Effect of feeding treated peat as a supplement to newborn piglets on the growth, health status and occurrence of conditionally pathogenic mycobacteria

M. Trckova[1], Z. Zraly[1], P. Bejcek[2], L. Matlova[1], V. Beran[1], A. Horvathova[1], M. Faldyna[1], M. Moravkova[1], J.E. Shitaye[1,3], J. Svobodová[4], I. Pavlik[1]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]Bohemia Vitae, Jindrichuv Hradec, Czech Republic
[3]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[4]Regional Institute of Public Health, Brno, Czech Republic

ABSTRACT: The first purpose of this experiment was to investigate the effect of *ad libitum* feeding of peat as a supplement to piglets from the age of five days up 23 days of age on their growth performance and health status. The second purpose was to assess the risk of the occurrence of conditionally pathogenic mycobacteria (CPM) in peat treated with ionizing radiation (Group PI) or per acetic acid (Group PP) and fed as a supplement to piglets. In respective experimental periods (at the age of 4, 23, 41 and 67 days), no significant differences in the average body weight between control group (C) and experimental Groups PI and PP were detected. Levels of selected biochemical (total protein, albumin, glucose, cholesterol, Ca, P, Fe and I) and haematological (erythrocytes, leukocytes and immunoglobulin – Ig) parameters of the health status of the piglets from all three Groups C, PI and PP were comparable at the age of 41 and 67 days. Mycobacteria were detected by culture in one diet sample (*Mycobacterium intracellulare*), in all 10 peat samples (7 *M. a. hominissuis* isolates, 2 *M. intracellulare* isolates and 1 *M. xenopi* isolate) and in 4 samples of biofilm from the drinking water pipeline system in the stables (*M. xenopi*, *M. a. hominissuis*, *M. gordonae* and *Mycobacterium* sp., one isolate in each). In 15 slaughtered pigs (at 67 days of age), no gross lesions that would give evidence of tuberculosis were found either in lymph nodes or parenchymatous organs. In Group C, mycobacteria were detected in tissues from two piglets (*Mycobacterium* sp. and *M. a. hominissuis*), Group PI in four piglets (*M. a. hominissuis*) and in Group PP in all five piglets (*Mycobacterium* sp., *M. a. hominissuis, M. terrae* and *M. intracellulare).* High positivity for CPM in both types of treated peat caused disseminated infection of the digestive tract of piglets from Groups PI and PP. Based on these results, feeding peat treated with ionisation or per acetic acid may be viewed as risky.

Keywords: pig; humate; body weight gain, blood biochemistry; tuberculosis; *Mycobacterium avium* complex; zoonosis; food safety; mycobacteria distribution

In association with the banned use of antibiotic stimulators in farm animal nutrition, many farmers search for alternatives to minimise health problems, potential losses and decreased performance in herds. According to our experience, a number of farmers try to use various non-traditional supplements in animal nutrition such as peat (Matlova et al., 2005; Trckova et al., 2005), kaolin, zeolites or bentonites (Matlova et al., 2004; Trckova et al., 2004) for economic reasons.

The prerequisite for beneficial effects of peat on health status and performance of animals is, above

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all, high contents of humic substances: humic, fulvic and ulmic acids and humins (Trckova et al., 2005). Peat contains up to 60 to 80% of these substances according to its humification stage (Andriesse, 1988; Lotosh, 1991; Anonymous, 2002). Positive effects of humic substances on the performance and health status of animals have been documented in various studies (Stepchenko et al., 1991; Zhorina and Stepchenko, 1991; Fuchs et al., 1995; Bailey et al., 1996; Kocabagli et al., 2002; Yoruk et al., 2004). Humic substances exert the following beneficial effects on organisms:

(1) improvement of particular nutrient uptake by an organism (Visser, 1973; Fuchs et al., 1990, 1995; Stepchenko et al., 1991; Anonymous, 2002; Kocabagli et al., 2002);

(2) due to this chelating capability, peat detoxifies organisms from a number of toxic agents such as heavy metals, moulds and toxins produced by pathogenic bacteria (Klocking, 1980; Kuhnert et al., 1982; Herzig et al., 1994);

(3) stimulation of digestion (Kuhnert et al., 1982, 1991; Beer and Lukanov, 1998);

(4) considerable anti-inflammatory and antiviral effects (Schultz, 1965; Klocking and Sprossig, 1972; Kuhnert et al., 1982, 1991; Sydow et al., 1986; Kuhnert et al., 1991; Jankowski et al., 1993);

(5) increased activity of the immune system (Riede et al., 1991).

