37.95 ± 0.30	37.25 ± 0.27		36.85 ± 0.16
44	36.95 ± 0.16	36.90 ± 0.12	36.81 ± 0.17		37.50 ± 0.18	37.90 ± 0.20		36.72 ± 0.18
52	36.93 ± 0.09	36.90 ± 0.17	36.88 ± 0.14		37.85 ± 0.10	38.15 ± 0.15		36.62 ± 0.15
60	36.83 ± 0.12	36.98 ± 0.10	36.95 ± 0.16		37.42 ± 0.21	37.90 ± 0.24		36.92 ± 0.13
68	36.47 ± 0.16	36.48 ± 0.17	36.82 ± 0.19			37.64 ± 0.21		36.52 ± 0.16

^{abc} – Different letters in superscript in a row indicate significant difference between the means at the level of $P < 0.05$

Table 4. Mean body weight (g) ± SE of isometamidium chloride resistant *Trypanosoma congolense* infected mice treated with different doses of diminazene diaceturate (DD) or isometamidium chloride (IC)

Exp days	7 mg.kg^{-1} DD	14 mg.kg^{-1} DD	28 mg.kg^{-1} DD	1 mg.kg^{-1} IC	2 mg.kg^{-1} IC	4 mg.kg^{-1} IC	Infected Untreated	Uninfected Control
0	26.00 ± 1.26	27.65 ± 1.10	31.82 ± 0.82	29.87 ± 2.76	31.65 ± 1.99	29.25 ± 0.64	28.23 ± 2.23	29.10 ± 2.39
4	27.15 ± 1.07	28.52 ± 1.12	31.51 ± 1.28	28.99 ± 2.09	29.73 ± 2.06	29.23 ± 1.76	27.42 ± 1.76	29.48 ± 2.36
8	26.47 ± 1.24	25.98 ± 0.98	30.95 ± 2.44	27.18 ± 2.22	29.60 ± 1.30	29.00 ± 0.97	27.57 ± 2.87	30.47 ± 2.37
12	26.00 ± 1.09	26.08 ± 1.03	31.70 ± 1.34	25.43 ± 1.49	28.72 ± 2.02	29.43 ± 1.45	22.05 ± 2.05	31.18 ± 2.41
16	26.83 ± 1.98^{ab}	26.70 ± 1.64^{ab}	31.57 ± 1.21^{a}	26.04 ± 1.57^{b}	27.43 ± 1.03^{ab}	30.52 ± 1.66^{a}	21.48 ± 0.98^{c}	31.27 ± 2.31^{a}
20	26.78 ± 1.10	27.10 ± 0.65	32.57 ± 0.80	26.28 ± 1.68	28.70 ± 0.10	29.73 ± 1.25		31.47 ± 2.32
24	26.92 ± 1.09	28.38 ± 0.65	32.60 ± 0.80	25.98 ± 0.97	28.40 ± 1.20	29.73 ± 1.25		31.48 ± 2.32
28	26.80 ± 1.03	28.28 ± 0.60	32.52 ± 0.85		27.95 ± 1.09	28.92 ± 1.18		31.27 ± 2.27
36	26.93 ± 1.03	28.57 ± 0.76	32.28 ± 0.67		27.75 ± 1.15	27.38 ± 1.45		32.08 ± 2.28
44	27.10 ± 1.04	28.62 ± 0.69	32.77 ± 0.79		27.05 ± 1.15	26.93 ± 1.45		32.52 ± 2.01
52	27.22 ± 1.14	28.70 ± 1.68	32.77 ± 0.76		26.42 ± 1.00	26.54 ± 1.35		32.59 ± 1.34
60	27.32 ± 1.02	28.57 ± 0.67	32.63 ± 0.78			24.48 ± 1.30		32.36 ± 1.46
68	27.43 ± 0.86	28.99 ± 1.06	32.45 ± 2.01					31.78 ± 1.92

^{abc} – Different letters in superscript in a row indicate significant difference between the means at the level of $P < 0.05$

IC treated groups increased significantly ($P < 0.05$) from day 44 PI, when compared with DD treated groups and uninfected untreated. There was no significant difference between the IC treated groups and the infected untreated group.

The infection led to a significant ($P < 0.05$) loss of weight on days 12 and 16 PI in the infected untreated group when compared with other groups. The weight of the treated groups did not vary significantly ($P > 0.05$) when compared with the infected untreated group.

DISCUSSION

The *Trypanosoma congolense* infection of mice was established by day 4 post infection (PI) in all infected groups. This is in agreement with the findings of Swell and Brocklessby (40) and Ezeokonkwo *et al.* (17). The trypanosome used in this study developed resistance to isometamidium chloride after prolonged use in experimental procedures which exposed it to IC, is in agreement with reports that widespread use of trypanocides, especially diminazene aceturate (diminazene) and isometamidium chloride (isometamidium), acts as a selection pressure for the development of resistant trypanosomes (6).

The treatment with diminazene diaceturate at all doses was able to clear parasitaemia without relapse, whereas the isometamidium chloride treated groups relapsed. Relapse after treatment has been reported by earlier workers (2, 3, 20). Relapse is usually considered to be due to resistance to the drug under test at the dose rate employed and also due to migration of the parasite into the brain tissue where some drugs cannot reach them (8, 21, 26). Relapse may also be due to inability of the hosts' immune response to clear the trypanosomes from the body even after administration of trypanocides (39). The $4\,mg.kg^{-1}$ IC treated group had the longest mean relapse interval when compared with 1 and $2\,mg.kg^{-1}$ body weight dosage. This agrees with reports that higher dose treatments offer the best opportunity for eliminating infections with trypanosomes, which express a high degree of resistance to drugs (4).

The infection led to a significant loss of weight which is consistent with the findings by other researchers (11, 36, 42). The weight loss could be attributed to the fact that trypanosomosis cause anorexia and general systemic disturbance; and for the fact that trypanosome-infected animals lose body fat (25, 42) due to lipolysis. The losses were however, reversed by trypanocidal treatment. The ability of diminazene treated groups to maintain steady weight gain until the end of the experiment could be due to the drug being able to clear the parasite without relapse. This was not so with the isometamidium treated group where the relapses occurred. Also the infection led to an increase in the mean rectal temperature of the infected mice. Fever in trypanosomosis has been attributed to the activity of the trypanosomes in the host (42).

The low PCV observed in the infected untreated group may be as a result of acute haemolysis due to the growing infection. Previous studies have shown that infection with trypanosomes resulted in an increased susceptibility of red blood cell membrane to oxidative damage, probably as a result of the depletion of reduced glutathione on the surface of the red blood cell (1, 23, 24, 44). The results suggest that administered drugs improved blood components, possibly, by depletion of proliferating parasites. The severity of the anaemia usually reflects the intensity and duration of proliferating parasites. The anaemia in the infected mice treated with isometamidium was less severe than the one recorded for the untreated infected mice. This may be partly due to the trypanocide clearing/lowering parasitaemia in the groups. Manifestations of trypanosomosis have been positively correlated with the onset and level of parasitaemia (15).

Experimental studies have demonstrated that the occurrence of resistance of trypanosomes to both diminazene and to isometamidium chloride at the same time is rare (4, 38). Thus, these two compounds have been termed a sanative pair (47), since in instances of resistance to one drug application, the other compound usually controls the disease. Nevertheless, recent reports on multiple drug resistance suggest that the concept of sanative pairs might no longer always be valid (12, 13, 34). The resistance of trypanosomes to drugs develop through: (i) under dosing, which may occur for a variety of reasons such as underestimation of animal body weight, over diluting the solutions or incorrect calculation of dose volume; (ii) incorrect (and therefore ineffective) injection or; (iii) an incorrect strategy of drug use (29). Although drug resistance reduces the efficiency of trypanocides, there is significant evidence from the field that the continuing use of trypanocides (to a limited extent and in combination with other control measures) might still provide beneficial effects to cattle.

From the results, the isolate expressed high levels of resistance to all doses of isometamidium chloride used, but was very sensitive to diminazene diaceturate. This is in support of the principle of sanative pairs and its applicability in the field may still be useful in treating drug resistant trypanosomes.

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SPERM OUTPUT AND SPERMIOGRAM OF DOMESTIC CHICKENS (*GALLUS GALLUS DOMESTICUS*) FOLLOWING SHORT TERM TREATMENT WITH HYALURONAN

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ABSTRACT

The sperm output and spermiogram of the normal feathered strain of 28-week old Nigerian indigenous chickens with a mean weight of 1 400 ± 200 *g* were evaluated following short term injections with hyaluronan (HA). Each rooster was first treated with 10 ml of normal saline (NST) intraperitoneally (i. p.) daily for 10 days. One week afterward, HA was administered daily to each of the roosters (HAT) at a dosage of 0.25 mg.kg^{-1} intraperitoneally (i. p.), for 10 days. The parameters measured were: semen volume; percentage of sperm motility; sperm concentration; sperm viability; and percentage of sperm abnormalities.

There was no significant difference (P > 0.05) in the semen volume of NST (0.37 ± 0.06 ml) and HAT (0.26 ± 0.04 ml) rooster chickens. Also, there was no significant difference in the sperm concentration and sperm viability of NST (9.38 × 10^9 ± 306.4 sperm.ml^{-1}; 91.38 ± 3.5 %) and HAT (1.01 × 10^9 ± 230.2 sperm.ml^{-1}; 94.75 ± 2.2 %) rooster chickens, respectively. The percentage of sperm motility (rapidly progressive motility) was significantly (P < 0.05) higher in HAT (91.88 ± 11.26 %) than NST (61.88 ± 3.53 %) roosters. In addition, the percentage of sperm abnormalities was significantly (P < 0.05) lower in HAT (0.03 ± 0.01 %) than in NST (0.08 ± 0.02 %) roosters. It was concluded that short term intra-peritoneal injection of chickens with hyaluronan improved sperm motility and sperm morphology.

Key words: hyaluronan; roosters; sperm abnormalities; spermiogram

INTRODUCTION

Artificial insemination (A. I.) has the potential of increasing productivity, to enhance biosecurity and aid the genetic management of animals for either agricultural or conservation purposes (12). It has become an important production method for the poultry industry. The primary limitation to artificial insemination appears to be the inability to consistently collect semen that is of sufficiently high quality to result in fertility. This is because there is a remarkable variation in ejaculate volume, sperm concentration and the quality of sperm among avian species (2). Thus, an assessment of the semen quality characteristics of poultry birds, gives an excellent indication of their reproductive potential and has been reported to be a major determinant of fertility and the subsequent hatchability of eggs (14).

For several decades, natural or synthetic hormones have been used to improve the reproductive potentials of animals. In the reproductive management of farm animals, human menopausal gonadotropin (Pergonal®) is often used in superovulatory protocols (9). Pergonal® is a lyophilized gonadotropin preparation that consists of follicle stimulating hormone and luteinizing hormone in ratio of 1 : 1 (4). The use of Pergonal® was reported to be effective in improving semen quality of local cocks (1) and did not have any deleterious effects on their haematological and serum biochemical parameters. However, toxicological and safety studies were not conducted (8).

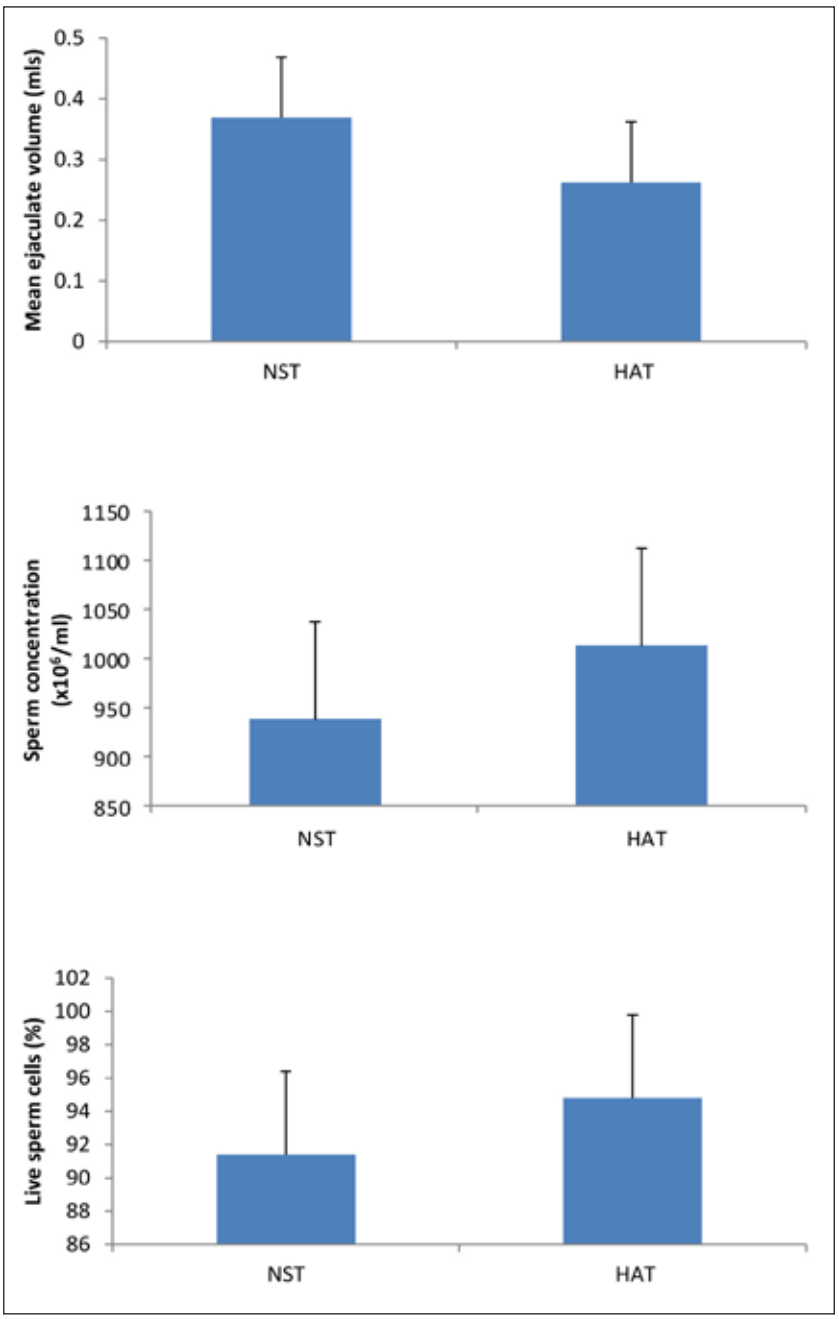

Fig. 1. Mean ejaculate volume, sperm concentration and percentage live sperm cells normal saline treated (NST) and hyaluronan treated (HAT) roosters

Glycosaminoglycans (GAGs) are essential components of the extracellular matrix, contributing to the cell recognition, cellular adhesion and growth regulation. Important aspects of sperm functions such as, motility and capacitation (6) appear to be mediated, at least partially, through hyaluronan (HA). The addition of HA to fresh (and frozen-thawed) sperms improves their motility (16). Interestingly, HA did not only improve sperm motility after freezing and thawing procedures, but it also stabilized the spermatozoa with already declining motility in frozen/thawed sperms (17). Up to now, there is a dearth of information on the *in vivo* effects of HA on sperm quality. We hypothesize that intra-peritoneal injection of hyaluronan will improve sperm output and the quality of the chicken. The aim of this study therefore, was to evaluate the effect of HA on the sperm output and spermiogram of domestic chickens.

MATERIAL AND METHODS

Twelve 28-week old intentionally bred roosters of the normal feathered strain of Nigerian indigenous chicken with a mean weight 1 400 *g* ± 200 *g* were used. The roosters were housed individually in a standard battery cage. They were fed with commercial grower mash (Growfast®, Animal care, Nigeria) at 125 *g* of feed per day per bird and water *ad libitum*. The roosters were preconditioned by deworm-

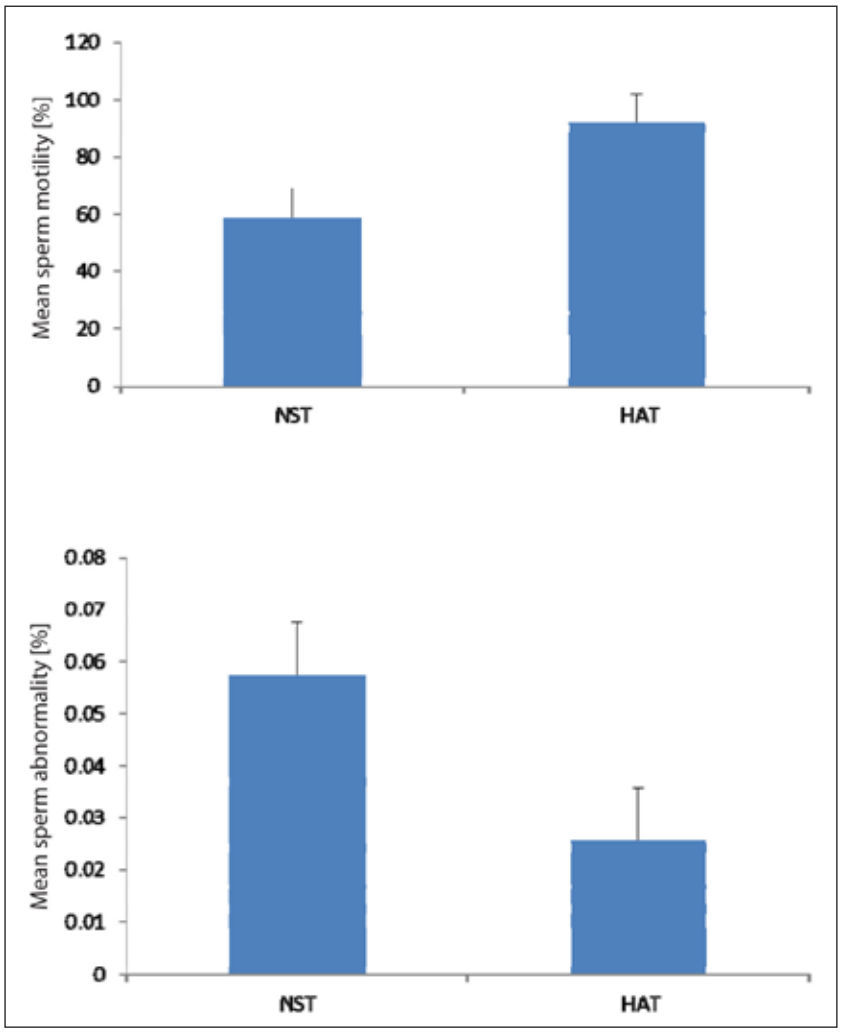

Fig. 2. Mean percentage sperm motility and mean percentage sperm abnormalities of normal saline treated (NST) and hyaluronan treated (HAT) roosters

ing with Ivermectin (Concimectin®, Animal care, Nigeria) at a dosage of 0.25 mg.kg^{-1} *per os* and vaccinated against Newcastle disease with Lasota (Indovax private limited, India) and were all assessed to be in good general health prior to the study. The roosters were also trained for four weeks with the abdominal massage methods of semen collection as described by Burrows and Quinn (3). Eight out of the twelve cocks were selected based on a positive reaction to the dorso-abdominal massage method of semen collection, resulting in semen ejaculation.

The protocol for the study was approved by the Poultry Management Committee of the University of Agriculture, Abeokuta. A cross over design was used for this study, in which a sequence of treatments was given to the subjects one after the other in a specific order. The roosters were first treated with 10 ml of normal saline which was administered intraperitoneally (i. p.) for 10 days to each of the roosters. Immediately after treatment, semen was collected from each rooster and was allowed to recover for one week. Thereafter, HA was administered to each of the roosters at a dosage of 0.25 mg.kg^{-1} intraperitoneally (i. p.) for 10 days after which semen was again collected from each rooster. The parameters measured were semen volume, percentage of sperm motility, sperm concentration, percentage of live sperm and percentage of sperm abnormalities after each of the treatment.

Immediately after collection, semen volume, sperm motility, percentage of live sperm cells, sperm concentration and percentage of sperm cell abnormalities were evaluated for each sample collected. The Live-Dead ratio of the semen was evaluated using Eosin-Nigrosin staining. In each case, a hundred spermatozoa per slide (using oil immersion magnification) were counted. Sperm concentration was measured using a haemocytometer (Neuber counting chamber), and the motility was estimated by microscopic observation of individual sperm in slightly diluted semen. The ejaculate volume was measured using a graduated ependuff tube. The determination of sperm cell abnormalities was carried out by staining with Giemsa stain and phosphate buffer saline in a ratio of 1 ml to 3 ml respectively. A thin smear was made from each of the ejaculate collected and was immediately fixed with methanol; it was thereafter stained with the prepared Giemsa Phosphate buffer saline (PBS) for 30 minutes after which it was rinsed off with PBS and allowed to dry and viewed using a light microscope (× 100); a

minimum of 200 cells were counted and were classified as head abnormality, mid-piece abnormality or tail abnormality.

Data obtained from this study were expressed as the mean ± standard deviation. The semen volume, percentage of sperm motility, percentage of sperm abnormalities and percentage of live sperm were compared between treatments using the paired *T*-test. The sperm concentration was compared using the Wilcoxon sign rank test. The P value <0.05 was considered significant in all cases.

RESULTS

There was no significant difference ($P > 0.05$) between the mean ejaculate volume, mean sperm concentration and percentage of live sperm of NST and HAT rooster chickens, although the mean sperm concentration was higher in HAT compared to NST rooster chickens (Fig. 1). However, the percentage of sperm motility (rapidly progressive motility) was significantly ($P<0.05$) higher in HAT than NST chicken roosters (Fig. 2). In addition, the percentage of sperm abnormalities was significantly ($P<0.05$) lower in HAT than in NST roosters (Fig. 2).

DISCUSSION

The result of this study showed that the short term intra-peritoneal injection of domestic chickens with HA significantly improved the sperm motility and morphology. HA has been localized in female reproductive tract of rats (10) and humans (5) and detected in the cervical mucus, uterine and oviductal secretions of ruminants (11). Important aspect of sperm functions such as motility (6) and capacitation have been shown to be mediated partially through HA.

The short term intra-peritoneal injection of HA improved sperm motility and also reduced the number of sperm abnormalities. Although, there was an increase in the mean semen volume, sperm concentration and sperm viability following treatment with HA, the difference was not significant. This seems to be in line with the result of the *in vitro* use of HA in sperm preservation in which HA improved post-thawed sperm motility and viability as reported by Mitra *et al.* (13). In this study, HA did not have a significant effect on viability which may be a dose dependent effect, since improved sperm viability as earlier reported was at a concentration of $750\,\mu g.ml^{-1}$ of HA *in vitro*.

The mechanism behind a HA induced increase in sperm motility remains to be determined. HA-receptor has been localized along the tail, mid-piece and head of human spermatozoa (15); blocking this receptor with non specific antibodies resulted in the inhibition of sperm motility. It may be that similar receptors are also present in the spermatozoa of domestic chickens, which increase the adenosine triphosphate (ATP) levels and thus may improve flagellar function. Moreover, enhanced phosphorylation of 34 kDa HA-binding protein (HABP) has been observed in HA supplementation, suggesting an important role of this glycosaminoglycan in initiating the signal transduction controlling the important process of sperm physiology such as sperm motility. In the present study, *in vivo* injection of HA also improved sperm morphology. This may be in line with the findings of Huszar *et al.* (7), who suggested the role of 70 KDa heat shock-related protein (HSP70.2) in elongating spermatids and events of late spermiogenesis, such as cytoplasmic extrusion and plasma membrane remodeling.

CONCLUSIONS

In conclusion, we suggest that intra-peritoneal injection of HA in domestic chickens could be an applicable approach to improve sperm motility of domestic chickens, especially aged toms that have been reported to have declining sperm motilities as well as in other avian species. However, more research work should still be carried out to determine the most appropriate dosage and to determine what the outcome of the long term administration would be.

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METABOLIC CHANGES AFTER *SALMONELLA* INFECTION AND *ENTEROCOCCUS FAECIUM* EF55 APPLICATION IN CHICKENS

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ABSTRACT

The effects of *Enterococcus faecium* EF55 and *Salmonella enterica* serovar Enteritidis phage type 4 (SEPT4) on the biochemical parameters and the weight of the organs were studied in chickens. Experiments were carried out on chickens of the hybrid ISA BROWN which were divided into four treatment groups (C – control, SE – *Salmonella Enteritidis* PT4, EF – *Enterococcus faecium* EF55, EFSE – *S. Enteritidis* PT4 + *E. Faecium* EF55) which lasted for 22 days. For analysis, the blood and internal organs were collected on days 8 and 22. The biochemical parameters were determined spectrophotometrically and the organs were weighed on laboratory scales. The *Salmonella* infections and probiotic cultures affected significantly ($P < 0.05$) the concentration of the total bilirubin (decrease in groups SE, EF, EFSE *vs.* C in 8-day old chickens; increase in groups SE, EFSE *vs.* C and in SE *vs.* EF in 22-day old chickens), glucose (decrease in groups SE, EFSE *vs.* C and in EFSE *vs.* EF in 8-day old chickens; decrease in EF *vs.* SE, increase in groups SE, EFSE *vs.* C and in EFSE *vs.* EF in 22-day old chickens), enzymatic activity of alanine aminotransferase (decrease in groups EF, EFSE *vs.* SE and increase in SE *vs.* C in 8-day old chickens; decrease in EF *vs.* SE, increase in group SE *vs.* C and in EFSE *vs.* EF in 22-day old chickens) and relative weight of the pancreas (decrease in EF group *vs.* SE, increase in groups SE, EFSE *vs.* C and EFSE *vs.* EF in 8 day-old chickens) and of the spleen (decrease in EF *vs.* SE, increase in groups SE, EFSE *vs.* C and in EFSE *vs.* EF in 22 day-old chickens). Our results demonstrated the appropriateness of the use of probiotics to improve digestive processes and metabolism in chickens.

Key words: biochemical parameters; chicken; *Enterococcus faecium; Salmonella Enteritidis*

INTRODUCTION

Salmonella enterica serovar Enteritidis causes gastroenteritis in humans and animals worldwide. *Salmonella* colonies are localized mainly in the crop and caecum, entering epithelial cells, where they can survive, reproduce and actively penetrate the intestinal mucosa to reach the mesenteric lymph nodes, lymph and blood circulation to the organs (especially the spleen and liver). Chickens are the most susceptible to the colonisation by pathogens during the early period, when a stable gut microflora has not yet been established. The stabilised gut microflora provides broiler chickens with protection against these undesirable organisms (2).

Probiotics optimize the gut microbiota of chickens and other animals, and thus create an effective barrier against pathogens. Levkut *et al*. (3) confirmed the inhibitory effects of the EF55 strain against *Salmonella enterica* serovar Enteritidis type 4 (SEPT4) in chickens by the significant reduction in the number of strain PT4 in the liver, spleen, caecum and faeces.

The aim of our study was to investigate the effects of bacteriocin-producing probiotic strains, *E. faecium* EF55 and *Salmonella* infection (strain PT4) on the biochemical parameters and weight of organs in chickens.

MATERIALS AND METHODS

Forty, one-day old chickens of the ISA BROWN hybrid were included in the experiments. They were divided into four groups of 10 chicks in each (C – control, SE – *Salmonella Enteritidis*, EF – *Enterococcus faecium* EF55 and EFSE – *E. faecium* and *S. Enteritidis*). The chickens were kept on wood shavings litter which was

changed daily, and they were provided an area of one m^2/group. The temperature was maintained according to age (32 °C first week with a gradual reduction of 2 °C weekly). Water and feed HYD-04/a were given *ad libitum*. The EF55 was applied at a dose of 10^9 CFU.ml^{-1}, 3 *g* per group and SEPT4 at a dose of 10^8 CFU per 0.2 ml PBS once, *per os*. The experiment lasted for 22 days.

The blood (on days 8 and 22) was collected from the wing vein and processed. We conducted two samplings (days 8 and 22) of the internal organs, such as the liver, spleen, pancreas and gizzard. The blood and organs were collected from five chickens (n = 5) from each group. The internal organs were weighed on laboratory scales (KERN EG, Kern & Sohn GmbH, Germany). The concentration of glucose was determined using a commercial glucose oxidase-peroxidase assay kit (GLU GOD 250, Lachema a. s., Czech Republic), total bilirubin by assay kit (T BIL, RANDOX Laboratories Ltd., United Kingdom), calcium by assay kit (Ca 100 KX, Biolatest, Pliva – Lachema Diagnostika s. r. o., Czech Republic) and enzymatic activity of alkaline phosphatase (ALP) using an assay kit (ALP 2 × 60, Biolatest, Pliva – Lachema Diagnostika s. r. o., Czech Republic), aspartate aminotransferase (AST) using an assay kit (AS 147, RANDOX Laboratories Ltd., United Kingdom), alanine aminotransferase (ALT) using an assay kit (AL 146, RANDOX Laboratories Ltd., United Kingdom).

The experiments were approved by the University of Veterinary Medicine and Pharmacy Ethical Committee, pursuant to Government Code No. 289/2003Vypočuť.

The statistical analysis of the results were performed using a one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparison test. Bacteria counts were evaluated by *t*-test. The differences between the mean values for the groups of chickens were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Results of our investigations of the effects of bacteriocin-producing probiotic strain *E. faecium* EF55 and *Salmonella* infection (strain PT4) on the biochemical parameters and weight of organs in chickens are summarised in Tables 1 to 5.

Table 1. Effect of SEPT4 and EF55 on plasma biochemical parameters in 8-day old chickens

Parameters	C	SE	EF	EFSE
Total bilirubin [µmol.l^{-1}]	5.168 ± 0.365^{abcd}	2.475 ± 0.28^{ac}	2.970 ± 0.571^{d}	3.590 ± 0.236^{b}
ALP [µkat.l^{-1}]	19.33 ± 12.71^{abc}	14.98 ± 6.77^{a}	16.78 ± 8.267^{b}	10.87 ± 16.78^{c}
AST [µkat.l^{-1}]	3.156 ± 0.173	3.775 ± 0.165	3.120 ± 0.05	3.01 ± 0.04
ALT ([µkat.l^{-1}]	0.29 ± 0.004^{a}	0.53 ± 0.004^{abc}	0.212 ± 0.003^{b}	0.25 ± 0.002^{c}
Glucose [mmol.l^{-1}]	17.26 ± 0.671^{ab}	11.14 ± 1.135^{a}	16.33 ± 0.483^{c}	12.61 ± 0.57^{bc}
Calcium [mmol.l^{-1}]	2.086 ± 0.123	2.07 ± 0.02	1.97 ± 0.085	2.34 ± 0.03

Significant differences within a row are marked with the same superscript letter; $P < 0.05$; mean ± SD; n = 5; C – control; SE – *S. Enteritidis* (SEPT4); EF – *E. faecium* (EF55); EFSE – *E. faecium* + *S. Enteritidis* (EF55 + SEPT4); ALP – alkaline phosphatase; AST – aspartate aminotransferase; ALT – alanine aminotransferase

Table 2. Effect of SEPT4 and EF55 on plasma biochemical parameters in 22-day old chickens

Parameter	C	SE	EF	EFSE
Total bilirubin [µmol.l^{-1}]	1.975 ± 0.005^{ab}	6.450 ± 0.495^{ac}	1.983 ± 0.003^{c}	3.718 ± 0.428^{b}
ALP [µkat.l^{-1}]	4.848 ± 0.260	6.556 ± 0.630	5.297 ± 0.164	4.725 ± 0.172
AST [µkat.l^{-1}]	3.032 ± 0.091	2.666 ± 0.260	3.052 ± 0.121	2.236 ± 0.191
ALT [µkat.l^{-1}]	0.395 ± 0.005^{a}	0.660 ± 0.005^{ab}	0.260 ± 0.003^{bc}	0.546 ± 0.004^{c}
Glucose [mmol.l^{-1}]	11.93 ± 0.317^{ad}	18.96 ± 0.219^{ab}	10.06 ± 0.363^{bc}	18.56 ± 0.552^{cd}
Calcium [mmol.l^{-1}]	2.136 ± 0.09	2.260 ± 0.115	2.122 ± 0.092	2.378 ± 0.091

Significant differences within a row are marked with the same superscript letter; $P < 0.05$; mean ± SD; n = 5; C – control; SE – *S. Enteritidis* (SEPT4); EF v *E. faecium* (EF55); EFSE – *E. faecium* + *S. Enteritidis* (EF55 + SEPT4); ALP – alkaline phosphatase; AST – aspartate aminotransferase; ALT – alanine aminotransferase

Table 3. *Salmonella* counts expressed in $\log_{10}$ CFU.g^{-1} after administration of SEPT4 and EF55 in the liver, spleen, and caecum of chickens on days 8 and 22

	Liver Day 8	Liver Day 22	Spleen Day 8	Spleen Day 22	Caecum Day 8	Caecum Day 22
SE	2.74^{b} ± 0.55	1.79^{ab} ± 0.47	2.92^{d} ± 0.47	1.50^{d} ± 0.44	2.24^{f} ± 0.78	1.13^{f} ± 0.79
EFSE	2.22^{c} ± 0.78	0.81^{ac} ± 0.76	2.24 ± 0.99	1.12 ± 0.75	1.94^{e} ± 0.39	0.76^{e} ± 0.70

Significant differences within a row are marked with the same superscript letter; $P < 0.05$; mean ± SD; n = 5; SE – *S. Enteritidis* (SEPT4); EFSE – *E. faecium* + *S. Enteritidis* (EF55 + SEPT4)

Table 4. Effect of SEPT4 and EF55 on the relative weight (%/ body weight) of liver, pancreas, spleen and gizzard in 8-day old chickens

	C	SE	EF	EFSE
Liver	3.310 ± 0.171	3.778 ± 0.96	3.298 ± 0.137	3.090 ± 0.245
Pancreas	0.350 ± 0.039^{ab}	0.708 ± 0.57^{ac}	0.394 ± 0.046 ^{cd}	0.636 ± 0.042 ^{bd}
Spleen	0.148 ± 0.003	0.156 ± 0.04	0.184 ± 0.027	0.176 ± 0.018
Gizzard	4.658 ± 0.191	3.40 ± 0.125	4.484 ± 0.262	3.370 ± 0.652

Significant differences within a row are marked with the same superscript letter; P < 0.05; mean ± SD; n = 5; C – control; SE – *S. Enteritidis* (SEPT4); EF – *E. faecium* (EF55); EFSE – *E. faecium* + *S. Enteritidis* (EF55 + SEPT4)

Table 5. Effect of SEPT4 and EF 55 on the relative weight (% /body weight) of liver, pancreas, spleen and gizzard in 22 day old chickens

	C	SE	EF	EFSE
Liver	2.706 ± 0.140	2.976 ± 0.143	2.524 ± 0.063	2.910 ± 0.053
Pancreas	0.3180 ± 0.019	0.498 ± 0.033	0.360 ± 0.024	0.534 ± 0.068
Spleen	0.114 ± 0.012^{ab}	0.244 ± 0.022^{ac}	0.102 ± 0.021^{cd}	0.198 ± 0.019^{bd}
Gizzard	3.426 ± 0.188	3.642 ± 0.288	3.64 ± 0.162	2.600 ± 0.401

Significant differences within a row are marked with the same superscript letter; P < 0.05; mean ± SD; n = 5; C – control; SE – *S. Enteritidis* (SEPT4); EF – *E. faecium* (EF55); EFSE – *E. faecium* + *S. Enteritidis* (EF55 + SEPT4)

In our experiments, the *Salmonella* infection and probiotic strain affected some biochemical parameters and the relative weight of organs in chickens.

Microbiological examination (Table 3) showed a significant reduction in the number of *Salmonella* in the liver of 22 day-old chickens in the group EFSE against SE. The reduction of *Salmonella* in the group EFSE compared to SE was evident also in other organs and individual samplings, but the differences were insignificant. The reduction of bacteria in the organs could be explained by the presence of EF55 strain. Individual samples (8 and 22 day-old chickens) taken from all three organs showed significant reduction in the number of *Salmonella* in both groups (SE and EFSE) in the second sampling compared to the first one. This decrease in bacterial counts could indicate the higher resistance of older chickens.

The higher concentrations of total bilirubin in the 22-day old chickens (Table 2) in the *Salmonella* groups (SE, EFSE) could be associated with haemorrhage that usually accompanies *Salmonella* infection. Santos *et al*. (9) in their experiment reported similarly high concentration of bilirubin after *Salmonella* infection.

The decreased glucose concentration in groups SE and EFSE compared with C and EF in 8-day old chickens (Table 1) could be associated with an enlarged pancreas (Table 4) just in these groups. In the EF group, we observed almost the same level of glucose as in the controls, which could be considered a proof of the positive impact of the probiotics. Some authors reported that probiotic supplementation could have affected some metabolic parameters that are under the strong control of pancreatic hormones (10). On the other hand, in 22-day old chickens (Table 2), the concentration of glucose had an upward trend in the S*almonella* groups (SE, EFSE). One explanation could be a longer exposure to infection, which could cause additional stress in these animals. Stress causes a general deterioration of the well-being of chickens, usually involving a cascade of physiological adaptive responses that include changes in the plasma levels of blood metabolites (5, 6, 7, 8, 11). Increased glucose levels in broilers and laying hens subjected to adrenocorticotropic hormone (ACTH) induced stress have been reported (4, 5, 6, 7).

The ALT enzyme activity was the highest in the SE group at both ages, apparently in relation to the localization of *Salmonella* in the liver. In the study of Gupta *et al*. (1), experimental salmonellosis caused a significant increase in the alanine aminotransferase activity.

In conclusion, the presented results reveal that the probiotic strain EF55 could be successfully used as a nutritional additive to poultry feeds for the promotion of improved metabolism.

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EVALUATION OF EFFECTS OF *THYMUS VULGARIS* EXTRACT ON METABOLISM AND GUT MUCUS IN CHICKS

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ABSTRACT

This study was to observe the effects of the essential oil from *Thymus vulgaris* L. on the performance of chicks; their plasma biochemical parameters; the thickness of the gut mucus adherent layer; and the quantity of neutral and acid mucins in the small intestine. One-day-old chicks of the Isa Brown breed were divided into three groups (n = 9) and fed 11 weeks as follows: Control 1 basal diet (BD) without sunflower oil (SO); Control 2 – BD + SO (1%); and Experimental – BD + SO + thyme extract (0.05%). The determination of the total body and internal organ weights (liver, spleen, pancreas, and gizzard) was without significant changes. The biochemistry of the plasma revealed an increase of total proteins, phosphorus and ALP ($P < 0.05$) and a decrease of calcium and AST in the experimental group ($P < 0.05$) compared to Control 1, as well as an increase of total protein and AST ($P < 0.05$) and a decrease of phosphorus, calcium and ALP ($P < 0.05$) in the experimental group compared to Control 2. Colorimetric examination of the mucus adherent layer thickness in the small intestine showed no differences. The neutral mucins, determined via PAS staining of goblet cells in the intestinal mucosa, were decreased in the jejunum ($P < 0.001$) and caecum ($P < 0.05$) of the experimental group compared to Control 1. The quantity of acid mucins, observed via alcian blue goblet cells staining, was increased in the duodenum of the experimental group ($P < 0.001$) when compared to both controls and a decreased in the jejunum ($P < 0.05$) compared to Control 2. Because acid mucins play a role in the local defence against pathogens, a significant increase of this type of mucins in the duodenum might be a beneficial effect of dietary thyme on the health of the chicks.

Key words: biochemical parameters; chick; gut mucus; neutral and acid mucins; *Thymus vulgaris* L.

INTRODUCTION

Various dietary herbs, plant extracts, and especially essential oils (EOs), have been studied for their antimicrobial and growth promoter abilities (7, 9). Eos: enhance the production of digestive secretions; stimulate blood circulation; exert antioxidant properties; reduce the levels of pathogenic bacteria; and may enhance the immune system (5). The positive effects observed *in vitro* justify further research in this area to determine the optimal dietary concentration level and the mode of action of these plant products to achieve the optimal growth performance and disease resistance in poultry (11). The entire surface of the chicken gastrointestinal tract is covered by a layer of mucus that functions as a diffusive barrier between the intestinal lumen and the absorptive cells. The mucins are the main component of the mucus layer, which are produced and secreted by goblet cells (20). The histochemical expression of mucins in the gut mucosa is well known, and the presence of neutral and acid mucins can be easily demonstrated (1). The proportions

between neutral and acid mucins are constant and may be modified in inflammatory bowel disease, ulcerative colitis and it has also been shown that such changes may even precede the development of neoplasia (16). The direct relationship between the changes in the intestinal structure and mucus gel quantity has not been adequately investigated (19). Morphological investigations has been carried out with staining methods using periodic acid-Schiff (PAS) for neutral mucins and alcian blue (AB) pH 2.5 for acid mucins, but quantitative investigation by such methods presents some difficulties (3).

The aim of this study was to examine the effect of feeding a diet supplemented by essential oil from thyme *Thymus vulgaris* L. on various aspects of a chick's development, such as: some plasma biochemical parameters; the thickness of the gut mucus adherent layer; and the quantity of neutral and acid mucins in the small intestine.

MATERIALS AND METHODS

Animals and experimental groups

Twenty-seven one-day-old chicks of the Isa Brown breed from the hatchery Párovské Háje, Slovakia, were used. They were divided into three groups of nine chicks each and fed as it is shown in Table 1. The birds were kept in wooden group cages, containing wood shavings. The temperature was kept according of the age of the chicks (32 °C in the first two weeks, then gradually decrease of 1.5 °C each week to 26 °C). The relative humidity was 65–75°%. The experiment lasted 11 weeks. All groups received feed and water *ad libitum*. The composition of the basal diet is shown in Table 2. The experimental group was supplemented with 0.05 % thyme essential oil (*Thymi aetheroleum* of *Thymus vulgaris* L.; declared parameters: cymene 48 ± 3 %, thymol 24 ± 2 %; producer: Calendula a.s., Nová Ľubovňa, SR).