Available literature concerning long-term feeding of original natural peat as a supplement is scarce. Most studies used peat in a form of various preparations, extracts or isolated humic substances. In our previous experiment with the short-term feeding of peat to pigs during the fattening period did not show a significant beneficial effect on growth and performance of animals (Trckova et al., 2006).

Feeding peat as a supplement in a natural form may adversely affect organisms. An increased occurrence of tuberculous lesions in head and intestinal lymph nodes was found in farm animal herds, above all in pigs, fed peat as a supplement from birth onwards (Pavlik et al., 2003, 2005). These were caused by mycobacteria contamination of fully mineralised "black" peat stored under unfavourable conditions (Kazda, 2000; Matlova et al., 2003, 2005). Secondary contamination of peat with conditionally pathogenic mycobacteria (CPM) mainly represented the highest potential risk during peat feeding (Matlova et al., 2003, 2005; Trckova et al., 2006). Counts of contaminating CPM have been markedly reduced on farms by disinfection of natural peat with per acetic acid or by purchase of ionised commercial peat packaged in sealed polyethylene bags (non-published data).

The purpose of the first section of the present study was to assess the effect of feeding treated peat to piglets during the pre-weaning period of growth under conditions specific by the pig industry. The second purpose of the present study was to assess the health risk of CPM occurrence in commercial peat fed as a supplement, treated with ionising radiation, in natural peat treated with per acetic acid, in the diet and the drinking water.

MATERIAL AND METHODS

Experimental animals

Fifteen litters of piglets on farm SNN were included in the study immediately after birth. The animals were allocated into three groups, five litters in each. Respective litters were housed separately, together with their dams, until weaning of the piglets ended. After weaning, piglets from five litters of each experimental group were housed together in three pens:

Group C – control group of piglets not fed peat as a supplement

Group PI – experimental group of piglets fed commercial peat (Baby Vig, INOBIO, Romilly Sur Andelle) treated with ionising radiation

Group PP – experimental group of piglets fed natural peat disinfected with per acetic acid

Feeding of pigs

Piglets from the Groups C, PI and PP were fed a conventional diet *ad libitum* intended for early weaned piglets (COS I) from the age of 7 days. The diet contained extruded cereals, wheat, barley, whey powder, soy-protein concentrate, fish meal, powder yeasts, soy oil and premix of supplements. Piglets in Group PI were fed commercial peat treated with ionising radiation as a supplement *ad libitum* from the age of 5 days to the end of weaning at the age of 23 days. Piglets of Group PP were fed natural peat treated with per acetic acid *ad libitum* at the same age.

After weaning, piglets from all three Groups C, PI and PP were fed the COS II diet. Both diets COS I and COS II were analysed for essential nutrients and metabolisable energy (Anonymous, 2000).

Part 1

Monitoring of the health status and growth of piglets. Health status was monitored daily by observation; occasional morbidity and mortality were recorded. Live piglet weight was taken four times with an accuracy of 0.1 kg:

(1) prior to the beginning of peat feeding at the age of 4 days

(2) at the end of weaning at the age of 23 days

(3) during pre-fattening at the age of 41 days

(4) after completing the experiment at the age of 67 days

Blood and blood plasma analyses. Blood samples were collected from the *vena cava cranialis* of five pigs from each group for biochemical analysis at the age of 41 and 67 days old.

Erythrocyte and leukocyte cell counts, differential leukocyte count and Ig levels were assessed. The total leukocyte count was determined using the Digicell 500 cell counter (Contraves AG, Switzerland). The differential leukocyte count was calculated from the values obtained by the evaluation of blood smears stained with May-Grünwald and Giemsa-Romanowski. Total serum Ig concentrations were determined spectrophoto-metrically by measuring the turbidity resulting from the addition of zinc sulphate to serum. The procedure was a modification of the method described by McEwan et al. (1970). Twenty-five microlitres of serum were mixed with 1.5 ml of 0.7mM solution of zinc sulphate, pH 5.8 and the resulting turbidity was measured at 590 nm after 2 h at room temperature. A blank (serum diluted in phosphate-buffered saline only) was run with each serum sample. The concentration of total Ig in the tested sera was calculated from the calibration curve prepared using four dilutions of standard serum (Precinorm Protein, Roche, France).