Weighing

At the end of the experiment, the live body weight of the chicks (n = 5) was measured and blood samples (n = 8) from *v. ulnaris* were taken. Then, the chicks were anaesthetized by intraperitoneal injection of xylazine (Rometar 2 %, SPOFA, Czech Republic) and ketamine (Narkamon 5 %, SPOFA, Czech Republic) in doses of 0.6 and 0.7 ml.kg^{-1} of live body weight respectively. The weight of the following internal body organs (n = 5) was determined: liver without gallbladder, spleen, pancreas and empty gizzard without cuticula.

Determination of biochemical parameters

For biochemical measurements, the samples of the plasma of eight chicks of each group were used. Analyses were done using the commercial kits (RANDOX Laboratories Ltd., UK and PLIVA-Lachema Diagnostika s. r. o., Czech Republic) and absorbances were measured by a spectrophotometer GENESYS 10 UV (Thermo Electronic Corporation, USA).

Determination of the mucus adherent layer of the gut

The samples of the gut of five chicks from each group were processed by using a method for quantification of mucus, modified by Smirnov *et al.* (20), in order to determine the thickness of the gut mucus adherent layer. The middle segments of the duodenum, jejunum, ileum and caecum were taken. Each segment was flushed with 0.15 M NaCl and cut into two parts of about 1 × 1 cm. The segments of gut were stained in solution of alcian blue (AB) (AppliChem GmbH, Germany), flushed with 0.25 M saccharose and incubated in 10 g.l^{-1} docusate sodium salt solution (Aldrich, Germany) overnight at room temperature. The samples were cleared by centrifugation (700 × g/2 min) and the supernatant was pipetted into 96-well plates in triplicate (100 µl of each sample as well as the set of standard solutions of AB). The optical density of the samples and standards were measured by ELISA READER (Opsys MR™ Microplate Reader, Dynex Technologies Inc., USA) at 630 nm. The amount of the adherent gut mucus was stained by alcian blue (AB) and calculated by the standard curve and expressed as µg AB.cm^{-2} of gut.

Determination of mucins

The guts of five chicks randomly taken from each group were used. One-cm long middle segments of the duodenum, jejunum, ileum and caecum were removed, gently flushed with 0.9 % (wt/vol) NaCl and fixed in 10 % (vol/vol) formaldehyde for further processing. These segments were dehydrated, cleared, embedded in paraffin and cut into 3 µm serial cross sections. Then, sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Finally, the sections were stained with periodic acid-Schiff (PAS) (Acros Organics, New Jersey, USA; Merck KGaA, Darmstadt, Germany), alcian blue (AB) and combined AB/PAS staining (14). Three sections for each staining were prepared to examine the individual intestinal segments.

System of image analysis

For the PAS-, AB- and AB/PAS-stained sections, the colour images were taken from a light microscope Nikon ECLIPSE E600 with Nikon digital camera DXM1200 and transferred to a TV monitor. From each section and each staining, 10 photos were taken (every photo was the photo of the same place in the three various stainings). All images were then stored in a personal computer and analysed with Adobe Photoshop CS2 9.0. The surface area recorded on each photo was 0.09045 mm^2.

Measurements

The goblet cells in the villi were counted on the area mentioned above within each section and each staining. The number of the goblet cells was converted to the area of 1 mm^2. The number of the goblet cells stained with AB and PAS was expressed as percentage of the total number of goblet cells per unit of the surface area (mm^2).

Statistical analysis

The results are expressed as the means ± SEM (standard error of the mean). The statistical significance was evaluated with one-way analysis of variance (ANOVA) and Tukey *post-hoc* test.

The experiments were carried out in accordance with the established standards for the use of experimental animals. The protocol was approved by the local ethic and scientific authorities.

Table 1. Feeding of the chicks

Control 1 BD without SO	Control 2 BD + SO (1%)	Experimental group BD + SO + Thyme (0.05%)
Day 1–10: 200 g of feed	Day 1–10: 200 g of feed + 2 ml SO	Day 1–10: 200 g of feed + 2 ml SO + 0.1 ml of thyme
Day 10–30: 400 g of feed	Day 10–30: 400 g of feed + 4 ml SO	Day 10–30: 400 g of feed + 4 ml SO + 0.2 ml of thyme
After 30 days: 630 g of feed	After 30 days: 630 g of feed + 6.3 ml SO	After 30 days: 630 g of feed + 6.3 ml SO + 0.315 ml of thyme

BD – basal diet; SO – sunflower oil

Table 2. Composition of basal diet – nutritional analysis per 1 kg

Ingredient	Amount	Ingredient	Amount
N	*min. 190 g*	Co	0.4 mg
Crude ME	min. 12 IU	Se	0.1 + 0.4 mg
Ash	max. 50 g	Vitamin A	min. 12 000 IU
Fibre	max. 35 g	Vitamin D3	min. 4000 IU
Lysine	min. 905 g	Vit. E (t-tocopherol)	min. 100 IU
Cystine	min. 3.5 g	Vitamin K3	4 mg
Methinone	*min. 4 g*	Vitamin B1	3 mg
Linoleic acid	min. 10 g	Vitamin B2	min. 9 mg
Ca	min. 7 g	Vitamin B6	6 mg
P	min. 5 g	Vitamin B12	min. 40 μg
Na	1.5 g	Vitamin C	200 mg
Mn	min. 110 mg	Nicotinic acid	60 mg
Fe	min. 120 mg	Calcium pantothenate	15 mg
Cu	min. 15 mg	Folic acid	2 mg
Zn	min. 100 mg	Biotine	0.2 mg
I	1 mg	Choline	500 mg

RESULTS

Performance of chicks

No significant changes were observed between the groups.

Table 3. Effect of feeding the diet supplemented with plant extract of thyme on total body weight (BW) (g) and the weight of internal body organs (% of live BW)

	Control 1	Control 2	Experimental group
Total body weight (g)	*1060 ± 32.25*	1028 ± *22.45*	988 ± *33.53*
Spleen (% of live BW)	0.278 ± *0.020*	0.244 ± *0.025*	0.280 ± *0.012*
Gizzard (% of live BW)	3.412 ± *0.180*	3.370 ± *0.281*	3.540 ± *0.124*
Pancreas (% of live BW)	0.260 ± *0.013*	0.258 ± *0.019*	0.290 ± *0.017*
Liver (% of live BW)	2.502 ± *0.219*	2.466 ± *0.204*	2.510 ± *0.218*

ns; mean ± SEM; n = 5

Biochemistry

There was a significant increase of total protein, phosphorus and ALP activity in the experimental group ($P < 0.05$)and a decrease of calcium and AST activity ($P < 0.05$) compared to Control 1 and a significant increase of AST activity and total protein in experimental group ($P < 0.05$) and a decrease of phosphorus, calcium and ALP activity ($P < 0.05$) compared to Control 2.

Measurement of the mucus adherent layer thickness

No significant changes were observed between the groups.

Table 5. Effect of feeding diet supplemented by plant extract of thyme on thickness of gut mucus layer of chicks (μg AB.cm^{-2} of gut)

Segment of gut	Control 1	Control 2	Experimental group
Duodenum	*5.34* ± 0.30	4.57 ± 0.17	4.26 ± 0.23
Jejunum	4.60 ± 0.25	3.89 ± 0.12	4.26 ± 0.20
Ileum	4.56 ± 0.21	4.11 ± 0.16	4.51 ± 0.29
Caecum	4.73 ± 0.22	4.18 ± 0.15	4.96 ± 0.26

ns; mean ± SEM; n = 5; AB – alcian blue

Mucin quantification

Duodenum: In the experimental group, the acid mucins significantly increased by 15 % compared to Control 1 ($P < 0.001$) and by 8 % compared to Control 2.

Jejunum: In the experimental group the acid mucins significantly increased by 9 % compared to Control 2 ($P < 0.05$). The neutral mucins in the experimental group significantly decreased by13 % compared to Control 1 ($P < 0.001$).

Ileum: In the experimental group the neutral mucins significantly increased by 16 % compared to Control 1 ($P < 0.001$) and decreased by 5 % compared to Control 2. In Control 2, the neutral mucins significantly increased by 21 % compared to Control 1 ($P < 0.001$).

Table 4. Effect of feeding diet supplemented by plant extract of thyme on biochemical parameters in plasma of chicks

Parameter	Control 1	Control 2	Experimental group
Mg (mmol.l^{-1})	1.28 ± 0.09	1.36 ± 0.07	1.42 ± 0.04
P (mmol.l^{-1})	2.3 ± 0.088^{ab}	4.013 ± 0.061^{ac}	3.1 ± 0.141^{bc}
Ca (mmol.l^{-1})	2.43 ± 0.114^{a}	2.013 ± 0.069	1.788 ± 0.118^{a}
AST (μkat.l^{-1})	1.36 ± 0.032^{ab}	1.034 ± 0.016^{a}	1.1 ± 0.042^{b}
ALP (μkat.l^{-1})	14.03 ± 0.353^{a}	21.13 ± 0.692^{ab}	15.88 ± 0.58^{b}
Albumin (g.l^{-1})	12.31 ± 0.689	12.86 ± 0.522	12.37 ± 0.255
Glucose (mmol.l^{-1})	15.53 ± 0.37	14.75 ± 0.196	15.79 ± 0.412
Cholesterol (mmol.l^{-1})	2.78 ± 0.11	2.96 ± 0.119	2.58 ± 0.07
Bilirubin (mmol.l^{-1})	5.063 ± 0.163	4.325 ± 0.142	4.95 ± 0.219
Total protein (g.l^{-1})	33.79 ± 0.878^{a}	35.62 ± 2.298	41.25 ± 1.264^{a}

Significant differences within a row are indicated by the same superscripts at $P < 0.05$ level; mean ± SEM; n = 8

Caecum: In the experimental group, the neutral mucins significantly decreased by 10 % compared to control 1 ($P < 0.05$). There was a significant decrease in Control 2 by 13 % ($P < 0.01$) compared to Control 1.

Table 6. Comparison between percentage expressions of acid and neutral mucins on the area of 1 mm² in small intestine of chicks

	Duodenum		Jejunum		Ileum		Caecum	
	%							
	AB	*PAS*	AB	PAS	AB	PAS	AB	PAS
Control 1	43^{a}	57	45	55^{d}	61	39^{ef}	47	53^{hi}
Control 2	50^{b}	50	49^{c}	51	40	60^{eg}	60	40^{h}
Exp. group	58^{ab}	42	58^{c}	42^{d}	45	55^{fg}	57	43^{i}

Significant differences within a column are indicated by the same superscripts at P < 0.05 level (c, i); P < 0.01 level (f, g, h); P < 0.001 level (a, b, d, e); mean ± SEM; n = 5; AB – alcian blue; PAS – periodic acid-Schiff

The effect of dietary thyme on the amount of acid mucins per mm² in small intestine of chicks is included in table 7. In the experimental group the acid mucins significantly increased in the duodenum compared to both controls ($P < 0.001$) and decreased in the jejunum compared to Control 2 ($P < 0.05$).

Table 7. Effect of dietary thyme on amount of acid mucins per mm²

	Duodenum	Jejunum	Ileum	Caecum
Control 1	*713.1* ± 84.501^{a}	1413.3 ± 101.91	1628.4 ± 96.705	631.56 ± 44.54
Control 2	865.12 ± 44.112^{b}	1321.2 ± 83.604^{c}	1378.3 ± 181.59	262.18 ± 31.614
Exp. group	1533.1 ± 76.045^{ab}	1235.1 ± 42.045^{c}	1276.2 ± 77.686	339.97 ± 3 7

Significant differences within a column are indicated by the same superscripts at P < 0.05 level (c); P < 0.001 level (a, b); mean ± SEM; n = 5

The effect of dietary thyme on the amount of neutral mucins per mm² in the small intestine of the chick is included in Table 8. In the experimental group, the neutral mucins decreased in the jejunum ($P < 0.001$) and in the caecum ($P < 0.01$) compared to Control 1 and in the ileum ($P < 0.05$) compared to Control 2 and increased in the ileum ($P < 0.05$) compared to Control 1. In Control 2 the neutral mucins significantly increased in the ileum ($P < 0.001$) and decreased in the caecum ($P < 0.05$) when compared to Control 1.

Table 8. Effect of dietary thyme on amount of neutral mucins per mm²

	Duodenum	Jejunum	Ileum	Caecum
Control 1	*959.65*± 76.947	1721 ± 80.42^{a}	1054.4 ± 31.957^{bc}	707.57 ± 50.503^{ef}
Control 2	874.99± 41.608	1358 ± 115.55	2041.6 ± 206.02^{bd}	176.89 ± 15.258^{e}
Exp. group	1103.1 ± 44.061	877.1 ± 62.062^{a}	1523.9 ± 116.91^{cd}	257.05 ± 24.433^{f}

Significant differences within a column are indicated by the same superscripts at P < 0.05 level (c, d, e); P < 0.01 level (f); P < 0.001 level (a, b); mean ± SEM; n = 5

DISCUSSION

In general, we observed no differences in total body weight or weight of internal organs in chickens fed the experimental diet or the control rations. The total body weight of the chicks in the experimental group after 11 weeks of age was 988 g which corresponds with the usual total body weight of this breed in this age (960–1000 *g*); although it was expected that supplementing the dietary plant extract (8) would stimulate the growth performance of the chicks. However, the results of the present study are in agreement with previous observations that indicated herbs, plant extracts, EOs (or the main components of the EOs) did not affect body weight gain, feed intake or feed efficiency in broilers (8, 11). No EO effects on growth performance were reported by Botsoglou *et al.* (6), Zhang *et al.* (23), or Jang *et al.* (13), whereas improved growth performance was observed in birds fed certain EO-supplemented diets by Jamroz *et al.* (12), Hernandez *et al.* (11) and Cross *et al.* (7). Al-Kassie (2) reported that chicks fed with 200 ppm EO derived from thyme and cinnamon had significantly higher (P0.05) feed intake, body weight gain and feed conversion ratio,followed by chicks fed with 100 ppm EO derived from thyme and cinnamon compared to the control group, which showed the lowest performance.

The biochemical analysis revealed the increase of the phosphorus level over the reference values as well as a decrease of the calcium level below the physiological values in all tested groups. The ALP activity in all tested groups corresponded with an increased level of phosphorus and decreased level of calcium. These findings indicated a demineralization of the bone. In Control 2 and the experimental group where the Ca : P ratio was the most unbalanced(0.5 and 0.57), the total body weight was lower when compared

to Control 1 (ratio 1.05). The total proteins (TP) in the experimental group significantly increased ($P < 0.05$) compared to both controls. Al-Kassie (2) reported that the TP increased significantly ($P < 0.05$) for the group of broilers consuming 200 ppm essential oil (EO) derived from thyme and cinnamon for 42 days. Moreover, the chicks fed with the feed ration containing EO derived from thyme and cinnamon had reduced ($P < 0.05$) serum cholesterol. Gerzilov *et al.* (10) did an experiment with Peking ducks fed a diet supplemented with herbs (rosemary, thyme, basil, oregano and cinnamon, each in an amount of 0.03 %) and reported a significant reduction in triglyceride concentrations ($P < 0.01$) and total cholesterol concentrations ($P < 0.01$) in the serum, which may be associated with the herbal anti-stress effect on Peking ducks. However, Toghyani *et al*. (22) reported that thyme powder at 10 $g.kg^{-1}$ level significantly ($P < 0.05$) increased HDL-cholesterol concentration but the protein, albumin, triglyceride, total and LDL cholesterol concentrations were not influenced which is in agreement with our study.

The presence of nutrients in the intestine is important for the maintenance of normal mucosal function (20). Factors such as, the frequency of feeding, the composition of the diet or stress have been reported to affect the mucosal thickness. The effect of dietary thyme supplementation on the thickness of the gut mucus layer is shown in Table 5. No significant changes were observed between the tested groups. However, changes in nutrition would be expected to influence the thickness of the adherent mucus layer. Similarly, Smirnov *et al*. (20) studied the effects of starvation on the thickness of the adherent mucus layer in the small intestine of chicks and observed no significant changes among the small intestine segments. However, some nutritional manipulations in chicks were found to change the profile of mucin types in the small intestine (20). The neutral mucins appear to be the predominant subtype expressed in the gastric mucosa (19). In densely populated regions of the GIT (ileum, large intestine), the goblet cells predominantly contain acid mucins (17), which are further distinguished by sulphated (sulphomucins) or non-sulphated (sialomucins) groups. The relationship of the different mucins is affected by age and microbial status (4), and, as the present study demonstrated, by dietary thyme supplementation. In the experimental group, the amount of PAS positive stained goblet cells was lower in the jejunum and the caecum and higher in the duodenum and ileum when compared to Control 1. Meslin *et al.* (15) reported the reduction in the neutral and sulphomucins in the caecum and colon in rats. It can be attributed to the potentially mucolytic activity of the intestinal flora which showed higher hydrolytic activity towards neutral and sulphomucins than towards sialomucins. In the acid mucins we can only consider the total amount of the AB positive stained goblet cells since we did not differentiate them into their respective subtypes. In the monitored area of the duodenum of the experimental group, we observed a significant increase of AB positive stained goblet cells when compared to both controls. Inasmuch as acid mucins play an important role in the local defence against pathogens, and our study revealed a significant increase of acid mucins in duodenum, we can suppose the beneficial effect of dietary thyme on the chick's health in this regard.

CONCLUSIONS

It can be concluded that dietary thyme supplementation did not influence the total body weight or the weight of internal organs of chicks. On the other hand, the experimental diet significantly increased the plasma concentration of total protein and decreased the level of calcium compared to both controls. The phosphorus level and ALP activity in the experimental group was higher compared to Control 1 and lower when compared to Control 2. The experimental diet significantly increased the amount of acid mucins in the duodenum compared to both controls. The results demonstrate that dietary thyme supplementation in chicks did not lead to a negative effect on the growth performance and had a positive effect on the gut mucus barrier in the duodenum, but the effect on the metabolism of calcium and phosphorus needs to be examined further.

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CONCURRENT *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI* CAUSED PERIPARTURIENT DISEASE IN OUTDOOR SOWS

Short communication

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ABSTRACT

The present study was performed in a large combined indoor/outdoor pig breeding unit which experienced a sudden outbreak of concurrent *Pseudomonas aeruginosa* and *Escherichia coli* caused periparturient diseases (mastitis, metritis, agalactia, MMA). Subsequently a high culling rate of the sows and preweaning mortality of piglets followed.

In this unit, a total of 173 late pregnant outdoor sows were randomly divided into three groups. Group one was kept outdoors and received prophylactic medication for 1 week prior and 1 week after farrowing with in-feed enrofloxacine. Groups two (prophylactic medicated as Group one) and three (non-medicated) were kept in individual farrowing crates indoors. The incidence of: MMA, sow mortality, preweaning piglet mortality, sows culling due to vulval discharge and chronic mastitis were evaluated.

Despite the prophylaxis with the antibiotic, the incidence of MMA and periparturient sow mortality were significantly higher ($P < 0.05$) in the outdoor sows compared with the indoor sows. Similarly, preweaning piglet mortality and culling of the sows were greater ($P < 0.05$) in the outdoor sows compared with the indoor sows.

Key words: culling; *Escherichia coli*; mortality; *Pseudomonas aeruginosa*; sow

INTRODUCTION

Outdoor sows have a higher incidence of infectious periparturient diseases (mastitis, metritis, agalactia, MMA) mortality, and culling compared to indoor sows (10). Profitability in outdoor production is highly dependant upon the management system (9); especially in outdoor sows, a failure to meet hygienic and animal friendly requirements may result in high infectious pressure and major financial losses (1). Climatic and environmental circumstances, pathogenic pressure, soil type, reproductive management, hut design and profitability are closely interrelated (5). Periparturient problems caused by concurrent infections with *Pseudomonas aeruginosa (P)* and *Escherichia coli (E)* in outdoor sows are not new in the veterinary literature (1, 2, 3, 5, 7, 9, 10, 11, 12). Karg and Bilkei (10) found, during a four year evaluation period, lower average annual mortality rate in indoor than in outdoor production units. In a published East-European study, outdoor sows had, under high infectious pressure with *P. aeruginosa* and *E. coli,* significantly higher losses due to MMA and preweaning piglet mortality compared to indoor sows (9).

During the last decade, an increase in outdoor pig production has taken place in many East-European countries. This has been due to a number of factors including; lower capital costs for housing, equipment, heating and ventilation. Further, animal welfare and environmental considerations (together with consumer requests) supported the trend towards less intensive and more "animal friendly" production (1).

The present paper describes an outbreak of periparturient disease in a large outdoor production unit and the subsequent clinical trials, in order to investigate the best possible preventive measures. The authors compared the influence of outdoor/indoor production and the effects of a prophylaxic antibiotic against MMA in outdoor *vs.* indoor sows.

MATERIALS AND METHODS

In October 2011, in an East-European large indoor/outdoor pig production unit, a sudden increase (from 15.4 % to 58.1 %) of urogenital tract infections of the sows accompanied with MMA and high mortality rate was diagnosed. The outdoor part of the unit had higher losses (outdoor 19.1 % *vs.* indoor 5.3 %). The sows were F1 or F2 genetic lines of Landrace × Large White, mated to Duroc or Landrace boars. The sows were fed with commercial gestational and lactational feed (Table 1).

Post-mortem bacteriological examination of uteri, urinary bladder and mammary tissue of sows having suffered urogenital disease revealed heavy growth of *P. aeruginosa* (10^4 colony forming units (CFU) per millilitre of tissue homogenate) and *E. coli* (10^5 CFU per millilitre of bladder and mammary tissue homogenates). Antibiotic sensitivity tests showed that both organisms were sensitive to gentamycine and enrofloxacine.

According to these findings a trial was conducted in order to compare the influence of outdoor/indoor production and the effects of antibiotic prophylaxis against concurrent infections with *P. aeruginosa* and *E. coli* in outdoor *vs.* indoor sows.

A total of 173 late pregnant outdoor sows were randomly divided into three groups. The sows of Group one (n = 55; average parity 3.01 ± 0.62; body condition 4.6 ± 1.1) were kept and farrowed outdoors and were medicated for one week prior and 1 week after farrowing with in-feed enrofloxacine (Baytril® 5 % feed additive, Bayer, Munich, Germany) at a dose of 5 mg.kg^{-1} body weight per day. The sows of Group two (n = 55; average parity 3.46 ± 0.51; body condition 4.1 ± 0.8) were moved to individual farrowing crates indoors and received the same prophylactic medication as Group one. Group three (indoor, n = 63; average parity 3.08 ± 0.4; body condition 4.1 ± 1.2) was given no medication.

The indoor groups of sows were kept in a high investment facility according to the standard requirements of the intensive breeding enterprises of the late 90's.

The following data were recorded:

- the incidence of MMA based on the early MMA diagnosis system (8)
- bacterial count of *P. aeruginosa* and *E. coli* in the urinary bladder and mammary tissue homogenates of 2 culled sows in each group (7)
- sow mortalities within 72 hours of diagnosing periparturient disease (8)
- preweaning piglet mortalities
- the number of sows culled as a result of having had vulval discharge post-weaning and/or chronic mastitis.

Data were assessed by analysis of variance using the procedure of GLM in SAS (Cary NC SAS/STATE AE User's Guide, Version 6, 1989, SAS Institute Inc.).

Table 1. Diet composition in different production sequence

	MJDE.kg^{-1}	Crude protein [%]	Lysine [%]	Phosphorus [%]
Gestation	12.2	12.5	0.65	0.4
Lactation	13	18	1.0	0.6

MJ DE – megajoul digestible energy

Table 2. Production parameters in sows kept outdoors and medicated with in-feed enrofloxacine compared with sows farrowed indoors with and without medication in a herd with a high incidence of *Pseudomonas aeruginosa* and *Escherichia coli* caused periparturient disease

Parameter	Outdoor medicated N/n [%]	Indoor medicated N/n [%]	Indoor non-medicated N/n [%]
A	55/34 (61.8)^a	55/9 (16.4^b	63/12 (19.0)^b
B	55/2	55/0	63/0
C	612/97 (15.8)^a	608/26 (4.3)^b	659/28 (4.2)^b
D	55/11 (20.0)^a	55/2 (3.6)^b	63/3 (4.8)^b

N – number of animals in a group; n – number of cases in a group; A – sows diagnosed with periparturient disease; B – Sow mortalities; C – preweaning piglet mortalities; D – sows culled; different superscripts within the same row indicate a significant difference (P < 0.05)

RESULTS

Table 2 shows the incidence of periparturient disease, the sow and preweaning piglet mortalities, and the culling rate of sows in the different groups. The incidence of periparturient disease was significantly higher (P < 0.05) in the outdoor medicated sows compared with indoor sows. Similarly, the number of preweaning piglet mortalities and the number of sow culled was greater (P < 0.05) in the outdoor medicated sows compared with the indoor sows regardless of treatment. Bacteriological examinations of both the urinary bladder and mammary tissues revealed in the outdoor sows, despite prophylactic treatment, a moderate growth of *P. aeruginosa* (10^3 CFU per millilitre of mammary tissue homogenate) and *E. coli* (10^3 CFU per millilitre) in the mammary and bladder mucosal tissue homogenates. In contrast to the outdoor sows, the bacteriological examinations of both the urinary bladder and mammary tissue of the indoor sows revealed a low growth of *P. aeruginosa* (10^2 CFU per millilitre of mammary tissue homogenate) and *E. coli* (10^2 CFU per millilitre of mammary tissue homogenate).

DISCUSSION

The results of the present study indicate that, although an outdoor environment may better satisfy the ethological needs of the animals, an indoor production system allows the

breeding sows to have lower infectious pressures and higher production levels (4). Sow mortality has multifactorial causes and consists of many components, such as; genetics, environmental, hygiene, nutritional, management, welfare and infections (3). High sow mortality increases economic losses such as, replacement costs and opportunity costs and elevates animal welfare concerns (7). It negatively influences employee's moral as well (6).

P. aeruginosa is a common ubiquitous organism found worldwide and frequently cultured from inflammatory conditions in the pig (2). However, there is little evidence to suggest it is a primary pathogen, although it can produce inflammatory changes by the production of toxins (9, 10, 11, 12, 13). *P. aeruginosa* is frequently found in skin wounds associated with injuries of mammary glands in outdoor sows (2). A number of reports have associated *P. aeruginosa* with abortion, stillbirths and mummification in pigs (1, 2, 6), agalactia (11), urinary tract infections (13) and MMA in sows (9).

The incidence of *P. aeruginosa* in sows diagnosed with periparturient disease in the present study herd suggest the organism can cause metritis, mastitis and cystitis most likely by ascending infections in sows reared and farrowed outdoors (7). It also appears to cause concurrent infection with *E. coli* in the urogenital tract (5). Consistent with literature data (3) the findings reported here also suggest that antibiotic prophylaxis alone does not prevent *P. aeruginosa* and *E. coli* caused concurrent infections at farrowing. The results of non treated, indoor kept sows shows that clean hygienic housing of sows during late pregnancy is more likely to prevent periparturient disease than antibiotic prophylaxis. The cumulative effect of stress (as weather changes, non supervised parturition, infected soil, less intensive husbandry, possible problems with water supply, rodents and birds in the environment) is often present in outdoor units. In the present case, higher infectious pressure in non hygienic outdoor huts may have negatively influenced *P. aeruginosa* caused MMA, sow and preweaning piglet mortality. Furthermore, it seems to be likely that reinfections with *P. aeruginosa* from the infected soil could have overwhelmed the Enrofloxacine treatment.

CONCLUSIONS

Despite medication, outdoor sows had higher infectious pressure with *P. aeruginosa* and also outdoor sows had significantly higher losses due to MMA from *E. coli* as well as higher preweaning piglet mortality compared to indoor sows.

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A CLINICAL CASE OF HEMIVERTEBRAE IN A PUG – DIAGNOSIS AND TREATMENT

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ABSTRACT

This short communication concerns a clinical case of hemivertebrae of the 7th and 8th thoracic vertebrae in a dog. It describes the clinical, neurological and radiological examinations as well as the therapeutic procedures in one male Pug, 6 months of age with a body weight of 14 kg. The patient displayed a spinal deformity and was referred to the University of Veterinary Medicine and Pharmacy in Kosice with neurological symptoms in its hind legs. It was successfully treated with a hemilaminectomy. The surgical intervention helped to eliminate the compression of the spinal cord and after a period of recovery and growth, the patient exhibited only very minor neurological deficits in the hind legs.

Key words: dogs; spinal diseases; vertebral anomalies

INTRODUCTION

Congenital anomalies of the vertebral column are frequently identified and have been described in the veterinary literature. In most cases, these developmental aberrations are an incidental finding (4). Anomalies such as hemivertebrae are relatively common, but they rarely cause clinical signs. Hemivertebrae are seen usually in the small, brachycephalic breeds (5). Vertebral anomalies of the thoracolumbal vertebral column are common in dogs with a screw tail, such as the English and French bulldogs and the Boston terrier (11), bulldog-related breeds, and Pugs (4). Failure of ossification of one half of the vertebral body may cause the development of unilateral, dorsal, or ventral hemivertebrae and dorsal displacement of a ventral hemivertebrae may induce chronic progressive spinal cord compression (11).

Clinical signs of hemivertebrae may be mild and non-progressive and stabilise once the dog has stopped growing at about 9 months of age. The condition is usually manifested in young dogs and the condition worsens as growth proceeds (2). Some patients may not exhibit clinical signs until adulthood (8). Myelography is essential to confirm that there is an associated spinal cord compression (10). Surgical stabilisation or decompression can result in improvement in many cases. However, surgical correction may be very difficult (6) and deterioration may also occur in some animals when these lesions are subjected to decompressing laminectomy (5).

CASE PRESENTATION

In the patient's history, the owners of the dog indicated no gait disturbances during the first 4 months of age, but they began to appear gradually after the fourth month of age. The owners observed the first problems probably when the spinal canal began to ossify and when neurological deficits occurred in dog's hind legs. The first clinical sign was uncoordinated walking in both hind legs with occasional weakness and inability to walk only on the hind legs. Walking on the thoracic legs was normal, with no neurological deficits discerned. The owners further stated that the dog was active, he stood also on hind legs at rest, but when he was moving, his hind legs dragged behind the body. The food intake, defecation and urination were normal. The first private veterinarian contacted prescribed NSAIDs for the dog.

When the dog was referred to the clinic, he was in good condition, well oriented in space and responded well to outside stimuli. The dog stood at rest on all four legs. By prolonged standing we observed the displacement of the pelvis from the left to the right

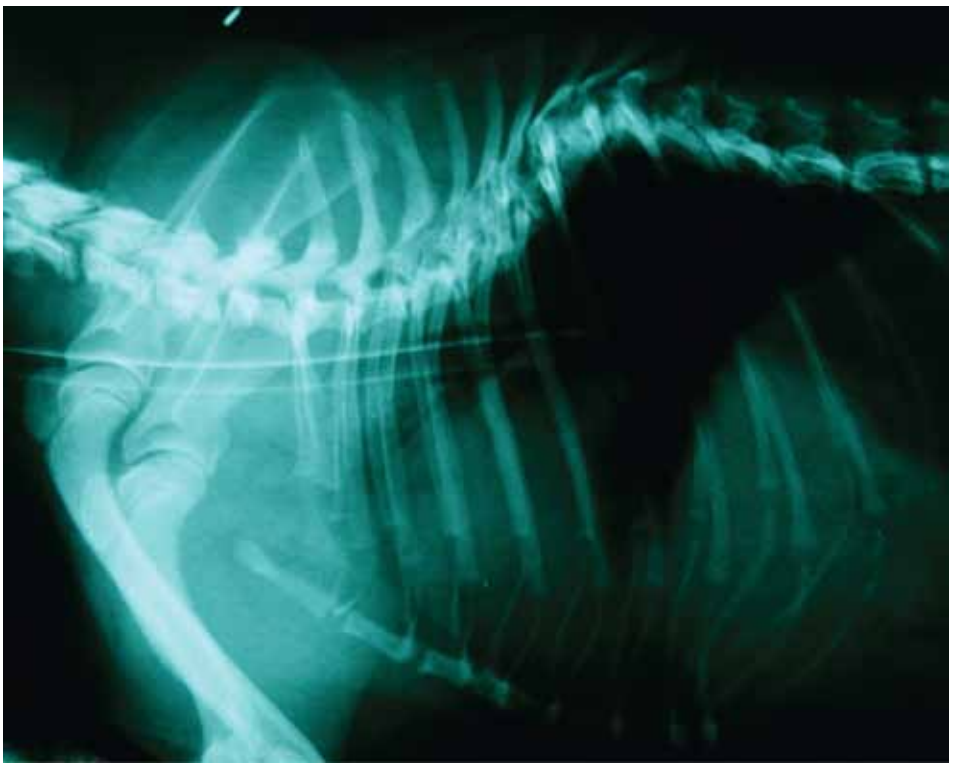

Fig. 1. Latero-lateral right projection of the dog's thorax. Deformed line of the spine and thorax-kyphosis

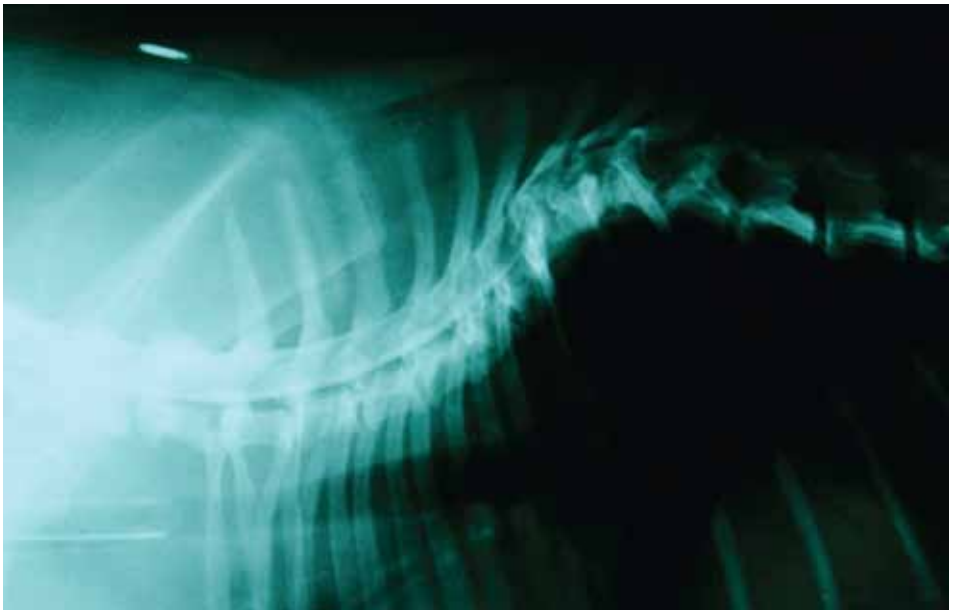

Fig. 2. Application of the contrast medium into the *cysterna magna* revealed the stoppage of the contrast medium in the level of 6th and 7th thoracic vertebrae, which indicated a compression of the spinal cord. This compression caused neurological deficits characteristic of the level Th3–L3. Neurological deficits deteriorate with age

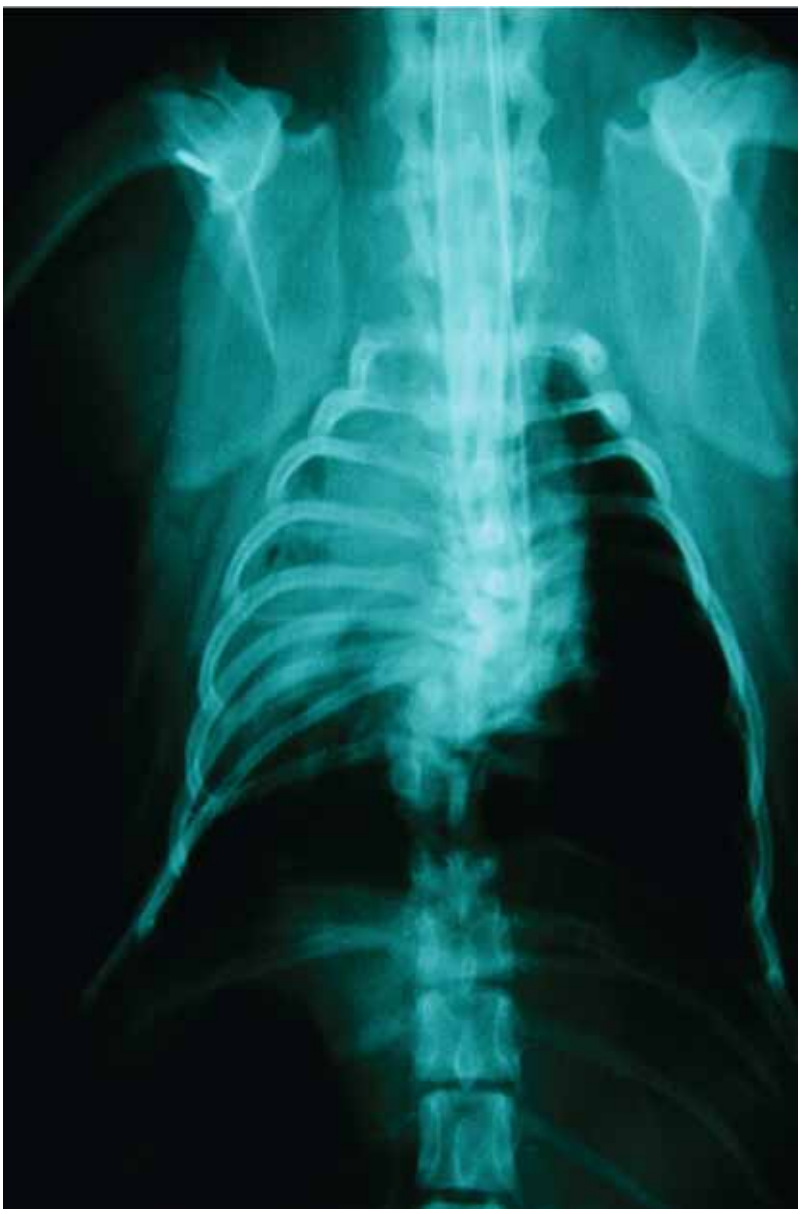

Fig. 3. Ventro-dorsal projection shows deviation of the spine, scoliosis, deformation of 8th thoracic vertebrae and also the stoppage of the contrast medium

side and vice versa and then the dog collapsed. The dog was standing for prolonged time only by external support in the caudal abdomen. He had bilateral bloody excoriations on the dorsal skin of his posterior limb digits.

The diagnosis of congenital vertebral anomalies relies on radiography to identify the bone development abnormality. However, because many of these dogs will have congenital vertebral anomalies in the absence of clinical sings, the definitive diagnosis that a vertebral anomaly is causing the problem relies on an accurate neurological examination, combined with demonstration of a spinal cord compression due to the hemivertebrae by myelography or advanced imaging (8).

The neurological examination in our case revealed no abnormalities in the thoracic legs. The patellar reflex on both sides was slightly enhanced, and the cranial tibial reflex was reduced. The proprioception, flexor reflex, as well as the corrective reflex was all reduced. The sensitivity and deep pain perception were present in the pelvic legs.

The radiographic examination revealed no pathological changes on the latero-lateral projection of the cervical spine. The radiograph of the thoracic spine showed a clear abnormality of the vertebral column (Fig. 1). In the region of Th6–Th9 we observed scoliosis of the spine with the body deformation of the 7th and 8th thoracic vertebrae which exhibited radiolucency and changed shape. Both hemivertebrae were wedge-shaped. This type of hemivertebrae is characteristic for this and other dog breeds.

A cranial myelographic examination was performed under total isoflurane (Isoflurane, Torrex Chiesi) inhalation anaesthe-

sia with propofol (Propofol 1 %, Fresenius Kabi) administered as an intravenous premedication. We administered iohexol into the cysterna magna (Omnipaque, Healthcare) at a dose of 0.5 ml.kg^{-1}. The radiograph showed the stoppage of the applied contrast medium at the level of the 7th thoracic vertebrae (Fig. 2 and Fig. 3). The diagnosis of hemivertebrae of the Th 8 was made according to these findings.

A dorsal hemilaminectomy was performed because of the increased movement disorders in the dog. The hemilaminectomy was performed using isoflurane (Torrex Chiesi) inhalation anaesthesia with premedication using diazepam 0.3 mg.kg^{-1} (Apaurin, Krka) and ketamine 4 mg.kg^{-1} (Narketan-10, Vétoquinol). The dog was placed in sternal recumbency and surgically approached from the left side. The hemilaminectomy was performed on the Th7–Th8. The exposed spinal cord was attached to the subcutaneous fat and the surgical wound closed in a standard way.

During the postoperative period, the dog was given opioid substances during 5 days after surgery and then NSAIDs for 5 days. The dog began to re-use its pelvic legs on the 3rd day after surgery. The strength in the muscles of his hind legs improved rapidly. The dog was able to move independently. The excoriations on the dorsal surface of the digits were cured in a few days and the skin eventually healed completely with regrowth of new hair.