Total protein, albumin, glucose, cholesterol, calcium, phosphorus, iron and iodine blood plasma levels were determined by spectrophotometry using Bio-La-Tests (PLIVA-Lachema Brno a.s., Czech Republic). Iodine levels were detected by the alkaline incineration method using spectrophotometry according to Sandell-Kolthoff (Bednar et al., 1964).

Part 2

Microscopic examination for the presence of acid-fast rods. Diets (10 samples), commercial (5 samples) and natural peat (5 samples), biofilm from the drinking water pipeline system in the stables for weaned piglets (5 samples) and tissues from 15 slaughtered piglets (300 samples), at the end of experimental period at 67 days (5 piglets from each groups were slaughtered), were examined for the presence of acid-fast rods (AFR) after Ziehl-Neelsen (ZN) staining. One hundred microscopic fields were examined in each sample by light microscopy under magnifying 1 000× using immersion oil.

Culture examination for the presence of mycobacteria. Samples of tissues, diets, peat and biofilm from the drinking water pipeline system (five swabs were collected after unscrewing the watering devices for piglets) were homogenised in water, decontaminated by the HCl/NaOH method and cultured at 25 and 37°C on three media: solid medium according to Stonebrink and Herrold and on liquid medium by Sula. The growth of mycobacteria was monitored every second week for two months (Fischer et al., 2000).

Identification of mycobacterial isolates. For species identification, 59 mycobacterial isolates from diets, peat, drinking water and tissues were examined by the PCR method for the detection of specific fragments IS*901* and IS*1245* (Bartos et al., 2006). The isolates lacking these two insertion sequences (IS) were examined by selected biochemical tests: test of growth speed, test of photochromogeneity, growth at various temperatures, growth on medium containing 5% NaCl, nitrate test, catalase thermoresistance test, test for Tween 80 hydrolysis, arylsulphatase test and telluride test (Wayne and Kubica, 1986).

Statistical assessment

The results obtained were processed and verified by the statistical and graphic software STAT Plus (Matouskova et al., 1992).

RESULTS

Part 1

Composition of diets, commercial and natural peat. The results of analyses of nutrient and metabolised energy contents in diets and peat are presented in Table 1. The levels of all parameters

Table 1. Nutrient and metabolisable energy contents in animal diets and peat and the presence of mycobacteria

Parameter	Feeding concentrate		Peat		Water biofilm
	COS I	COS II	PI	PP	
Nutrient (g/kg) and metabolisable energy (MJ) contents					
Dry matter	906.5	894.5	391.6	519.5	nt
Crude protein	204.4	191.6	59.1	79.6	nt
Lipids	41.6	34.5	6.8	8.4	nt
Fibre	25.2	29.5	41.1	44.9	nt
Ash	68.4	58.8	40.4	75.0	nt
Nitrogen – free extract	566.9	580.1	244.2	311.6	nt
Organic matter	838.1	835.7	351.2	444.5	nt
Metabolisable energy	14.0	13.6	4.7	6.1	nt
Culture detection of mycobacteria (species identification)					
Sample No. 1	1 (*MI*)	0	1 (*MAH*)	1 (*MI*)	1 (*MX*)
Sample No. 2	0	0	1 (*MAH*)	1 (*MI*)	1 (*MAH*)
Sample No. 3	0	0	1 (*MAH*)	1 (*MAH*)	0
Sample No. 4	0	0	1 *(MX)*	1 (*MAH*)	1 (*MG*)
Sample No. 5	0	0	1 (*MAH*)	1 (*MAH*)	1 (*M.* sp.)
Mycobacterial isolates (total)	1	0	5	5	4
M. intracellulare (3)	1	0	0	2	0
M. a. hominissuis (8)	0	0	4	3	1
M. xenopi (2*)*	0	0	1	0	1
M. gordonae (1*)*	0	0	0	0	1
Mycobacterium sp. (1)	0	0	0	0	1

COS I = diet for early weaned piglets I
COS II = diet for early weaned piglets II
PI = commercial peat treated with ionising radiation
PP = natural peat treated with per acetic acid
MI = Mycobacterium intracellulare
MAH = M. a. hominissuis
MX = M. xenopi
MG = M. gordonae
M. sp. = non-identified mycobacterial species, IS*901* and IS*1245* PCR negative
nt = not tested

were higher in natural peat in comparison with commercial peat.