During the first check-up (two months after surgery), the owners were satisfied with the dog's progress during the postoperative period; the dog was not given any anti-inflammatory drugs. The second check-up (six months after surgery), did not find any changes in the patient's condition. The dog was moving normally. Only during fast walking or running was there observed any minor uncoordinated movements of the pelvic legs. The skin on the dorsal surface of the digits was fully covered with hair. The length of the claws was the same on both rear legs. Walking in the fast-moving mode exhibited only a slight uncoordinated aspect and proprioception in general appeared only slightly affected.

DISCUSSION

Congenital spinal deformities in companion animals are reported as hereditary disorders (**7**). Hemivertebrae is a common abnormality in most small breeds of dog. It is most frequently described in French Bulldogs, English Bulldogs and Boston Terriers but other breeds can be also affected, including West Highland White Terriers, Pekingeses, Yorkshire Terriers, Fox Terriers, and German Pointers (12). Chondrodystrophic breeds of dogs are predisposed to formation of vertebral abnormalities. Unfortunately, there are scant or incomplete information available concerning the clinical outcomes after surgical treatment of hemivertebrae in dogs.

In human medicine for determining the prognosis and treatment models of this condition, the NASCA classification of spinal deformity is used (1). Congenital scoliosis and kyphosis often involve the process of somitogenesis and can be the result of different vertebral malformations, including wedge vertebrae, isolated hemivertebra, or butterfly vertebra (9). According to the wedge shape of two adjacent vertebrae, we included this type of Type 3 according to the NASCA classification.

Congenital spinal deformities manifests differently depending on the localization and the involvement of neural structures and in general tends to be progressive (3). After seeing the dog at first clinically without the application of NSAIDs, the dog in our case was unable to walk or move. After application of the NSAIDs we noticed that the dog could move with the pelvic limbs but quickly got tired and could not use them again. When performing the initial neurological examination, NSAIDs were not used for 48 hours. In this case, the surgical intervention relieved the pressure from the hemivertebrae condition to the spinal cord and the neurological deficits declined dramatically with no serious recurrence.

At 12 months of age, there was only a very mild residual neurological deficit discernible in the dog's pelvic legs. Walking was almost normal, except for occasionally there may be seen some minor uncoordinated movements of the pelvic legs when running.

Our result confirms that the surgical decompression was fully helpful and was successful from the point of view of locomotion and improving the patient's comfort.

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LOW-LEVEL LASER THERAPY AT 808 NM INCREASES TENSILE STRENGTH OF HEALING SKIN WOUNDS IN RATS

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ABSTRACT

The optimal parameters of low-level laser therapy (LLLT) have not been completely standardized and are still open for discussion. Therefore, our study was aimed at comparing the effects of different power densities of LLLT at 808 nm, in excisional and incisional models of wound healing in rats. Accordingly, one round full thickness skin wound and one incision site were performed on the backs of 15 rats which were randomly divided into three groups (control, laser-treated at 0.9 J.min^{-1}.cm^{-2}, and laser-treated at 1.2 J.min^{-1}.cm^{-2}). Rats from both laser-treated groups were stimulated daily with a diode laser, whereas the control group was sham irradiated. Seven days after surgery, all animals were sacrificed and samples removed for histological and biomechanical evaluation. No remarkable differences in the granulation tissue formation between groups were observed. In contrast, the highest wound tensile strengths were measured in rats treated with the lower tested power density. In conclusion, our results demonstrate that LLLT is able to improve wound healing. However, the laser parameters should be considered with the respect to wound type – incision vs. excision.

Key words: infrared laser; LLLT; power density; skin wound healing

INTRODUCTION

It is well known that complications associated with delayed wound healing significantly prolongs the treatment period of the patient. Several techniques, such as: VAC – vacuum assisted wound closure (20); magnetic field (14); phytotherapy (19); growth factors (10), etc., have been used to improve wound healing. Of note, it has already been shown that low-level laser therapy (LLLT) is also capable of improving wound healing (5, 18). Although, the exact mechanism of LLLT action on wound healing has not been fully clarified, it has already been documented that lasers in the red and near infra-red spectrum at the dose-range of approximately 5 J.cm^{-2} are able to: accelerate wound closure; increase collagen deposition; reduce inflammation; and in other ways may positively modulate the healing process (8, 15).

In contrast to those studies, it has also been shown that lasers induce deceleration of proliferation/healing rates after increasing the dose up to 20 J.cm^{-2} (11). Moreover, we showed that red lasers at 635 and 670 nm improve open wound healing in a power density dependent manner (8, 13). Furthermore, do Nascimento *et al.* (3) showed that LLLT at 670 and 685 nm is more effective when higher intensity is combined with shorter wavelength and lower intensity with longer wavelength. We confirmed this inverse relationship in another study where an incisional wound model was used and the healing rate was assessed by tensile strength measurements (21).

Whereas the incisional (sutured) skin healing model is preferred for wound tensile strength measurement (2), the excisional model is more appropriate for histological examination (6). Since significant differences in the healing course of both wound types do exist it may be hypothesized that the therapy of an excisional and an incisional wound should be different.

In general, it is well known that the infrared laser has significantly higher depth of tissue penetration when compared to a red one. Accordingly, it may be suggested that for deeper wounds as well as for granulation tissue formation, the infrared may be found more suitable. Therefore, in our recent experiments, we compared the influence of the different power densities of an infrared laser at

808 nm on skin wound healing using both incisional (wound tensile strength measurement) and excisional (histological evaluation) models of skin healing in rats.

MATERIALS AND METHODS

Animal model

This experiment was approved by the Ethic Committee of the Faculty of Medicine of P. J. Šafárik University and by the State Veterinary Administration of the Slovak Republic.

Ten-months-old male Sprague-Dawley rats (n = 15), weighing 500–550 g, were included in the experiments and randomly divided into three groups of 5 animals each: control sham-irradiated group; regular laser-treated group; and more powerful laser-treated group. Utilizing general anesthesia, ketamine 40 $mg.kg^{-1}$ (Narkamon a.u.v., Spofa, Prague, Czech Republic), xylazine 15 $mg.kg^{-1}$ (Rometar a.u.v., Spofa, Prague, Czech Republic), and tramadol 5 $mg.kg^{-1}$ (Tramadol-K, Krka, Novo Mesto, Slovenia), one incision (4 cm length) and two round full thickness skin excisions (4 mm in diameter) were performed on the back of each rat.

Low-level laser therapy

Wounds of each rat from the laser-treated groups were irradiated daily using a commercially available diode laser (B-Cure Laser, Good Energies, Haifa, Israel; λ = 808 nm; shape of beam oval, S = 4.5 cm^2, energy density of standard laser (SL) = 0.9 $J.min^{-1}.cm^{-2}$, energy density of the more powerful laser (PL) = 1.2 $J.min^{-1}.cm^{-2}$, treatment time 5 min.). The control group was sham irradiated to assure the same stress levels for all animals involved. During the wound treatments, rats were restrained in a Plexiglas cage, with an oval opening over the stimulated wound.

Wound tensile strength measurement – incisions

The device for measuring the wound-breaking strength was designed and constructed in our laboratory (7). Briefly, one side of a sample (wound) was fixed to the measuring tip of a force meter unit (Omega Engineering Inc., Stamford, Conn., USA). The second side of the wound was pulled horizontally by a slider arm driven by a high-precision stepper motor MDI-17 (Intelligent Motion Systems Inc., Marlborough, Conn., USA). The control unit supplies the motor and contains also an RS-422/USB converter and input/output connectors for manual control and a computer. Both motor and force meter are controlled by a computer via the USB serial ports. This device guarantees certified 0.2 % precision in the whole dynamic range of 0–5 kg.

The measurement technique utilized has been described in detail in our previous study (9). Briefly, samples including the skin wound, were removed from the body and trimmed to the optimal dimensions of 40 x 10 mm. The skin strip was lengthwise placed between the two clamps of the tensile strength testing device. The clamps were secured to avoid any slippage of the sample. Pulling was performed perpendicularly to the original direction of the incision.

The maximal breaking strength was measured for each sample. Tensile strength was calculated by using the following formula:

$$TS = MBS/A$$

where

TS – tensile strength ($g.mm^{-2}$), MBS – maximal breaking strength (g), and A – wound area (mm^2).

Histopathological evaluation – excisions

Five animals from all groups were sacrificed by ether inhalation seven days after the surgery. The tissue specimens were processed routinely for light microscopy (fixing, dehydrating, embedding, cutting, staining with hematoxylin and eosin, HE – basic staining). The following histological structures/processes were evaluated: PMNL (absence or presence of polymorphonuclear leukocytes); re-epithelization (migration of keratinocytes above the granulation tissue and process of differentiation detected by the presence of a keratin layer); fibroblasts (their presence near the injury site and in the newly formed granulation tissue); new vessels (presence of newly formed lumenized vessels in the granulation tissue); and collagen (absence or presence of collagen fibres detected by polarized light).

Statistical analysis

Data obtained from the quantitative evaluation of wound tensile strength were compared using the one-way ANOVA followed by Tuckey-Kramer post-hoc test. Significance was accepted at $P < 0.05$.

RESULTS

During the post-surgery period, animals remained healthy, without clinical evidence of infection. The results of our biomechanical and histological investigations are shown in Fig. 1 and 2, respectively.

Wound tensile strength – incisions

The wound tensile strengths of all groups are shown in Fig. 1.

The wound tensile strengths were similar in the control (325 ± 79 $g.mm^{-2}$) and PL-treated (321 ± 75 $g.mm^{-2}$) groups. In contrast, the SL demonstrated significantly increased wound tensile strength (389 ± 81 $g.mm^{-2}$, $P < 0.05$) when compared to both other groups.

Histology – excisions

Representative histological photomicrographs from all groups are shown in Fig. 2.

The histological analysis in these experiments demonstrated very low evidence of any inflammatory process in all wounds. Keratinocytes migrated beneath the scab, but did not completely bridge the excisions. This time period (seven days after surgery) showed a typical histological picture of the proliferation phase with expressive representation of fibroblasts and new vessels in all wounds, with no remarkable differences between the wounds (Fig. 2). The organization of collagen into fibres was not observed in the control group or in the laser-treated groups.

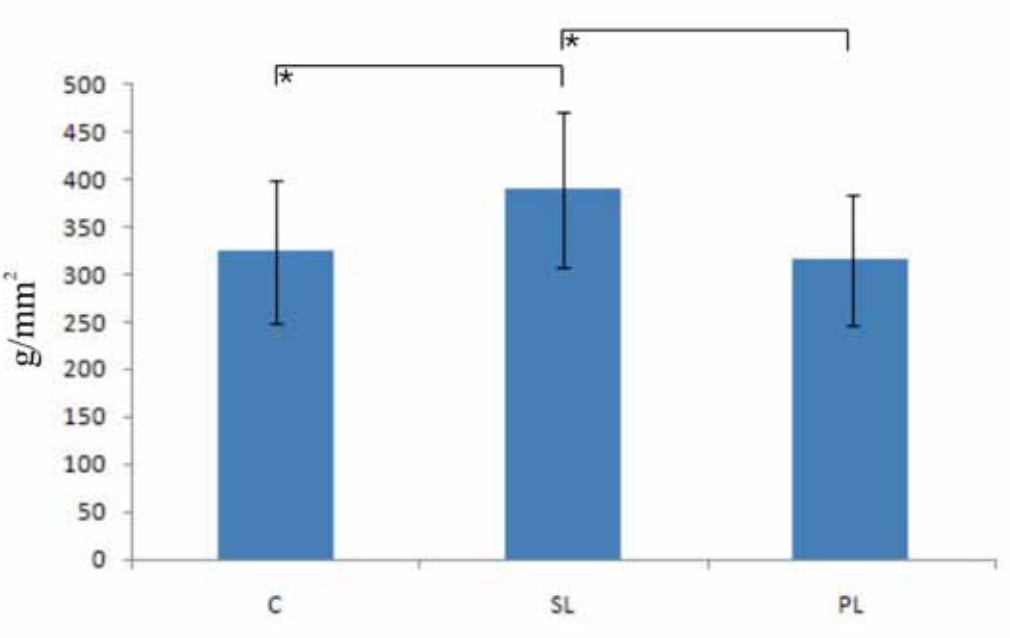

Fig. 1. Wound tensile strength of skin wounds removed 7 days after surgery from sham-treated control rats (n = 5), SL-treated rats (n = 5), and PL-treated rats (n = 5); (SL – standard laser with 0.9 J.min^{-1}.cm^{-2}; L – powerful laser with 1.2 J.min^{-1}.cm^{-2}; * P < 0.05)

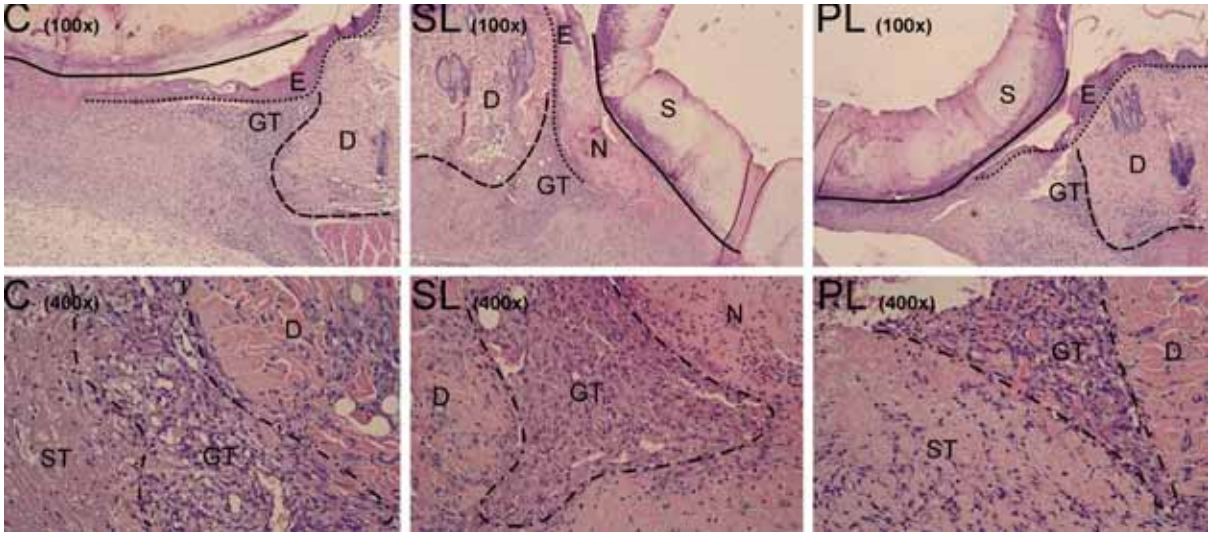

Fig. 2. Histology of skin wounds removed 7 days after surgery in sham-treated control (C) rats (n = 5), SL-treated rats (n = 5), and PL-treated rats (n = 5); (SL – standard laser with 0.9 J.min^{-1}.cm^{-2}; PL – powerful laser with 1.2 J.min^{-1}.cm^{-2}); (D – dermis, E – epidermis, GT – granulation tissue, N – tissue necrosis, S – scab, ST – subcutaneous tissue)

DISCUSSION

Although, the exact mechanism of LLLT is still not fully understand, it can be hypothesized that following a laser treatment the oxidative metabolism in mitochondria increases. This suggestion is supported by an experiment performed on HeLa cells where it was shown that cytochrome-c oxidase became more oxidized following irradiation at 633, 670, and 820 nm (12). However, by comparing the effects of different wavelengths and intensities on healing skin wounds, LLLT has demonstrated rather parameter-dependent effects. In this context, we showed that LLLT at 635 and 670 nm during the proliferative phase acted in a power density-dependent manner by means of accelerating re-epithelization and granulation tissue formation (8). In contrast to open wound healing, however, the 635 nm laser was more effective on wound tensile strength at the higher tested power density than the 670 nm laser at the lower power density (21). This is in agree-

ment with our results where we showed that the infrared laser at 808 nm significantly increased wound tensile strength at the lower tested power density.

The main difference between primary (incision – sutured) and secondary (excision/open – not sutured) wound healing is in the amount of granulation tissue that is formed during the proliferation phase of the healing. Of note, an open wound needs new tissue formation to restore the integrity of skin that includes extensive granulation tissue development (6). It is known that lasers have a wavelength-dependent tissue penetration, i.e. the absorbed energy for respective skin layers differs for each type of laser. In addition, depending upon the absorbed doses of LLLT, it has been shown that cells might be stimulated either to; proliferate (4), differentiate (16), produce collagen (1), and/or undergo apoptosis (17). From this point of view, in order to achieve the best outcome in a clinical practice, both the LLLT parameters, as well as, the wound type needs to be taken into consideration.

CONCLUSIONS

In conclusion, our results extend and reinforce the positive effects of LLLT on wound healing. Since inter-species differences do exist, our results obtained in the rat model need to be tested also on other animals before they can be introduced into the general veterinary clinical practice.

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SERUM ENZYME ACTIVITIES OF SIX DIFFERENT PRODUCTION CATEGORIES OF PIGS

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ABSTRACT

The aim of this investigation was to analyze the serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), and gamma glutamyl transferase (GGT) of six different production categories in pigs. The data presented were obtained from evaluating the blood samples of healthy pigs from a breeding herd. The samples were taken at six different points of the physiological production process: pregnant sows 1 week before farrowing; lactating sows 1 week after farrowing; weaned sows 1 week after weaning (artificially inseminated); piglets aged 14–21 days; weaned piglets 2 weeks after weaning; and fattening pigs 2 weeks prior to slaughter. The results showed significant differences in the values of the serum enzyme activities when comparing the six different production groups. The results obtained confirmed the importance of the age and production phase in determining more relevant and appropriate serum enzyme activities.

Key words: activity; ALP; ALT; AST; blood serum; CK; GGT; LDH; pigs

INTRODUCTION

Measuring the serum biochemical parameters of farm animals can provide important information on health and metabolism (4) and is a practical diagnostic tool for assessing pathological conditions in the live animal or for monitoring the health status of groups of animals (14). However, to be of actual significance as a useful diagnostic tool, it is necessary firstly to identify values that are sensitive enough to detect changes in the health status of the pigs, and secondly to serve as reliable reference values (14). The haematological and biochemical parameters of swine are influenced by a wide range of environmental and physiological factors including; diet, age, gender and housing (5). The detection of enzymes in serum by their catalytic activity as an indication of tissue damage, is a cornerstone of medical laboratory analyses (11).

The aim of this experimental work was to analyze and compare serum activities of AST, ALT, ALP, LDH, CK, and GGT of six different production categories of pigs.

MATERIALS AND METHODS

Animals

Thirty six clinically healthy pigs (Landrace) from one pig herd located in Eastern Slovakia were divided to six categories based on their different ages and production phases. Six pregnant sows 1 week before farrowing; six lactating sows 1 week after farrowing; six weaned sows 1 week after weaning (artificially inseminated); six sucking piglets aged 14–21 days; six weaned piglets 2 weeks after weaning; and six fattening pigs 2 weeks prior to slaughter were selected. The housing and feeding of all production categories were up to standard.