Weight and health status of piglets. No significant differences in the average weight of piglets from control Group C and experimental Groups PI and PP were found in the respective periods of the experiment (4, 23, 41 and 67 days of age; Table 2). Average weight gains in Groups C, PI and PP were 0.31 kg, 0.32 kg and 0.34 kg, respectively. The health status of animals was monitored by observation; no serious health problems were detected. On day 67 of the experiment, the following animals were excluded due to weakness (dwarfism) or developmental disabilities of locomotive organs: one piglet before weaning from Group C, one piglet after weaning and two piglets before weaning from Group PI and three piglets after weaning from Group PP.

Table 2. Average body weight of pigs (kg)

Day		Group		
		C	PI	PP
4	$\bar{x}$	2.45	2.38	2.36
	SD	0.45	0.16	0.25
23	$\bar{x}$	6.78	6.90	7.25
	SD	1.10	0.41	0.63
41	$\bar{x}$	10.20	11.90	11.30
	SD	1.83	2.07	1.75
67	$\bar{x}$	22.10	22.70	23.70
	SD	2.10	1.94	1.53

C = control group without feeding peat as a supplement
PI = experimental group fed commercial peat sterilised by ionising radiation as a supplement
PP = experimental group fed natural peat treated with per acetic acid as a supplement

Blood and blood plasma analyses. Selected biochemical parameters of health status of piglets in all three Groups C, PI and PP were comparable at the age of 41 and 67 days. Blood plasma glucose levels in piglets from Group PP were significantly higher ($P < 0.05$) on day 41 in comparison with Group PI. Differences in erythrocyte and leukocyte counts and Ig levels between groups were non-significant (Table 3).

Part 2

Analysis of diets, peat and biofilms from water pipelines for the presence of mycobacteria. No AFR were detected by microscopy in 10 samples of diets COS I and COS II or in 10 peat samples. Occasional AFR were observed in two of the five biofilm samples by microscopy. Mycobacteria were detected by culture in one sample of the COS I diet (*M. intracellulare*), in all of the 10 peat samples (7 *M. a. hominissuis* isolates, 2 *M. intracellulare* isolates and 1 *M. xenopi* isolate) and in four biofilm samples: one isolate of *M. xenopi*, *M. a. hominissuis*, *M. gordonae* and *Mycobacterium* sp. in each sample (Table 1).

Veterinary-meat inspection after slaughter of pigs. By veterinary-meat inspection of all 15 pigs, no gross lesions that would give evidence of tuberculosis were detected either in lymph nodes or parenchymatous organs.

Distribution of mycobacterial infection in the organisms of 15 piglets. No AFR were found in any tissue sample by microscopy after ZN staining. Mycobacteria were detected in two piglets from Group C: from the submandibular lymph node (*Mycobacterium* sp.) in piglet No. 1 and from the ileal lymph node (*M. a. hominissuis*) in piglet No. 2. In piglets from Group PI, mycobacteria were isolated from 4 piglets: *M. a. hominissuis* was detected in one tissue sample from piglet No. 7 and 10; two and three tissue samples infected with *M. a. hominissuis* were found in piglet No. 9 and No. 8, respectively. In Group PP, mycobacteria were isolated from all piglets in 3 to 10 tissue samples with prevailing detection of *M. a. hominissuis* (Table 4).

DISCUSSION

Part 1

Composition of diets, commercial and natural peat. Chemical analysis of the diets COS I and COS II showed that supplementation of the piglets with metabolisable energy was sufficient. The contents of N-substances in diet COS I was slightly lower (204.4 g/kg) than usually recommended: 245.0 g/kg for piglets with a live weight of 4 to 7 kg (Simecek et al., 1993). Availability of nutrients, present in peat, is very low (Enueme et al., 1987, 1990).

Analysis of nutrient content in peat samples showed differences in the content of N-substances, lipids, ash and metabolisable energy, which confirms previously described differences in peat composition from various sources (Bozkurt et al., 2001; Pereverzev, 2005). The chemistry of peat results from a combination of the chemical composition of mire plants and microorganisms, the soil water quality and the secondary substances produced during the decomposition process (Enueme et al., 1987; Kazda, 2000; Wise et al., 2000; Bozkurt et al., 2001).

The organic matter composition of peat depends on the degree of humification of plant residues. During humification, stable humic substances are formed by microbial transformation of non-humic substances such as hemicellulose, cellulose, lignin, pectins, bitumens, waxes, resins, nitrogenous materials, lipids, amino acids, non-saturated and saturated fatty acids, organic sulphur, various types of carbohydrates, starch compounds, ethereal oils, balsam, bioterin and tannic acid (Andriesse, 1988;

Table 3. Erythrocyte and leukocyte counts, blood immunoglobulin levels and selected biochemical parameters of blood plasma in pigs (n = 5)

Parameter		Group C		Group PI		Group PP	
		day 41	day 67	day 41	day 67	day 41	day 67
Blood							
Leukocytes × 10^6/1 l	$\bar{x}$	12 980	14 540	14 280	15 960	14 180	12 580
	SD	3 560	2 513	3 223	2 741	6 339	2 563
Erythrocytes × 10^9/1 ml	$\bar{x}$	4.87	5.23	5.37	5.41	4.57	5.55
	SD	0.88	0.33	0.94	0.17	1.80	0.53
Lymphocytes (%)	$\bar{x}$	54.9	62.80	49.50	71.50	52.00	64.20
	SD	12.7	9.28	6.59	9.44	11.10	6.12
Neutrophils (%)	$\bar{x}$	39.7	31.40	46.20	23.20	37.10	31.40
	SD	12.2	10.40	5.95	9.26	8.84	6.19
Monocytes %	$\bar{x}$	3.46	3.20	2.40	3.70	2.40	2.78
	SD	1.43	0.84	0.65	1.40	1.02	0.98
Eosinophils (%)	$\bar{x}$	1.96	1.50	1.70	1.40	2.20	1.50
	SD	1.42	0.79	1.68	0.55	1.60	1.12
Basophils (%)	$\bar{x}$	0	0.70	0.30	0.10	0.30	0
	SD	0	1.04	0.67	0.22	0.45	0
Immunoglobulin (mg/ml)	$\bar{x}$	11.30	12.50	12.00	12.80	12.80	14.60
	SD	1.00	2.73	1.19	1.69	1.85	3.54
Blood plasma							
Total protein (g/l)	$\bar{x}$	48.47	50.35	49.16	47.75	48.68	53.03
	SD	1.58	7.13	3.47	4.93	2.55	2.85
Albumin (g/l)	$\bar{x}$	31.03	29.19	33.41	31.02	33.19	28.06
	SD	4.28	4.18	3.91	4.04	3.78	7.39
Glucose (g/l)	$\bar{x}$	5.42	3.21	5.15*	4.55	6.11*	3.44
	SD	0.43	2.27	0.55	0.81	0.59	0.76
Cholesterol (mmol/l)	$\bar{x}$	1.80	2.53	1.56	2.46	1.59	2.18
	SD	0.30	0.44	0.41	0.96	0.31	0.37
Ca (mmol/l)	$\bar{x}$	3.07	2.72	2.94	2.47	2.89	2.61
	SD	0.15	0.40	0.23	0.33	0.36	0.37
P (mmol/l)	$\bar{x}$	3.55	3.58	3.50	3.35	3.43	3.66
	SD	0.07	0.20	0.37	0.18	0.26	0.21
Fe (mmol/l)	$\bar{x}$	38.64	48.13	47.45	43.79	40.20	46.42
	SD	3.60	4.05	8.89	17.50	6.71	8.45
I (mmol/l)	$\bar{x}$	118.52	nt	94.91	nt	137.35	nt
	SD	16.64		17.98		46.04	

C = control group without feeding peat as a supplement
PI = experimental group fed commercial peat sterilised by ionising radiation as a supplement
PP = experimental group fed natural peat treated with per acetic acid as a supplement
nt = not tested