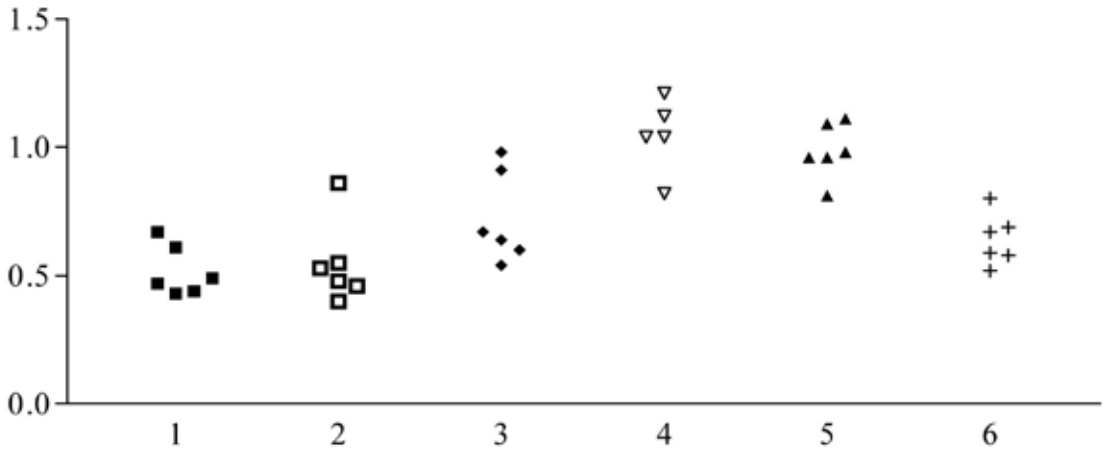

Fig. 1. Individual serum AST activities in pigs (μkat.l^{-1})

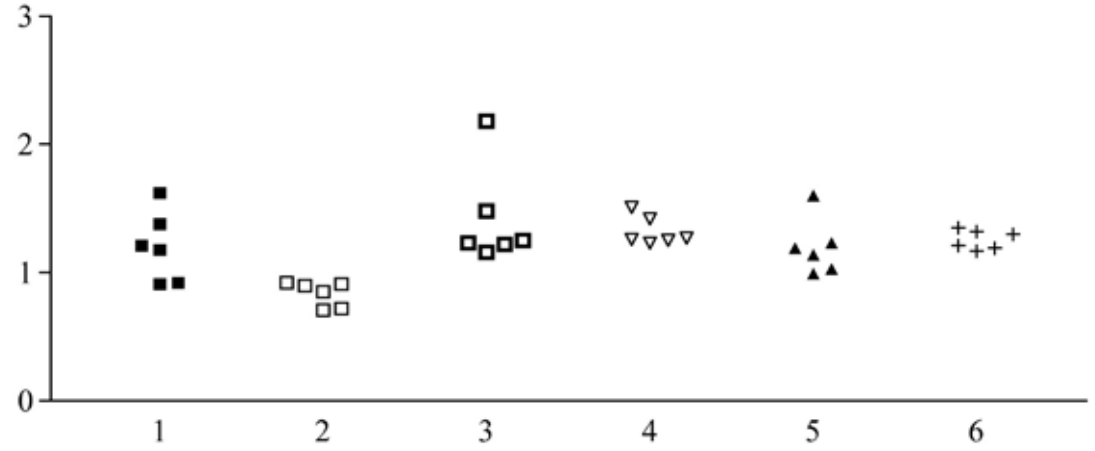

Fig. 2. Individual serum ALT activities in pigs (μkat.l^{-1})

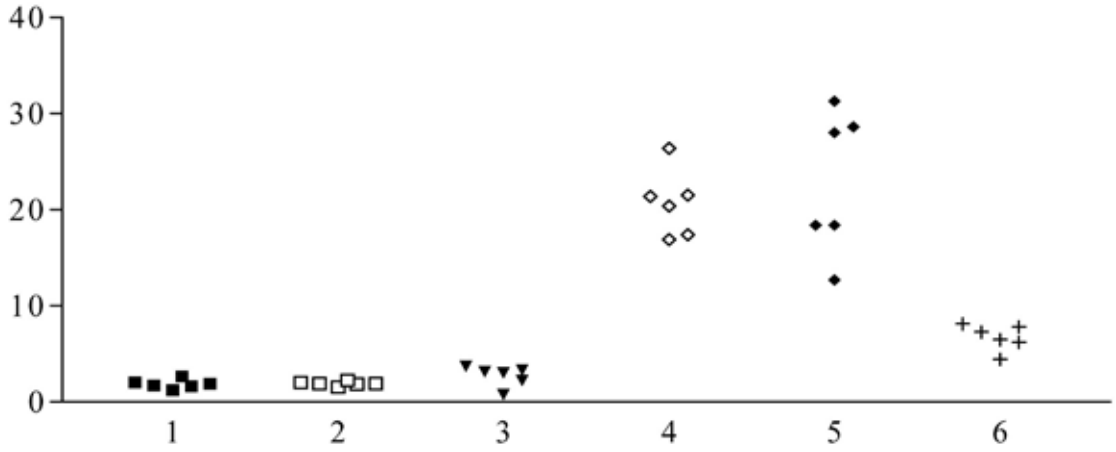

Fig. 3. Individual serum ALP activities in pigs (μkat.l^{-1})

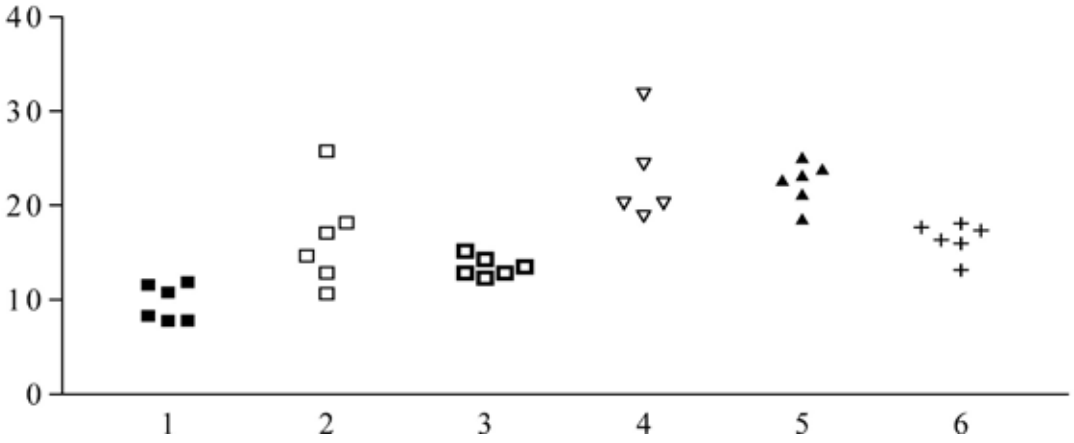

Fig. 4. Individual serum LDH activities in pigs (μkat.l^{-1})

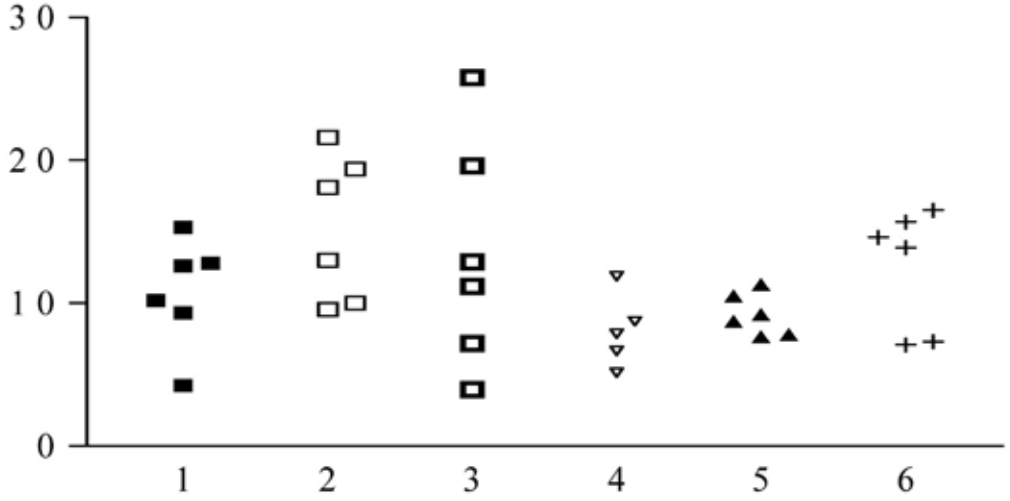

Fig. 5. Individual serum CK activities in pigs (μkat.l^{-1})

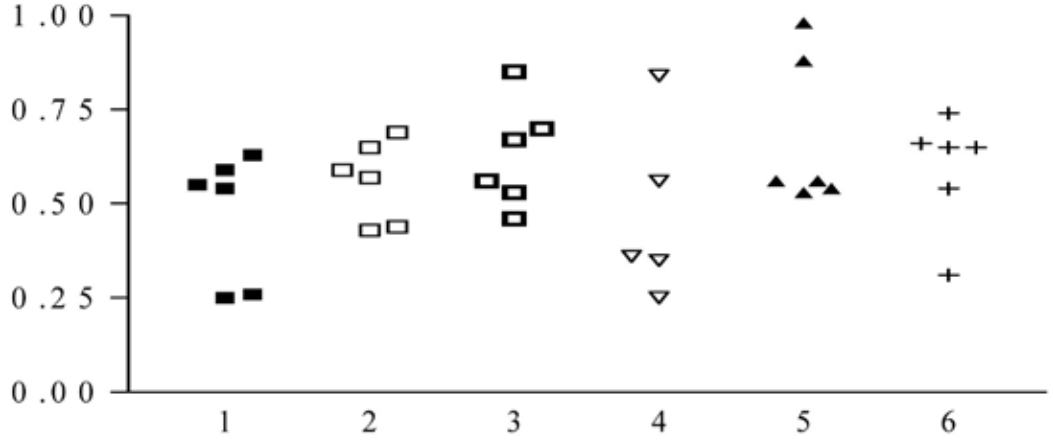

Fig. 6. Individual serum GGT activities in pigs (μkat.l^{-1})

Experimental design

Blood was collected from the *vena cava cranialis* (sows and fattening pigs), and the *sinus ophtalmicus* (sucking piglets and weaned piglets). Adult animals were restrained by a wire loop in the standing position. Sucking piglets and weaned pigs were restrained in a supine position; head and neck was straightened out and front limbs were drawn backwards. An adjustable V shaped cushioned restrainer, helped hold the animals in the correct position. The samplings were carried out aseptically according to common methods (7, 12).

Determination of activities of enzymes in blood serum

The enzyme activities of ALT, AST, ALP, CK, GGT and LDH were determined by the spectrophotometric method using commercial diagnostic tests (Enzyline®) and an automatic biochemical analyser ALIZE (Lisabio, France).

Statistical processing of results

The statistical processing of results was performed by assessment of means(x) and standard deviations (SD) in each group of pigs. The significance (P) of differences in the means of corresponding variables was evaluated by one way analysis of variance (ANOVA). The significance of differences between the groups using Tukey's Multiple Comparisons Test was evaluated at the same time. Statistical analyses were done with the GraphPad Prism 3.0 software (9).

RESULTS

The highest mean activity of AST (1.05 ± 0.13 μkat.l^{-1}; Table 1) was recorded in sucking piglets. This activity was significantly higher ($P < 0.001$) than the mean values of pregnant sows, lactating sows, fattening pigs and significantly higher ($P < 0.01$) than values of weaned sows. Significant differences were also found between pregnant sows and weaned pigs ($P < 0.001$), weaned sows and weaned piglets ($P < 0.05$), lactating sows and fattening pigs ($P < 0.01$).

The highest mean activity of ALT (1.42 ± 0.39 μkat.l^{-1}; Table 1) was recorded in weaned sows. This value was significantly higher ($P < 0.001$) than the value in lactating sows. Statistically significant differences were also found between lactating sows and weaned pigs ($P < 0.05$), lactating sows and fattening pigs ($P < 0.05$).

The highest mean activity of ALP (22.90 ± 7.40 μkat.l^{-1}; Table 1) was recorded in weaned pigs. This value was significantly higher ($P < 0.001$) than values in pregnant sows, lactating sows, weaned sows, and fattening pigs. Significant differences were also found between pregnant sows and sucking piglets ($P < 0.001$), lactating sows and sucking piglets ($P < 0.001$), weaned sows and sucking piglets ($P < 0.001$), sucking piglets and fattening pigs ($P < 0.001$).

The highest mean activity of LDH (23.1 ± 4.7 μkat.l^{-1}; Table 1) was recorded in sucking piglets. This activity was significantly higher ($P < 0.001$) than values in pregnant and weaned sows; and significantly higher ($P < 0.05$) than the values of lactating sows and fattening pigs. Significant differences were also found between pregnant and lactating sows ($P < 0.05$), pregnant sows and weaned pigs ($P < 0.001$), pregnant sows and fattening pigs ($P < 0.05$), lactating sows and weaned pigs ($P < 0.05$), weaned sows and weaned pigs ($P < 0.001$), and between weaned and fattening pigs ($P < 0.05$).

The highest mean activity of CK (15.28 ± 5.11 μkat.l^{-1}; Table 1) was recorded in lactating sows. A statistical analysis did not show any significant changes.

The highest mean activity of GGT (0.68 ± 0.20 μkat.l^{-1}; Table 1) was recorded in weaned piglets. A statistical analysis also did not show any significant differences between the examined groups.

DISCUSSION

Because the purpose of this study was to evaluate and compare parameters of enzymatic profiles in different pig

Table 1. Mean activities (x ± SD) of serum enzymes in pigs

	1 Pregnant sows	2 Lactating sows	3 Weaned sows	4 Sucking piglets	5 Weaned piglets	6 Fattening pigs
AST	$0.52 \pm 0.10^{1,4}$	$0.55 \pm 0.16^{2,B}$	$0.72 \pm 0.18^{A,a}$	$1.05 \pm 0.13^{1,2,3,A}$	$0.99 \pm 0.11^{4,a}$	$0.64 \pm 0.10^{3,B}$
ALT	1.20 ± 0.27	$0.84 \pm 0.10^{A,a,b}$	1.42 ± 0.39^{A}	1.34 ± 0.11	1.20 ± 0.22^{a}	1.26 ± 0.08^{b}
ALP	$1.85 \pm 0.48^{1,5}$	$1.90 \pm 0.24^{2,6}$	$2.70 \pm 1.07^{3,7}$	$20.72 \pm 3.4^{5,6,7,8}$	$22.90 \pm 7.4^{1,2,3,4}$	$6.73 \pm 1.33^{4,8}$
LDH	$9.71 \pm 1.9^{1,c,3,d}$	$16.57 \pm 5.3^{a,c,e}$	$13.52 \pm 1.07^{2,4}$	$23.1 \pm 4.7^{1,2,a,b}$	$22.5 \pm 2.3^{3,e,4,f}$	$16.47 \pm 1.8^{b,d,f}$
CK	10.75 ± 3.82	15.28 ± 5.11	13.45 ± 8.06	8.06 ± 2.27	9.21 ± 1.45	12.52 ± 4.21
GGT	0.47 ± 1.17	0.56 ± 0.11	0.63 ± 0.14	0.47 ± 0.21	0.68 ± 0.20	0.59 ± 0.15

Results with the same superscripts within a row differ significantly at $P < 0.05$ (a; b; c; d; e; f); $P < 0.01$ (A; B); $P < 0.001$ (1; 2; 3; 4; 5; 6; 7; 8)

categories, we deliberately chose only clinically healthy animals. So, the selected animals had no symptoms of local and/or systemic diseases (e.g. diarrhoea, mastitis, lameness, fever, apathy, etc.). Also, at the time of blood sampling, these animals had not been subjected to any veterinary treatment or intervention (e.g. vaccination, castration, dehelminthisation, etc.), because that might have influenced the activities of the analysed enzymes.

It is well known that one of the most relevant problems in the veterinary diagnostic laboratory is the establishment of reference values for haematological and haemato-chemical parameters. It is assumed that, if the animals are healthy, their parameters will follow a given distribution, while non-healthy subjects will show a different distribution (8).

Reference data often appear outdated due to changes in laboratory methods and apparatus. Furthermore, after weaning there is a sudden change in the environment and nutritional regime and so it is important to monitor the health status of gilts in this transition period. Moreover, reference data for consultation are frequently published with scant information on the number of animals involved, the methods of analysis adopted and the statistical methodologies; therefore these reference limits are often not comparable with values obtained for a given class of animals (3). Differences in biochemical and haematological parameters in swine of different ages are well documented by Friendship *et al.* (5), Reese *et al.* (10), Tumbleson *et al.* (13), Heath *et al.* (6), Dubreuil and Lapierre (1), and Egeli *et al.* (2). From these results it appears clear that age plays a key role.

The statistical evaluation showed significant differences between examined categories of pigs in serum AST activity ($P<0.05$; $P<0.01$; $P<0.001$), serum ALT activity ($P<0.05$; $P<0.01$), serum ALP activity ($P<0.01$), and serum LDH activity($P<0.05$; $P<0.001$). On the other hand, serum CK and GGT activities did not differ significantly. Sucking piglets had the highest AST and LDH activity (1.05 ± 0.13 and 23.1 ± 4.7 μkat.l^{-1}, respectively), weaned pigs ALP and GGT activity (22.90 ± 7.4 and 0.68 ± 0.20 μkat.l^{-1}, respectively), lactating sows CK activity (15.28 ± 5.11 μkat.l^{-1}), and weaned sows ALT activity (1.42 ± 0.39 μkat.l^{-1}). These results demonstrated that the activity of serum enzymes is diverse in different production categories of pigs. Reference values should be established specifically for every category of pigs. It results from different level of metabolism, growth and development of animal, different stages of gestation and many another factors which create differences between categories of pigs.

Our study confirmed previous conclusions (3),that age is a determining factor for blood reference intervals and those reference values should be determined by each laboratory, taking into account the age of subjects, the sample size and methods of analysis.

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OCCURRENCE OF DERMATOMYCOSES IN FARM ANIMALS IN EAST SLOVAKIA

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ABSTRACT

The aim of our study was to monitor the occurrence of dermatomycoses in farm animals in the Košice region in East Slovakia. We collected samples of hair and skin from 193 patients, which suffered from dermatitis. The samples originated from farms near Košice and the majority of them were from cattle (n = 107), but there were also samples from sheep (n = 45) and pigs (n = 41). Our monitoring lasted one year. Of 43 calves suspected of dermatophytosis the infection was proven in ten calves (23.3%). The causative agent was *Trichophyton verrucosum*. There were also two positive samples from cows (6.3%) affected by *Trichophyton verrucosum*. *Microsporum nanum* was found in one pig. The examined samples contained also saprophytic micromycetes of *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Alternaria alternata*, *Absidia* sp., *Candida* sp., *Cladosporium* sp. and *Aspergillus* sp., from all animal species in the study. The most frequent occurrence from all samples was recorded for *Aspergillus* sp. (21.6 %). Saprophytic fungi, e. g. *Alternaria* sp., *Aspergillus* sp. *Penicillium* sp., can also cause conjunctivitis and skin lesions, especially in sick animals. Our results showed that the correct etiological diagnosis of dermatitis should be based on collection of samples for mycological testing.

Key words: animals; dermatomycoses; *Microsporum nanum*; *Trichophyton verrucosum*

INTRODUCTION

An important group of micromycetes with an affinity for keratinized tissues of humans and other animals, causing superficial infections, are dermatophytes. They are significant pathogens in animals due to their zoonotic potential, the economic consequences of infection in farm animals and fur production systems and the distressing lesions they cause in small domestic pets (2).

Dermatophytes were known to Greeks and Romans. Thrush was noted in the Hippocrates writings as aphthae, and the Romans, according to Celsus, knew favus under the name porrigo (1).

Contagiousness among animal communities, the high cost of treatment, difficulty with control measures, and the public health consequences of animal ringworm explain the great importance of dermatophytoses. Certain *Trichophyton* species are cosmopolitan while others have a limited geographical distribution. There are twenty-two species under the genus *Trichophyton*. Out of the total number of species, eleven are commonly associated with tinea of the scalp, the nails, and the skin in humans, while only four are isolated from animals. *Trichophyton* is a keratinophilic filamentous fungus which has the ability to invade keratinized tissues. Possession of several enzymes, such as acid proteinases, lactase, keratinases, and other proteinases are the major virulence factors of *Trichophyton* species.

Infection has usually an enzootic character with a higher prevalence in young animals. Although a wide variety of dermatophytes have been isolated from animals, a few zoophilic species are responsible for the majority of the cases, e. g. *Microsporum canis, M. nanum*, *Trichophyton mentagrophytes*, *T. equinum* and *T. verrucosum*, and also the geophilic species *Microsporum gypseum* (3).

The aim of our study was to monitor the occurrence of dermatophytoses in farm animals in the Košice region in East Slovakia.

MATERIALS AND METHODS

The investigated set of animals included 107 cattle, 45 sheep and 41 pigs, originating from the farms located in the Košice region (Table 1). Our monitoring lasted one year. We collected 35 samples in spring, 38 in summer, 57 in autumn and 63 in winter. There were 144 samples from females and 49 from males.

Table 1. Number of samples from different animal species and categories

Calves	43
Heifers	32
Cows	32
Sheep	45
Weaned piglets	41
Total	193

The animals showed skin changes in the form of lesions typical for mycotic diseases. The focus of infection was cleaned with 70 % ethanol and skin scrapings and some hair were taken from the edge of the lesions with a sterile scalpel and placed into sterile sampling vessels.

One portion of the sample was used for direct microbiological examination of the pathogens, based on visualisation of fungal structures in the samples treated with 20 % KOH. The second portion was cultured on Sabouraud dextrose agars containing chloramphenicol and on Dermatophyte agar (HiMedia Laboratories, Mumbai, India) at laboratory temperature for 3–4 weeks.

The pathogens were identified on the basis of their macro- and microscopic appearance. The microscopic detection of the pathogen was carried out using lactophenol with cotton blue.

RESULTS

During our monitoring, we discovered that some severe cases of dermatitis were caused by dangerous fungi. Of 43 calves suspected of dermatophytosis, the infection was proven in ten calves (23.3 %). Their causative agent was *Trichophyton verrucosum*. There were also two positive samples with *Trichophyton verrucosum* revealed in cows. The percentage of positive *T. verrucosum* samples was 6.3 % out of 32 cows. *Microsporum nanum* was detected in one sample from a pig. Skin lesions were found on the head, particularly close to the eyes and ears, and on the neck and chest of the animals (Table 2).

The majority of the positive samples were collected in the winter, but we found *T. verrucosum* also in the summer and autumn. Saprophytic fungi were evident throughout the year, but predominantly in the summer and winter (Table 3).