Table 4. Mycobacteria isolation (CFU counts) from 15 pigs

Tissue samples	Group C: pig No.					Positive	Group PI: pig No.					Positive	Group PP: pig No.					Positive
	1	2	3	4	5		6	7	8	9	10		11	12	13	14	15	
Submandibular ln	1[a]	0	0	0	0	1	0	0	0	10[b]	0	1	50[b]	7[a]	0	15[b]	14[b]	4
Retropharyngeal ln	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung ln	0	0	0	0	0	0	0	4[b]	7[b]	0	0	2	0	7[b]	0	1[b]	0	2
Tracheobronchial ln	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver tissue	0	0	0	0	0	0	0	0	0	0	0	0	10[b]	0	0	0	0	1
Liver ln	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	P[c]	0	1
Spleen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Duodenal ln	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Duodenal m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jejunal ln (beginning)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3[b]	1
Jejunal m (beginning)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jejunal ln (middle)	0	0	0	0	0	0	0	0	0	0	0	0	30[b]	0	0	P[a]	20[b]	3
Jejunal m (middle)	0	0	0	0	0	0	0	0	30[b]	0	0	1	0	0	40[b]	1[a]	0	2
Jejunal ln (end)	0	0	0	0	0	0	0	0	0	0	0	0	50[a]	20[a]	30[d]	P[a]	50[b]	5
Jejunal m (end)	0	0	0	0	0	0	0	0	0	0	0	0	30[b]	0	0	5[d]	0	2
Ileal ln	0	20[b]	0	0	0	1	0	0	0	50[b]	10[b]	2	1[b]	6[a]	50[b]	40[b]	30[a]	5
Ileal m	0	0	0	0	0	0	0	0	0	0	0	0	0	6[b]	0	10[b]	0	2
Ileocecal valve ln	0	0	0	0	0	0	0	0	0	0	0	0	30[b]	40[b]	0	P[b]	P[a]	4
Ileocecal valve m	0	0	0	0	0	0	0	0	11[a]	0	0	1	14[b]	16[b]	0	0	10[b]	3
Inguinal ln	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isolates (Total)	1	1	0	0	0	2	0	1	3	2	1	7	8	7	3	10	7	35
Mycobacterium sp. (11)	1	0	0	0	0	1	0	0	1	0	0	1	1	3	0	3	2	9
M. a. hominissuis (30)	0	1	0	0	0	1	0	1	2	2	1	6	7	4	2	5	5	23
M. terrae (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
M. intracellulare (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2

[a]non-identified mycobacterial species
[b]*Mycobacterium avium* subsp. *hominissuis*
[c]*M. terrae*
[e]*M. intracellulare*
ln = lymph nodes
m = mucosa
C = control group without feeding peat as a supplement
PI = experimental group fed commercial peat sterilised by ionising radiation as a supplement
PP = experimental group fed natural peat treated with per acetic acid as a supplement
P = non-countable Colony Forming Units

Hruska, 1988; Riede et al., 1992; Banaszkiewicz and Drobnik, 1994; Anonymous, 2002). The organic carbon content of peat exceeds 50% of dry matter (Bozkurt et al., 2001; Pereverzev, 2005). The carbon content in peat increases with depth. Therefore, the older the peat layer, the more carbonised its organic matter regardless of its composition.

By contrast, the concentration of nitrogen varies significantly depending on the proportion of sphagnum in the peat (Pereverzev, 2005). There is generally a wide variation in mineral composition between different peat sources. Inorganic substances get into peat as earthy mineral ingredients by water, wind or as mineral substances, originally bound in plant organisms (Sanovec, 1947; Andriesse, 1988; Anonymous, 2002).

The weight and health status of piglets. Feeding peat as a supplement to piglets from 5 to 23 days of age did not result in a significant effect on the body weight gain in piglets (Table 2). The highest body weight was detected in an experimental Group PP fed natural peat as a supplement. This contrasts with control Group C, average body weight of piglets from Group PP was higher by 6.9% at the end of peat feeding (day 23) and by 7.2% at the end of the monitored period (day 67). The body weight in piglets from Group PI was higher by 1.8% (day 23) and 2.7% (day 67) in comparison with control Group C. Non-significant growth stimulation was recorded in our previous study focused on feeding peat to pigs with the body weight of 25 kg for 30 days (Trckova et al., 2006).

A stimulating effect of humic substances present in peat may have been suppressed to a certain degree by the higher fibre content in the diet for piglets fed natural, non-processed peat as a supplement. A significant stimulating effect, as described in literature, was reached only if peat was used in the form of various peat extracts, preparations and humic isolates (Stepchenko et al., 1991; Fuchs et al., 1995; Bailey et al., 1996; Kocabagli et al., 2002).