The examined samples contained also saprophytic micromycetes of genera *Penicillium*, *Mucor*, *Rhizopus*, *Alternaria*, *Absidia*, *Candida*, *Cladosporium* and *Aspergillus* from all categories of cattle. *Mucor* sp. was the micromycetes most frequently occurring in calves (67.4 %), followed by *Rhizopus* sp. (55.8 %) and *Penicillium* sp. (48.8 %).

Table 2. Localization of fungi on animal bodies

	Regions of body					
	Udder	**Head**	**Trunk**	**Ears**	**Legs**	**Nose**
Trichophyton verrucosum		5	7			
Trichophyton terestre				2		
Microsporum nanum				1		
***Penicillium* sp.**	1	3	24	33	1	3
Alternaria alternata		2	22	1		
Geotrichum candidum		1	2	3	1	
***Mucor* sp.**	4	4	36	43	3	3
***Absidia* sp.**	1		4	19	1	1
Aspergillus parasiticus	1	4	10	20	2	
Aspergillus flavus	1	1	15	18		
Aspergillus fumigatus	2		9	14	2	
Aspergillus niger			7	1		
***Cladosporium* sp.**		2	8	3		
***Candida* sp.**	2	2	6	26	4	1
***Rhizomucor* sp.**			1	2	1	
Trichoderma viride		2	8			
Doratomyces stemonitis			2			
***Chaetomium* sp.**			1			
***Rhizopus* sp.**	2	1	7	61	2	2
***Fusarium* sp.**			5	1		

We found saprophytic micromycetes also in the samples from cows. The most frequent were *Mucor* sp. (71.9 %), followed by *Penicillium* sp. and *Aspergillus fumigatus* (50.0 % both).

In pigs the most frequent saprophytic fungi were *Rhizopus* sp. (34.1 %) and *Mucor* sp. (26.8 %). The findings of *Candida* sp., which belong to yeasts, were also frequent (36.6 %).

Rhizopus sp. and *Mucor* sp. were the most frequent (37.8 % and 35.6 %, respectively) in the samples from sheep (Table 4).

Table 3. Occurrence of fungi according to seasons during the year

	Spring	Summer	Autumn	Winter
Trichophyton verrucosum		4	2	6
Trichophyton terestre				2
Microsporum nanum				1
***Penicillium* sp.**	7	25	8	25
Alternaria alternata	2	13	4	6
Geotrichum candidum		4		3
***Mucor* sp.**	14	33	31	15
***Absidia* sp.**	4	13	2	7
Aspergillus parasiticus	8	6	8	15
Aspergillus flavus	7	17	4	7
Aspergillus fumigatus		14	5	8
Aspergillus niger		6		2
***Cladosporium* sp.**		8	3	2
***Candida* sp.**	7	15	8	14
***Rhizomucor* sp.**	3	1		
Trichoderma viride		7	1	2
Doratomyces stemonitis		2		
***Chaetomium* sp.**			1	
***Rhizopus* sp.**	14	20	11	30
***Fusarium* sp.**		5	1	

DISCUSSION

The occurrence of dermatophytes in our study was not as high as in the study by Papini *et al.* (9). The epidemiology of the zoonotic dermatophyte *Trichophyton verrucosum* was investigated in central Italy. The total prevalence rate of *T. verrucosum* infection was found to be high, with 87.7 % of the samples yielding positive results and 100 % of the farms being infected. The prevalence rates were higher in calves under 6 month of age, than in older calves (89.8 % *versus* 85.7 %). *T. verrucosum* infection may be more widespread in calves from central Italy than it was previously thought to be.

Our findings indicated that farmers in East Slovakia should also pay attention to the prevention of dermatomycoses. In our study, we found *Trichophyton verrucosum* in cattle and *Microsporum nanum* in pigs. The most frequent agent of dermatophytoses in ruminants was *Trichophyton verrucosum* and in some cases, also other species were isolated, such as *T. mentagrophytes*, *T. rubrum*, *T. equinum*, *Microsporum gypseum*, *M. canis* and *M. nanum* (7, 4). *Microsporum nanum* was detected in pigs.

Our results are in accordance with other authors. They reported that dermatophyte infections had a higher prevalence in young animals (9). The disease incidence was higher in cattle than in sheep and goats (10). In our study, a higher number of positive samples originated from calves than from older cattle and no positive sample were collected from sheep.

Mycotic infections caused by dermatophytes are presented in affected calves as well-defined, circular, grayish-white, thick lesions found on the head, around the eyes, on the neck and forelegs (5). Also in our study, most of the skin lesion were observed on the head and chest.

According to the experience of mycologists and veterinarians, saprophytes present in soil and air and on plants and other material that animals came into contact with, are potential causative agents of animal dermatomycoses (6, 8). Our results with the high percentage of findings of the genera *Penicillium*, *Mucor*, *Rhizopus*, *Candida* and *Aspergillus* showed that also saprophytic fungi can be potential pathogens.

CONCLUSION

Our study revealed that the prevalence of *Trichophyton verrucosum* in calves was 23.3 % and in cows 6.3 %. *Microsporum nanum* was found in one pig (prevalence 2.4 %). In addition to the genera Trichophyton and Microsporon, we detected many saprophytic fungi, i. e. *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Alternaria alternata*, *Absidia* sp., *Candida* sp., *Cladosporium* sp. and *Aspergillus* sp. from of all of the investigated farm animal species.

The results obtained indicate that examination for the causative agent of animal dermatitis is justified, particularly in animals suspected of mycotic infections. Despite the fact that dermatophytes are regarded to be the primary pathogens of mycoses, one must also consider the possible participation of saprophytic mycoflora.

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THE STUDY OF ARTERIAL ARRANGEMENT OF THE CERVICAL SPINAL CORD IN RABBIT USING DISSECTION TECHNIQUE

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ABSTRACT

The aim of this study was to describe the arrangement of the arteries supplying the cervical spinal cord in the rabbit. The cervical region in the rabbit is the experimental model for the study of several types of the spinal cord damage. The study was carried out on 20 adult New Zealand white rabbits. The arterial system of the cervical spinal cord was injected by using Batson's corrosion casting kit No. 17. The presence of branches entering the ventral spinal artery in the cervical region was observed in 46.2 % of the cases on the right side and in 53.8 % of cases on the left side. We found no origin of the dorsal spinal arteries in the area of the formation of the basilar artery. When the two irregular dorsal spinal arteries are present, they are formed only by fusion of the small cranial and caudal branches arising from the dorsal branches. Our results confirm the need for more detailed knowledge of the blood supply in laboratory animals which are frequently used as experimental models.

Key words: dissection; dorsal spinal artery; segmental artery; ventral spinal artery

INTRODUCTION

Experimental studies on laboratory animals, and the detailed knowledge of the anatomy of the spinal cord blood supply with all existing variations, can contribute to the essential knowledge of the spinal cord. Rabbits are the laboratory animals frequently used in the studies of spinal cord ischemic damage. The cervical spinal cord is often used as an experimental model for the study of: spondylotic myelopathy (1, 3); axonal degeneration; spinal arachnoiditis; ischemic stroke (5, 7); and some other pathological conditions.

We describe some variations in the arrangement of the segmental spinal arteries in the cervical region of the rabbit.

MATERIALS AND METHODS

This study was carried out on 20 adult rabbits (age 140 days). We used New Zealand white rabbits (breed HY+) of both sexes (female n = 10; male n = 10) with an average weight 2.5–3 kg 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-hour light period), and fed with a granular feed mixture (O-10 NORM TYP). Drinking water was available to all animals ad libitum. 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 through the ascending aorta, the right atrium of the heart was opened in order to lower pressure in the vessels to ensure a good injection. Batson's corrosion casting kit No. 17 using a quantity of 35 ml (Dione, České Budějovice, Czech Republic) was used as a casting medium. After polymerization, 10 % formaldehyde was injected into the vertebral canal between the occipital bone and the first cervical vertebra and between the sixth and seventh cervical vertebra to fix the spinal cord. After 1-week fixation, the vertebral canal was opened by removing the vertebral arches in the cervical spinal region. Also, the occipital bone was partly removed. The prepared spinal cord was fixed in 10 % formalin. This study was carried under authority decision No. 2647/07-221/5.

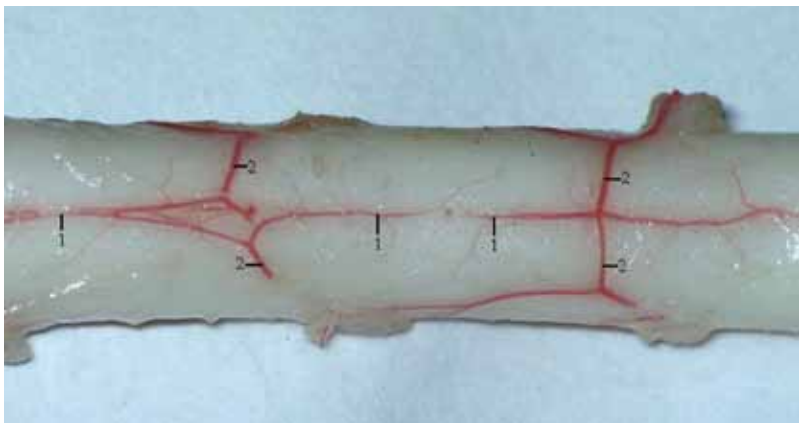

Fig. 1. Ventral branches of spinal arteries entering the ventral spinal artery. 1– Ventral spinal artery; 2 – Ventral branch of spinal artery; Ventral view. Magn. × 8

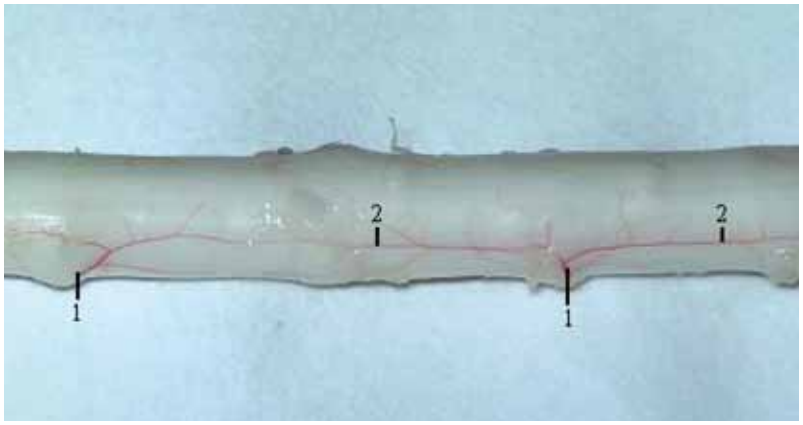

Fig. 2. Dorsal branches of the spinal arteries entering the irregular dorsal spinal arteries. 1 – Dorsal branch of spinal artery; 2 – Irregular dorsal spinal artery. Dorsolateral view. Magn. × 5

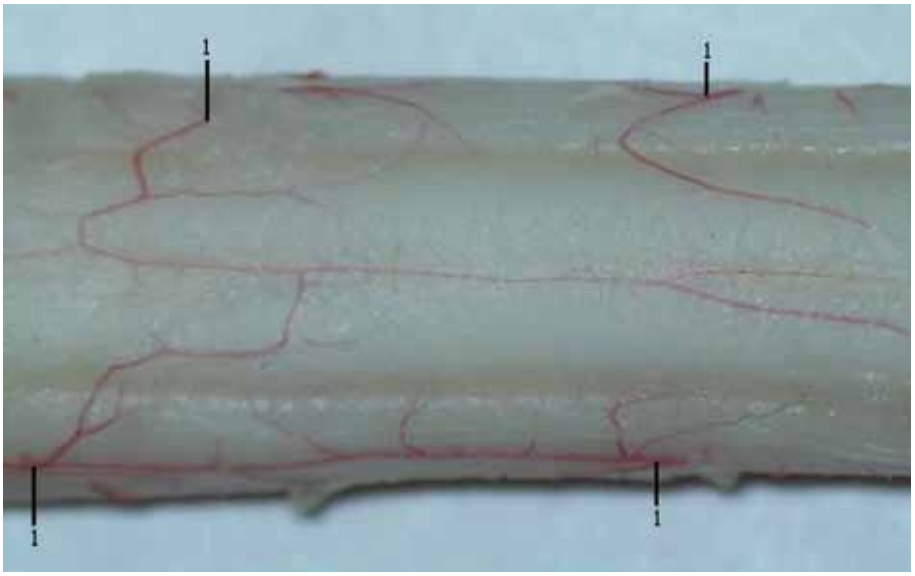

Fig. 3. Dorsal branches of the spinal arteries forming irregular loops. The typical longitudinal dorsal spinal arteries are absent. 1 – Dorsal branch of spinal artery. Dorsal view. Magn. × 12.5

Table 1. Frequency of occurrence of spinal arteries of the cervical spinal cord (%)

	Right	Left
C1	0	0
C2	70	50
C3	50	30
C4	50	50
C5	30	50
C6	20	70
C7	30	50
C8	50	50

C – cervical segment of the spinal cord

RESULTS

The ventral spinal artery runs along the ventral median fissure of the spinal cord. Spinal arteries as segmental branches of bilateral vertebral arteries enter the vertebral canal at the level of the intervertebral foramina. The spinal arteries after entering the vertebral canal send ventral and dorsal branches to the spinal cord. The ventral branches enter the ventral spinal artery (Fig. 1). The frequency of occurrence of individual ventral branches is shown in Table 1. The presence of branches entering the ventral spinal artery in the cervical region was observed in 46.2 % of cases on the right side and in 53.8 % of cases on the left side.

On the dorsal surface we found two irregular longitudinal arteries receiving dorsal branches of spinal arteries (Fig. 2) or they were absent (Fig. 3). These two longitudinal arteries were highly variable in their arrangement. We found no origin of dorsal spinal arteries in the area of the formation of the basilar artery. When the two irregular dorsal spinal arteries are present, they are formed only by fusion of the small cranial and caudal branches arising from the dorsal branches. The frequency of occurrence of dorsal branches parallels that of the ventral branches. They formed irregular loops between each other on the same and on the opposite side.

DISCUSSION

Based on the results of this study, it can be concluded that the blood supply of the cervical spinal cord in the rabbit has high variability. Dogs, rats, pigs and rabbits are often used in the study of cervical spinal cord injuries. The dog is the only one of these species whose cervical spinal cord has been studied in detail, with description of variations in the origin of the ventral spinal artery and the frequency of occurrence of spinal arteries (6). The rat is also one of the most studied species, but the results of several studies differ (9, 12). In the pig, only variations and the presence of extrasegmental arteries of the spinal cord blood supply have been described (10, 11). The frequency of the occurrence of segmental spinal arteries was higher on the left than on the right side, i.e., opposite to the situation in dogs (6).

C h a k r a v o r t y (1) studied the arterial blood supply of the spinal cord in the cervical region in monkeys, dogs, rabbits and rats. The ventral spinal artery was also described as paired vessels. We found this artery to be a single trunk. In this latter study, the presence of one single segmental spinal artery was observed in 64.5 % of cases, and in our study in only one case (5 %).

In humans, the anterior spinal artery is formed at the level of the foramen magnum only by fusion of the anterior spinal branches of the vertebral arteries (8). On the dorsal surface we found two irregular or absent dorsal spinal arteries (in human the posterior spinal arteries). The posterior spinal arteries in human are normally continuous rostral to caudal and supply the posterior third of the spinal cord (2).

We found right-sided ventral branches entering the ventral spinal artery in 46.2 % and left-sided in 53.8 % of cases. Only 2 or 3 ventral branches entering the anterior spinal artery were described in humans (4). The part of the spinal cord at the level of the first cervical vertebra was supplied by small branches arising from the posterior inferior cerebellar artery.

CONCLUSIONS

The arterial arrangement of the cervical spinal cord in the rabbit is more segmental than in humans. The results of our study indicate a higher resistance to ischemic damage by the interruption of ventral and dorsal spinal arteries because of the presence of radicular arteries in almost every segment.

Until the cervical spinal cord arterial arrangement in the species of laboratory animals is described in detail, it will be very difficult to determine the appropriate species for experiments in this field. Variations in arterial arrangement can produce biased or erroneous results in studies.

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IMPACT OF DIETARY INTAKE OF OREGANO ESSENTIAL OIL ON PARAMETERS OF DIGESTION AND CAECAL MICROFLORA OF BROILER CHICKENS

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ABSTRACT

This study focused on the evaluation of the effects of the dietary intake of oregano (*Origanum vulgare* L.) essential oil on: digestive characteristics; some digestive enzyme activities in the chyme of the jejunum; and the selected bacterial microflora in the caecum of chickens. Seventy (one-day-old) broiler chickens (Ross 308) were allocated into two equal groups (control and experimental) for 42 days. The experimental group received a diet supplemented with the essential oil at the level of 1.179 g.kg^{-1} of the feed mixture. The concentration of carvacrol, the main component of the oregano essential oil, was 600 g.kg^{-1} of the essential oil. The digestibility coefficient of crude fibre increased by day 16 (46.32 ± 0.787, P < 0.001), day 29 (50.58 ± 1.518, P < 0.001) and day 42 (47.18 ± 1.180, P < 0.01). The apparent assimilable mass coefficient of crude protein, corrected for protein catabolism, decreased on day 16 (0.19 ± 0.026, P < 0.01), day 29 (0.19 ± 0.027, P < 0.05) and day 42 (0.20 ± 0.019, P < 0.05). The amylolytic and cellulolytic activities in the chyme of the jejunum increased by day 29 [(0.14 ± 0.013, P < 0.001) and (0.13 ± 0.03, P < 0.001), respectively]. The cellulolytic activity increased by day 42 (0.21 ± 0.025, P < 0.01), as well. The proteolytic activity decreased by day 16 (0.36 ± 0.038, P < 0.01). The counts of *Escherichia coli* in the caecum decreased (P < 0.05) by day 29 (3.1 × 10^6) and day 42 (2.3 × 10^6). The dietary supplementation with oregano essential oil beneficially increased the crude fibre digestibility, the jejunal amylolytic and cellulolytic activities, as well as decreased the counts of *E. coli* in the caecum.

Key words: essential oil; carvacrol; chickens; digestibility measurement; oregano

INTRODUCTION

Feed additives have been used successfully for decades to secure and enhance the performance of food animals. During recent years, the search for new substances has been intensified. The use of secondary metabolites of medicinal, aromatic and spicy plants containing essential oils with inhibitory characteristics against pathogens represents a significant alternative approach.

Plant extracts contain a vast source of different molecules which have intrinsic bioactivities on animal physiology and metabolism. Many of these compounds have been used in the form of whole plant extracts for food or medical applications in man (31). The explanatory power of many studies is rather limited due to the use of blends of different phytogenic feed additives, making it difficult to assess the individual effects. On the one hand, oregano (*Origanum vulgare* L.) is a common ingredient of herbal feed additives but on the other hand, only few studies are available using oregano as a single supplement (25). The assumption that phytogenic compounds might improve the palatability of feed has not yet been confirmed by choice-feeding studies. Although numerous studies have demonstrated antioxidative and antimicrobial efficacy *in vitro*, respective experimental *in vivo* evidence is still quite limited. The same applies to the supposition that phytogenic compounds may specifically enhance activities of digestive enzymes and nutrient absorption (32). However, the influence of the oregano essential oil on digestive parameters in relation to enzyme activities and intestinal microflora population is not clear.

From this reason, the objective of this study was to evaluate the influence of the dietary intake of the oregano essential oil on: digestive characteristics; some digestive enzyme activities in the chyme

of the jejunum; and selected bacterial microflora in the caecum of broiler chickens.

MATERIALS AND METHODS

Chickens, diets and oregano essential oil

For the trial, 70 one-day-old broiler chickens of Ross 308 hybrid were obtained from a commercial hatchery. Individually weighed chickens were divided at random into 2 groups of 35 animals each (control and oregano). The average initial body weights of the individual chickens on the first day in the control and experimental groups were 38.86 ± 6.71 g and 39.29 ± 8.31 g, respectively. The chicks were housed in two floor pens located in one hall of a commercial broiler chicken fattening farm (Michalovce, Slovak Republic). The two pens were identical, with the same direction and the same covered area (0.12 m^2 per broiler chicken). The animals had constant access to feed and water. The broiler chicken flock was fed with mash diets: starter, grower and finisher (DOMICA Ltd., Slovak Republic) for 42 days (Table 1).

The essential oil was isolated from oregano tops (*Origanum vulgare* L., family *Lamiaceae*) by steam distillation of the plant materials in Calendula Inc. (Nová Ľubovňa, Slovak Republic). The percentage range of the main active component (carvacrol) was analysed by gas chromatography (GC) using Hewlet-Packard 5890 Series II (injection input split splitless, capillary column HP-5, detector FIF, automatic injector HP 7673) with nitrogen as the carrier gas (11). The experimental group received a diet supplemented with oregano essential oil at a level of 1.179 $g.kg^{-1}$. The control group of the trial was fed with an identical ration without the essential oil. During the experiment, the body weights of the chickens were assessed once a week. Feed was weighed to evaluate the feed consumption.