Higher effects may be obtained after long-term peat supplementation of a diet. Fuchs et al. (1995) documented a significant ($P < 0.05$) stimulating effect when peat preparation was fed to piglets from the age of 6 days for 100 days. No significant differences in the live body weight of piglets were observed during the first 28 days of peat feeding. Marked growth stimulation was recorded on days 29 to 59, which resulted in higher daily body weight gains. Despite the fact that the differences in daily body weight gains were not significant from day 60, the body weight of pigs fed peat as a supplement was higher due to faster growth up to the age of 60 days.

Likewise, Stepchenko et al. (1991) and Bailey et al. (1996) observed significantly increased body weight in broilers fed diets supplemented with humic substances only at the end of fattening. Kocabagli et al. (2002) documented increased body weight gains in the second half of a fattening period (22 and 42 days of age) when broilers were fed diets supplemented with humic substances. During the entire fattening period or only in the first half of fattening, growth stimulation was also recorded, however, as non-significant.

Blood and blood serum testing. The values of selected biochemical parameters in blood plasma from pigs from control and experimental Groups C, PI and PP ranged within reference values (Tluchor, 2001). A significantly higher ($P < 0.05$) average pig blood plasma glucose level in Group PP, in comparison with Group PI, might have been affected by different stages of satiation of respective pigs at the blood sample collection (Table 3).

Haematological parameters – total and differential counts – found in our study were in range of the physiological values described by Jain (1993). Numbers of lymphocytes prevailed over numbers of neutrophils. No alteration was found when experimental Groups C, PI and PP of animals were compared. Although, no statistically significant serum samples obtained from piglets in Group PP contained a higher concentration of total Ig. How this fact correlates with the infection of such animals with mycobacteria (as shown by bacteriology in Table 4) is not clear.

Part 2

Examination of diets, peat and biofilms from drinking water pipelines for the presence of mycobacteria. CPM were detected in all diets and water that piglets ingested *per os*: COS I diet, both types of peat fed as a supplement and biofilms from drinking water pipelines. Despite the fact that all peat samples were negative by microscopic examination according to ZN, we assumed that peat fed as a supplement was the main source of infection; almost all peat samples were positive by culture (Table 1). Due to the fact that CPM were also detected in other components (diet and biofilms from drinking water pipelines), mycobacterial infection was also detected in tissue samples from pigs in

control Group C. High positivity for CPM was also found in both of the peat samples. Hence, a disseminated infection was then found in various lymph nodes, above all in intestinal lymph nodes from animals of both experimental Groups PI and PP (Table 4).

Mycobacterial species composition in samples of the diets, peat and biofilms was comparable to the species composition of mycobacteria isolated from pig organs. CPM *M. xenopi* is usually isolated from water mains (Dauendorffer et al., 2001); that may explain what might have been the source of this species for one of the analysed diet samples (Table 1).

Veterinary meat inspection after slaughter. During veterinary meat inspection of all 15 pigs after slaughter, no gross lesions either in lymph nodes or parenchymatous organs were detected that would give evidence of tuberculosis. This status was noted due to the fact that piglets were slaughtered at the age of 67 days, which was not a sufficient time for the formation of apparent tuberculous lesions.

Distribution of mycobacterial infection in piglet organisms. No AFR were detected in any of the tissue samples by microscopy after ZN staining; this confirms relatively low amounts of mycobacteria in tissues. It follows from the present study (Table 4) that peat fed as a supplement was the most significant source of infection for piglets from both Groups PI and PP. CPM were identified in only two tissue samples from two piglets of control Group C in contrast to 4 and 5 infected piglets from Groups PI and PP, respectively.

CONCLUSIONS

Feeding peat as a supplement to piglets at the age of 5 to 23 days did not significantly affect their growth performance. Despite this fact, the average body weights of experimental Groups PI and PP animals, fed treated peat as a supplement, were by 2.7% and 7.2% higher, respectively, at the end of the monitored period (day 67), in comparison with the control Group C that was not fed peat as a supplement. Both types of peat were highly positive for CPM; that caused a disseminated mycobacterial infection in different lymph nodes, above all, those of the intestinal tract in piglets fed peat as a supplement. Based on the present results, feeding peat treated with ionising radiation or per acetic acid may be viewed as risky.

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Corresponding Author:

Prof. MVDr. Ivo Pavlik, CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 601, fax +420 541 211 229, e-mail: pavlik@vri.cz