Feed analysis

Samples of the experimental diets were analyzed (Table 1) according to the methods of the Association of Official Analytical Chemists (8). The analysis were conducted for dry matter DM, crude protein CP, crude fat, ash, starch and total carbohydrates. Crude fibre was analyzed by the common method (29). The amino acid analysis of the experimental diet was determined by high performance liquid chromatography. The AAA 400 amino acid analyser (INGOS, Czech Republic, www.instruments.ingos.cz) is a special compact liquid chromatographic designed for the analysis of amino acids on an ion exchanger column with a post-column derivatisation by means of ninhydrin. Lysine was determined after hydrolysis for 24 h at 110 °C with 6 $mol.l^{-1}$ HCl. Sulphur-containing amino acids, methionine and cystine, were analyzed after cold formic oxidation for 16 h before acid hydrolysis. The mineral composition of the feed (the content of calcium and sodium) was determined by atomic absorption spectrophotometry (AAS) with Shimadzu AA 6200, following the feed sample ashing in a muffle furnace (28). The content of phosphorus was determined spectrophotometricaly (7). The insoluble portion of ash in HCl was determined in the feed mixture as the residue of ash, after dissolving ash in diluted hydrochloric acid by weighing (9).

Blood sampling

When the chickens reached 16, 29 and 42 days of age, 10 broilers from each treatment were selected at random and were anaesthetized with intraperitoneal injections of xylazine (Rometar 2 %, SPOFA, Czech Republic) and ketamine (Narkamon 5 %, SPOFA, Czech Republic) at doses 0.6 and 0.7 $ml.kg^{-1}$ body weight, respectively. Therefore, before anesthesia the numbers of birds in the groups on day 16, 29 and 42 were 35, 25 and 15, respectively. After laparotomy, blood samples were taken by intracardial punction. The samples for preparation of the sera were collected into anticoagulant-free tubes. The blood sera were separated by centrifugation and kept at 20 °C until examined by the tested procedures. The serum total protein concentration was determined as $g.l^{-1}$ by the Bradford method at 595 nm wavelength (3). The serum content of calcium, magnesium and potassium was determined by AAS (28). The content of phosphorus was determined spectrophotometrically (7).

Sampling of intestinal content and analytical studies

During the necropsy, the samples of chyme from the jejunum and the caecum were placed into sterile tubes immediately after slaughtering, for digestive enzyme analyses and microbiological assays. The preparation of the samples of chyme from the jejunum for the quantification of digestion enzymes activities was performed as follows. One gram of the fresh sample was diluted with 49 ml sterile TBS buffer (TRIS-hydroxymethyl aminomethane 10 $mmol.l^{-1}$, HCl 0.5 $mol.l^{-1}$, pH 7.0). After homogenization, the samples were taken for the measurement of the nonspecific proteolytic activity (4) with the substrate azocasein (Merck, Germany). The cellulolytic and the amylolytic activity (22) were analysed with the substrates methylhydroxyethylcellulose (Merck, Germany) and starch (Fisher Slovakia Ltd.), respectively. The quantification of the total protein concentration was performed by the Bradford method (3).

For the microbiological examination, the caecal digesta were diluted 10 fold (1 : 9 w/v) by blending them with the anaerobically sterilized TBS buffer. Then a 0.1 ml sample was diluted by 10^{-2}—10^{-7} and spread onto sterilized selective nutrient media – *Escherichia coli* (McConkey agar, Merck Ltd., Germany), *Enterococcus* spp. (Slanetz-Bartley agar, Merck Ltd., Germany) and *Lactobacillus* spp. (Rogosa agar, Merck Ltd., Germany) for cultivation. The numbers of Colony Forming Units ($CFU.g^{-1}$) of wet caecal digesta of *E. coli* and *Enterococcus* spp. were optically enumerated after aerobic cultivation for 24 h, whereas *Lactobacillus* spp. after stationary anaerobical cultivation for 48 h at 37 °C.

Digestibility measurement

A check of digestibility was performed on days 16, 29 and 42. The samples of excreta were collected directly from the cloaca into sterile glass containers on the designated day.

The content of crude protein was measured in the excreta. The individual parameters of crude fibre, ash and portion of insoluble ash in HCl were analysed in the excreta according to the methods of D a n e k *et al.* (9).

The digestibility coefficient (DC) was calculated according to the formula:

$$100 - \frac{\text{measured parameter in sample of excreta/ash insoluble in HCl in excreta}}{\text{measured parameter in sample of feed mixture/ash insoluble in HCl in feed mixture}}$$

The digestibility was determined by calculating the analyzed content of nutrients in feed and excreta concerning the content of the insoluble portion of ash.

Because a part of the crude protein in the excreta originates from uric acid, the faecal crude protein should be corrected for uric acid nitrogen. At first, the correction for endogenous losses resulting from protein catabolism was performed by determining the crude protein balance (N_b) of the animal ($N_b = Q_iN_i - Q_eN_e$). Where N_i represents the crude protein content ($g.kg^{-1}$) of the food, and N_e the crude protein content ($g.kg^{-1}$) of the excreta, Q_i and Q_e are the rates of food intake and excretal output ($g.kg^{-1}.day^{-1}$). Subsequently, the crude protein losses to the moles of uric acid were converted to calculate the mass losses associated with uric acid excretion (26). The mass correction for uric acid nitrogen is 3 $g.g^{-1}$ N (19). Given these values, the equation for the apparent assimilable mass coefficient of crude protein corrected for protein catabolism (AMC_N) is: $AMC_N = 1 - ([Q_e + 3(N_b)]/Q_i)$ (13, 15).

The data are expressed as means ± standard deviation (SD) of single values (SAS, Version 8.2; SAS Institute Inc., 1999, Cary, NC USA). Means of the results from the treatments were compared by one-way analysis of variance. Treatment means were statistically compared by Tukey-Kramer multiple comparison test. Significance was declared at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

The concentration of the main active volatile compound of the oregano essential oil, carvacrol, was at the level of 600 ± 3 $g.kg^{-1}$. The analysed density of the essential oil was 0.949 ± 0.001 $g.cm^{-3}$.

The digestibility data of crude fibre and ash, as well as the apparent assimilable mass coefficient of crude protein, corrected are summarized in Table 2. The digestibility coefficients demonstrated a better utilisation of the experimental feed mixture, as far as the crude fibre is concerned. The intake of the oregano essential oil increased the digestibility of crude fibre on days 16, 29 ($P < 0.001$) and 42 ($P < 0.01$). The AMC_N value decreased in the experimental group on days 16 ($P < 0.01$), 29 and 42 ($P < 0.05$).

Digestive enzyme activities (amylolytic, cellulolytic and proteolytic) were assessed in the chyme of the jejunum (Table 3). The intake of the oregano essential oil increased the amylolytic activity ($P < 0.001$) on day 29. The cellulolytic activity was increased on days 29 ($P < 0.001$) and 42 ($P < 0.01$). The decrease of the proteolytic activity ($P < 0.01$) was observed on day 16.

The changes of total protein and macroelements (phosphorus, potassium, calcium, magnesium) in the serum are shown in Table 4. The level of serum total protein was higher ($P < 0.05$) in the experimental group on day 29. The essential oil caused an increase of calcium ($P < 0.001$) and magnesium ($P < 0.01$) on day 42.

The microbial counts in the caecal chyme of broiler chickens are summarized in Table 5. The total counts of *E. coli* in the caecum were lower in the experimental group on days 29 and 42 ($P < 0.05$).

The average final body weights of the individual chickens in the control and experimental groups were 1746.67 ± 340.52 g and 1840.00 ± 231.00 g on day 42. No significant differences in the average daily feed intake were observed between the groups during the experiment.

DISCUSSION

The chemical composition of the oregano essential oil depends on: the seasonal changes of the plants; extraction methods (steam distillation); the method of recovery of the extractions, standard samples of volatile compounds used; and the sensitivity of the analytical method applied for the quantitative and qualitative analysis (GC). One method used, contains the following components; carvacrol (40–70 %), gamma-terpinene (8–10 %), p-cymene (5–10 %), α-pinene, myrcene, thymol, flavonoids, and caffeic acid derivatives (10).

The positive effects of the oregano essential oil on the digestibility of the crude fibre were observed in all of the sampling periods. This fact is supported by the increased values of the cellulolytic activity.

Table 1. Analyzed chemical composition of the experimental diets ($g.kg^{-1}$ diet)

	Experimental diets		
Ingredients	**Starter**	**Grower**	**Finisher**
Dry mater	881.90	890.27	898.04
Crude protein	248.20	223.10	214.40
Crude fat	26.15	38.62	46.30
Crude fibre	44.10	35.20	31.30
Crude ash	66.00	57.90	55.30
Starch	340.34	398.02	416.55
Total carbohydrates	42.92	59.85	52.20
Calcium	9.68	9.41	9.19
Phosphorus	7.59	7.38	7.21
Sodium	1.82	1.80	1.41
Methionine	3.92	4.56	4.23
Lysine	10.05	13.39	12.05
Cystine	2.98	2.86	2.60
Metabolizable energy*	11.78 MJ	12.42 MJ	12.71 MJ

* – Calculation based on Kirchgessner and Roth (20).
Ingredient composition of the experimental diets ($g.kg^{-1}$ diet) (starter/ grower/ finisher): wheat 150/160/150; maize 453/482/495; soybean meal 300/221/213; soya 30/59/57; sunflower meal 20/20/20; rape-seed oil 5/17/28; calcium carbonate 13/14.5/14; sodium chloride 4/4/4. Minerals ($mg.kg^{-1}$ diet): Mg 100, Mn 80, Zn 60, Fe 60, Cu 5, Co 0.2, J 1, Se 0.15. Vitamins ($mg.kg^{-1}$ diet): retinol (A) 2.4, cholecalciferol (D3) 30, tocopherol (E) 20, menadione (K3) 4, thiamine (B1) 6, riboflavin (B2) 3, pyridoxine (B6) 5, cobalamin (B12) 0.02, folic acid (B9) 1, D-biotin (B7) 0.05, calcium D-panthothenate (B5) 10, niacin (B3) 25. Anticoccidial agent in diets: starter – Robenidin, grower – Narasin, finisher – absent

Table 2. Apparent assimilable mass coefficient of crude protein and digestibility of crude fibre and ash (mean ± SD)

Age (day)	Group	AMC_N	Crude fibre (dc)	Ash (dc)
16	Control	$0.25^{a} \pm 0.023$	$28.48^{a} \pm 0.712$	$39.04^{a} \pm 2.467$
(n = 8)	Oregano	$0.19^{c} \pm 0.026$	$46.32^{d} \pm 0.787$	$40.94^{a} \pm 1.556$
29	Control	$0.28^{a} \pm 0.019$	$33.99^{a} \pm 0.51$	$55.8^{a} \pm 3.471$
(n = 8)	Oregano	$0.19^{b} \pm 0.027$	$50.58^{d} \pm 1.518$	$48.82^{a} \pm 2.289$
42	Control	$0.25^{a} \pm 0.018$	$33.6^{a} \pm 0.874$	$46.18^{a} \pm 3.048$
(n = 8)	Oregano	$0.20^{b} \pm 0.019$	$47.18^{c} \pm 1.18$	$45.84^{a} \pm 3.897$

AMC_N – apparent assimilable mass coefficient of crude protein corrected for protein catabolism; dc – digestibility coefficient; means with different superscript letters differ significantly: $^{a, b}$ – $P < 0.05$, $^{a, c}$ – $P < 0.01$, $^{a, d}$ – $P < 0.001$

Table 3. Digestive enzyme activities in the chyme of the jejunum of broiler chickens (mean ± SD)

Age (day)	Group	Amylolytic (glucose) [$mmol.l^{-1}.min^{-1}$]	Cellulolytic activity (glucose) [$mmol.l^{-1}.min^{-1}$]	Proteolytic activity (azocasein) [$mg.ml^{-1}.min^{-1}$]
16	Control	$0.17^{a} \pm 0.011$	$0.11^{a} \pm 0.025$	$0.77^{a} \pm 0.063$
(n = 16)	Oregano	$0.17^{a} \pm 0.062$	$0.12^{a} \pm 0.017$	$0.36^{c} \pm 0.038$
29	Control	$0.10^{a} \pm 0.013$	$0.07^{a} \pm 0.015$	$0.41^{a} \pm 0.048$
(n = 16)	Oregano	$0.14^{d} \pm 0.013$	$0.13^{d} \pm 0.03$	$0.40^{a} \pm 0.055$
42	Control	$0.14^{a} \pm 0.017$	$0.16^{a} \pm 0.026$	$0.60^{a} \pm 0.066$
(n = 16)	Oregano	$0.13^{a} \pm 0.021$	$0.21^{c} \pm 0.025$	$0.56^{a} \pm 0.085$

Means with different superscript letters differ significantly: ^{a,c} – $P < 0.01$, ^{a,d} – $P < 0.001$

Table 4. Biochemical parameters in the serum of broiler chickens (n = 8, mean ± SD)

Age (day)	Group	Total protein [$g.l^{-1}$]	P [$mg.dl^{-1}$]	K [$mg.dl^{-1}$]	Ca [$mg.dl^{-1}$]	Mg [$mg.dl^{-1}$]
16	Control	$154.85^{a} \pm 4.94$	n. d.	n. d.	n. d.	n. d.
(n = 8)	Oregano	$154.52^{a} \pm 4.59$	n. d.	n. d.	n. d.	n. d.
29	Control	$115.08^{a} \pm 5.65$	$15.41^{a} \pm 2.98$	$19.07^{a} \pm 1.4$	$11.70^{a} \pm 3.33$	$3.09^{a} \pm 0.44$
(n = 8)	Oregano	$128.94^{b} \pm 15.68$	$15.69^{a} \pm 2.3$	$18.24^{a} \pm 3.06$	$9.73^{a} \pm 2.73$	$2.48^{a} \pm 0.52$
42	Control	$176.88^{a} \pm 12.18$	$14.54^{a} \pm 2.74$	$20.32^{a} \pm 2.72$	$9.03^{a} \pm 2.52$	$2.68^{a} \pm 0.48$
(n = 8)	Oregano	$174.24^{a} \pm 5.43$	$13.16^{a} \pm 1.16$	$21.39^{a} \pm 2.01$	$15.14^{d} \pm 0.53$	$3.48^{c} \pm 0.18$

n. d. – not determined; Means with different superscript letters differ significantly: $^{a, b}$ – $P < 0.05$, $^{a, c}$ – $P < 0.01$, $^{a, d}$ – $P < 0.001$

Table 5. Microbial counts in the caecal chyme of broiler chickens [CFU. g^{-1} wet digesta]

Age [day]	Group	*Lactobacillus* spp.	*Enterococcus* spp.	*E. coli*
16	Control	$1.0^{a} \times 10^{8}$	$0.4^{a} \times 10^{6}$	$1.1^{a} \times 10^{7}$
(n = 6)	Oregano	$1.6^{a} \times 10^{8}$	$2.1^{a} \times 10^{6}$	$6.0^{a} \times 10^{6}$
29	Control	$2.1^{a} \times 10^{8}$	$1.6^{a} \times 10^{6}$	$1.4^{a} \times 10^{7}$
(n = 6)	Oregano	$7.1^{a} \times 10^{8}$	$7.5^{a} \times 10^{6}$	$3.1^{b} \times 10^{6}$
42	Control	$3.0^{a} \times 10^{8}$	$7.9^{a} \times 10^{6}$	$2.0^{a} \times 10^{7}$
(n = 6)	Oregano	$1.4^{a} \times 10^{8}$	$1.5^{a} \times 10^{6}$	$2.3^{b} \times 10^{6}$

Means with different superscript letters are significantly different
$^{a, b}$ – $P < 0.05$, CFU – colony forming units

Three different methods are usually used for the measurement of nutrient digestibility in broilers. The first one, is the faecal digestibility measurement in the intact birds. The second one, is the faecal digestibility analysis using caecectomized birds (caeca are removed to decrease the influence of the hindgut fermentation on the nutrient digestibility). The last method, is an ileal digestibility when digesta are obtained at the terminal ileum (18). However, the effects of the essential oils on the digestibility of the crude protein, determined as the apparent assimilable mass coefficient of crude protein corrected for protein catabolism, was contrary. The feed additive used diminished this parameter. The values were in agreement with the proteolytic activities in the jejunum. The definition of digestibility is the difference between the intake and the faecal excretion. Excreta collected in the faecal digestibility assay contain not only the proteins from the faeces but also those excreted with the urine. Although Terpstra (27) showed that renal amino acid excretion is negligible.

The significant increase of amylolytic and cellulolytic activity, as well as the decrease of proteolytic activity in the jejunum, were observed in several sampling periods.

The enhancement of amylolytic activity, was in agreement with the results of Jang *et al.* (17). They observed that the activities of pancreatic trypsin and the total activities of pancreatic alpha-amylase and intestinal maltase were significantly elevated in broiler chickens fed a blend of essential oils extracted from herbs. According to Jamdar and Harikumar (16) the chicken intestine possesses proteolytic activities (cathepsin B, D, H, L, aminopeptidases and alkaline proteases). Kinetic studies employing specific inhibitors indicated that the degradation (90–94 %) of proteins at acidic pH is governed largely by pepstatin sensitive proteases. However, the cellulolytic activity is not an endogenous activity of the gastrointestinal tract of poultry. Therefore, the significant increase of cellulolytic activities in the jejunum of experimental chickens probably resulted from the favourable stimulation of a limited population of bacteria for this enzymatic activity with the essential oil used. The exact mechanism of the stimulation of cellulolytic activity of bacterial microflora in the jejunum with the oregano essential oil is not clear. Therefore, further experimental studies are needed. A wide variety of different metabolic activities due to bacteria can be demonstrated in the chicken gut. For example, as a result of bacterial metabolic activity, bacterial enzymes acting on cellulose (14) are present in the gut. While most of the fibre digestion probably occurs in the caecum, there is some evidence that fibre digestion occurs anterior to the caecum (12). For most birds, it has been suggested that little cellulose enters the caeca (30). In the chicken on a commercial diet, there is little evidence of fibre digestion but in chicks on high-fibre diets it can occur (14). According to Basmacioglu *et al.* (2) the supplementation of feed enzymes with essential oil in the diets of broiler chickens is likely more effective in regards to performance, nutrient digestibility, enzyme activities and the immune system.

Our experiment demonstrated the positive increase of the total protein level in the serum after the intake of the oregano essential oil.

Our data are in agreement with that of previous findings (1). The aqueous crude extracts of 5 plants (*Alchornea cordifolia*, *Emidosolus acontifolius*, *Phyllanthus amarus*, *Ph. Muellerianus*, *Segurinega virosa*) were used in a study with rats. There was observed a significant increase in the levels of total protein and albumin in the serum.

The dietary intake of the oregano essential oil favourably influenced the levels of calcium and magnesium in the serum of experimental animals in the finisher period.

Similarly, the increased level of minerals may be the consequence of increased availability of fatty acids for incorporation into biliary micelles and, thus improved fat digestibility (33).

According to Mahoney *et al.* (23), there is a connection between the serum level of Ca and Mg as well as

the thyroid gland function. Serum Ca diminished only when dietary Mg was 250 ppm or less and very young chicks fed a Mg-deficient diet had lower serum 3,5,3´-triiodthyronine (T3). The growth and the feed intake decreased progressively as the deficiency of Mg became more severe.

According to our observations reported here, the *in vivo* experiments demonstrated a significant decrease of the *E. coli* population in the caecal chyme of broiler chickens after the intake of the oregano essential oil.

The results of J a n g *et al.* (17) showed that the dietary addition of a blend of essential oils extracted from herbs, decreased *E. coli* population in ileo-caecal digesta. They demonstrated that the CFU of lactobacilli were unaffected by dietary supplementation of either essential oils or antibiotics. Generally, the essential oils with the strongest antibacterial activities against foodborne pathogens contain a high percentage of phenolic compounds such as carvacrol (6). Carvacrol-containing essential oils are biostatic and /or biocidal against many bacteria, yeast, and fungi in laboratory media (5, 21).

Results obtained in the *in vitro* study (24) with the agar spot tests and agar diffusion paper disc test demonstrated the higher antimicrobial activity of the essential oil from oregano than from sage against the bacterial strains tested (*E. coli* – pig isolate, haemolytic, K antigen positive; *Salmonella enterica* var. *enteritidis* – pig isolate, *Enterococcus faecium* M-74 – probiotic strain). The promising fact of essential oils was that oregano essential oil inhibited the growth of pathogenic *E. coli* isolated from the pig intestine.

CONCLUSIONS

The dietary supplementation of broiler chickens with the oregano essential oil: beneficially increased; the amylolytic and cellulolytic enzyme activities in the chyme of the jejunum, the digestibility of crude fibre, the concentration of total protein, calcium and magnesium in the serum, but decreased the counts of *E. coli* in the content of the caecum.

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